Cortical Structure and Function in Euglenoids with Reference to Trypanosomes, Ciliates, and Dinoflagellates

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The membrane skeletal complex (cortex) of euglenoids generates and maintains cell form. In this review we summarize structural, biochemical, physiological, and molecular studies on the euglenoid membrane skeleton, focusing specifically on four principal components: the plasma membrane, a submembrane layer (epiplasm), cisternae of the endoplasmic reticulum, and microtubules. The data from euglenoids are compared with findings from representative organisms of three other protist groups: the trypanosomes, ciliates, and dinoflagellates. Although there are significant differences in cell form and phylogenetic affinities among these groups, there are also many similarities in the organization and possibly the function of their cortical components. For example, an epiplasmic (membrane skeletal) layer is widely used for adding strength and rigidity to the cell surface. The ER/alveolus/amphiesmal vesicle may function in calcium storage and regulation, and in mediating assembly of surface plates. GPI-linked variable surface antigens are characteristic of both ciliates and the unrelated trypanosomatids. Microtubules are ubiquitous, and cortices in trypanosomes may relay exclusively on microtubules and microtubule-associated proteins for maintaining cell form. Also, in agreement with previous suggestions, there is an apparent preservation of many cortical structures during cell duplication. In three of the four groups there is convincing evidence that part or all of the parental cortex persists during cytokinesis, thereby producing mosaics or chimeras consisting of both inherited and newly synthesized cortical components.

KEY WORDS: Euglenoids, Membrane skeleton, Epiplasm, Cytokinesis, Articulins, Protists, Alveolus

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I. Introduction

The euglenoids are a relatively ancient group of mostly free-living unicellular protists that probably share a common lineage with the mostly parasitic trypanosomatids (Willey *et al.*, 1988; Cavalier-Smith, 1993). Other protists, such as the ciliates and dinoflagellates, have less in common with euglenoids and trypanosomes, but evidence from structural, biochemical, immunological, and molecular studies indicates that there may be sufficient similarities among all these groups—particularly with respect to the cell surface—that might justify a comparative review of this specialized region of the cell (Fleury *et al.*, 1992; Baroin-Tourancheau *et al.*, 1992). This chapter focuses on studies of the euglenoid cell surface complex, and also considers the surface complexes of the trypanosomes, ciliates, and dinoflagellates. Specifically, we compare the organization and biochemistry of (1) the surface coat and plasma membrane, (2) the membrane skeleton (epiplasm), (3) the alveolar–endoplasmic reticulum (ER) system, and (4) the microtubules, all of which appear to have broadly similar surface-associated functions among some or all of these protists. These four systems or their apparent analogs are general features of many protists that lack a cell wall, and we argue that they not only act in concert to provide a partially conserved, peripheral framework that maintains cell form and stability, but they may also include a universal system (alveolus–ER) for the regulation of surface assembly, surface motility, or possibly other functions that are mediated by calcium, such as trichocyst discharge, enzyme activation, and surface coat release.

Many of the detailed studies on the protist surface complex have been carried out on cells that were fragmented by sonication in the presence or absence of neutral detergents. Often the surface complex remains insoluble after fractionation and it can then be separated from other cell components by zonal or isopycnic centrifugation. The fact that the heterogeneous components of the cell surface complex remain together strongly suggests that they form part of a structural and probably functional unit. This unit has been referred to as the cortex, pellicle, membrane skeleton, surface complex, or surface isolate, and it may consist of more or less repetitive elements such as the parallel longitudinal strips characteristic of the euglenoids or the "scales" of ciliates such as *Paramecium*. Repetitive elements are not readily apparent, however, on the surfaces of dinoflagellates. An interesting property of the repetitive elements in many (possibly all) of these cortices is their continuity during cytokinesis; that is, the surface complex does not disassemble and then reassemble, but new cortical components are inserted among all or some of the unaltered parental units in preprogrammed patterns, as has been documented in the euglenoids and ciliates and which

probably also occurs in trypanosomes and dinoflagellates. This concept of "cortical inheritance" has been exhaustively analyzed in ciliates (Frankel, 1989), and remains an area of considerable current interest and experimentation (Iftode *et al.,* 1989).

Because of the variation in terminology used for the surface complex by different groups working with the same or different organisms, it is difficult to adopt nomenclature applicable to all the organisms under consideration in this review. Moreover, the isolated surface complex used for most biochemical studies will not be the same as the complex *in vivo.* Soluble or loosely bound proteins will certainly be lost during fractionation and labile structures will not survive the sonication and neutral detergents frequently used during surface isolation. As a reminder of these important differences, we refer to the particulate surface complex as the "surface isolate" and to the undisturbed complex *in vivo* by the terminology generally in use for each organism.

II. Organization of the Cell Surface Complexes

The structural details of the somatic (i.e., excluding the oral apparatus, flagellum, reservoir, and canal) surface complex in each of the four groups under consideration have been well documented. The choice of the specific genera from each group was based on the availability of relatively recent or seminal studies rather than selection as a prototypical representative of that group.

A. Euglenoids

In euglenoids the continuous plasma membrane of the exposed cell surface is underlaid by a series of separate strips (membrane skeleton, epiplasm). The maximum number of strips is fairly constant for a given genus: e.g., 40 in *Euglena gracilis,* 16 in *Distigma proteus,* and 32 in *Cyclidiopsis acus.* There are fewer strips at the cell posterior, where individual strips terminate at different longitudinal positions. Each strip articulates or overlaps with its neighbor. The overall surface architecture, generally described as ridges and grooves, is the result both of the overlapping strips and the organization of the strip itself. In the simplest cases such as *E. gracilis,* the strips are basically "S"-shaped in transverse view (Fig. 1a) whereas in others such as the large species *E. ehrenbergii* and *E. oxyuris,* strips are elaborately modified, and strip projections may subtend adjacent strips (Fig. 1b). The minor fold that appears in the surface between strip overlaps in these latter

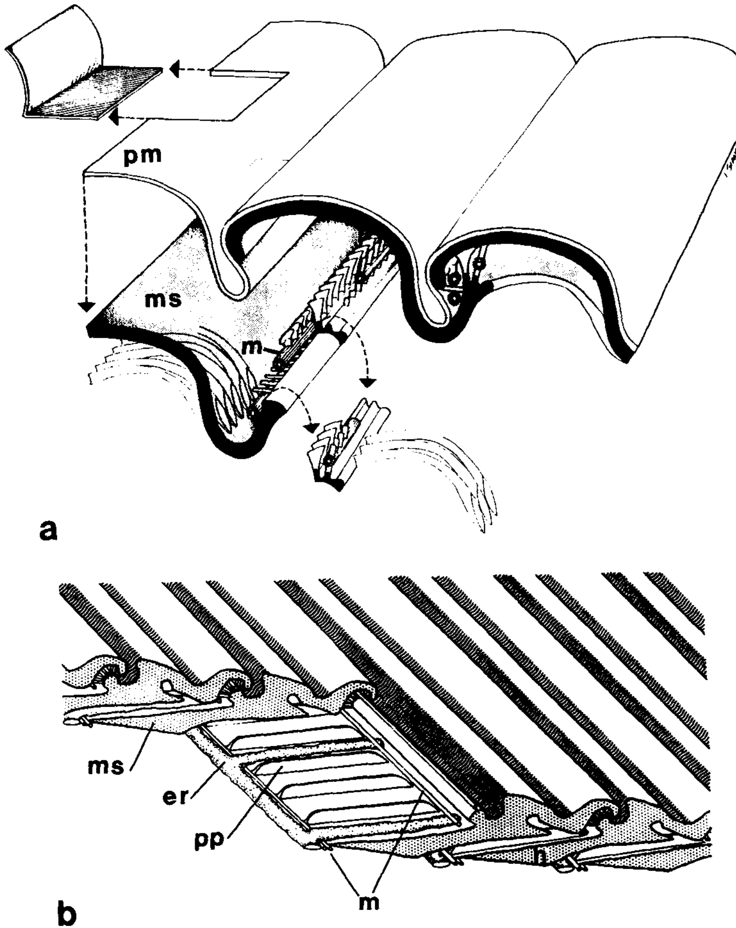

FIG. 1 (a) Schematic diagram of the surface complex of *Euglena gracilis* illustrating the arrangements of the articulating membrane skeletal layer (ms), microtubules (m), and the plasma membrane (pm). The endoplasmic reticulum is not illustrated. The membrane skeleton directly abuts the plasma membrane in euglenoids. [From Dubreuil and Bouck. Reproduced from *The Journal of Cell Biology,* 1985, Vol. 101, pp. 1884–1896 by copyright permission of The Rockefeller University Press.] (b) The surface complex in *Euglena ehrenbergii.* The membrane skeletal strips (ms) are structurally complex and overlap each other laterally for considerable distances. The plasma membrane (not shown) covers the entire surface. The endoplasmic reticulum (er) forms a complex network between the longitudinal strips and extending laterally along the transverse plate-like projections (pp). [From Suzaki and Williamson (1986a), with permission.]

species is the result of strip morphology and not strip overlap. There is generally, however, one well-defined membrane skeletal (epiplasmic) strip associated with each ridge and groove combination.

The two other major structural components of the euglenoid surface complex are microtubules and cisternae of the endoplasmic reticulum. The latter are closely associated with each strip and may be organized in two dimensions as an extensive interconnected network of tubular or flattened cisternae (Fig. 1b). Microtubules (MTs) are also associated with the strips, and their number and position are more or less characteristic for each euglenoid species. At least one MT is always located at the site of strip articulation (Figs. 1a and 1b); others may be positioned along the anterior margin of the strip, and still another set is often found at the cytoplasmic "heel" or bend in the strip. The heel MTs are the most variable in number in the surface complex of a single organism, and often are the most labile during preparation of surface isolates.

B. Trypanosomes

In trypanosomatids, both cell form and the surface complex vary according to the specific stage (e.g., procyclic, bloodstream) in the life cycle. Nonetheless, in many forms, such as *Trypanosoma,* the surface architecture has several striking parallels with the euglenoid pattern, although it is structurally less complex. The plasma membrane overlies a framework (basket, corset) which consists primarily of parallel arrays of single, cross-linked MTs (Seebeck *et al.,* 1988a).

Portions of the MT corset still attached to the plasma membrane via 100-nm links are readily prepared from whole cells. Microtubule links to the plasma membrane almost certainly exist in the euglenoids (Gallo and Schrével, 1982; Murray, 1983), but are generally obscured by the intervening amorphous, membrane skeletal layer (Fig. 1a). Microtubules of the corset of trypanosomatids are more or less evenly spaced and helically arranged; their numbers are greatest in the central expanded region of the cell and are less numerous toward the poles (Sherwin and Gull, 1989). On the anterior longitudinal surface, the corset of MTs is interrupted by a flagellar adhesion zone (FAZ), consisting of four specialized MTs and a closely adhering segment of endoplasmic reticulum. Cisternae of the ER are also associated with the MT corset (De Souza, 1986), although the association is generally not as precise as that found with the strips of many euglenoids. Absent, however, from all the trypanosomatids is a visible membrane skeletal (epiplasmic) layer, and pronounced ridges and grooves. A flagellar pocket invaginates from the surface at the pole or in a more lateral position (Vickerman and Preston, 1976). The arrangement of the flagellar pocket

and the origin of flagella in trypanosomes appear to be analogous to the canal and reservoir from which the flagella generally arise in the euglenoids.

C. Ciliates

In ciliates, a distinguishing feature of the surface complex is the alignment of cilia and basal bodies in rows or kineties. The intricate cortex of the ciliate *Paramecium* is further differentiated into cortical "epiplasmic scales" or cortical "units" arranged in longitudinal rows; each unit is centered around a ciliary basal body (Allen, 1988). The scales are surrounded laterally by a continuous "outer lattice" of fine fibers (Fig. 2a, ol), and the entire cell surface is enclosed by the plasma membrane. Just internal to the plasma membrane is a flattened cisternum known as the alveolus, and just internal to the alveolus and closely associated with the inner alveolar membrane is the amorphous or fibrous epiplasmic layer (Fig. 2a, e). Encountered further inward are various collections of MTs and kinetodesmal fibers that originate near the base of the kinetosome (basal body), and finally a network of fibers (Fig. 2a, il) that comprise the Ca^{2+}-binding, contractile infraciliary lattice (Garreau de Loubresse *et al.,* 1991). In many ciliates the alveolar sacs, the epiplasm, and the plasma membrane are each a continuum, albeit with interruptions to accommodate organelles such as the cilia, basal bodies, and trichocysts. Most other ciliates are not delineated or restricted by units or scales (e.g., Fig. 2b). The alveolar sac is structurally comparable to the endoplasmic reticulum of the euglenoids except that its position is now inserted between the plasma membrane and the epiplasm and MTs, whereas in euglenoids the ER cisternae lie below (centripetal to) the epiplasm and microtubules. The position of the alveolar sac external to the epiplasm in ciliates may be an adaptation to accommodate the

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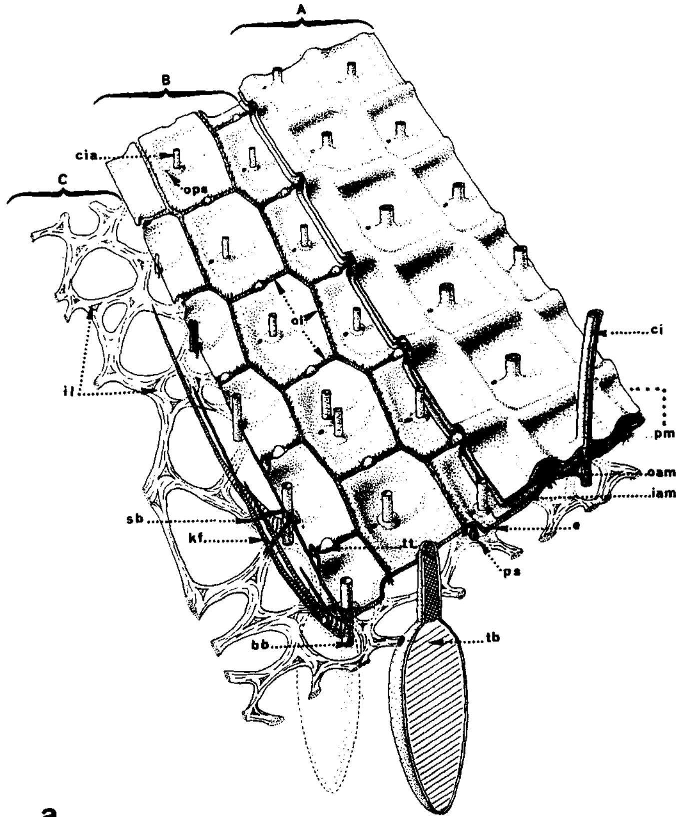
FIG. 2 (a) The complex cortex of the ciliate *Paramecium* viewed at three levels (A, B, C). The outermost level (A) consists of the plasma membrane (pm) underlaid by the inner (iam) and outer (oam) membranes of the alveolus. Cilia (ci) protrude through these membranes. Further inward (B level), the epiplasm (e) is partitioned into scales or units delineated by the fibers of the outer lattice (ol). At the innermost C level, basal bodies (bb) with their associated kinetodesmal fibers (kf) and microtubules (not shown) are surrounded by the meshwork of the inner lattice (il). [From Keryer *et al.* (1990) reprinted with permission.] (b) Cortex of the ciliate *Pseudomicrothorax dubius.* The thick epiplasm (e) lies between the microtubules and the alveolus (a). The cortex is not divided into scales, but rows of basal bodies (bb) are arranged linearly into kineties. Postciliary (p) and transverse (t) microtubules arise near the basal bodies. [From Peck (1977), reprinted with permission from the *Journal of Cell Science* and Company of Biologists, Ltd.]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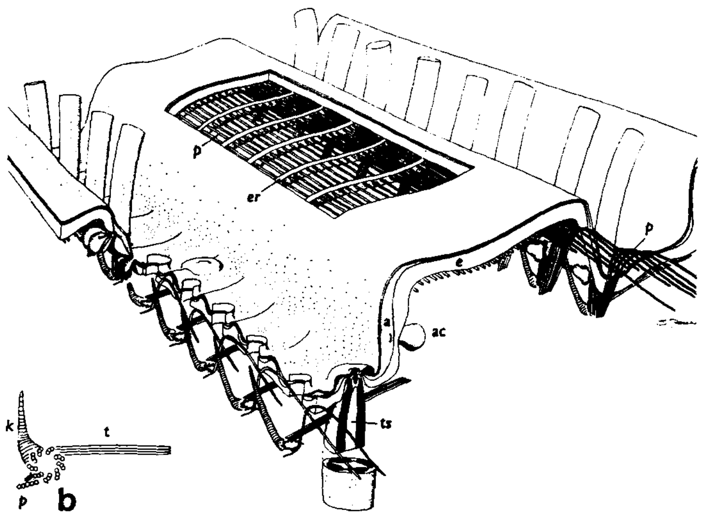
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remodeling of the cell surface similar to that proposed for dinoflagellates during the development of surface plates. In the ciliate *Euplotes,* for example, surface plates are deposited within the alveolar sacs as the alveolus is partitioned into discrete polygonal sectors.

D. Dinoflagellates

Dinoflagellates are thought to be closely related to the ciliates (Lee and Kugrens, 1992) but the cortical pattern is not divided into the regular scales or kineties (rows of basal bodies and attendant fibers) that characterize some ciliates. In dinoflagellates the cortex consists of a multiple membranous cell covering (amphiesma) subtended by MTs. The amphiesma includes a plasma membrane to which is fused a single layer of amphiesmal vesicles. In some cells a layer of epiplasm is assembled between the amphiesmal vesicles and the cortical MTs (Cachon and Cachon, 1974; Höhfeld and Melkonian, 1992). In armored (thecate) cells, the amphiesmal vesicle contains a thick, ornamented cellulosic plate, whereas in athecate (naked) cells the vesicle encompasses no formed structures. Thecate cells are generally rigid in comparison to athecate cells, which may undergo dynamic shape changes. As part of the cell cycle in some dinoflagellates, most of the amphiesmal vesicle is shed (via ecdysis), and the epiplasmic layer thickens to strengthen the cell surface; the inner amphiesmal vesicle membrane then becomes the new plasma membrane.

The structural studies summarized here suggest that there may be significant similarities between the organization of the cortex of euglenoids and that of at least some members of three other groups of protists. For example, in addition to the universal plasma membrane, these commonalities include an alveolar/ER-like component, an epiplasmic layer, and microtubules. Although there is significant variation among different organisms within each group, the basic cortical structures can generally be recognized. In the following sections these four cortical components are examined in more detail to assess whether these structural similarities extend to the biochemical and functional levels as well.

III. Biochemistry, Physiology, and Molecular Biology of the Surface Complex

A. Plasma Membrane and Surface Coats

1. Euglenoids

"Mucilage" secretion reportedly occurs to some extent in all euglenoid flagellates (Leedale, 1967), but the biochemistry of extracellular carbohy-

drates has not been extensively analyzed in this group. Certain euglenoids such as *Trachelemonas* develop complex envelopes in which mucilaginous secretions become impregnated with manganese and/or iron. The size, ornamentation, shape, and collar (through which the locomotory flagellum extends) characteristics are species specific. The cell is initially naked and either secretes a mucilaginous "skin" that is later lost (Leedale, 1975; Barnes *et al.,* 1986; Dunlap *et al.,* 1986), or it secretes mucilage that is mineralized directly with iron and/or manganese (Dunlap and Walne, 1985). The mucilage envelope of several *Trachelemonas* species and of the related euglenoid *Strombomonas conspera* stain with alcian blue at pH 2.5 and 1.3, suggesting the presence of sulfated aminoglucans, possibly specialized for Mn^{2+} binding in some cases (Dunlap *et al.,* 1986). Early studies on the cytochemistry of the extracellular coats are summarized by Willey *et al.* (1977), who also conclude from their own studies that the mucilaginous stalk of the euglenoid *Colacium mucronatum* consists of neutral or slightly acidic carbohydrates with widely spaced anionic groups in low concentration. The central stalk region is periodic acid Schiff (PAS) positive and alcian blue negative, suggesting that at least two types of carbohydrates are secreted during stalk formation.

The "mucus" of *E. gracilis* var. *bacillaris* extracted using a salt/EDTA solution (Marmur's solution) was separated into an SDS-soluble (sodium dodecyl sulfate at 100° C for 4 hr) and an SDS-insoluble gelatinous fraction (Cogburn and Schiff, 1984). The insoluble fraction, which comprised about 20% of the total mucus, stained with alcian blue and contained little covalently bound sulfate as judged from its infrared spectrum and from the absence of incorporation of ^{35}S. Resistance to hydrolysis in 1 *N* HCl together with its staining properties suggested that this SDS-insoluble gelatinous fraction contained uronic acids. The mucus fraction soluble in hot SDS (alcian blue negative) was separated by SDS–polyacrylamide gel electrophoresis (PAGE) and yielded 18 polypeptides of which 16 were also PAS positive and thus designated as glycoproteins. Pentose sugars released after acid hydrolysis of the total SDS-soluble mucus fraction were identified after chromatography on silica gel thin-layer plates (one-dimensional) as rhamnose, fucose, and xylose. Unidentified hexoses were also present (Cogburn and Schiff, 1984). Surprisingly, these results are remarkably similar to those derived from analyses (Nakano *et al.,* 1987) of saccharides by gas-liquid chromatography of hydrolyzates of surface isolates (pellicles) of *E. gracilis* Z strain, which has little or no surface mucus (Rosowski, 1977). For example, rhamnose was the most abundant saccharide (23.3 mol%), followed by xylose (21.1 mol%), fucose (20.4 mol%), and arabinose (10.7 mol%). Hexoses (glucose, galactose) together comprised 10.3 mol% of the total sugars; trace amounts of 3.4 mol% glucosamine and 1.2 mol% uronic acid were also reported. No sialic acids could be detected,

nor was there evidence for galactosamine—a common component of many biomembranes (Nakano *et al.*, 1987). At least some of the glucose reported by Nakano *et al.* (1987) must be associated with glycolipids because UDP-[^{3}H] glucose was readily incorporated into chloroform–methanol extracts of deflagellated whole cells and of isolated cell surface complexes (Chen and Bouck, 1984; Bouck and Chen, 1984). Since UDP-glucose probably does not enter these cells, it seemed likely that surface-associated glucosyltransferases glycosylated surface lipids. Cations greatly enhanced UDP-[^{3}H] incorporation. Two radiolabeled peaks were eluted from LH-60 columns; each of these peaks separated with different M_rs after thin layer chromatography (TLC), suggesting that there were at least two distinct glycolipids on the plasma membranes of *Euglena gracilis.*

As *E. gracilis* var. *bacillaris* enters the stationary phase of growth, the amount of mucus, estimated by quantitative alcian blue binding, continues to increase (Cogburn and Schiff, 1984), whereas in *E. gracilis* Z, starvation under most conditions has little effect on mucus production or encystment. If, however, the media is depleted of nitrogen for 48–72 hr in the dark, cells rapidly develop into cysts, with a concomitant secretion of mucilaginous material apparently via the Golgi system and reservoir (Triemer, 1980). This mucilage has not been characterized except for a positive reaction with the periodic acid–silver methenamine reaction—a general test for polysaccharides.

Because there is no detectable mucilage coat in log phase cultures of *E. gracilis* strain Z, plasma membrane-associated glycoproteins and glycolipids can be assayed directly. Using fluoresceinylated lectins, Bré *et al.* (1984) reported evidence for galactose and *N*-acetylgalactoseamine. The apparent number of lectin sites determined from Scatchard plots of fluorescinyl–HpA binding varied between exponential and stationary cells, the latter having two- to threefold fewer high-affinity lectin binding sites. Contrary to earlier reports (Vannini *et al.*, 1981) of concanavalin A (Con A) (mannose–glucose-specific) binding, Bré *et al.* (1984) found no accessible Con A binding sites in their studies. A more complex pattern of surface glycoconjugates was apparent when the surface isolates of these same cells were separated by SDS–PAGE and tested in the gels for lectin binding, for carbohydrate content (PAS procedure) and for covalently bound lipids (Sudan black B staining). At least 8 PAS-positive polypeptides were identified and their localization over the entire cell surface confirmed *in situ* with the PAS-methenamine method. This pattern of localization and the observation that the relative migration of some of the surface complex polypeptides were altered in B_{12}-starved cells, suggested (Bré *et al.*, 1984) that such surface changes might be functional homologs to the antigenic variation triggered in surface coats of some ciliates by environmental factors (see below). From the lectin-binding properties of the polypeptide bands and from analyses by

gas-liquid chromatography, Bré *et al.* (1986a) concluded that the pentoses, rhamnose, xylose, and fucose were the most abundant sugars, whereas the hexoses, mannose, galactose, and the hexosamine *N*-acetylglucosamine were present in smaller amounts. The predominance of pentose sugars thus seems to be a general characteristic of euglenoid surfaces, and these sugars may be associated with mucus coats, glycoproteins, or glycolipids.

Two specific integral membrane proteins of the plasma membrane of euglenoids have been studied in some detail—a high-molecular weight protein from *Distigma* and a 39-kDa protein from *E. gracilis.* Surface isolates from *Distigma proteus,* negatively stained with uranyl acetate, displayed crystalline arrays of particles arranged in paired rows positioned at about a 35-degree angle to the direction of the surface folds (Murray, 1983). The clarity of the EM images in this study and the ordered particle arrangement permitted the reconstruction by Fourier techniques of a three-dimensional model of the particles and their positions relative to underlying MTs. Each particle appeared to be roughly pentagonal and protrude as a ring or annulus about 2.5 nm from the outer membrane surface. The protein was described as almost featureless on the cytoplasmic surface of the plasma membrane and its molecular weight was estimated from the reconstructions as about 540 kDa. Because it resisted extraction after repeated washes in solutions of pH 4–9, and in 1 *M* salt, the protein was considered to have properties consistent with those of an integral membrane protein. Labeling of intact cells with diazotized [^{125}I]iodosulfanilic acid produced after SDS–PAGE high-molecular-weight, iodinated polypeptide that barely entered the gel, and a series of iodinated lower molecular weight polypeptides (Murray, 1983). Unfortunately, no additional work with this interesting protein has yet been published, and nothing further is known of its biochemistry or function. It may be unique to the cell surface of *Distigma,* as membrane proteins of similar size have not yet been recorded in other euglenoids.

The 39-kDa protein (IP39) of *E. gracilis* is an integral membrane protein because it is resistant to extraction from the plasma membrane by 100 m*M* NaOH and by 1 *M* NaCl (Dubreuil and Bouck, 1985). A diagnostic peculiarity of IP39 is its propensity to form higher order oligomers of approximately 68 kDa, and often at 110 and 140 kDa even under the extreme denaturing conditions used for standard SDS–PAGE (Dubreuil *et al.,* 1988). Radioiodination of intact cells did not label IP39 whereas IP39 was heavily iodinated when radioiodination was carried out on surface isolates (Dubreuil *et al.,* 1988). These results suggested that IP39 lacked an iodinatable external domain but did contain an exposed cytoplasmic region (Fig. 3). Recent experiments indicate, however, that IP39 separated by SDS–PAGE is PAS positive, and therefore probably has an external glycosylated domain (S. Sodin and G. B. Bouck, unpublished observations). Digestion of plasma membranes with papain resulted in loss of the iodinated

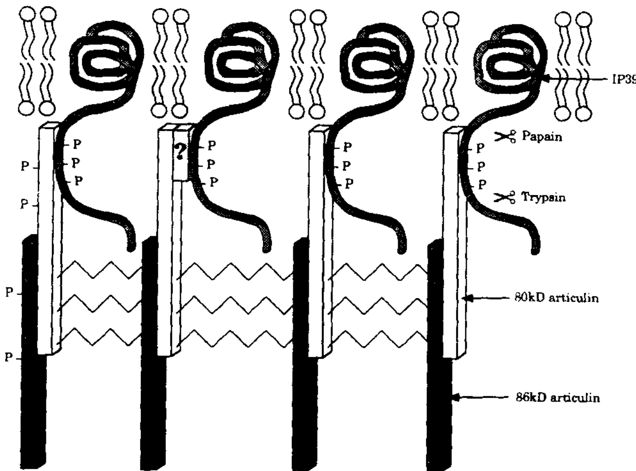


FIG. 3 Proposed arrangement of articulins and IP39 in *Euglena gracilis*. Potential phosphorylation sites (P) on IP39 are based on ^{32}P-labeling and proteolysis experiments. Phosphorylation (P) of articulins has been demonstrated but sites are arbitrary. (Drawing courtesy of Dr. Marc Fazio.)

domain and a loss in the ability of plasma membranes to bind membrane skeletal proteins (Dubreuil and Bouck, 1988; Rosiere *et al.,* 1990). The cytoplasmic domain was phosphorylated by endogenous protein kinases both *in vivo* and *in vitro* when whole cells or the surface were incubated respectively with γ-[^{32}P]ATP or [^{32}P]orthophosphate (Fazio *et al.,* 1995). The radiolabeled phosphoamino acids were identified as phosphothreonine and phosphotyrosine, suggesting that IP39 is a substrate for at least two endogenous protein kinases (Fazio *et al.,* 1995). Detergent-solubilized IP39 can itself phosphorylate casein, indicating that it is a threonine–serine protein kinase. The significance of IP39 both as a kinase and as a protein kinase substrate is not yet known. It seems likely, however, that phosphorylation is involved in the interactions between IP39 and the major membrane skeletal proteins (articulins), since the phosphorylation sites in IP39 are mostly within the specific domain that interacts with articulins (Fig. 3) (Fazio *et al.,* 1995). This cytoplasmic domain is defined by sequential proteolysis of surface isolates; i.e., it is resistant to trypsin but is lost after subsequent papain digestion (Dubreuil and Bouck, 1988; Rosiere *et al.,* 1990).

The 38- and 64-kDa chloroform–methanol-soluble proteins identified by Bré *et al.* (1986a) in *E. gracilis* as Sudan black B positive (lipoproteins) are probably identical to IP39 and its 68-kDa oligomer, suggesting that lipids may be covalently bound to IP39. In *E. acus* integral plasma membrane, proteins

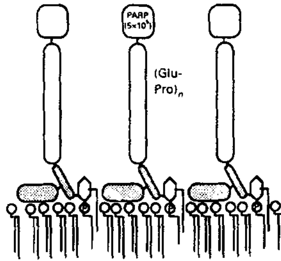
of 64 and 140 kDa (probably oligomers of IP39) were glycosylated (dansyl chloride positive) and reportedly iodinated in intact cells (Bricheux and Brugerolle, 1986). Except for the inability to demonstrate iodination in whole *E. gracilis* (Dubreuil *et al.,* 1988), *E. gracilis,* and *E. acus* appear to have similar integral membrane proteins.

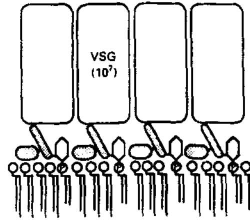
2. Trypanosomes, Ciliates, and Dinoflagellates

In trypanosomes, the plasma membrane may have a continuous coat of variant surface glycoproteins (VSGs); these have received a great deal of attention. The VSGs may represent up to 10% of the total cell protein, and the composition of the coat plays an important role in the survival of the parasite. The repertoire of coat proteins, which may have similar secondary and tertiary structures (Blum *et al.,* 1993), in parasitic trypanosomes may be encoded by more than 1000 specific genes. When coupled to their capacity for conversion by homologous recombination, these genes can produce a potentially inexhaustible supply of different coat proteins (antigenic variation) for evasion of the host immune system (Caron and Meyer, 1989; Pays *et al.,* 1994). In the mammalian bloodstream form of *Trypanosoma brucei,* the expression of a specific member of this VSG repertoire is regulated both by the telomeres and by DNA rearrangements that transpose the gene to the telomeric region of the chromosome. The VSG genes are expressed as polycistronic units which also encode unrelated but surface-associated proteins, the expression-site associated genes or ESAGs (see, for review, Vanhamme and Pays, 1995). In the procyclic or insect-dwelling form of *Trypanosoma,* a different kind of surface protein (procyclic acidic repeat protein or PARP, Fig. 4a) is expressed by genes that are not associated with the telomeres (Hehl *et al.,* 1994).

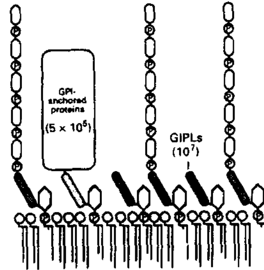
In contrast to the nearly limitless varieties of VSGs of trypanosomes, the surface antigens of ciliates [ca. 300-kDa Sags or i-ags in *Paramecium* (Prat *et al.,* 1986; Preer, 1986; Preer *et al.,* 1987; Nielsen *et al.,* 1991) and the 25-59-kDa i-ags or immobilization antigens in *Tetrahymena* (Williams *et al.,* 1985; Love *et al.,* 1987; Smith *et al.,* 1992)] are restricted to less than a dozen antigenic variants for each strain of *Paramecium,* and up to 40 variants in *Tetrahymena* (Doerder *et al.,* 1996). DNA rearrangements are not required for ciliate surface antigen expression (Forney *et al.,* 1983; Gilley *et al.,* 1990; Leeck and Forney, 1994; Martin *et al.,* 1994), and coat switching has no known function. Variable surface antigens from both ciliates and trypanosomes do, however, share several interesting properties: (1) generally, only one type of antigen is expressed at one time on the cell surface (mutual exclusion), and (2) the coat protein is bound to the plasma membrane by means of a glycosyl-phosphatidylinositol (GPI) anchor (see, for reviews, Cross, 1990; Weinhart *et al.,* 1991; Englund, 1993; McConville and Ferguson, 1993).

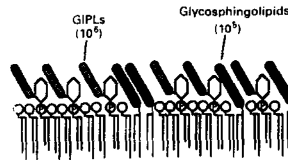
(a) *Trypanosoma brucei*



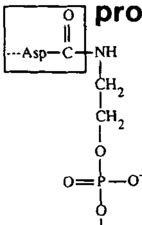


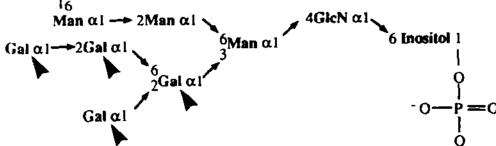
(b) *Leishmania* spp.

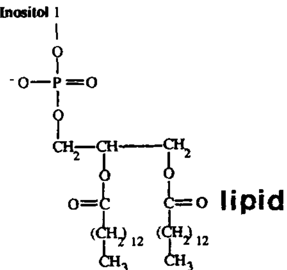




a







b

The GPI anchor consists of a more-or-less conserved core glycan of three mannose residues and one *N*-glucosamine attached to *myo*-inositol, which in turn is coupled via a phosphate ester to dimyristoyl glycerol in the specific VSGs of *Trypanosoma brucei*. At the other end of the glycan core, the first mannose is linked to ethanolamine through a phosphate ester (Fig. 4b). Among the trypanosomatids, there may be wide variations in this general theme, particularly with respect to sugar substitutions to the glucan core, and the type of lipid associated with the membrane (see, for review, McConville and Ferguson, 1993). In the ciliate *Tetrahymena mimbres*, the specific link between the second and third mannose of the core is α1-3 instead of the usually conserved α1-6 (Weinhart *et al.*, 1991). In the ciliate *Paramecium primaurelia*, the core glycan is substituted with mannosylphosphate, and the anchoring lipid is ceramide based (Azzouz *et al.*, 1995). During assembly of the surface coat in trypanosomes, the putative GPI-linked protein is first modified by removal of a carboxy-terminal hydrophobic domain that lacks strong sequence identity among different proteins, and then the protein is covalently coupled at its cleaved carboxy-terminal to the completely or nearly completely assembled GPI anchor in the endoplasmic reticulum (Ferguson and Williams, 1988; Masterson *et al.*, 1989; Undenfriend and Kodukula, 1995). The fully assembled complex is then transported to the plasma membrane (Duszenko *et al.*, 1988). GPIs of leishmanias may be inserted into the plasma membrane without attached proteins (Fig. 4a, GPILs) (McConville and Ferguson, 1993).

The possible functions of GPI-linked proteins in trypanosomatids have been considered (Cross, 1990; McConville and Ferguson, 1993): GPIs-linked proteins, or GPIs alone with their bulky glucans and saccharide substitutions, can provide an impenetrable glycocalyx (Fig. 4a), making it difficult for degradative enzymes of the host phagosome or components of the immune system to access the plasma membrane. In addition, the absence of a transmembrane domain isolates GPI-linked proteins from the interior of the cell, thereby preventing the effects of damaged surface proteins or transmission of inappropriate signals to the cytoplasm. And GPI-anchored proteins can undergo high levels of protein packing (e.g., 10^7 molecules/

FIG. 4 (a) Representation of the cell surfaces of different developmental stages of *Trypanosoma brucei* and *Leishmania*. A dense glycocalyx of GPIs or GPI-linked proteins is evident, although the specific molecules of the glycocalyx differ for each stage. Numbers correspond to the approximate quantity of each molecule per cell. [From McConville and Ferguson (1993), reprinted with permission.] (b) Structure of GPI anchor from *T. brucei* (variant 117) illustrating the membrane-associated lipid, the core glycan, and protein attachment site. The core glycan is substituted with galactose residues (arrowheads) in this specific variant. [Modified from Englund (1993).]

cell in trypanosomes) while occupying limited membrane space that might be better used for functional transmembrane channels and transmembrane enzymes (McConville and Ferguson, 1993). Finally, proteins with strictly extracellular functions are localized to their sites of activity. For example, GPI-linked *trans*-sialidases of trypanosome trypomastigotes can transfer sialic acids from host membranes to GPI-anchored mucins of the parasite—strictly extracellular events that do not require signals from the cytoplasm to help generate a highly negatively charged parasite cell surface (Schenkman *et al.*, 1994) through acquisition of sialic acids.

In summary, GPI-linked proteins are not restricted to the ciliates and trypanosomatids, but they appear to be more common among protozoa than in other groups (Cross, 1990). Although well suited to manage extracellular processes, GPI-linked proteins cannot provide direct support or anchorage for the internal membrane skeleton because they lack cytoplasmic domains. Thus unlike the role of IP39 in *Euglena*, it is unclear how the plasma membrane remains bound to the alveolar or epiplasmic regions in the cortex of ciliates with GPI-proteins. Despite their prevalence in trypanosomatids and some ciliates, GPI-linked proteins and variant surface antigens have not yet been identified in the euglenoids or in the dinoflagellates. The discovery, however, that the actin-binding protein ponticulin found in the plasma membrane of *Dictyostelium* is both a transmembrane and a GPI-linked protein provides precedence for more complex arrangements for GPI-linked proteins (Hitt *et al.*, 1994). Perhaps it would be revealing to assay IP39 from *Euglena* for GPI-type anchorage, since IP39 appears to have covalently bound lipids (Bré *et al.*, 1986a). The major surface-associated proteins are listed in Table I.

B. Membrane Skeleton (Epiplasm)

1. Euglenoids

Two proteins with molecular masses of about 80 and 86 kDa were identified as the principal components (>60%) of the membrane skeletal layer (epiplasm) of *Euglena gracilis* (Dubreuil and Bouck, 1985). Studies with *E. acus* demonstrated that the epiplasm in this species also consisted in part of two major proteins (80 and 70 kDa) termed "euglenidines" (Bricheux and Brugerolle, 1986). An additional protein of 180 kDa was also shown by immunogold labeling to be associated with the epiplasmic layer. These proteins were judged by Bricheux and Brugerolle (1986) to be rich in disulfide bonds because the reducing agent β-mercaptoethanol was required in all extraction solutions that solubilized the epiplasmic layer. In further studies (Bricheux and Brugerolle, 1987), proteins with broadly similar mo-

TABLE I
Plasma Membrane and Surface Coats

Organism	Principal (glyco)protein	Molecular mass (kDa)	Function	Antigenic variations	GPI anchored
Euglenoids	IP39	39	Anchor for articulins	No	Not known
	Mucins	Heterogeneous	Barrier?	No	Not known
Trypanosomes	VSGs	50–60	Barrier	Yes, infinite	Yes
Tetrahymena	Immobilization antigens (i-ags)	300	?	Yes, few variations	Yes
Paramecium	Immobilization antigens (Sags)	100	?	Yes, few variations	Yes
Dinoflagellates	Not known	Not known	—	—	—

lecular weights (70–90 kDa) were identified in eight additional species of euglenoids. In some species the epiplasmic proteins could be resolved into several isoforms: most migrated as fairly basic proteins after isoelectric focusing. Epiplasmic proteins from different species of euglenoids were recognized by the same antibodies and thus appeared to share immunological determinants (Bricheux and Brugerolle, 1987).

cDNAs for both the 80- and 86-kDa proteins from *E. gracilis* were cloned and sequenced (Marrs and Bouck, 1992), and they appeared to be related members of a novel class of membrane skeletal proteins that were termed "articulins." Although only 37% identical in amino acid content, both the 80- and 86-kDa articulins had similar tripartite organizations, which consisted of amino- and carboxy-terminal domains separated by a central region of over thirty 12-amino acid repeats. The repeats were characterized by a VPVPV motif; proline and valine together made up about 40 mol% of the total amino acid composition of each articulin. Unexpectedly, in view of the requirement for β-mercaptoethanol in solubilizing the membrane skeleton of various euglenoids (Bricheux and Brugerolle, 1987), there was only one cysteine residue in the 80-kDa articulin and two cysteines in the 86-kDa articulin, making it unlikely that these proteins were cross-linked to each other by disulfide bonds. Possibly, articulins are embedded in a disulfide-mediated matrix consisting of other, less abundant proteins of the membrane skeleton, such as the 180-kDa protein or others. The molecular mass predicted for both articulins from the amino acid composition was about 72 kDa (Marrs and Bouck, 1992), which was at variance

with the estimates (80 and 86 kDa) made from protein migration after SDS–PAGE. Thus either the proteins are extensively modified post-translationally, or more likely, they migrate anomalously in SDS–polyacrylamide gels. The latter interpretation was supported by experiments in which 86-kDa articulin was overexpressed in bacteria (Li, 1996), where it undergoes no post-translational additions. The overexpressed 86-kDa articulin still migrated near 86 kDa after SDS–PAGE. The 80- and 86-kDa articulins can, however, also be post-translationally modified, as both proteins were phosphorylated *in vivo* (whole cells) and *in vitro* (surface isolates) by endogenous protein kinases (Fazio *et al.*, 1995). The significance of this phosphorylation is not yet known.

2. Ciliates and Dinoflagellates

Biochemical and immunological data on the properties of the epiplasm (see Peck, 1977, for review of early work and definition of epiplasm) has been steadily accumulating for a number of different ciliates, particularly *Paramecium, Tetrahymena, Euplotes, Pseudomicrothorax,* and the entodiniomorphids. The epiplasm of ciliates generally lies below (centripetally to) the alveolar sacs and forms a distinct, continuous layer in the somatic cell cortex (i.e., exclusive of the oral apparatus and cilia). There are considerable variations on this theme. In *Paramecium,* for example, the epiplasm is not continuous, but is restricted to "scales" that collectively constitute the somatic cortex (Fig. 2a). Fractions enriched in somatic cortices of *Paramecium* displayed no especially prominent polypeptide that might be compared with the two abundant articulins in the euglenoid membrane skeletal complex. An antibody (CTR 211), however, originally raised against human lymphoblast centrosomes, fortuitously recognized a 45-kDa polypeptide on immunoblots of cortical polypeptides from *Paramecium tetraurelia,* and localized to the epiplasm after immunofluorescent and immunogold labeling (Keryer *et al.*, 1990).

More recently, monoclonal antibodies generated against a Triton-X 100-soluble fraction of purified cortices identified in immunoblots a collection of about 20 polypeptides ranging in molecular mass from 33 to 45 kDa (Nahon *et al.*, 1993). After two-dimensional isoelectric focusing and SDS–PAGE, these were further resolved into about 60 immunopositive spots. The polypeptides, collectively termed "epiplasmins," all responded similarly to extraction with 1 *M* KI and 4 *M* urea, and were localized to the epiplasm by immunofluorescent and immunogold labeling. One of the monoclonal antibodies against these epiplasmins cross-reacted in immunoblots with the ciliates *Pseudomicrothorax dubius* and *Euplotes aediculatus* and the flagellate, *Euglena acus,* but did not bind to polypeptides from the ciliates *Tetrahymena pyriformis, Colpidinium campylum,* or *Paraurostyla*

weissei (Nahon *et al.*, 1993). The molecular masses of the cross-reacting bands were not provided, so it is not known whether the polypeptides recognized in the blots correspond to the 80- and 86-kDa articulins of *Euglena.* Additional studies with monoclonal antibodies suggested that the biochemical heterogeneity of the epiplasmins of *Paramecium* might also reflect organizational heterogeneity within the epiplasmic layer (Curtenaz and Peck, 1992; Jeanmaire-Wolf *et al.*, 1993); that is, different monoclonal antibodies labeled different regions of the epiplasm in EM sections of the cell cortex.

Unlike the multiple, related polypeptides that were isolated from the epiplasm of *Paramecium,* the epiplasm of the closely related ciliate *Tetrahymena* consisted of three major high-molecular-weight proteins—A, B, and C (Williams *et al.*, 1987, 1989a, 1995). Band A (235 kDa) was conserved among all tested species of *Tetrahymena,* whereas bands B and C varied in molecular masses among different species (Williams *et al.*, 1984). Each of these proteins (A, B, and C) had different but overlapping distributions within the epiplasm, suggesting that (1) they were not heterodimers or heterotrimers, and (2) the epiplasm was not of uniform composition (Williams *et al.*, 1995). Moreover, the more or less protein-specific recognition by different monoclonal antibodies indicated that proteins A, B, and C each had distinct epitopes (Williams *et al.*, 1995). Peptide maps and sequencing of each protein should be particularly useful in further resolving the relatedness among this well-defined set of major epiplasmic proteins. Another family of proteins termed "K" antigens with molecular masses of 39–44 kDa were localized to asymmetric epiplasmic domains surrounding the basal bodies in *Tetrahymena* (Williams *et al.*, 1990). These K antigen-rich domains seem to exclude or render inaccessible antibodies against the A, B, and C epiplasmic proteins. Studies carried out on metabolically radiolabeled cortical proteins in *Tetrahymena* suggested that there may be significant turnover of epiplasmic proteins (Vaudaux and Williams, 1979); similar data on the stability of the epiplasm are not yet available for any of the other cortical systems.

In the entodiniomorphid ciliates (e.g., *Entodinium, Eudiplodinium, Epidinium,* and *Polyplastron),* which live in the digestive tract of various mammalian hosts, the fibrous-appearing epiplasm lies directly in contact with the plasma membrane without an intervening alveolar sac. Studies with monoclonal antibodies demonstrated that there were both similarities and differences among the 31-, 58-, 62-, and 68-kDa epiplasmic proteins extracted from different species in this group (Sghir and David, 1995). The 58-kDa polypeptide, which was distinct from tubulin (Vigues and David, 1989), appeared to be the major epiplasmic protein. A 43-kDa protein with some antigenic similarity to the intermediate filament protein vimentin has been localized to the epiplasmic/plasma membrane interface; antibodies

against this protein did not cross-react on immunoblots with polypeptides from *Euglena acus* or *Euglena gracilis* (Sghir *et al.*, 1994).

Dinoflagellate epiplasm, when present, has not been well characterized. A single biochemical study (Métivier *et al.*, 1987) on the epiplasm of *Noctiluca scintillans* identified 45-, 95-, and 220-kDa proteins in detergent-resistant fractions of whole cells. The 45-kDa protein migrated with actin in two-dimensional isoelectric focusing (IEF)/SDS gels, but produced peptide maps that were different from actin, did not decorate with heavy meromyosin, did not inhibit DNase I activity, and were not recognized by antibodies against actin. Antibodies against the 45-kDa protein recognized the epiplasmic layer after immunogold staining. The two other cortical proteins at 95 and 220 kDa were not further characterized. Proteins from the dinoflagellate *Noctiluca* and epiplasmic proteins from ciliates and euglenoids were shown to have common epitopes by antibody labeling (Vigues *et al.*, 1987). Antibodies generated against the *Euglena acus* 78-kDa articulin localized to both

TABLE II
Epiplasms of Selected Protists

Organism	Position	Proteins (name)	Sequence	Antiepiplasm ab cross-reactivity
Euglenoids	Subplasma membrane	80 and 86 kDa (articulins)	VPVP--repeats	eu x Ps, d (Vigues *et al.*, 1987)
Trypanosomatids	—	—	—	—
Paramecium (ciliate)	Subalveolus	33 to 45 kDa (epiplasmins)	—	P x Ps, eu, Ep (Nahon *et al.*, 1993)
Pseudomicrothorax (ciliate)	Subalveolus	60, 76–80 kDa (articulins)	VPVP--repeats	Ps x P, t, Ep (Curtenaz *et al.*, 1994)
Tetrahymena (ciliate)	Subalveolus	235 kDa, etc. Bands A, B, C	—	
Euplotes (ciliate)	Intra-alveolus	97, 125 kDa (plateins)	—	
Entodiniomorphid ciliates	Subplasma membrane	31, 43, 58, 62 and 68 kDa	—	
Dinoflagellates	Intra-alveolus, subplasma membrane	43 kDa	—	

Ep, *Euplotes;* eu, euglenoids; d, dinoflagellates; P, *Paramecium;* Ps, *Pseudomicrothorax;* t, *Tetrahymena.*

the epiplasmic layer and the thecal plates in the dinoflagellate, *Glenodinium* (Bricheux *et al.,* 1992). Naked (athecal) dinoflagellates contain a number of contractile and noncontractile filamentous systems that are either restricted to a specific region of the cortex or are transcellular (Cachon *et al.,* 1994). Some of these filaments appear to be noncontractile actin filaments; others are centrin-based contractile fibers (Höhfeld *et al.,* 1994; Godart and Huitorel, 1992; Roberts and Roberts, 1991).

The summation of these and other studies (Table II) supports the generally accepted notion that the proteins of the epiplasm among these different genera or even within different species of the same genus may vary widely in molecular masses and biochemical properties. However, reports of immunological cross-reactivities among different epiplasmic proteins, often from distantly related genera (Vigues *et al.,* 1987; Nahon *et al.,* 1993; Curtenaz *et al.,* 1994) have suggested that there may be conservation of secondary structure or of the primary sequence of specific protein domains. A striking confirmation of epiplasmic protein conservation was evidenced when the cDNA for a 60-kDa (p60) epiplasmic protein of the ciliate *Pseudomicrothorax* was sequenced (Huttenlauch *et al.,* 1995). From the deduced amino acids, this protein was found to have regions with remarkable similarities to the 12-amino acid VPVPV-----repeats of the articulins of the flagellate *Euglena gracilis* (Marrs and Bouck, 1992). There were 24 repeats in the fully sequenced 60-kDa epiplasmic protein in *Pseudomicrothroax,* in contrast to the >30 repeats in the articulins of *E. gracilis* (Table III). The 60-kDa protein was a minor component of the epiplasm (Huttenlauch and Peck, 1991); a group of polypeptides around 76–80 kDa and a second group of 11–13 kDa were quantita-

TABLE III
Sequences and Distribution of Articulins

Organism[a]	Size of protein[b] (kDa)	Size of protein[c] (kDa)	Proline + valine (%)	No. of repeats	Consensus sequence of repeats[d]
Euglena gracilis	80	72.1	40	33	V P V P V e v i V - v -
Euglena gracilis	86	71.9	39	33	V P V P y - V - - - V e
Pseudomicrothorax dubius	60[e]	60.9	36	24	V P V P - - V -V - V p
		OVERALL CONSENSUS =			V P V P - - V - V - V -

[a] *Euglena* data from Marrs and Bouck (1992); *Pseudomicrothorax* data from Huttenlauch *et al.* (1995).
[b] Determined by SDS-PAGE.
[c] Deduced from amino acid sequence.
[d] Upper case, present in >50% of repeats. Lower case, present in >33% of repeats.
[e] Partial sequence of peptides of 78–80 kDa have similar repeats.

tively more abundant. Partial sequence analysis, however, of the 76–80-kDa epiplasmic proteins of *Pseudomicrothorax* confirmed the presence of similar VPVPV---repeats (Huttenlauch *et al.*, 1995). If articulins are also found in dinoflagellates, as might be tentatively inferred from the immunological data (Vigues *et al.*, 1987), and in other protists as predicted by Huttenlauch *et al.* (1995), then the rather wide range of molecular weights of epiplasmic proteins found among different organisms might be the result of variable numbers of articulin repeats and/or modifications of the less conserved amino- and carboxy-terminal nonrepeat domains (Huttenlauch *et al.*, 1995). Obviously, only after sequencing epiplasmic proteins from dinoflagellates and other protists will the extent and degree of identity among epiplasmic proteins of the protist membrane skeletons be fully known.

C. The ER/Alveolar System

1. Euglenoids

Early EM studies on *Euglena* showed that a tubular membrane system was always associated with each ridge of the surface complex (Leedale, 1967). Other than noting its presence, relatively little attention was given to this putative endoplasmic reticulum until Murray (1981) found that when the euglenoid *Astasia longa* grown in medium containing calcium chloride was incubated with potassium oxalate, deposits of calcium oxalate appeared within this ER cisternae. This ER consisted of parallel tubes within each surface ridge with occasional cross-connections and other connections into deeper parts of the cytoplasm. Direct binding assays with $^{45}Ca^{2+}$ using isolated surface complexes of *Astasia* revealed high-affinity Ca^{2+} binding sites, and Ca^{2+} binding was enhanced with $MgCl_2$. A "crude" fraction of isolated microsomes (ER) accumulated Ca^{2+} in an ATP-dependent manner and released Ca^{2+} when incubated with caffeine, an alkaloid that also causes shape changes in intact cells (Murray, 1981). Based on the amount of calcium oxalate deposited in the ER, calcium concentration within the cisternae was estimated at 2 m*M*. These findings were of potential functional significance because shape changes (euglenoid movements) were found in the same study to be sensitive to exogenously added calcium. Murray suggested that each strip must be an independent motor unit which in some way was affected by calcium stores in the ER or elsewhere. Further support for this hypothesis came from detailed studies that clearly demonstrated that (1) strips slide relative to one another during cell movements in *Euglena fusca* (Suzaki and Williamson, 1985, 1986a,c), and (2) low levels, of calcium (10^{-7} *M*) were sufficient to cause irreversible cell contraction, presumably

by strip sliding, in detergent-permeabilized models of *Astasia longa;* ATP strongly enhanced this effect (Suzaki and Williamson, 1986b).

Several calcium-binding proteins have been cloned from euglenoids. Calmodulin, a small (ca. 17 kDa) Ca^{2+}-binding protein, well characterized in other eukaryotes, was directly purified from *Euglena gracilis* Z, digested with trypsin and V8 protease, and the released peptides sequenced (Toda *et al.,* 1992). The amino acid sequence was 92% identical to calmodulin from *Trypanosoma,* but differed from all previously sequenced calmodulins in trimethylation of the terminal lysine. The intracellular localization of calmodulin in euglenoids is not yet known. cDNA for a second calcium-binding protein was recovered while screening a *Euglena* cDNA expression library with antibodies against an unrelated protein (Gumpel and Smith, 1992). The amino acid sequence deduced from the cDNA predicted a 74-kDa protein which had sequence similarities with known calcium-binding proteins. Direct demonstration of calcium binding was carried out after overexpression of the cDNA in *Escherichia coli.* The calcium-binding protein contained 30 repeats with an acidic 23-amino acid motif in which aspartic acids (24.9%) predominated (Gumpel and Smith, 1992). The amino-terminal consisted of hydrophobic amino acids suggestive of a signal sequence; consistent with this interpretation, the polypeptide was processed to a lower molecular weight from after *in vitro* translation of the cDNA in the presence of pancreatic microsomal vesicles. Although no ER retention signal (e.g., KDEL) was identified, it seems likely that this protein is either transported to the cell surface or retained in one of the compartments (ER, Golgi) en route to the cell surface. Unusual targeting to other organelles, however, cannot be ruled out in euglenoids (Kishore *et al.,* 1993; Sulli and Schwartzbach, 1995). Nonetheless, this 74-kDa protein remains an intriguing candidate for a calcium modulating protein within the calcium-accumulating ER system of the surface complex of *Euglena.*

The close proximity and often the molding of ER to the conformation of the membrane skeletal strips in euglenoids, as seen especially well in *E. ehrenbergii* (Fig. 1b), is strikingly reminiscent of the alveolar system of other protists, as discussed later. In trypanosomatids there may be "extensive sheet-like cisternae of ER close to the microtubules" in many forms (Vickerman and Preston, 1976; Pimenta and De Souza, 1985; De Souza, 1989). Thus, although the ER of the cell surface may be less well defined than in the related euglenoids, it is positioned in trypanosomes to modulate cortical functions that are calcium dependent such as invasion of host cells (Docampo and Moreno, 1995); microtubule–membrane linker proteins (MAPs) of the cortex may also be calcium regulated (see later discussion). Reports that the relatively stable peripheral microtubules are selectively depolymerized by calcium (50–100 m*M*) in *Trypanosoma* (Dolan *et al.,* 1986) and in the euglenoid *Distigma* (Murray, 1984) indicated that calcium

could influence the microtubule–tubulin equilibria. Whether calcium affects MAPs or microtubules directly was not determined in these experiments.

2. Ciliates and Dinoflagellates

In *Paramecium,* the alveolus lies just internal to the plasma membrane, and is defined by inner and outer alveolar membranes which in fact are the margins of a continuous flattened sac-like system (Fig. 2a). One of the proposed functions of the alveolus, calcium accumulation, has been experimentally tested in a series of definitive experiments using intact *Paramecium* for localization, and isolated surfaces from *Paramecium* for physiological studies (Stelly *et al.,* 1991). These latter authors conclude that the alveolus in these cells is a "vast submembranous calcium storage compartment" which could mediate three major calcium-responsive organellar systems: cilia, trichocysts, and cytoskeletal networks of the cortex (Stelly *et al.,* 1991). The intraciliary lattice, for example, functions as a Ca^{2+}-sensitive contractile system in *Paramecium* (Garreau de Loubresse *et al.,* 1988, 1991). Direct evidence for calcium accumulation within the alveolus was obtained by incubating cells with antimonate and osmic acid, which produced dense deposits, presumably calcium antimonate, along the inner margin of the alveolus as well as in mitochondria and trichocyst tips, as expected. Isolated cell surfaces from Percoll gradients with more or less intact alveoli were assayed for uptake of $^{45}Ca^{2+}$. Calcium accumulation in these fractions required both ATP and $MgCl_2$, and uptake was related to calcium concentration. The calcium ionophore A23817 caused almost immediate release of the stored calcium (Stelly *et al.,* 1991). Thus in *Paramecium* it can be inferred that one of the functions of the alveolus parallels that proposed for the cortical membrane skeletal ER in euglenoids, i.e., calcium regulation and/or storage.

The alveolus in other ciliates such as *Tetrahymena* and *Pseudomicrothorax* has not been extensively analyzed although it seems reasonable to expect that they also will be found to mediate calcium release and sequestration. In *Pseudomicrothorax dubius,* however, there are striated deposits associated with the lumen side of the inner alveolar membrane, suggesting that processes in addition to calcium regulation can also take place within this compartment (Hausmann, 1979). More dramatic examples of intra-alveolar assembly have been found in the ciliate *Euplotes* in which the alveolus is divided by septa into individual compartments. Each compartment contains a distinct flattened polygonal structure that together with its neighbors forms an "epiplasmic layer" of separate articulating plates (Williams *et al.,* 1989a). Biochemically, the plates consist in part of polypeptides of approximately 97 kDa and another distinct set at 125 kDA; the term "plateins" was proposed for these proteins (Kloetzel, 1991). Plateins from *E. eurystomus* and *E. aediculatus* were probed on immunoblots with different monoclonal antibodies (Wil-

liams, 1991); individual plateins were found to have varying degrees of corecognition, suggesting that there were both similar and unique epitopes among the plateins from different species of Euplotes.

Dinoflagellates display perhaps the most dramatic and dynamic modification of an alveolus-like compartment (amphiesmal vesicle, thecal vesicle) which is located between the plasma membrane and the underlying microtubules. Thecal plates, when present, are initially assembled within this compartment, but during development or environmental stress the plasma membrane, outer amphiesmal membrane, and thecal plates are shed. The inner amphiesmal membrane then becomes the new plasma membrane (Bricheux *et al.*, 1992; Höhfeld and Melkonian, 1992), and a new amphiesmal vesicle appears between the subtending microtubules and the new plasma membrane. The vesicles are reportedly derived from an ER network that lies adjacent to the epiplasm (Cachon and Cachon, 1984). During the assembly of a new thecal plate within the new amphiesmal vesicle, a temporary layer—the "pellicle," encloses the ecdysed cell as a temporary cyst (Bricheux *et al.*, 1992; Höhfeld and Melkonian, 1992). The four-layered pellicle is derived from expansion of material that initially subtended the amphiesmal plate, and was exposed after ecdysis of the thecal plate. Thus the amphiesmal layer with enclosed plates interchanges with the pellicle layers (four distinct layers) in thecate cells as the protective cell covering. Polyclonal antibodies generated against the 70-kDa articulin of *Euglena acus* were incubated with cells at various stages of recovery after forced ecdysis (shedding) of the mature thecal layer in the dinoflagellate *Glenodinium foliaceum* (Bricheux *et al.*, 1992). The inner homogeneous layer of pellicle formed immediately after ecdysis was recognized by *Euglena* anti-78-kDa antibodies, but in mature cells the thecal plate within the amphiesmal vesicle was recognized. Unfortunately, proteins could not be recovered from the insoluble thecal plates for identification of immunoblots (Bricheux *et al.*, 1992), so that the specific moiety identified by these *Euglena* antiarticulin antibodies could not be determined. Earlier studies using iodine staining, partial digestion with cellulase (Loeblich, 1970), calcofluor white, and Congo red staining (Klut *et al.*, 1988) suggested that the thecal plates and/or other portions of the amphiesma contained glucose polymers (Loeblich, 1984), possibly cellulose or hemicellulose. Acid mucopolysaccharides (Klut *et al.*, 1985) and the plant terpenoid sporopollenin (Morrill and Loeblich, 1981) may also be components of the amphiesmal plates or membranes.

In summary, the alveolus or alveolus-like (membrane skeletal ER, amphiesmal vesicles) chambers that are common to many protists (Table IV) appear to be regions where cations (Ca^{2+}) and/or perhaps proteins and glycoproteins are sequestered either reversibly (presumably Ca^{2+}) or irreversibly (e.g., plateins or *Euplotes;* thecal plate proteins and carbohydrates of dinoflagellates). Embedding the alveolus within the cell cortex ensures that its

TABLE IV
Cortical Chambers

Organism	Cortical vesicle	Ca^{2+} storage	Plates within	Position in cortex
Euglenoids	ER	Yes	No	Internal to membrane skeleton
Trypanosomes	ER	Yes	No	Internal to corset
Paramecium[a]	Alveolus	Yes	No	Between pm[b] and epiplasm
Euplotes[a]	Alveolus	?	Yes	Between pm and epiplasm
Dinoflagellates	Amphiesma	?	Yes/no[c]	Between pm and epiplasm

[a] Ciliates.
[b] pm, plasma membrane.
[c] Only armored (thecate) forms with plates.

products are immediately accessible to the surface complex. The presence in some organisms of epiplasmic proteins within alveolus-like compartments would not be inconsistent with a general function. That is, if the epiplasm consisted of formed structures such as plates (e.g., *Euplotes*), then their modeling might require templates determined by the form and contents of the alveolar sacs or imprints in the membrane of the alveolus. Thus far there are no biochemical details available on purified alveolar membranes, and few decisive biochemical studies on alveolar contents. Studies such as those carried out with *Paramecium* (Stelly *et al.*, 1991), when extended to other organisms, particularly the dinoflagellates, should help to verify whether the alveolus is a general organelle used by many different protists as we speculate here, or a specialized compartment that is restricted to a specific subset of protists, the alveolates (Cavalier-Smith, 1993).

D. Microtubules

1. Euglenoids

As in other eukaryotes, MTs in euglenoids assemble from α- and β-tubulins; γ-tubulins have been identified in some protists (Oakley, 1994), but not yet in the euglenoids. Consistent with the well-known conservation of tubulin structure, bovine brain tubulins readily polymerized on presumptive microtubule organizing centers of the euglenoid plasma membrane (Murray, 1984). Reports of a single incomplete MT in the region of strip overlap in the euglenoid *Distigma proteus* (Gallo and Schrével, 1982; Murray, 1984) appear to be an exception to the 13-protofilament tubular wall that is generally found in MTs of euglenoids, and in MTs from most other eukaryotes (See, for review, Burns and Surridge, 1994). *In vitro* reassembly of *Distigma* MTs (Murray, 1984) under conditions in which tubulins assemble into partial MTs with di-

rectional hooks (Heidemann and McIntosh, 1980) revealed that the faster growing MT end (+ end) was directed toward the cell posterior. This finding is in accord with the anterior to posterior growth of daughter strips during surface duplication (see later discussion). Microtubules of the surface complex are more stable than those of most other eukaryotic cytoplasmic microtubules. In *Distigma,* for example, MTs in isolated surface complexes were resistant to depolymerization by colchicine, *p*-fluorophenylaline, griseofulvin, nocodazole, benomyl, and 0°C (Murray, 1984); in surface isolates of *E. gracilis,* MTs were also structurally unaffected by colchicine and 0° C (Dubreuil and Bouck, 1985). It is not yet known whether this stability is the result of tubulin modifications or their association with specific MT-associated proteins (MAPS). Soluble tubulins of *Euglena gracilis* were phosphorylated *in vitro* by endogenous protein kinase(s), but *in vivo* microtubules of the membrane skeleton were not phosphorylated after incubation with *P*-orthophosphate (Fazio *et al.,* 1995). Whether soluble pools of tubulins become phosphorylated in living cells remains to be determined.

Surface isolates from *Euglena gracilis* Z yielded PAS-positive 54–56-kDa polypeptides after SDS–PAGE; immunoblots of similar preparations revealed 54–56 kDa polypeptides that were recognized by four antitubulin antibodies (Bré *et al.,* 1986a). These polypeptide bands also bound several lectins [concanavolin A (Con A) peanut agglutinin (PA), and soybean agglutinin (SA)]. The putative glycosylated tubulins were fairly resistant to extraction with the neutral detergent N-40, raising doubts as to whether they were membrane associated and therefore similar to the membrane-associated, glycosylated tubulins reported in several other systems (see, for review, Bré *et al.,* 1986a). N-40 alone, however, does not effectively solubilize plasma membrane proteins of *Euglena* (Dubreuil and Bouck, 1985), so that the glycosylated tubulins could be membrane associated. No further details of the location, or of the types of sugar moieties associated with these tubulins is known. The relative mobility of the putative glycosylated tubulin bands is altered, however, in vitamin B_{12} starved cells (Bré *et al.,* 1986a,b). Thus tubulin modifications might be related in some way to the growth patterns of the membrane skeletal ridges which are also affected by vitamin B_{12} starvation (Bré and Lefort-Tran, 1978; Lefort-Tran *et al.,* 1980; Bré *et al.,* 1986a). Because of their insensitivity to the more commonly used pharmacological MT poisons, the role of MTs in development of the surface complex in euglenoids has not been experimentally tested; a systematic application of other reagents (e.g., trifluralin, rhizoxin, oryzalin) that have proved effective against the stable MTs of the trypanosomids (Chan and Fong, 1990; Robinson *et al.,* 1995) and other organisms (Schnepf *et al.,* 1990) might prove to be useful for euglenoids as well.

The relative positions and helical-to-longitudinal orientation (i.e., parallel to the strips) of MTs is fairly constant for a given species of euglenoid. In

Distigma proteus, MTs lie at an angle of about 35 degrees to the direction of the rows of membrane particles, and from electron diffraction studies it was evident that the "position of each microtubule is not independent of its neighbor, rather, all are fixed relative to some common coordinate system. A likely candidate is the array of particles embedded in the membrane" Murray (1983). This latter study demonstrated that there was a correspondence between the regular pattern of the membrane particle arrays and the predicted position of MAPs attached to MTs. The position of a hypothetical MAP appeared to coincide exactly with the position of a membrane particle in every second row. The adjacent row of membrane particles had binding sites only for every second MT. Thus each MT was capable of independent movement from its neighbor, since they were never attached to the same row of membrane particles (Murray, 1983). This rather satisfying hypothesis predicts that during development the arrangement of membrane particles precedes rather than follows the arrangement of the MTs and their MAPs. Other euglenoids do not generate the large membrane particles described for *Distigma,* but all euglenoid plasma membranes studied by freeze-fracture display an intramembrane organization consisting of regularly spaced striations or particles oriented at about 35 degrees to the direction of the MTs (Miller and Miller, 1978); these striations might represent membrane protein arrays that are subtle versions of the large *Distigma* particles.

Both β-tubulin (Schantz and Schantz, 1989) and α-tubulin (Levasseur *et al.,* 1994) of *Euglena gracilis* have been cloned and sequenced. Each of the deduced amino acid sequences is >90% identical to its counterpart among other protist tubulins; the greatest overall identity is with the tubulins of trypanosomes. Generally, the carboxy-terminal regions of tubulins from different species show the greatest divergence in their amino acid composition (see, for review, Burns and Serridge, 1994), but the carboxy-terminal regions of both α- and β- tubulins in *Euglena gracilis* have only a few conservative substitutions compared with those of *Trypanosoma brucei* (see Levasseur *et al.,* 1994, for further discussion). The carboxy-terminal is often the binding domain for microtubule associated proteins; possibly MAPs of *Euglena,* which have not yet been identified, and the more thoroughly studied MAPs of *Trypanosoma* will prove to have similar MT binding sites.

2. Trypanosomes, Ciliates, and Dinoflagellates

Microtubules are the principal structural elements of the membrane skeletal complex of trypanosomes, and therefore must provide the major mechanical support for the plasma membrane, and rigidity for the whole cell. Two-dimensional polyacrylamide gel electrophoresis revealed that the peripheral MTs of *Trypanosoma* consist of one β- and two α-tubulin isoforms; one of the α-tubulin isoforms is the primary translation product, the other isoform

appears to be an acetylated derivative of the primary polypeptide (Schneider *et al.,* 1987). As with the euglenoids, the peripheral MTs are unusually resistant to extraction by a variety of buffers, are not affected by low temperatures, and are insensitive to many MT-depolymerizing reagents.

Among possible mechanisms that affect MT stability are reversible post-translational modifications such as acetylation (L'Hernault and Rosenbaum, 1983), tyrosination (Thompson, 1982), glutamylation (Audebert *et al.,* 1993), glycylation (Redeker *et al.,* 1994), phosphorylation (Serrano *et al.,* 1987; Gurland and Gundersen, 1993), and/or association with MAPs (see, for review, Hemphill *et al.,* 1992). Using a monoclonal antibody, acetylated MTs were localized to both transient (mitotic) and to the more permanent peripheral and flagellar MTs in *Trypanosoma* (Schneider *et al.,* 1987; Sasse and Gull, 1988), suggesting that acetylation alone does not ensure MT stability (see, for review, Gelfand and Bershadsky, 1991). Tyrosinated MTs have been found in different MT fractions, but antibody localization studies revealed distinct zones of tyrosinated tubulins at the ends of individual MTs, indicating that tyrosination may be a mechanism to distinguish old from new MT, or MT regions (Sherwin *et al.,* 1987; Sasse and Gull, 1988), rather than a modification uniquely associated with MTs of defined stability, position, or function (Sasse and Gull, 1988). Tyrosinalated MTs have in fact been useful for following the assembly of new MTs during cell division in trypanosomes (Sherwin and Gull, 1989).

The trypanosome membrane skeleton contains a number of proteins that have been implicated in MT–MT or MT–membrane interactions (Seebeck *et al.,* 1988a,b). Most of these MAPs are structural (see Mandelkow and Mandelkow, 1995, for recent general review of MAPs), although Burns *et al.* (1995) have identified a kinesin-related MAP in the trypanosomid *Leishmania.* The function and localization of the *Leishmania* protein have not yet been determined. The most thoroughly characterized MAPs are those of *Trypanosoma brucei,* especially the microtubule-associated, repetitive proteins, or MARPs. MARP-1 and MARP-2 are two closely related, high-molecular-weight proteins (320 kDa), each with a large acidic central domain consisting of 38 amino acid repeats arranged in tandem to comprise over 90% of the protein mass (Affolter *et al.,* 1994). The carboxy-terminal domain as well as individual central repeats bind tubulins *in vivo* and *in vitro* (Affolter *et al.,* 1994; Hemphill *et al.,* 1992). The two potential MT binding sites in MARPs suggested a model in which MARPs stabilize MTs by anchorage with their carboxy-domains, and by binding with their long repeat domains along the MT axis (Hemphill *et al.,* 1992). MARPs have been localized to the membrane-facing region of MTs in the membrane skeletal complex (Hemphill *et al.,* 1992).

Cross-linking of adjacent MTs and binding of MTs to the plasma membrane can also contribute to MT stability in the membrane skeletal complex.

Two MAPs that may be involved with cross-linking MTs in *Trypanosoma brucei* are p15 and p52, since they can promote polymerization and bundling of MTs *in vitro* (Balaban and Goldman, 1992; Balaban *et al.,* 1989). A 210-kDa putative MAP has been localized to sites on MTs facing the plasma membrane and close to MT cross-bridges (Woods *et al.,* 1992). This protein coprecipitates with polymerized tubulins, but unlike other structural MAPs, it is heat stable and has a predicted globular conformation. Since it is heavily phosphorylated, p210 may play a role in regulating the cross-linking of MTs via other associated proteins (Woods *et al.,* 1992). In *Crithidia fasciculata,* a 41- and 61-kDa protein can induce MT bundling through periodic cross-linking (Bramblett *et al.,* 1989). The 61-kDa protein has not been localized, but the 41-kDa (COP-41) was identified as the glycosome enzyme glyceraldehyde 3-P-dehydrogenase (GADPH); GADPH from brain extracts also stimulates MT polymerization and bundling (Huitorel and Pantaloni, 1984; Kumagai and Sakai, 1983).

Two proteins have been identified that may link MTs of the membrane skeleton to the plasma membrane in *Trypanosoma brucei.* The MAP protein p41, isolated from the membrane skeletal complex (Schneider *et al.,* 1988a), carries thioester-linked fatty acids which presumably can interact hydrophobically with lipids of the plasma membrane, as is the case with other palmitoylated proteins. Binding of p41 to microtubules requires Ca^{2+}. P60 is a second MAP also isolated from the membrane skeletal complex of the same organism. P60 copolymerized with MTs and induced extensive cross-linking between *Trypanosoma* MTs and membrane vesicles (Seebeck *et al.,* 1988b). Ca^{2+} may also mediate the binding of P60 to MTs because P60 was initially purified by phenothiazine affinity chromatography similar to that used for the isolation of the calcium-binding protein, calmodulin (Steiger and Seebeck, 1986).

Microtubules of the membrane skeleton of trypanosomes converge at the anterior of the cell at a capping structure of unknown composition. At the opposite pole (posterior), a novel 28-kDa protein (Gb4) has been localized to a ring-shaped opening in the MT array (Rindisbacher *et al.,* 1993). Transcript analysis indicated that the 28-kDa protein was a processed form of a large polycistronic RNA. The interactions of Gb4 with MTs has not yet been determined, although judging from its position in the cell, it is a good candidate for a membrane skeletal MAP with specialized location and function.

Overall, progress in understanding the nature and function of MAPs in trypanosomes has far outpaced that of other protist groups. In part this work has been stimulated by the search for a pharmacological compound that might specifically disrupt the propagation or transformations of these medically important parasites; in part the work has progressed rapidly because the relatively uncluttered surface isolates, consisting of the plasma

membrane and MTs reduce the number of potential proteins that might serve as MAPs among the proteins present in the surface isolate. MAPs characterized in this group may be relevant to the euglenoids which have retained portions of a MT corset, but have superimposed an additional layer of articulin-rich proteins, presumably for increased stiffness and stability. Perhaps the need for dynamic remodeling of cell form during their cell and life cycles, often in different hosts, has dictated a more pliable cortical architecture in trypanosomes, as opposed to the free-living euglenoids, which assume only one vegetative form from generation to generation.

In the ciliate *Paramecium* at least 12 MT arrays can be distinguished cytologically (Cohen and Beisson, 1988); four of these—the basal bodies, cilia, ribbons (rootlet MTs), and the cytospindle—are associated with the cortex. One of the MT ribbons (postciliary MTs) arises from the right posterior quadrant of the basal body and extends upward toward the epiplasm, where it ends (Allen, 1988); a transverse ribbon of MTs arises from the anterior left side of the basal body and crosses to the left and extends upward and ends at the epiplasm. In addition, an extensive set of acetylated MTs is associated with a specific region in the left, anterior portion of the interphase cell known as the A-paratene. The latter extends around the oral apparatus and to the right of the anterior and posterior suture lines; the A-paratene is identified by the lateral as well as longitudinal alignment of the epiplasmic scales, resulting in hexagonal arrangements of the cortical units in this region (Fleury and Laurent, 1995; Fleury *et al.*, 1995). Microtubules are more abundant in this A-paratene region than elsewhere in the cortex. These MTs originate from the posterior kinetosome of each cortical unit's basal body pair, and may form bundles with MTs from adjacent kineties. The bundles progress superficially under the cell cortex in the A-paratene region (Fleury and Laurent, 1995).

The number of tubulin genes in protists is generally small (see, for reviews, Grain 1986; Silflow 1991), but in *Paramecium* there are four α-tubulin and three β-tubulin genes. Nonetheless, it has been argued (Fleury *et al.*, 1995) that the biochemical heterogeneity of tubulins in *Paramecium* is not the result of genetic diversity, but is produced by post-translational modifications. As summarized in Table V, application of a battery of monoclonal antibodies that recognized acetylated, polyglutamylated, or polyglycylated MTs indicated that most of the cortical MTs have one or more of these modifications (Fleury *et al.*, 1995). Moreover, at least in the case of rootlet MTs, there are significant changes in antibody recognition, and therefore presumably post-translational modifications, during cell division. Microtubule-associated proteins have not yet been identified for cytoplasmic or cortical MTs in ciliates.

The dinoflagellates also utilize a stable array of cortical MTs that appear to function in morphogenesis and in maintaining cell form. The general

TABLE V
Paramecium Cortical Microtubules[a]

Location	Noc. sen.[b]	αS, βS[c]		Ac[d]		GT 335[e]		TAP 952[f]		AXO 58[g]		AXO 49[h]	
		Antibodies tested											
Cell cycle[i]	I	I	D	I	D	I	D	I	D	I	D	I	D
Rootlet MTs	–	+	–	+	+	+		–	+	+/–	+/–	+	–
Postoral MTs	–	+	+	+	+	+		+	+	+	+	+/–	–
Vacuolar MTs	–	+	+	+	+	+		+	+	+	+	+/–	–
Ciliary tip MTs	–	+	+	+	+	+		+	+	+	+	–	–
Ciliary body MTs	–	–	–	–	–	+/–		+/–	+/–	+/–	+/–	+	+
Cytospindle MTs			+		+		+		+		+/–		–

[a] Data from Fleury *et al.* (1995).
[b] Nocodazole sensitive.
[c] Recognizes carboxy-terminal of α-, β-tubulin.
[d] Recognizes acetylated tubulins.
[e] Recognizes glutamylated tubulin.
[f] Recognizes carboxy-terminal of α-, β-tubulins.
[g] Recognizes β-tubulin of cytoplasmic microtubules.
[h] Recognizes polyglycylated tubulins.
[i] I, interphase, D, dividing cell.

organization of the cortical MT in predominantly longitudinal arrays is fairly constant among different genera. These MTs are arranged in three zones; the epicone, hypocone, and girdle (Roberts *et al.,* 1988; Brown *et al.,* 1988). The longitudinal MTs are braced by two lateral bands of 3–5 MTs that are positioned at the transverse cingulum. The posterior MTs converge and abut each other at the posterior pole (Roberts and Roberts, 1991). In some dinoflagellates, the longitudinal MTs of the epicone appear to radiate from transverse MT bands at the anterior pole; these appear as a complex three-pronged fork in *Cryptothecodinium* (Perret *et al.,* 1993; Kubai and Ris, 1969), or as a simple helical band in *Gymnodinium* (Roberts and Roberts, 1991). Thus a stable cortical system of longitudinal and transverse MTs appears to outline and define dinoflagellate topology.

In some dinoflagellates such as *Gymnodinium, Cochlodinium,* and *Amphidinium,* the cortical, longitudinal MTs are organized as a single, evenly spaced layer (Brown *et al.,* 1988; Roberts *et al.,* 1988) that is similar in distribution to the MT corset of the trypanosomatids. In more complex cells (e.g., *Oxyrrhis* and *Gyrodinium),* the longitudinal MTs in the ventral cortex are evenly spaced and unlinked, whereas on the dorsal and lateral sides they are arranged in cross-linked bundles of 10–100 MTs (*Gyrodin-*

ium), or 3–4 cross-linked MTs (*Oxyrrhis,* Brown *et al.,* 1988). Some reorganization of MTs occurs during cell division and during ecdysis.

In summary, cortical MTs of trypanosomes and dinoflagellates appear to have a major role in maintaining cortical integrity, whereas in cells with well-developed epiplasms, such as the euglenoids and ciliates, MT function is less certain. In *Paramecium* the A-paratene MTs may be involved in stabilizing the invariant region of the cell cortex (Fleury *et al.,* 1995); MT rootlets probably anchor the ciliary–basal body during ciliary beat (Allen, 1988). In euglenoids, MTs are strategically positioned to mediate surface movements, to direct the development of new strips during cytokinesis, and/or to carry out transport of cell materials. Experiments with new classes of MT poisons and further exploration with the numerous antibodies available for MAPs and post-translational modifications will help resolve the function of these universal cortical components.

IV. Reuse of the Parental Surface Complex during Surface Duplication

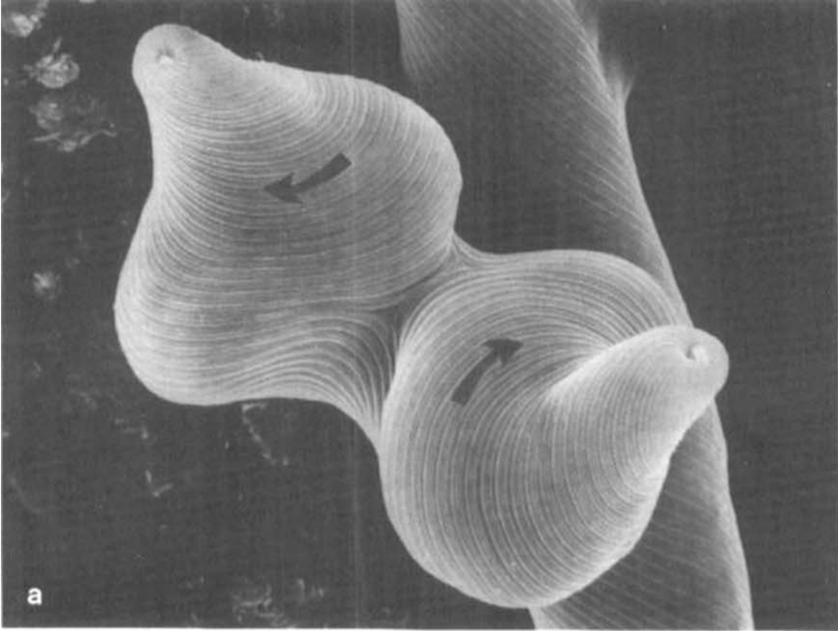
A. The Membrane Skeleton during Cytokinesis in Euglenoids

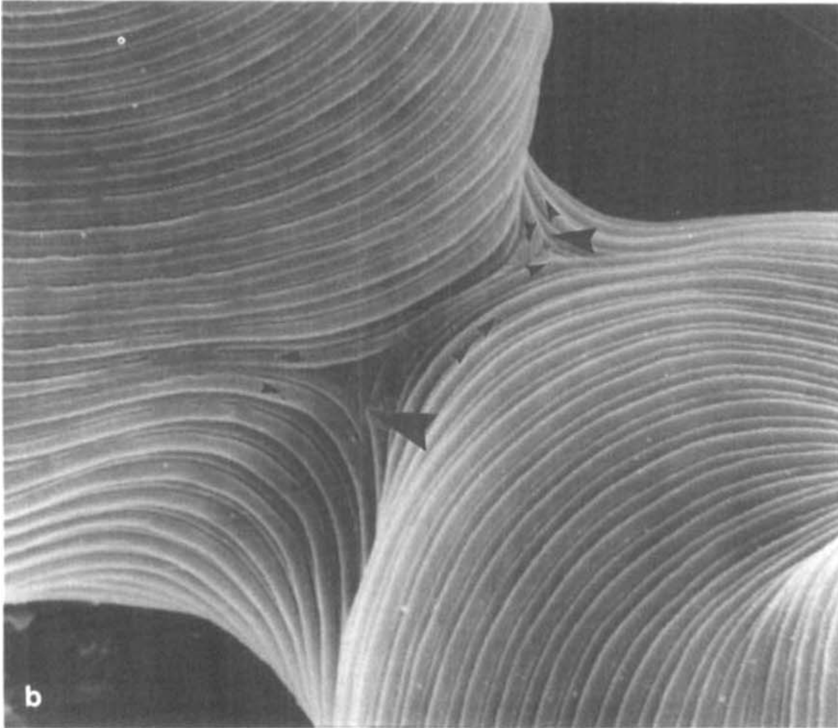
The mechanics of surface duplication in euglenoids was firmly established in early electron micrographs of dividing cells of *Astasia longa* (Sommer and Blum, 1965). In these cells, a small ridge (presumptive daughter strip) appeared between each of the larger parental surface strips during cytokinesis. From these and other structural studies it was postulated that the intercalated daughter ridge (strip) would double the number of strips, for example, from 40 to 80 in *E. gracilis,* and that after cytokinesis each of these daughter cells would again consist of 40 strips. But of these 40 postdivision strips, 20 were assembled *de novo,* and each of these alternated with a strip that was conserved from the original predivision cell. This hypothesis was tested by pulse immunolabeling strips in predivision cells, and then following the distribution of bound antibodies after cytokinesis (Hofmann and Bouck, 1976). As expected, the immunolabeling on the postdivision cells was restricted to alternate (parental) strips, indicating that new strips with unlabeled antigens were assembled and inserted intussuscepted after the initial pulse labeling. The clear separation between labeled and unlabeled strips demonstrated that (1) there was little or no intermixing of exposed antigens between adjacent strips, and (2) the surface as a whole consisted of half old (parental) and half new strips after cytokinesis.

Generally, presumptive daughter strips first appear at the cell anterior and progressively extend more or less coordinately toward the posterior. In *Euglena gracilis,* new strips were present at the cell anterior by early prophase and had extended halfway down the cell by prometaphase (Pickett-Heaps and Weik, 1977); in the euglenoid *Ploeotia costata,* new strips were evident by anaphase (Triemer and Fritz, 1988), but strip insertion occurred later in cell division in the euglenoid *Entosiphon sulcatum* (Triemer, 1988). The division furrow proceeds by infolding between two opposing pairs of strips approximately 180 degrees apart, necessitating the rupture of the dividing cell and then the rejoining or "suturing" (Pochmann, 1953) of the daughter cell margins. This process appears to take place within a slice/suture zone (Fig. 5b) in which there is sequentially (1) separation (tearing apart) of a pair of adjacent strips at two opposing regions of the cell (Fig. 5b, arrowheads), (2) lateral joining (zippering) of nonadjacent strips (from opposing sides of the cell; Fig. 5b, arrows), and (3) systematic progression of this zone toward the cell posterior. The plasma membrane must be sufficiently fluid to accommodate these submembrane processes since it remains visibly undamaged and continuous in the slice/suture zone (Fig. 5b). The effects of the temporary weakness in surface structure that must result from separation of adjacent strips seem to be minimized by restricting the size of the splice/suture zone to a few micrometers, and by rapid rezippering of heterologous strip margins. The general position of the cleavage furrow will presumably be determined by the position of the duplicated canal and reservoir (Mignot *et al.,* 1987), but whether specific pairs of strips are predesignated as sites of cell separation is not known.

How new surface strips are initiated, and what controls initiation remains largely unexplained. Bré and Lefort-Tran (1978) and Lefort-Tran *et al.* (1980) observed that when cell division was inhibited by vitamin B_{12} starvation in *Euglena gracilis,* surface strips expanded laterally to accommodate the greatly enlarged cell, and new strips were not intercalated between parental strips unless vitamin B_{12} was restored to the medium. These experiments suggested that initiation and growth of daughter strips was in some

FIG. 2 *Euglena gracilis* viewed from the anterior pole undergoing cytokinesis. In the upper panel, the two putative daughter cells are separating parental strips by clockwise rotation. The zone of strip separation and rejoining is seen between the large arrowheads in the lower panel. At the upper large arrowhead, separated strips are rejoining (suturing) to construct the cell on the left. The lower large arrowhead indicates strips that are suturing (zippering) to form the cell on the bottom right. This zone of separation and rejoining progresses toward the cell posterior. The daughter strips intussuscepted between every second parental strip are more evident in the right-hand cell as a narrow strip. Top micrograph, ×3500; lower micrograph, ×8500. (Micrographs courtesy of Ron Wibel.)


a


b

way coregulated with, or was dependent on cell division and/or specifically, cytokinesis. In the euglenoid *Cyclidiopsis acus*, the surface ridges extend deep into the canal. Thus the formation of new surface ridges and their MTs, which appear before or simultaneously with strip assembly, can be traced from their presumed origins within the reservoir and basal body region (Mignot *et al.*, 1987). From EM studies using semiserial sections, however, it was evident that strips developed in two directions from a region close to the anterior invagination that gives rise to the canal and reservoir (collectively the flagellar pocket). In the nondividing cell, the 32 strips of the external surface were more or less uniform in width, whereas in the canal there were 16 major strips and 16 smaller strips. The latter developed into major strips during strip duplication and new minor strips appeared from "morphogenetic centers" located between major and minor strips. Interestingly, at the onset of cytokinesis, new strips (32 new plus 32 parental = 64) appeared in the external surface before they were evident in the canal (Mignot *et al.*, 1987), suggesting that surface development did not progress from canal to external surface, as might be expected from the presumed origin of the MTs (reservoir, basal body region; Willey and Wibel, 1985; Solomon *et al.*, 1991). Thus strips either arose independently of the canal MTs, or developed first outward over the external surface and then extended inward toward the flagellar pocket to form new strips within the canal. The transition region (i.e., the lip) between the canal and exterior surface seems to be the site of strip initiation, the site of the start of cytokinesis, and possibly the site of the generation of strips within the canal.

B. The Cortical Complex in Trypanosomes

As with the euglenoids, the trypanosomes undergo an ordered duplication and separation of the cortical region during cytokinesis that probably results in a similar retention of parental surface during the production of daughter cells. However, the surface of trypanosomes is not organized into visible strips, so it is not evident from direct observations how and where new cortical MTs are assembled, and how they are segregated during division. The question of cortical duplication has been addressed by using antibodies that recognize tyrosinated tubulins in order to identify new vs old microtubule regions (Sherwin *et al.*, 1987; Sherwin and Gull, 1989). In general, tubulins newly added to a growing MT were carboxy-tyrosinated, whereas as they matured within the MT, the tubulins were detyrosinated. Immunolabeling of extracted membrane skeletons from *T. brucei* showed that tyrosinated microtubule ends (i.e., the positive or fast-growing ends of the MTs) were located at the cell posterior (Sherwin and Gull, 1989). This is consistent with the modest elongation of the cell that accompanies the duplication

of the nucleus, basal body, and kinetoplast—all of which are arranged longitudinally before cytokinesis (Fig. 6). The demonstration of MT polarity was confirmed and extended with additional assays, including direct seeding of cytoskeletons with tubulin and observing the appearance and growth rates of "flumes" of new MTs at the posterior pole. These flumes also were decorated with antibodies against tyrosinated tubulins, again indicating preferential addition of new subunits to MT ends at the posterior cell pole (Robinson *et al.,* 1995). In addition to establishing overall MT polarity, Sherwin and Gull (1989) also demonstrated that near the cell center, short MTs were present between the MTs that comprised the microtubule corset. These short MTs labeled heavily with the antityrosinated MT antibody, suggesting that they were newly assembled and hence were increasing the MT population by intercalation—a process with obvious parallels to the intussusceptive strip development in the euglenoids. Cleavage of the cell during cytokinesis proceeds from anterior to posterior poles by means of a 'helical, biased longitudinal cleavage" (Sherwin and Gull, 1989; Robinson *et al.,* 1995) which partitions the linearly arranged pairs of duplicated nuclei and kinetosomes into the daughter cells. Since there is no evidence for

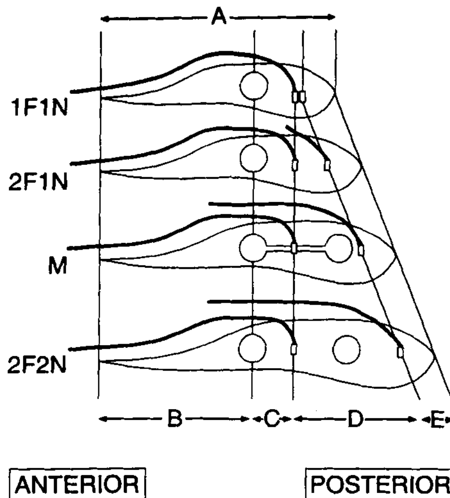


FIG. 6 Early stages in cell division in *Trypanosoma brucei* showing the linear arrangement of nuclei, basal bodies, and flagella. The cleavage furrow (not shown) follows a longitudinal helical path beginning at the anterior pole to separate the two set nuclei and basal bodies during cytokinesis. Cell expansion occurs mostly in the longitudinal axis, and predominantly in the zone labeled D before cytokinesis. [From Robinson *et al.* Reproduced from *The Journal of Cell Biology,* 1985, Vol. 128, 1163–1172 by copyright permission of The Rockefeller University Press.]

disassembly of parental MTs, the surface complex of each daughter cells presumably contains half of the original MT corset within which are intercalated a new MT between each of the parental MTs. As with the euglenoids, the predominantly longitudinal cleavage furrow forces a separation (tearing apart) of adjacent MTs of the corset followed by rejoining (suturing) of the new corset margins to generate two intact daughter cells.

It is not known whether the short intercalated MTs are initiated at microtubule organizing centers at their sites in the cell center, or whether they are initiated at the cell posterior (the major site of MT assembly) and are then transported to other regions along tracks of existing MTs. Cortical microtubules converge at a capping structure at the anterior pole of the cell, but at the posterior pole they end abruptly, leaving an opening in the membrane skeleton. A 28-kDa protein (Gb4) is localized to the posterior pole of the MT corset; Gb4 appears to be an MT capping structure (Rindisbacher *et al.*, 1993), although this has yet to be demonstrated experimentally. Most of the corset MTs are not directly associated with the basal body of the flagellum, but basal segregation during cell division appears to be an MT-dependent process, as indicated by its inhibition by the maytansine group of antimicrotubule agents (Robinson and Gull, 1991).

The position of the basal body and its flagellum is an important landmark in cytokinesis and in the longitudinal partitioning of the MT corset. The flagellum in *Trypanosoma* is directed from its origins in the flagellar pocket toward the cell pole, maintaining firm contact with the cell surface throughout much of its length. This contact region, termed the "flagellum attachment zone" (FAZ), is a desmosome-like complex, and includes a specialized set of four MTs and a narrow cisternum of endoplasmic reticulum. The FAZ represents the only obvious longitudinal asymmetry in the MT corset. Experiments with rhizoxin, which "inhibits microtubule dynamics" (Robinson *et al.*, 1995), have led to the suggestion that FAZ may define the axis of cleavage in trypanosomes.

C. The Cortical Complex in Ciliates

Cortical duplication and pattern formation in the ciliates, particularly *Paramecium*, have received considerable attention. The segregation of the cortex into thousands of asymmetric units with a defined position and orientation provides visible markers for genetic and developmental studies. Moreover, early stages of new unit assembly are readily identified either by duplication (basal bodies), expansion (epiplasm, cytospindle), insertion and elongation (outer lattice), and regression and expansion (kinetodesmal fibers). Each of these processes occurs more or less independently during cortical assembly. The arrangement of units is generally in curved longitudinal rows, but

Paramecium, Tetrahymena, and most other ciliates are asymmetric in both the dorsal–ventral axis and anterior–posterior axis. This asymmetry is defined in part by the position of the oral apparatus as well as a suture line along the ventral surface. Cytokinesis occurs transversely to the anterior–posterior (long) axis so that a new half-cell cortex must be generated, not as a simple extension of the existing cortex, i.e., as an infinitely elongating and segmenting cylinder (Frankel and Nelsen, 1981; Frankel, 1989), but as a complete reconstruction of an asymmetric half-cell. In fact, the newly regenerated cells are mosaics of invariant (conserved) regions and other areas that exhibit more or less independent behavior (Iftode *et al.,* 1989).

Fluorescein-labeled antibodies visualized by confocal microscopy of permeabilized cells have been particularly useful in identifying specific components of the cortex during cell division. In *Paramecium,* application of these techniques together with conventional silver staining has helped produce a fate map (Fig. 7) for several key structures of the cortical surface (Iftode *et al.,* 1989). The pattern of assembly is both spatially and temporally regulated in that specific cortical components are stimulated in successive "waves" that originate from a single or from two epicenters, located around the oral apparatus and the fission furrow. The nature of the waves is not known but they result in global regulation of morphogenesis, while at the local level individual scales respond more or less independently. The fate map provides graphic illustration of the independent timing of the development of basal bodies, epiplasm, and kinetodesmal fibers relative to the putative stimulatory waves. One documented effect of these waves is the activation of a protein kinase that hyperphosphorylates the structural proteins of the ciliary rootlets just before their disassembly (regression). A monoclonal antibody, MPM-2, specific for phosphoproteins was localized to ciliary rootlet fibers (Fig. 2a, kf, kinetodesmal fibers) before rootlet regression, but MPM-2 did not recognize rootlets in interphase cells, suggesting that phosphorylation and dephosphorylation cycles may be regulating factors in the regression and regrowth of ciliary rootlets (Sperling *et al.,* 1991). The lost by regression of this "rigid rope-like anteroposterior scaffold" (ciliary rootlets) appears to be compensated for by the appearance of a cytospindle consisting of bundles of longitudinal MTs, which generate a temporary scaffold to maintain cell integrity (Sperling *et al.,* 1991). Phosphorylation of other cortical components, such as the infraciliary lattice proteins, is probably also critical to cortical development (Kaczanowska *et al.,* 1995).

One of the interesting facts to emerge from the fate maps was that certain regions were invariant, that is, they remained essentially unchanged during each cell division. Other regions are extensively remodeled. Yet the absolute number of parental basal bodies is faithfully maintained in both daughter cells (Iftode *et al.,* 1989). The basis for and control of this remarkable

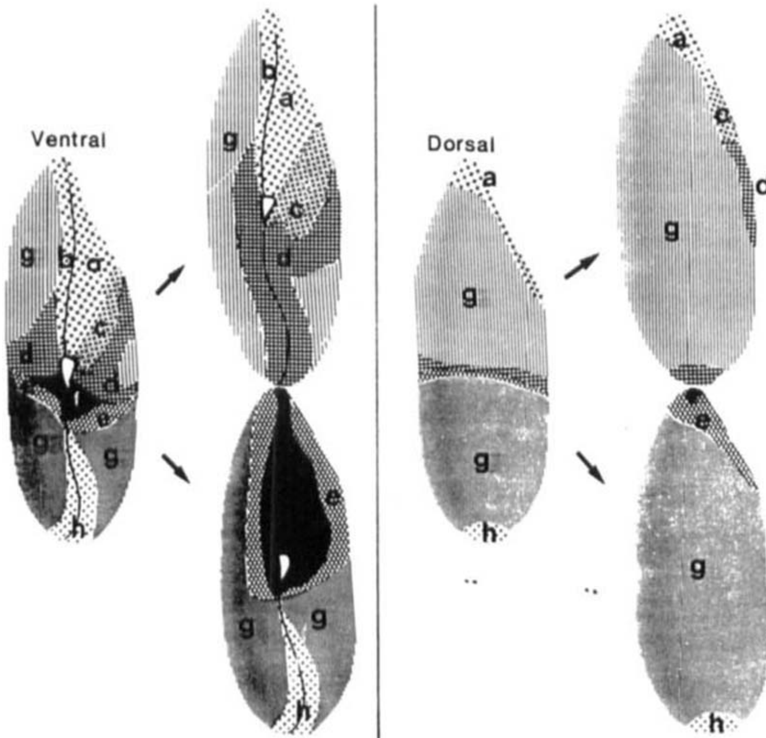


FIG. 7 Fate map of cell territories in *Paramecium* cell division. The division plane is transverse to the long axis, necessitating reconstruction of an asymmetric upper and lower half. The origin and fate of units (scales) is shown on both ventral and dorsal surfaces. Territories are delineated according to the extent and method of proliferation of basal bodies. Areas designated as a, b, and h are essentially invariant fields that are transmitted without obvious changes to daughter cells. Region f is transformed mostly into an invariant field in the posterior daughter cell. Area e is completely remodeled into anterior fields; g are territories that expand greatly by elongation. [Slightly modified from Iftode *et al.* (1989), reprinted with permission of Development Company of Biologists, Ltd.]

global control of cortical reproduction remains to be determined. Cortical duplication in the ciliate *Tetrahymena* also seems to be regulated at both the local and whole cell (global) levels since severe disruption of cortical organization in a *disorganized* mutant did not radically change the pattern of cytokinesis in these cells (Jerka-Dziadosz *et al.*, 1995).

D. The Cortical Complex in Dinoflagellates

The MT cytoskeleton of dinoflagellates consists of two groups of stable MTs: (1) the cortical microtubules and (2) those of the desmose and three-

pronged fork. Perret *et al.* (1991, 1993) reported that in *Cryptothecodinium* the latter two sets of MTs duplicate early in prophase and segregate into the two putative daughter cells. It is the migration of the two three-pronged forks that seems to separate the cortical MTs because all the cortical MTs are anchored on the forks. It is not yet known whether the two sets of epiplasmic MTs are semiconserved or whether they are all formed *de novo*. The actin–tubulin-rich cleavage furrow is thought to recruit the stable MTs of the transverse cingulum. The duplication and assembly of the kinetosome and centrosome-like regions, the desmose, three–pronged fork, epiplasmic MTs and extranuclear spindle MTs are carefully timed and interdependent events that precede the appearance of the cleavage furrow.

Cell cleavage begins at either or both poles (Schnepf, 1988; Perret *et al.*, 1993), and follows an oblique course from posterior left to anterior right, as suggested by the fission line of the parental amphiesma (Dodge, 1988). In some dinoflagellates (e.g., *Peridinium),* the growth of the cleavage furrow "proceeds like a draw string being tightened" (Tippit and Pickett-Heaps, 1976). Cytokinesis may require both MTs and actin microfilaments, as evidenced by the localization of β-tubulin and actin to the cleavage furrow (Perret *et al.*, 1993; Schnepf, 1988). In several studies cortical MTs were shown to line the two surfaces of the cleavage furrow in close association with the newly recruited amphiesmal vesicle (Weatherbee, 1975; Dürr, 1979; Tippit and Pickett-Heaps, 1976; Perret *et al.*, 1993), suggesting that epiplasmic MTs of the cingulum were directly involved with the formation of the furrow. In the absence of experimental evidence, however, it remains uncertain whether MTs function actively in cell cleavage, or whether they are associated with transport of the new amphiesmal vesicle, or both.

Cell cleavage in some dinoflagellates can be divided into an actin-dependent and an actin-independent stage. For example, when thecate and naked dinoflagellates were treated with the antimicrofilament drug cytochalasin D, the cleavage furrow passed through and separated the daughter hypocones, but cleavage did not progress through the epicone (Schnepf, 1988). After prolonged treatment with cytochalasin (several days), the binucleate cell completed the next nuclear divisions—resulting in groups of four or more cells still attached apically (Schnepf *et al.*, 1990). Similar results were reported with the drug isopropyl-*N*-phenyl carbamate, which reportedly affects the microtubule organizing center (Matthys-Rochon, 1980). Possibly, the biphasic cleavage in these cells is a reflection of the bipolar organization of the cell, i.e., the epicone and hypocone separated by the cingulum and containing the kinetosomes. Regardless of the method of cleavage in dinoflagellates, the resulting daughter cells probably each consist of conserved MTs (Perret *et al.*, 1993) and other portions of the original parental cell cortex, although this conservation has not yet been directly demonstrated.

In summary, euglenoids, trypanosomes, ciliates, and dinoflagellates seem to reuse ("conserve") all or part of their existing cortical components during cell duplication. Among these groups, ciliates such as *Paramecium* exhibit the most complex pattern of cortical duplication. It is not yet clear how the cortical mosaics are locally regulated and how the various mosaics are globally controlled to yield an exact copy of the original cell. Recent experiments with protein kinase inhibitors show promise for developing models (e.g., tensegrity, Kaczanowska *et al.,* 1995) of global control, but cortical remodeling and the resulting mosaics produced at the regional level are still difficult to explain. In euglenoids and trypanosomes, the patterns of duplication are more easily visualized as a relatively simple intussusception of whole strips (euglenoids) or the insertion of single MTs (trypanosomes) between each of the existing strips or MTs. Longitudinal fission then separates the integrated (with old and new components) cell into two equivalent halves.

Interestingly, in trypanosomes the daughter nuclei are arranged along the long axis and the cleavage furrow must pass as a steep, almost transverse spiral to separate the two cells (Robinson *et al.,* 1995) perhaps presaging the transverse division of the ciliates, whereas in euglenoids the nuclei are side by side and are readily cleaved by a longitudinal or spiral furrow (Mignot *et al.,* 1987). In both cases intussusception permits surface duplication without extensive disruption of the existing cortical architecture. Cortical duplication in dinoflagellates is still largely conjecture, but the pattern of fission, and the probable absence of parental MT breakdown (Perret *et al.,* 1993), would suggest that portions of the cell are conserved, and that there is no large-scale cortical disassembly accompanying cytokinesis.

V. Concluding Remarks

The surface complex of protists has been of long-standing interest, because (1) the cortex is relatively stable, (2) the cortical patterns are inherited by succeeding generations, (3) portions of the cortex remain intact during and after cytokinesis and (4) the cortical components are often readily visualized and followed during duplication and cytokinesis. The biochemical, physiological, molecular, and cellular bases for these cortical properties have been studied to varying degrees in the four different groups of organisms considered here. It is evident that each group of protists offers different and often overlapping opportunities for resolving the properties of different components of the cortex. Thus the epiplasm is relatively easily isolated and characterized in euglenoids; MT stability and MAP function can be readily approached in the corset of MTs of the trypanosomes; surface

glycoconjugates and surface coats are accessible in ciliates and trypanosomes that undergo coat switches and antigenic variation, and alveoli are best examined in ciliates and dinoflagellates, where these chambers are highly developed.

An implicit assumption of this review has been that the cortical components such as the epiplasm, alveoli, MTs and some membrane coats of these very different protists have common functions. However, are the cortical systems of protists analogous? The evidence for similar functions and/or composition is in most cases incomplete, and commonality of cortical components among phylogenetically distant groups should be viewed primarily as a working hypothesis. It is worth noting, however, that whereas the functional MTs and actin of higher eukaryotes are also found in protists (Grain, 1986), at least one group of important and ubiquitous membrane skeletal proteins (the spectrins) of vertebrates and invertebrates (Bennett and Gilligan, 1993) is either absent or has no established function in lower eukaryotes. Spectrin-like proteins have been identified in several protists (Schneider *et al.*, 1988b; Alcina *et al.*, 1988; Williams *et al.*, 1989b; Hemphill *et al.*, 1991; Kwiatkowska and Sobota, 1992; Ghazali *et al.*, 1995; Lorenz *et al.*, 1995) by size and immunological criteria, but spectrin-based membrane skeletons have not been demonstrated in protists, and most protists lack spectrin altogether.

Conversely, higher organisms lack obvious homologs to articulin-based epiplasms and to the continuity of cortical structures during cell division. Whether these differences are the result of multicellularity vs the single-cell life style, or the result of evolutionary selection is not clear. In any case, the protists offer unparalleled opportunities for resolving the mechanisms of control and assembly of the supramolecular complexes that comprise the cell cortex. With powerful molecular, biochemical, and immunological and genetic tools now available, we can look forward to their increasing application to protists as interesting systems in their own right as well as approachable models for understanding how the cell surface is generated, maintained, and duplicated faithfully with each cell division.

References

Affolter, M., Hemphill, A., Roditi, I., Müller, N., and Seebeck, T. (1994). The repetitive microtubule-associated proteins MARP-1 and MARP-2 of *Trypanosoma brucei*. *J. Struct. Biol.* **112,** 241–251.

Alcina, A., Hargreaves, A. J., Avila, J., Hesketh, J. E., and Fresno, M. (1988). The detection of a spectrin-like protein in *Trypanosoma cruzi* with a polyclonal antibody. *Cell Biol. Int. Rep.* **12,** 979–985.

Allen, R. D. (1988). Cytology. *In* "Paramecium" (H.-D. Görtz, ed), pp. 4–40. Springer-Verlag. Berlin.

Antony, C., and Capdeville, Y. (1989). Uneven distribution of surface antigens during antigenic variation in *Paramecium primaurelia. J. Cell Sci.* **92,** 205–215.

Audebert, S., Desbruyeres, E., Gruszczynski, C., Koulakoff, A., Gros, F., Denoulet, P., and Edde, B. (1993). Reversible polyglutamylation of α- and β-tubulin and microtubule dynamics in mouse brain neurons. *Mol. Biol. Cell* **4,** 615–626.

Azzouz, N., Striepen, B., Gerold, P., Capdeville, Y., and Schwarz, R. T. (1995). Glycosylinositol-phosphoceramide in the free-living protozoan *Paramecium primaurelia:* modification of core glycans by mannosyl phosphate. *EMBO J.* **14,** 4422–4433.

Balaban, N., and Goldman, R. (1992). Isolation and characterization of a unique 15 kilodalton trypanosome subpellicular microtubule-associated protein. *Cell Motil. Cytoskeleton* **21,** 138–146.

Balaban, N., Waithaka, H. K., Njogu, A. R., and Goldman, R. (1989). Isolation of a subpellicular microtubule protein from *Trypanosoma brucei* that mediates crosslinking of microtubules. *Cell Motil. Cytoskeleton* **14,** 393–400.

Barnes, L. S. D., Walne, P. L., and Dunlap, J. R. (1986). Cytological and taxonomic studies of the euglenales. I. Ultrastructure and envelope elemental composition in *Trachelomonas. Br. Phycol. J.* **21,** 387–397.

Baroin-Tourancheau, A., Delgado, P., Perasso, R., and Adoutte, A. (1992). A broad molecular phylogeny of ciliates: Identification of major evolutionary trends and radiations within the phylum. *Proc. Natl. Acad. Sci. U.S.A.* **89,** 9764–9768.

Bennett, V., and Gilligan, D. M. (1993). The spectrin-based membrane skeleton and micron-scale organization of the plasma membrane. *Annu. Rev. Cell Biol.* **9,** 27–66.

Blum, M. L., Down, J. A., Gurnett, A. M., Carrington, M., Turner, M. J., and Wiley, D. C. (1993). A structural motif in the variant surface glycoproteins of *Trypanosoma brucei. Nature* **362,** 603–609.

Bouck, G. B., and Chen, S.-J. (1984). Synthesis and assembly of the flagellar surface. *J. Protozool.* **31,** 21–24.

Bramblett, G. T., Kambadur, R., and Flavin, M. (1989). Immunocytochemical studies with antibodies to three proteins prominent in the isolated microtubule cytoskeleton of a trypanosomatid. *Cell Motil. Cytoskeleton* **13,** 145–157.

Bré, M.-H., and Lefort-Tran, M. (1978). Induction et réversibilité des événements cuticulaires par carence et réalimentation en vitamine B_{12} chez *Euglena gracilis. J. Ultractruct. Res.* **64,** 362–376.

Bré, M.-H., Lefort-Tran, M., Obrenovitch, A., and Monsigny, M. (1984). Detection of *Euglena* cell surface carbohydrates by lectins: alterations related to vitamin B_{12} deficiency. *Eur. J. Cell Biol.* **35,** 273–278.

Bré, M.-H., Delpech-Lafouasse, S., and Adoutte, A. (1986a). Cortical tubulin in *Euglena:* modifications related to a cell cycle arrest. *Biol. Cell.* **58,** 239–244.

Bré, M.-H., Philippe, M., Fournet, B., Delpech-Lafouasse, S., Pouphile, M., and Lefort-Tran, M. (1986b). Identification of cell surface glycoconjugates in a unicellular organism: modifications related to vitamin B_{12} deficiency. *Eur. J. Cell Biol.* **41,** 189–197.

Bricheux, G., and Brugerolle, G. (1986). The membrane cytoskeleton complex of euglenids. I. Biochemical and immunological characterization of the epiplasmic proteins of *Euglena acus. Eur. J. Cell Biol.* **40,** 150–159.

Bricheux, G., and Brugerolle, G. (1987). The pellicular complex of euglenoids. II. A biochemical and immunological comparative study of major epiplasmic proteins. *Protoplasma* **140,** 43–54.

Bricheux, G., Mahoney, D. G., and Gibbs, S. P. (1992). Development of the pellicle and thecal plates following ecdysis in the dinoflagellate *Glenodinium foliaceum. Protoplasma* **168,** 159–171.

Brown, D. L., Cachon, J., Cachon, M., and Boillot, A. (1988). The cytoskeletal microtubular system of some naked dinoflagellates. *Cell Motil. Cytoskeleton* **9,** 361–374.

Burns, J. M., Shreffler, W. G., Benson, D. R., Ghalib, H. W., Badaro, R., and Reed, S. G. (1993). Molecular characterization of a kinesin-related antigen of *Leishmania chagasi* that detects specific antibody in African and American visceral leishmaniasis. *Proc. Natl. Acad. Sci. U.S.A.* **90,** 775–779.

Burns, R. G., and Surridge, C. D. (1994). Tubulin: Conservation and structure. *In* "Microtubules" (J. S. Hyams and C. W. Lloyd, eds.), pp. 3–31. Wiley, New York.

Cachon, J., and Cachon, M. (1974). Le systeme stromopharyngien de *Kofoidinium* Pavillard. Comparaisons avec de divers peridiniens libres et parasites. *Protistologica* **10,** 217–222.

Cachon, J., and Cachon, M. (1984). An unusual mechanism of cell contraction (Leptodiscinae Dinoflagellates). *Cell Motil. Cytoskeleton* **4,** 41–55.

Cachon, J., Cachon, M., Greuet, C., and Huitorel, P. (1994). Nanofilament dependent motility in dinoflagellates. *Biol. Cell.* **81,** 1–10.

Caron, F., and Meyer, E. (1989). Molecular basis of surface antigen variation in paramecia. *Annu. Rev. Microbiol.* **43,** 23–42.

Cavalier-Smith, T. (1993). Kingdom protozoa and its 18 phyla. *Microbiol. Rev.* **57,** 953–994.

Chan, M. M.-Y., and Fong, D. (1990). Inhibition of leishmanias but not host macrophages by the antitubulin herbicide trifluralin. *Science* **249,** 924–926.

Chen, S. J., and Bouck, G. B. (1984). Endogenous glycosyltransferases glucosylate lipids in flagella of *Euglena. J. Cell Biol.* **98,** 1825–1835.

Cogburn, J. N., and Schiff, J. A. (1984). Purification and properties of the mucus of *Euglena gracilis* (euglenophyceae). *J. Phycol.* **20,** 533–544.

Cohen, J., and Beisson, J. (1988). The cytoskeleton. *In* "Paramecium" (H.-D. Görtz, ed.), pp. 363–392. Springer-Verlag, Berlin.

Cross, G. A. M. (1990). Glycolipid anchoring of plasma membrane proteins. *Annu. Rev. Cell Biol.* **6,** 1–39.

Curtenaz, S., and Peck, R. K. (1992). A monoclonal antibody study of protein distribution in the membrane skeleton of the ciliate *Pseudomicrothorax. J. Cell Sci.* **103,** 1117–1125.

Curtenaz, S., Nahon, P., Iftode, F., and Fleury, A. (1994). Interspecific immunological cross-reactions among cortical proteins of four ciliates. *Eur. J. Protistol.* **30,** 440–450.

De Souza, W. (1989). Components of the cell surface of trypanosomatids. *In* "Progress in Protistology" (D. J. Patterson and J. O. Corliss, eds.), pp. 87–184. Biopress, Bristol, England.

Docampo, R., and Moreno, S. N. J. (1996). The role of Ca^{2+} in the process of cell invasion by intracellular parasites. *Parasitol. Today* **12,** 61–65.

Dodge, J. D. (1988). An SEM study of thecal division in *Gonyaulax* (Dinophyceae). *Phycologia* **27,** 241–247.

Doerder, F. P., Arslanyolu, M., Said, Y., Kaczmarek, M., Mendoza, M., and Mita, B. (1996). Ecological genetics of *Tetrahymena thermophila:* Mating types, i-antigens, multiple alleles and epistosis. *J. Euk. Microbiol.* **43,** 95–100.

Dolan, M. T., Reid, C. G., and Voorheis, H. P. (1986). Calcium ions initiate the selective depolymerization of the pellicular microtubules in bloodstream forms of *Trypanosoma brucei, J. Cell Sci.* **80,** 123–140.

Dubreuil, R. R., and Bouck, G. B. (1985). The membrane skeleton of a unicellular organism consists of bridged, articulating strips. *J. Cell Biol.* **101,** 1884–1896.

Dubreuil, R. R., and Bouck, G. B. (1988). Interrelationships among the plasma membrane, the membrane skeleton and surface form in a unicellular flagellate. *Protoplasma* **143,** 150–164.

Dubreuil, R. R., Rosiere, T. K., Rosner, M. C., and Bouck, G. B. (1988). Properties and topography of the major integral plasma membrane protein of a unicellular organism. *J. Cell Biol.* **107,** 191–200.

Dunlap, J. R., and Walne, P. L. (1985). Fine structure and biomineralization of the mucilage in envelopes of *Trachelomonas lefevrei* (Euglenophyceae). *J. Protozool.* **32,** 437–441.

Dunlap, J. R., Walne, P. L., and Kivic, P. A. (1986). Cytological and taxonomic studies of the euglenales. II. Comparative microarchitecture and cytochemistry of envelopes of *Strombomonas* and *Trachelomonas. Br. Phycol. J.* **21,** 399–405.

Dürr, G. (1979). Elektronenmikroskopische Unteruchengen am Panzer von Dinoflagellaten. I. *Gonyaulax polyedra. J. Phycol.* **31,** 395–400.

Duszenko, M., Ivanov, I. E., Ferguson, M. A. J., Plesken, H., and Cross, G. A. M. (1988). Intracellular transport of a variant surface glycoprotein in *Trypanosoma brucei. J. Cell Biol.* **106,** 77–86.

Englund, P. T. (1993). The structure and biosynthesis of glycosyl phosphatidylinositol protein anchors. *Annu. Rev. Biochem.* **62,** 121–138.

Fazio, M. J., Da Silva, A. C., Rosiere, T. K., and Bouck, G. B. (1995). Membrane skeletal protein anchors are targets for tyrosine and threonine kinases in *Euglena. J. Euk. Microbiol.* **42,** 570–580.

Ferguson, M. A. J., and Williams, A. F. (1988). Cell-surface anchoring of proteins via glycosyl-phosphatidylinositol structures. *Annu. Rev. Biochem.* **57,** 285–320.

Fleury, A., and Laurent, M. (1995). Microtubule dynamics and morphogenesis in *Paramecium. Eur. J. Protistol.* **31,** 190–200.

Fleury, A., Delgado, P., Iftode, F., and Adoutte, A. (1992). Molecular phylogeny of ciliates: What does it tell us about the evolution of the cytoskeleton and of developmental strategies? *Dev. Genet.* **13,** 247–254.

Fleury, A., Callen, A.-M., Bré, M.-H., Iftode, F., Jeanmaire-Wolf, R., Levilliers, N., and Clérot, J.-C. (1995). Where and when is microtubule diversity generated in *Paramecium?* Immunological properties of microtubular networks in the interphase and dividing cells. *Protoplasma* **189,** 37–60.

Forney, J. D., Epstein, L. M., Preer, L. B., Rudman, B. M., Widmayer, D. J., Klein, W. H., and Preer, J. R. (1983). Structure and expression of genes for surface proteins in *Paramecium. Mol. Cell. Biol.* **3,** 466–474.

Frankel, J. (1989). "Pattern Formation." Oxford Univ. Press, New York.

Frankel, J., and Nelsen, E. M. (1981). Discontinuities and overlaps in patterning within single cells. *Philos. Trans. R. Soc. London, Ser. B* **295,** 525–538.

Gallo, J.-M., and Schrével, J. (1982). Euglenoid movement in *Distigma proteus. Biol. Cell.* **42,** 139–148.

Garreau de Loubresse, N., Keryer, G., Vigues, B., and Beisson, J. (1988). A contractile cytoskeletal network of *Paramecium:* the infraciliary lattice. *J. Cell Sci.* **90,** 351–364.

Garreau de Loubresse, N., Klotz, C., Vigues, B., Rutin, J., and Beisson, J. (1991). Ca^{2+}-binding proteins and contractility of the infraciliary lattice in *Paramecium. Biol. Cell.* **71,** 217–223.

Gelfand, V. I., and Bershadsky, A. D. (1991). Microtubule dynamics: mechanism, regulation, and function. *Annu. Rev. Cell Biol.* **7,** 93–116.

Ghazali, M., Rodier, M.-H., El Moudni, B., Babin, P., Fernandez, B., and Jacquemin, J.-L. (1995). Detection and immunolocalization of human erythrocyte spectrin immunoanalogues in *Toxoplasma gondii* (Protozoan, Parasite). *J. Euk. Microbiol.* **42,** 427–433.

Gilley, D., Rudman, B. M., Preer, J. R., and Polisky, B. (1990). Multilevel regulation of surface antigen expression gene in *Paramecium tetraurelia. Mol. Cell. Biol.* **10,** 1538–1544.

Godart, H., and Huitorel, P. (1992). Effects of calcium on the longitudinal flagellum of *Oxyrrhis marina. Biol. Cell.* **76,** 365–372.

Grain, J. (1986). The cytoskeleton in protists: Nature, structure, and functions. *Int. Rev. Cytol.* **104,** 153–249.

Gumpel, N. J., and Smith, A. G. (1992). A novel calcium-binding protein from *Euglena gracilis. Eur. J. Biochem.* **210,** 721–727.

Gurland, G., and Gundersen, G. G. (1993). Protein phosphatase inhibitors induce the selective breakdown of stable microtubules in fibroblasts and epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* **90,** 8827–8831.

Hausmann, K. (1979). Characteristics of the membranes of the pellicle of the ciliate *Pseudomicrothorax dubius. Protoplasma* **100,** 199–213.

Hehl, A., Vassella, E., Braun, R., and Riditi, I. (1994). A conserved stem-loop structure in the 3′ untranslated region of procyclin mRNAs regulates expression in *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. U.S.A.* **91,** 370–374.

Heidemann, S. R., and McIntosh, J. R. (1980). Visualization of the structural polarity of microtubules. *Nature* **286,** 517–519.

Hemphill, A., Seebeck, T., and Lawson, D. (1991). The *Trypanosoma brucei* cytoskeleton: Ultrastructure and localization of microtubule-associated and spectrin-like proteins using quick-freeze, deep-etch, immunogold electron microscopy. *J. Struct. Biol.* **107,** 211–220.

Hemphill, A., Affolter, M., and Seebeck, T. (1992). A novel microtubule-binding motif identified in a high molecular weight microtubule-associated protein from *Trypanosoma brucei*. *J. Cell Biol.* **117,** 95–103.

Hitt, A. L., Lu, T. H., and Luna, E. J. (1994). Ponticulin is an atypical membrane protein. *J. Cell Biol.* **126,** 1421–1431.

Hoffmann, C., and Bouck, G. B. (1976). Immunological and structural evidence for patterned intussusceptive surface growth in a unicellular organism. *J. Cell Biol.* **69,** 693–715.

Höhfeld, I., and Melkonian, M. (1992). Amphiesmal ultrastructure of dinoflagellates: A reevaluation of pellicle formation. *J. Phycol.* **28,** 82–89.

Höhfeld, I., Beech, P. L., and Melkonian, M. (1994). Immunolocalization of centrin in *Oxyrrhis marina* (Dinophyceae). *J. Phycol.* **30,** 474–489.

Huitorel, P., and Pantaloni, D. (1985). Bundling of microtubules by glyderaldehyde-3-phosphate-dehydrogenase and its modulation by ATP. *Eur. J. Biochem.* **150,** 265–269.

Huttenlauch, I., and Peck, R. K. (1991). The membrane skeleton of *Pseudomicrothorax*. II. Biochemical and immunological characterization of the glycosylated cytoskeletal proteins. *J. Cell Sci.* **100,** 707–715.

Huttenlauch, I., Geisler, N., Plessmann, U., Peck, R. K., Weber, K., and Stick, R. (1995). Major epiplasmic proteins of ciliates are articulins: Cloning, recombinant expression, and structural characterization. *J. Cell Biol.* **130,** 1401–1412.

Iftode, F., Cohen, J., Ruiz, F., Rueda, A. T., Chen-Shan, L., Adoutte, A., and Beisson, J. (1989). Development of surface pattern during division in *Paramecium*. I. Mapping of duplication and reorganization of cortical cytoskeletal structures in the wild type. *Development* **105,** 191–211.

Jeanmaire-Wolf, R., Clérot, J.-C., Nahon, P., Iftode, F., Fleury, A., and Adoutte, A. (1993). Isolation and characterization of monoclonal antibodies to cytoskeletal and membrane proteins or the *Paramecium* cortex. *Eur. J. Protistol.* **29,** 311–333.

Jerka-Dziadosz, M., Jenkins, L. M., Nelsen, E. M., Williams, N. E., Jaeckel-Williams, R., and Frankel, J. (1995). Cellular polarity in ciliates: Persistence of global polarity in a *disorganized* mutant of *Tetrahymena thermophila* that disrupts cytoskeletal organization. *Dev. Biol.* **169,** 644–661.

Kaczanowska, J., Iftode, F., Jeanmaire-Wolf, R., Clérot, J.-C., Kiersnowska, M., and Adoutte, A. (1995). Tensegrity model of pattern formation during cytokinesis of a ciliate, *Paramecium:* Effects of an inhibitor of phosphorylation, 6-dimethylaminopurine, and of adenine. *J. Exp. Zool.* **273,** 494–510.

Keryer, G., Adoutte, A., Ng, S. F., Cohen, J., Garreau de Loubresse, N., Rossignol, M., Stelly, N., and Beisson, J. (1990). Purification of the surface membrane-cytoskeleton complex (cortex) of *Paramecium* and identification of several of its protein constituents. *Eur. J. Protistol.* **25,** 209–225.

Kishore, R., Muchhal, U. S., and Schwartzbach, S. D. (1993). The presequence of *Euglena* LHCPII, a cytoplasmically synthesized chloroplast protein, contains a functional endoplasmic reticulum-targeting domain. *Proc. Natl. Acad. Sci. U.S.A.* **90,** 11845–11849.

Kloetzel, J. A. (1991). Identification and properties of plateins, major proteins in the cortical alveolar plates of *Euplotes*. *J. Protozool.* **38,** 392–401.

Klut, M. E., Bisalputra, T., and Antia, N. J. (1985). Some cytochemical studies on the cell surface of *Amphidinium carterae* (Dinophyceae). *Protoplasma* **129,** 93–99.

Klut, M. E., Bisalputra, T., and Antia, N. J. (1988). The use of fluorochromes in the cytochemical characterization of some phytoflagellates. *Histochem. J.* **20,** 35–40.

Kubai, D. F., and Ris, H. (1969). Division in the dinoflagellate *Gyrodinium cohnii* (schiller). A new type of nuclear reproduction. *J. Cell Biol.* **40,** 508–528.

Kumagai, H., and Sakai, H. (1983). A porcine brain protein (35 K protein) which bundles microtubules and its identification as glyceraldehyde 3-phosphate dehydrogenase. *J. Biochem. (Tokyo)* **93,** 1259–1269.

Kwiatkowska, K., and Sobota, A. (1992). 240 kDa immunoanalogue of vertebrate α-spectrin occurs in *Paramecium* cells. *Cell Motil. Cytoskeleton* **23,** 111–121.

Lee, R. E., and Kugrens, P. (1992). Relationship between the flagellates and the ciliates. *Microbiol. Rev.* **56,** 529–542.

Leeck, C. L., and Forney, J. D. (1994). The upstream region is required but not sufficient to control mutually exclusive expression of *Paramecium* surface antigen genes. *J. Biol. Chem.* **269,** 31283–31288.

Leedale, G. F. (1967). "Euglenoid Flagellates." Prentice-Hall, Englewood Cliffs, New Jersey.

Leedale, G. F. (1975). Envelope formation and structure in the euglenoid genus *Trachelomonas. Br. Phycol. J.* **10,** 17–41.

Lefort-Tran, M., Bré, M. H., Ranck, J. L., and Pouphile, M. (1980). *Euglena* plasma membrane during normal and vitamin B_{12} starvation growth. *J. Cell Sci.* **41,** 245–261.

Levasseur, P. J., Meng, Q., and Bouck, G. B. (1994). Tubulin genes in the algal protist *Euglena gracilis. J. Euk. Microbiol.* **41,** 468–477.

L'Hernault, S. W., and Rosenbaum, J. L. (1983). *Chlamydomonas* α-tubulin is posttranslationally modified in the flagella during flagellar assembly. *J. Cell Biol.* **97,** 258–263.

Li, D. (1996). Genomic organization and phosphorylation of the major membrane skeletal proteins in *Euglena gracilis.* Ph. D. Thesis. University of Illinois at Chicago.

Loeblich, A. R. (1970). The amphiesma or dinoflagellate cell covering. *In* "Proceedings of the North American Paleontological Convention" (E. L. Yochelson, ed.), Vol. 2, Part G, pp. 867–929. Allen Press, Lawrence, Kansas.

Loeblich, A. R. (1984). Dinoflagellate physiology and biochemistry. *In* "Dinoflagellates" (D. L. Spector, ed.), pp. 299–342. Academic Press, New York.

Lorenz, M., Bisikirska, B., Hanus-Lorenz, B., Strzalka, K., and Sikorski, A. F. (1995). Proteins reacting with anti-spectrin antibodies are present in *Chlamydomonas* cells. *Cell Biol. Int.* **19,** 83–90.

Love, H. D., Allen-Nash, A., Zhao, Q., and Bannon, G. A. (1988). mRNA stability plays a major role in regulating the temperature-specific expression of a *Tetrahymena thermophila* surface protein. *Mol. Cell Biol.* **8,** 427–432.

Mandelkow, E., and Mandelkow, E.-M. (1995). Microtubules and microtubule-associated proteins. *Curr. Opinion Cell Biol.* **7,** 72–81.

Marrs, J. A., and Bouck, G. B. (1992). The two major membrane skeletal proteins articulius of *Euglena gracilis* define a novel class of cytoskeletal proteins. *J. Cell Biol.* **118,** 1465–1475.

Martin, L. D., Pollack, S., Preer, J. R., and Polisky, B. (1994). DNA sequence requirements for the regulation of immobilization antigen A expression in *Paramecium tetraurelia. Dev. Genet.* **15,** 443–451.

Masterson, W. J., Doering, T. L., Hart, G. W., and Englund, P. T. (1989). A novel pathway for glycan assembly: Biosynthesis of the glycosyl-phosphatidylinositol anchor of the trypanosome variant surface glycoprotein. *Cell* **56,** 793–800.

Matthys-Rochon, E. (1980). Action de l'*Amphidinium carterae* Hulburt. Effects sur la croissance et la dinomitose. *Cryptogamie: Algol.* **1,** 3–17.

McConville, M. J., and Ferguson, A. J. (1993). The structure, biosynthesis and function of glycosylated phosphatidylinositols in the parasitic protozoa and higher eukaryotes. *Biochem. J.* **294,** 305–324.

Métivier, C., de Billy, F., and Soyer-Gobillard, M.-O. (1987). Biochemical characterization and immunological localization of a 45 kDa cytoskeletal protein in the dinoflagellate *Noctiluca scintillans*. *Eur. J. Cell Biol.* **44,** 42–49.

Mignot, J. P., Brugerolle, G., and Bricheux, G. (1987). Intercalary strip development and dividing cell morphogenesis in the euglenid *Cyclidiopsis acus*. *Protoplasma* **139,** 51–65.

Miller, K. R., and Miller, G. J. (1978). Organization of the cell membrane in *Euglena*. *Protoplasma* **95,** 11–24.

Morrill, L. C., and Loeblich, A. R. (1981). The dinoflagellate pellicular wall layer and its occurrence in the division Pyrrhophyta. *J. Phycol.* **17,** 315–323.

Murray, J. M. (1981). Control of cell shape by calcium in the euglenophyceae. *J. Cell Sci.* **49,** 99–117.

Murray, J. M. (1983). Three-dimensional structure of a membrane-microtubule complex. *J. Cell Biol.* **98,** 283–295.

Murray, J. M. (1984). Disassembly and reconstitution of a membrane-microtubule complex. *J. Cell Biol.* **98,** 1481–1487.

Nahon, P., Coffe, G., Le Guyader, H., Darmanaden-Delorme, J., Jeanmaire-Wolf, R., Clérot, J.-C., and Adoutte, A. (1993). Identification of the epiplasmins, a new set of cortical proteins of the membrane cytoskeleton in *Paramecium*. *J. Cell Sci.* **104,** 975–990.

Nakano, Y., Urade, Y., Urade, R., and Kitaoka, S. (1987). Isolation, purification, and characterization of the pellicle of *Euglena gracilis* z. *J. Biochem.* (*Tokyo*) **102,** 1053–1063.

Nielsen, E., You, Y., and Forney, J. (1991). Cysteine residue periodicity is a conserved structural feature of variable surface proteins from *Paramecium tetraurelia*. *J. Mol. Biol.* **222,** 835–841.

Oakley, B. R. (1994). γ-Tubulin. *In* "Microtubules" (J. S. Hyams and C. W. Lloyd, eds.), pp. 33–45. Wiley, New York.

Pays, E., Vanhamme, L., and Berberof, M. (1994). Genetic controls for the expression of surface antigens in african trypanosomes. *Annu. Rev. Microbiol.* **48,** 25–52.

Peck, R. K. (1977). The ultrastructure of the somatic cortex of *Pseudomicrothorax dubius*: structure and function of the epiplasm in ciliated protozoa. *J. Cell Sci.* **25,** 367–385.

Perret, E., Alberts, M., Bordes, N., Bornens, M., and Soyer-Gobillard, M.-O. (1991). Microtubular spindle and centrosome structures during the cell cycle in a dinoflagellate *Crypthecodinium cohnii* B: an immunological study. *Biosystems* **24,** 53–65.

Perret, E., Davoust, J., Albert, M., Besseau, L., and Soyer-Gobillard, M.-O. (1993). Microtubule organization during the cell cycle of the primitive eukaryote dinoflagellate *Crypthecodinium cohnii*. *J. Cell Sci.* **104,** 639–651.

Pickett-Heaps, J. D., and Weik, K. L. (1977). Cell division in *Euglena* and *Phacus*. I. Mitosis. *In* "Mechanisms and Control of Cell Division" (T. L. Rost and E. M. Gifford, eds.), pp. 308–336. Dowden, Hutchinson & Ross, Stroudsburg, Pennsylvania.

Pimenta, P. F. P., and De Souza, W. (1985). Fine structure and cytochemistry of the endoplasmic reticulum and its association with the plasma membrane of *Leishmania mexicana amazonensis*. *J. Submicrosc. Cytol.* **17,** 413–419.

Pochmann, A. (1953). Struktur, Wachstum und Teilung der Körperhülle bei den Eugleninen. *Planta* **42,** 478–548.

Prat, A., Katinka, M., Caron, F., and Meyer, E. (1986). Nucleotide sequence of the *Paramecium primaurelia* G surface protein. *J. Mol. Biol.* **189,** 47–60.

Preer, J. R., Jr. (1986). Surface antigens of *Paramecium*. *In* "The Molecular Biology of Ciliated Protozoa" (J. G. Gall, ed.), pp. 301–339. Academic Press, Orlando, Florida.

Preer, J. R., Jr., Preer, L. B., Rudman, B., and Barnett, A. (1987). Molecular biology of the genes for immobilization antigens in *Paramecium*. *J. Protozool.* **34,** 418–423.

Redeker, V., Levilliers, N., Schmitter, J. M., Le Caer, J. P., Rossier, J., Adoutte, A., and Bré, M. H. (1994). Polyglycylation of tubulin: a post-translational modification in axonemal microtubules. *Science* **266,** 1688–1691.

Rindisbacher, L., Hemphill, A., and Seebeck, T. (1993). A repetitive protein from *Trypanosoma brucei* which caps the microtubules at the posterior end of the cytoskeleton. *Mol. Biochem. Parasitol.* **58,** 83–96.

Roberts, K. R., and Roberts, J. E. (1991). The flagellar apparatus and cytoskeleton of the dinoflagellates. A comparative overview. *Protoplasma* **164,** 105–122.

Roberts, K. R., Lemoine, J. E., Schneider, R. M., and Farmer, M. A. (1988). The microtubular cytoskeleton of three dinoflagellates: an immunofluorescence study. *Protoplasma* **144,** 68–71.

Robinson, D. R., and Gull, K. (1991). Basal body movements as a mechanism for mitochondrial genome separation in the trypanosome life cycle. *Nature* **342,** 731–733.

Robinson, D. R., Sherwin, T., Ploubidou, A., Byard, E. H., and Gull, K. (1995). Microtubule polarity and dynamics in the control of organelle positioning, segregation, and cytokinesis in the trypanosome cell cycle. *J. Cell Biol.* **128,** 1163–1172.

Rosiere, T. K., Marrs, J. A., and Bouck, G. B. (1990). A 39-kD plasma membrane protein (IP39) is an anchor for the unusual membrane skeleton of *Euglena gracilis. J. Cell Biol.* **110,** 1077–1088.

Rosowski, J. R. (1977). Development of mucilaginous surfaces in euglenoids. II. Flagellated creeping and palmelloid cells of *Euglena. J. Phycol.* **13,** 323–328.

Sasse, R., and Gull, K. (1988). Tubulin post-translational modifications and the construction of microtubule organelles in *Trypanosoma brucei. J. Cell Sci.* **90,** 577–589.

Schantz, M.-L., and Schantz, R. (1989). Sequences of a cDNA clone encoding β-tubulin from *Euglena gracilis. Nucleic Acids Res.* **17,** 6727.

Schenkman, S., Eichinger, D., Pereira, M. E. A., and Nussenzweig, V. (1994). Structural and functional properties of *Trypanosoma* trans-sialidase. *Annu. Rev. Microbiol.* **48,** 499–523.

Schneider, A., Sherwin, T., Sasse, R., Russell, D. G., Gull, K., and Seebeck, T. (1987). Subcellular and flagellar microtubules of *Trypanosoma brucei brucei* contain the same α-tubulin isoforms. *J. Cell Biol.* **104,** 431–438.

Schneider, A., Eichenberger, W., and Seebeck, T. (1988a). A microtubule-binding protein of *Trypanosoma brucei* which contains covalently bound fatty acid. *J. Biol Chem.* **263,** 6472–6475.

Schneider, A., Lutz, H. U., Marugg, R., Gehr, P., and Seebeck, T. (1988b). Spectrin-like proteins in the paraflagellar rod structure of *Trypanosoma brucei. J. Cell Sci.* **90,** 307–315.

Schnepf, E. (1988). Cytochalasin D inhibits completion of cytokinesis and affects theca formation in dinoflagellates. *Protoplasma* **143,** 22–28.

Schnepf, E., Winter, S., Storck, I., and Quader, H. (1990). A complementary experimental study of cell division in the dinoflagellate *Prorocentrum micans. Eur. J. Protistol.* **25,** 234–242.

Seebeck, T., Küng, V., Wyler, T., and Müller, M. (1988a). A 60-kDa cytoskeletal protein from *Trypanosoma brucei brucei* can interact with membranes and with microtubules. *Proc. Natl. Acad. Sci. U.S.A.* **85,** 1101–1104.

Seebeck, T., Schneider, A., Kueng, V., Schlaeppi, K., and Hemphill, A. (1988b). The cytoskeleton of *Trypanosoma brucei*—the beauty of simplicity. *Protoplasma* **145,** 188–194.

Serrano, L., Díaz-Nido, J., Wandosell, F., and Avila, J. (1987). Tubulin phosphorylation by casein kinase II is similar to that found *in vivo. J. Cell Biol.* **105,** 1731–1739.

Sghir, A., and David, D. (1995). Immunological comparisons of major cortical cytoskeletal proteins in four entodiniomorphid ciliates. *Eur. J. Protistol.* **31,** 16–23.

Sghir, A., David, D., and Ferrier, M. (1994). A 43 kDa protein inserted at the plasma membrane-cytoskeleton interface in rumen entodiniomorphid ciliates. *Protoplasma* **182,** 149–159.

Sherwin, T., and Gull, K. (1989). Visualization of detyrosination along single microtubules reveals novel mechanisms of assembly during cytoskeletal duplication in trypanosomes. *Cell* **57,** 211–221.

Sherwin, T., Schneider, A., Sasse, R., Seebeck, T., and Gull, K. (1987). Distinct localization and cell cycle dependence of COOH terminally tyrosinalated α-tubulin in the microtubules of *Trypanosoma brucei brucei. J. Cell Biol.* **104,** 439–446.

Silflow, C. D. (1991). Why do tubulin gene families lack diversity in flagellate/ciliate protists? *Protoplasma* **164,** 9–11.

Smith, D. L., Berkowitz, M. S., Potoczak, D. , Krause, M., Raab, C., Quinn, F., and Doerder, F. P. (1992). Characterization of the T, L, I, S, M, and P cell surface (immobilization) antigens of *Tetrahymena thermophila:* Molecular weights, isoforms, and cross-reactivity of antisera. *J. Protozool* **39,** 420–428.

Solomon, J. A., Walne, P. L., Dawson, N. S., and Willey, R. L. (1991). Structural characterization of *Eutreptia* (Euglenophyta). II. The flagellar root system and putative vestigial cytopharynx. *Phycologia* **30,** 402–414.

Sommer, J. R., and Blum, J. J. (1965). Cell division in *Astasia longa. Exp. Cell Res.* **39,** 504–527.

Sperling, L., Keryer, G., Ruiz, F., and Beisson, J. (1991). Cortical morphogenesis in *Paramecium:* A transcellular wave of protein phosphorylation involved in ciliary rootlet disassembly. *Dev. Biol.* **148,** 205–218.

Stieger, J., and Seebeck, T. (1986). Monoclonal antibodies against a 60 kDa phenothiazine-binding protein from *Trypanosoma brucei* can discriminate between different trypanosome species. *Mol. Biochem. Parasitol.* **21,** 37–45.

Stelly, N., Mauger, J.-P., Claret, M., and Adoutte, A. (1991). Cortical alveoli of *Paramecium:* a vast submembranous calcium storage compartment. *J. Cell Biol.* **113,** 103–112.

Sulli, C., and Schwartzbach, S. D. (1995). The polyprotein precursor to the *Euglena* light-harvesting chlorophyll a/b-binding protein is transported to the Golgi apparatus prior to chloroplast import and polyprotein processing. *J. Biol. Chem.* **270,** 13084–13090.

Suzaki, T., and Williamson, R. E. (1985). Euglenoid movement in *Euglena fusca:* Evidence for sliding between pellicular strips. *Protoplasma* **124,** 137–146.

Suzaki, T., and Williamson, R. E. (1986a). Pellicular ultrastructure and euglenoid movement in *Euglena ehrenbergii* Klebs and *Euglena oxyuris* Schmarda. *J. Protozool.* **33,** 165–171.

Suzaki, T., and Williamson, R. E. (1986b). Reactivation of euglenoid movement and flagellar beating in detergent-extracted cells of *Astasia longa:* Different mechanisms of force generation are involved. *J. Cell Sci.* **80,** 75–89.

Suzaki, T., and Williamson, R. E. (1986c). Ultrastructure and sliding of pellicular structures during euglenoid movement in *Astasia longa* Pringsheim (Sarcomastigophora, Euglenida). *J. Protozool.* **33,** 179–184.

Thompson, W. C. (1982). The cyclic tyrosination/detyrosination of alpha tubulin. *Methods Cell Biol.* **24,** 235–255.

Tippit, D. H., and Pickett-Heaps, J. D. (1976). Apparent amitosis in the binucleate dinoflagellate *Peridinium balticum. J. Cell Sci.* **21,** 273–289.

Toda, H., Yazawa, M., and Yagi, K. (1992). Amino acid sequence of calmodulin from *Euglena gracilis. Eur. J. Biochem.* **205,** 653–660.

Triemer, R. E. (1980). Role of Golgi apparatus in mucilage production and cyst formation in *Euglena gracilis* (euglenophyceae). *J. Phycol.* **16,** 46–52.

Triemer, R. E., (1988). Ultrastructure of mitosis in *Entosiphon sulcatum* (Euglenida). *J. Protozool.* **35,** 231–237.

Triemer, R. E., and Fritz, L. (1988). Ultrastructural features of mitosis in *Ploeotia costata* (Heteronematales, Euglenophyta). *J. Phycol.* **24,** 514–519.

Udenfriend, S., and Kodukula, K. (1995). How glycosyl-phosphatidylinositol anchored membrane proteins are made. *Annu. Rev. Biochem.* **64,** 563–591.

Vanhamme, L., and Pays, E. (1995). Control of gene expression in trypanosomes. *Microbiol. Rev.* **59,** 223–240.

Vannini, G. L., Bonora, A., and Dall'Olio, G. (1981). Distribution of the receptors for concanavalin A on the surface of *Euglena gracilis* as revealed by fluorescence microscopy. *Plant Sci. Lett.* **22,** 23–28.

Vaudaux, P. E., and Williams, N. E. (1979). Cytoskeletal proteins of the cell surface in *Tetrahymena.* II. Turnover of major proteins. *Exp. Cell Res.* **123,** 321–331.

Vickerman, K., and Preston, T. M. (1976). Comparative cell biology of the kinetoplastid flagellates. *In* "Biology of the Kinetoplastida" (W. H. R. Lumsden and D. A. Evans, eds.), pp. 35–130. Academic Press, New York.

Vigues, B., and David, D. (1989). Demonstration of a 58 kd protein as a major component of the plasma membrane skeleton in the ciliate *Entodinium bursa. Protoplasma* **149,** 11–19.

Vigues, B., Bricheux, G., Métivier, C., Brugerolle, G., and Peck, R. K. (1987). Evidence for common epitopes among proteins of the membrane skeleton of a ciliate, a euglenoid and a dinoflagellate. *Eur. J. Protistol.* **23,** 101–110.

Wetherbee, R. (1975). The fine structure of *Ceratium tripos,* a marine armored dinoflagellate. II. Cytokinesis and development of the characteristic cell shape. *J. Ultrastruct. Res.* **50,** 65–76.

Weinhart, U., Thomas, J. R., Pak, Y., Thompson, G. A., and Ferguson, A. J. (1991). Structural characterization of a novel glycosyl-phosphatidylinositol from the protozoan *Tetrahymena mimbres. Biochem. J.* **279,** 605–608.

Willey, R. L., and Wibel, R. G. (1985). The reservoir cytoskeleton and a possible cytostomal homologue in *Colacium (Euglenophyceae). J. Phycol.* **21,** 570–577.

Willey, R. L., Ward, K., Russin, W., and Wibel, R. (1977). Histochemical studies of the extracellular carbohydrate of *Colacium mucronatum* (euglenophyceae). *J. Phycol.* **13,** 349–353.

Willey, R. L., Walne, P. L., and Kivic, P. (1988). Phagotrophy and the origins of the euglenoid flagellates. *CRC Crit. Rev. Plant Sci.* **7,** 303–340.

Williams, N. E. (1991). A comparison of alveolar plate proteins in two species of *Euplotes. Eur. J. Protistol.* **27,** 21–25.

Williams, N. E., Buhse, H. E., and Smith, M. G. (1984). Protein similarities in the genus *Tetrahymena* and a description of *Tetrahymena leucophrys* n.sp. *J. Protozool.* **31,** 313–321.

Williams, N. E., Doerder, F. P., and Ron, A. (1985). Expression of cell surface immobilization antigen during serotype transformation in *Tetrahymena thermophila. Mol. Cell Biol.* **5,** 1925–1932.

Williams, N. E., Honts, J. E., and Jaeckel-Williams, R. F. (1987). Regional differentiation of the membrane skeleton in *Tetrahymena. J. Cell Sci.* **87,** 457–463.

Williams, N. E., Honts, J. E., Lu, Q., Olson, C. L., and Moore, K. C. (1989a). Identification and localization of major cortical proteins in the ciliated protozoan, *Euplotes eurystomus. J. Cell Sci.* **92,** 433–439.

Williams, N. E., Honts, J. E., and Stuart, K. R. (1989b). Properties of microtubule-free cortical residues isolated from *Paramecium tetraurelia. J. Cell Sci.* **92,** 427–432.

Williams, N. E., Honts, J. E., and Kaczanowska, J. (1990). The formation of basal body domains in the membrane skeleton of *Tetrahymena. Development* **109,** 935–942.

Williams, N. E., Honts, J. E., Dress, V. M., Nelsen, E. M., and Frankel, J. (1995). Monoclonal antibodies reveal complex structure in the membrane skeleton of *Tetrahymena. J. Euk. Microbiol.* **42,** 422–427.

Woods, A., Baines, A. J., and Gull, K. (1989). Evidence for a M_r 88 000 glycoprotein with a transmembrane association to a unique flagellum attachment region in *Trypanosoma brucei. J. Cell Sci.* **93,** 501–508.

Woods, A., Baines, A. J., and Gull, K. (1992). A high molecular mass phosphoprotein defined by a novel monoclonal antibody is closely associated with the intermicrotubule cross bridges in the *Trypanosoma brucei* cytoskeleton. *J. Cell Sci.* **103,** 665–675.