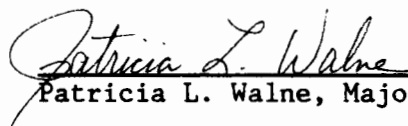
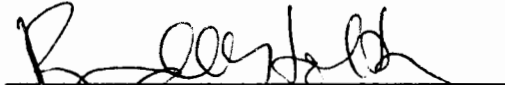
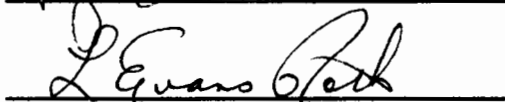


To the Graduate Council:

I am submitting herewith a dissertation written by Nancy Adams Dawson entitled "Fine Structure of Eutreptia pertyi (Euglenophyceae) and Analytical Ultrastructural Characterization of the Flagellar Paraxial Rods: Functional and Phylogenetic Implications." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Botany.


Patricia L. Walne, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:


Vice Provost
and Dean of the Graduate School

FINE STRUCTURE OF EUTREPTIA PERTYI (EUGLENOPHYCEAE) AND ANALYTICAL
ULTRASTRUCTURAL CHARACTERIZATION OF THE FLAGELLAR PARAXIAL RODS:
FUNCTIONAL AND PHYLOGENETIC IMPLICATIONS

A Dissertation
Presented for the
Doctor of Philosophy
Degree
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Nancy Adams Dawson

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ABSTRACT

The photosynthetic marine protist, Eutreptia pertyi (Prings.), has been suggested as the model "primitive green" organism in the Euglenophyceae. As a member of the order Eutreptiales, Eutreptia is characterized by two emergent flagella. Previous cytological and taxonomic studies have been based primarily on light-microscopic observations. This is the first ultrastructural investigation of the genus Eutreptia. For the species, E. pertyi, the following major cytological characters are reported: two nearly equal-length, heterodynamic flagella, ribbon-shaped chloroplasts that extend from a pyrenoid center, a relatively smooth pellicle, a presumptive vestigial cytopharynx associated with the canal cytoskeletal complex, and presumptive intracellular reservoirs for flagellar mastigonemes, termed "mastigoneme vesicles."

The flagella can be distinguished at the ultrastructural level by different complexities in an accessory component, termed the paraxial rod (PAR). Structural characterization of the PAR in the dorsal flagellum (PAR1) shows one major substructural component -- the oblique filaments, which are arranged in reciprocal axes and give a "lattice-like" appearance to PAR1. Structural characterization of the PAR in the ventral flagellum (PAR2) shows 3 major substructural components-- oblique, horizontal and longitudinal filaments which are arranged in three axes and give PAR2 a paracrystalline "plate-like" appearance. The different substructures of PAR1 and PAR2 may contribute to heterodynamic flagellar motion.

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LIST OF ABBREVIATIONS

Å	= Angstrom
AC	= accummulation body
ATP	= adenosine triphosphate
Ax	= axoneme
Ax1	= axoneme of dorsal or F1 flagellum
Ax2	= axoneme of ventral or F2 flagellum
B/W	= black-white film
C	= canal
Ch	= chloroplast
CP	= cytopharynx, cytopharyngeal pocket
CV	= contractile vacuole
DIC	= differential interference
DMT	= dorsal band of microtubules
DNA	= deoxyribonucleic acid
DPMB	= dorsal pellicular microtubular band
DR	= dorsal flagellar root
EL	= epiplasmic dense layer of pellicle
EM	= extracellular matrix of mucilage
ER	= endoplasmic reticulum
F1	= dorsal or anterior flagellum
F2	= ventral or trailing flagellum
FFT	= Fast Fourier Transform
g	= groove region of a pellicular stria
GA	= Golgi apparatus
h	= heel region of a pellicular stria
hr	= hour
IFS	= intraflagellar structure
IR	= intermediate flagellar root
kDNA	= kinetoplastid deoxyribonucleic acid
keV	= kiloelectron volts
KPTA	= phosphotungstic acid
LD	= light/dark cycle
LM	= light microscope
lx	= lux
M	= meter
m	= mitochondrion
m ₁	= microtubule 1 of a pellicular stria
m ₂	= microtubule 2 of a pellicular stria
m ₃	= microtubule 3 of a pellicular stria
m ₄	= microtubule 4 of a pellicular stria
mb	= mucocyst-like body
mg	= milligram
min	= minute
ml	= milliliter
mm	= millimeter
MTR	= microtubular root
mv	= mastigoneme vesicle

LIST OF ABBREVIATIONS (continued)

MW	= molecular weight
N	= nucleus
nm	= nanometer
Nu	= nucleolus
O _s O ₄	= osmium tetroxide
P	= pyrenoid center
PAR	= paraxial rod(s)
PAR1	= paraxial rod of dorsal or F1 flagellum
PAR2	= paraxial rod of ventral or F2 flagellum
PFB	= paraflagellar swelling, paraflagellar body
PFR	= paraflagellar rod
PG	= paramylon granule
PM	= plasma membrane
P _s	= striated plaque
ri	= ridge region of a pellicular stria
rm	= reservoir hairs
s	= second
S	= stigma
SEM	= scanning electron microscopy
TEM	= transmission electron microscopy
VR	= ventral flagellar root
w/v	= weight/volume
°	= degrees
°C	= degrees Centigrade
μE	= micro Einsteins
μm	= micrometer

PART I. INTRODUCTION

I. GENERAL INFORMATION

The euglenoid flagellates are protists with both plant- and animal-like attributes, and although they appear to be closely related to one another, their phylogenetic affiliations with other groups have remained uncertain (See Table 1¹). One of their closest phytoflagellate relatives has been suggested to be the dinoflagellates on the basis of similar nuclear division, and flagellar hairs and grooves (Chadefaud, 1937; Cavalier-Smith, 1975; Loeblich, 1976; Scagel et al., 1982); however, they have also been suggested to be related to the kinetoplastid zooflagellates on the basis of a number of apparent structural homologies (Leedale, 1978; Corliss, 1981; Kivic & Walne, 1984) (See the partial listing of shared characteristics of euglenoids and kinetoplastids and of euglenoids and dinoflagellates, in addition to the PAR, Appendix to Part I). One shared structural feature in the flagellum of all of these protists is a paraxial rod, that appears, at least superficially, similar in all of them. This investigation describes the fine structure of Eutreptia pertyi and investigates the extent of structural similarity and possible structural homology of the paraxial rod within and among the above-named taxa and assesses the utility of this structural character as a phylogenetic marker for a larger super-phyletic assemblage that would include euglenoids, dinoflagellates and kinetoplastid zooflagellates.

¹All tables referenced in Part I are found in the appendix at the end of Part I unless otherwise noted.

II. OVERVIEW OF FLAGELLAR STRUCTURES

Euglenoids

Although some euglenoid genera have only one emergent flagellum and others are multiflagellated, most euglenoids are biflagellated. Both flagella are inserted in the posterior or lateral reservoir wall of the flagellar pocket (Leedale, 1967a). Of the two flagella, only one is primarily used in propelling the cell in most species. In most phagotrophs, the second flagellum is characteristically long and usually trails passively as a ventral 'skid'; in the phototrophic and osmotrophic euglenoids, this flagellum may be reduced to a nonemergent stub within the flagellar pocket. In addition to the axoneme, the emergent flagella usually contain a paraxial rod (PAR) (see next section for details). In the non-emergent flagella, a PAR may be amorphous or absent.

In the intrareservoir region of the photosynthetic euglenoids, the emergent flagellum usually has an ovoid basal swelling, termed the paraflagellar body (PFB) next to the PAR. Like the PAR, it is also enclosed by the flagellar membrane and has a (quasi-) crystalline substructure. The PFB is suggested to be the primary photoreceptor and reportedly contains the major photoreceptor pigment (flavin or flavoprotein) for phototaxis (Checcucci et al., 1976).

The "9 + 2" arrangement of axonemal microtubules occurs in the distal region of the flagellum; the peripheral axonemal doublet microtubules become triplets in the flagellar base. Unlike most eukaryotes with a stellate pattern in the transition zone (the area

separating the emergent portion of the flagellum from the basal body), the euglenoids reportedly have no structural elements other than the nine outer doublets of the axoneme in this transition zone (Kivic & Walne, 1984; Hilenski & Walne, 1985b). Non-tubular fibrillar mastigonemes are arranged in a single helical row along the length of the flagellum. In addition to the relatively long mastigonemes (2-3 μm), there are also short non-tubular mastigonemes, arranged in a 4-start helix along the flagellum (Melkonian et al., 1982). The long non-tubular mastigonemes appear to link directly (Leedale, 1967a; Bouck et al., 1978) or indirectly (Melkonian et al., 1982) to the PAR. (See Table 2 for general ultrastructural information on euglenoid flagella and associated structures of the reservoir and canal).

Dinoflagellates

The typical flagellar arrangement in dinokont dinoflagellates is that of a wavy transverse flagellum lying in a transverse groove (cingulum) and a "free" longitudinal flagellum that projects out from a longitudinal groove (sulcus) perpendicular to the cingulum. The flagella are inserted in the intersecting region of the cingulum and sulcus (Lee, 1980).

In addition to the helically or hemihelically coiled axoneme along the outer margin in the transverse flagellum, there is, on its inner margin, a striated strand which runs outside the coils of the axoneme. The striated strand is banded, with an overall periodicity of 66 nm (Leadbeater & Dodge, 1967a) and follows a straight path in the cingulum. The striated strand has been proposed to be a banded paraxial rod (Taylor, 1975; Moestrup, 1982). The sheath of the transverse

flagellum is expanded to enclose both the axoneme and the striated strand and bears long, fine, uniseriate hairs along the outer margin (Pitelka & Schooley, 1955; Leadbeater & Dodge, 1967b; Taylor, 1975). Regularly organized packing material (similar in appearance to the euglenoid PAR) occupies space not filled by the axoneme and the striated strand (Leadbeater & Dodge, 1967b). The axonemal waves of the transverse flagellum appear to follow a helical (Berdach, 1977; Rees & Leedale, 1980) or hemi-helical path (Taylor, 1975; Leblond & Taylor, 1976; Herman & Sweeney, 1977).

The longitudinal flagellum usually has a wide basal portion and a thinner distal portion. In the basal portion the flagellar sheath surrounds packing material plus the axoneme, whereas in the distal portion the flagellar sheath surrounds only the axoneme. Fibrillar hairs (0.5 μm long and 10 nm wide) may cover the entire flagellum (Lee, 1980). The motion of the longitudinal flagellum is a planar sine wave (Maruyama, 1981).

A different flagellar arrangement is found in the dinoflagellate, Oxyrrhis marina, in which the two heterokont flagella are not confined to grooves; rather, they originate near the base of a projection called the tentacle and extend in a posterior or latero-posterior direction.

The shorter, ventral flagellum is strongly coiled and bears fibrils of three different lengths (Clarke & Pennick, 1972); one set (10 nm thick and up to 3 μm long) is arranged in a unilateral row along the entire flagellar length. Internally, this flagellum contains a paraxial rod in addition to the axoneme.

The longer, dorsal flagellum arises at an angle of 110 degrees from the ventral flagellum. This flagellum beats in a planar sine wave and bears hairs, 0.75 μm in length (Clarke & Pennick, 1972), except for the flagellar tip where $<1 \mu\text{m}$ long, $<10 \text{ nm}$ thick short hairs are arranged bilaterally (Dodge & Crawford, 1971b).

Both flagella taper to a point but in the dorsal flagellum, the terminal 6-8 μm is much thinner than the usual flagellar thickness of 300-700 nm. Both flagella are covered with scales except at their proximal ends (Clarke & Pennick, 1972). (See Table 2 for the general ultrastructural information on dinoflagellate flagella).

Kinetoplastids

The kinetoplastid zooflagellates include both the bodonids and trypanosomatids. The bodonine flagellates are heterokonts--one flagellum is forward projecting and locomotory, the other flagellum is recurrent along the body and attached to it in cryptobids, Trypanophis, and Trypanoplasma. The trypanosomatids have a single flagellum that is homologous with the anterior flagellum of bodonines; the flagellum is attached to the body for part of its length (Vickerman & Preston, 1976). Trypanosomatids can propagate waveforms from tip to base as well as from base to tip (Vickerman & Preston, 1976; Sleight, 1981). (Bend initiations can occur at any point along the flagellum of Crithidia oncopelti which lacks a PAR but has a septum on doublet B tubules). The recurrent flagellum of bodonids serves as a steering device and "skid" on which the flagellate moves over the substratum and does not beat in synchrony with the anterior flagellum. Like the euglenoids, the transition zone of the emergent flagellum of both trypanosomes and

bodonids is electrontransparent and devoid of discernible structure (Kivic & Walne, 1984). Long, unilateral mastigonemes are present on the flagella of some kinetoplastids (Kivic & Walne, 1984). (See Table 2 for a partial listing of general ultrastructural information on kinetoplastid flagella).

III. OVERVIEW OF THE PARAXIAL ROD

The eukaryotic flagellum is surrounded by a "unit" membrane that is continuous with the plasmalemma and contains a cylinder of 9 outer-doublet and 2 central-pair microtubules that forms the typical "9 + 2" axonemal structure. In addition to the axoneme, protistan flagella may contain a number of accessory structures also surrounded by the flagellar membrane. One such inclusion, an elaborate basketweave structure termed a paraxial rod, parallels the axoneme for most, if not all, of the flagellar length.

A PAR-like structure is present in at least some members of the algal classes of euglenoids and dinoflagellates and in the kinetoplastid zooflagellate groups of trypanosomatids and bodonids (See Tables 1, 2, and 3, Appendix to Part III). Early descriptive studies of flagella in the above groups gave rise to different names for the PAR-like structure: paraxial rod (PAR) and paraflagellar rod (PFR) in euglenoid flagellates; packing material, "cordon spirale" and striated strand in the dinoflagellates; and intraflagellar structure (IFS), paraxial fibre, "bande striee du flagelle" or "accessory filament" in the kinetoplastid zooflagellates. The extent of PAR complexity and precise substructure, however, varies from species to species within each class, and from flagellum to flagellum in heterodynamic biflagellates.

The morphological resemblance of the PAR in these groups is that of a 3-dimensional, paracrystalline lattice composed of helical filaments. Projections either from axonemal doublet microtubule(s) or from the helically arranged filaments of the rod connect the axoneme and the

PAR. Physical connections between the rod and the flagellar membrane have also been reported in Euglena and the trypanosomes. Variations in the PAR-axonemal complex include the number, size and arrangement of individual helical filaments, as well as the orientation of the PAR to the central pair of axonemal microtubules. At the molecular level, there are two or three major constituent proteins that form the filaments. Only two of the major PAR proteins of Euglena gracilis and a few trypanosomes have been studied comparatively. The two major proteins that comprise the rod in these two groups have similar molecular weights (between 65-80,000), similar isoelectric points (~6.1), and share at least one common antigenic determinant (epitope) that may represent a conserved functional protein domain, responsible for similar supramolecular assembly into a paracrystalline network of filaments. The native protein may also exist as a dimer, i.e., a subunit of PFR1 and a subunit of PFR2 (terminology of Hyams, 1982).

The precise structural and chemical composition of this unique flagellar component has been critically examined only in Euglena gracilis and a very few trypanosomes; moreover, no comparative study of the PAR in the several taxa indicated has ever been carried out. Thus, it is the intent of this investigation 1) to structurally characterize the PAR in both flagella of the euglenoid Eutreptia pertyi, 2) to compare the detailed substructure of the PARs in Eutreptia with the known PAR substructure in selected euglenoids, dinoflagellates and kinetoplastids and to determine the extent of structural similarity and possible homology among the PARs in these organisms, and 3) to develop a three-dimensional model of the PAR in one euglenoid organism.

IV. GENERAL MICROSTRUCTURE OF THE PAR IN EUGLENIDS, DINOFLAGELLATES AND KINETOPLASTIDS

Only the microstructure of the PAR in E. gracilis, has been described for the **euglenoids**. In this species, the PAR is a hollow lattice-like helix (Hyams, 1982) composed of filaments associated with the axonemal doublet microtubules by goblet-shaped projections with a periodicity of 53 μm along the axoneme (Russell et al., 1983). The rod is ~ 90 nm in diameter and the oblique filaments cross at a 45 degree angle to the long axis of the axoneme, generating a 7-start left-handed helix (Hyams, 1982) with a periodicity of 54 nm (Hyams, 1982).

In the **dinoflagellates**, PAR-like "packing material" (PM) has been reported in several genera, but the only detailed investigation has been the characterization of the PAR in Oxyrrhis (Cachon et al., 1988); there are no detailed investigations of the PM. Some descriptive information is available on the longitudinal flagellum of Ceratium (Maruyama, 1981) and on the flagella of Woloszynskia (Leadbeater & Dodge, 1967a, b) and Oxyrrhis (Dodge & Crawford, 1971b, Cachon et al., 1988).

In Ceratium, the PM of the longitudinal flagellum is morphologically similar to the PAR of the euglenoids, and is composed of a central core of 0-20 tubules and a radiating fin-like structure. The PM connects to the axonemal microtubules on one side and to the "R-fibre" on the other side at intervals of 5.8 μm (Maruyama, 1981)

In Woloszynskia, the transverse flagellum contains an axoneme plus PM and a striated strand ("a cross-banded rod", Moestrup, 1982). PM fills space between the axoneme and the striated strand. The

longitudinal flagellum contains the axoneme and a large amount of PM composed of regularly organized longitudinal rows 360 Å apart; the PM is absent from its narrow tip (Leadbeater & Dodge, 1967b; Taylor, 1975). (The PM in this dinoflagellate is similar to the PAR structure in euglenoids).

In Oxyrrhis marina both flagella are of equal length and contain typical axonemes; in transverse section, both have an oval profile. The axoneme is situated to the side of the area enclosed by the flagellar sheath, and in the case of the dorsal flagellum, the remaining space is occupied by finely granular PM. In addition to the axoneme and PM, the ventral flagellum also contains a paraxial rod, a hollow basket-like cylinder composed of 4 or 5 helically arranged strands of dark-staining material. The diameter of the rod is ~200 nm, and the individual strands of which it is composed are ~10-15 nm thick, spaced at 40 nm intervals. The rod and the axoneme appear to fuse just above the transition zone of the flagellum. "Like the differently constructed striated strand in the transverse flagellum of other dinoflagellates (Leadbeater & Dodge, 1967a, b), the paraxial rod appears to take a straighter course along the flagellum than the axoneme (Dodge & Crawford, 1971b).

A PAR is present in **kinetoplastid** flagella, except for those bodonids or trypanosomatids which harbor a bacterium-like endo-symbiont in their cytoplasm (Freymuller & Camargo, 1981) or spheromastigote forms of Trypanosoma cruzi (Brugerolle et al., 1979; De Souza & Souto-Padron, 1980). In the trypanosomatids, the rod is always subtended by axonemal doublets 4-7 (De Souza & Souto-Padron, 1980) and lies in a plane

bisecting the axoneme through its two central tubules. In the bodonine flagellates, the rod has a similar relationship to the axoneme in the anterior flagellum, but in the recurrent flagellum, it is subtended by doublets 2-5 or 3-6 (Vickerman & Preston, 1976). It is formed of longitudinal filaments ~5-7 nm thick, longitudinally oriented in relation to the axoneme and spaced at intervals of 18 nm, 23-25 nm, or 27 nm intervals (Fuge, 1969; Russell et al., 1983; De Souza & Souto-Padron, 1980, respectively) and arranged at 45° angles to the longitudinal filaments (De Souza & Souto-Padron, 1980). The PAR runs alongside the axoneme from the terminal plate to near the end of the axoneme in bodonines; in trypanosomatids, the rod is often not present until after the flagellum emerges from the flagellar pocket (Vickerman & Preston, 1976). The relative position of the paraxial structure to the axoneme remains constant; however, its position relative to the cell body varies considerably; sometimes it is in touch with the cell body or sometimes at its side in epimastigote and trypomastigote forms (De Souza & Souto-Padron, 1980). In Bodo and Cephalothamnium the PAR's are oriented one-to-another in the emergent flagella and an interflagellar connection links the two PARs at the level of the terminal plate; in Cryptobia and Rhynchomonas the actual membranes of the two emergent flagella are apposed, integrating the two heterodynamic flagella (Vickerman & Preston, 1976).

There appear to be structural variations of the PAR and the PAR-like material in members of different taxa as well as in different species within the same taxon.

LIST OF REFERENCES

- Anderson, W.A. and R.A. Ellis. 1965. Ultrastructure of Trypanosoma lewisi: flagellum, microtubules, and the kinetoplast. J. Protozool. 12: 483-499.
- Berdach, J.T. 1977. In situ preservation of the transverse flagellum of Peridinium cinctum (Dinophyceae) for scanning electron microscopy. J. Phycol. 13: 243-251.
- Berman, T. and I.L. Roth. 1979. The flagella of Peridinium cinctum fa. westii: in situ fixation and observation by scanning electron microscopy. Phycologia 18: 307-311.
- Boisson, C., X. Mattei, M.E. Boisson. 1965. Le flagelle de Trypanosoma gambiense etudie au microscope electronique. C. R. Soc. Biol. 159: 228-230.
- Bouck, G.B., A. Rogalski and A. Valaitis. 1978. Surface organization and composition of Euglena. II. Flagellar mastigonemes. J. Cell Biol. 77: 805-826.
- Bradbury, P., J.A. Westfall and J.W. Townsend. 1983. Ultrastructure of the dinoflagellate Polykrikos II. The nucleus and its connections to the flagellar apparatus. J. Ultrast. Res. 85: 24-32.
- Brugerolle, G. 1985. Des trichocystes chez les bodonides, un caractere phylogenetique supplementaire entre Kinetoplastida et Euglenoida. Protistologica 21: 339-348.
- Brugerolle, G., J. Lom, E. Nohynkova and L. Joyon. 1979. Comparison et evolution des structures cellularies chez plusieurs especes de bodonides et cryptobiides appartenant aux genres Bodo, Cryptobia et Trypanoplasma (Kinetoplastida, Mastigophora). Protistologica 15: 197-221.
- Bujak, J.P. and G.L. Williams. 1981. The evolution of the dinoflagellates. Can. J. Bot. 59: 2077-2087.
- Burzell, L.A. 1973. Observations on the proboscis-cytopharynx complex and flagella of Rhynchomonas metabolita Pshenin (Zoomastigophorea: Bodonidae). J. Protozool. 20: 385.
- Burzell, L.A. 1975. Fine structure of Bodo curvifilus Griessmann (Kinetoplastida: Bodonidae). J. Protozool. 22: 35-39.
- Cachon, J. and M. Cachon. 1974. Le systeme stomopharyngien de Kofoidinum Pavillard. Comparaisons avec celui de divers peridiniens libres et parasites. Protistologica 10: 217.

- Cachon, M., M. Cachon and P. Salvano. 1979. The nuclear division of Oxyrrhis marina: an example of the role played by the nuclear envelope in chromosome segregation. Arch. Protistenk. 122: 43-54.
- Cachon, M., J. Cosson, M. Cosson, P. Huitorel and J. Cachon. 1988a. Ultrastructure of the flagellar apparatus of Oxyrrhis marina. Biol. Cell 63: 159-168.
- Cachon, M., M. Cachon, M. Cosson and J. Cosson. 1988b. The paraflagellar rod: a structure in search of a function. Biol. Cell 63: 169-181.
- Cavalier-Smith, T. 1975. The origin of nuclei and of eukaryotic cells. Nature 256: 463-468.
- Chadefaud, M. 1937. Recherches sur l'anatomie comparee des Eugleniens. Le Botaniste 28: 85-185.
- Checucci, A., G. Colombetti, R. Ferrara, F. Lenci. 1976. Modelli molecolari per la fototassi in Euglena gracilis. Atti III Congresso Nazionale Cibernetica E Biofisica, S. Marino, 3-5 Ottobre 1974.
- Clarke, K.J. and N.C. Pennick. 1972. Flagellar scales in Oxyrrhis marina Dujardin. Br. Phycol. J. 7: 357-360.
- Corliss, J.O. 1981. What are the taxonomic and evolutionary relationships of the Protozoa to the Protista? Biosystems 14: 445-459.
- Cosson J., M. Cachon, J. Cachon and M.P. Cosson. 1988. The swimming behaviour of the unicellular biflagellate O. marina: in vivo and in vitro movement of the two flagella. Biol. Cell 63: 117-126.
- Deflandre, G. 1934. Sur la structure des flagelles. Ann. Protistenk. 106: 442.
- Dentler, W.L. 1981. Microtubule-membrane interactions in cilia and flagella. Int. Rev. Cytol. 72: 1-47.
- De Souza, W. and T. Souto-Padron. 1980. The paraxial structure of the flagellum of trypanosomatidae. J. Parasitol. 66: 229-235.
- Dodge, J.D. 1971. Fine structure of the Pyrrhophyta. Bot. Rev. 37: 481-508.
- Dodge, J.D. 1975. The fine structure of Trachelomonas (Euglenophyceae). Arch. Protistenkd. 117: 65-77.
- Dodge, J.D. and B.T. Bibby. 1973. The Prorocentrales (Dinophyceae). I. A comparative account of fine structure in the genera Prorocentrum and Exuviella. Bot. J. Linn. Soc. 67: 175-187.

- Dodge, J.D. and R.M. Crawford. 1970. The morphology and fine structure of Ceratium hirundinella (Dinophyceae). J. Phycol. 6: 137-149.
- Dodge, J.D. and R.M. Crawford. 1971a. Fine structure of the dinoflagellate Oxyrrhis marina I. The general structure of the cell. Protistologica 7: 295-304.
- Dodge, J.D. and R.M. Crawford. 1971b. Fine structure of the dinoflagellate Oxyrrhis marina II. The flagellar system. Protistologica 7: 399-409.
- Dragesco, J. 1952. Le flagelle Oxyrrhis marina: cytologie trichocysts, position systematique. Bull. Microscop. Appl. 2E Serie 1-2: 148-157.
- Dynesius, R.A. and P.L. Walne. 1973. Ultrastructure of the euglenoid flagellate, Phacus pleuronectes. J. Phycol. 9 (suppl.): 6.
- Dynesius, R.A. and P.L. Walne. 1975. Ultrastructure of the reservoir and flagella in Phacus pleuronectus (Euglenophyceae). J. Phycol. 11: 125- 130.
- Freymuller, E., E.P. Camargo. 1981. Ultrastructural differences between species of trypanosomatids with and without endosymbionts. J. Protozool. 28: 175-181.
- Fuge, H. 1969. Electron microscopic studies on the intra-flagellar structures of trypanosomes. J. Protozool. 16: 460-466.
- Gaines, G. & F.J.R. Taylor. 1985. Form and Function of the dinoflagellate transverse flagellum. J. Protozool. 32: 290-296.
- Hall, R.P. 1925. Binary fission in Oxyrrhis marina. Dujardin. Univ. Calif. Publ. Zool. 26: 281-324.
- Hanson, E.D. 1976. Major evolutionary trends in animal protists. J. Protozool. 23: 4-12.
- Herman, E.M. and B.M. Sweeney. 1977. Scanning electron microscopic observations of the flagellar structure of Gymnodinium spendens (Pyrrophyta, Dinophyceae). Phycologia 16: 115-118.
- Heywood, P. D. Weinman & M. Lipman. 1974. Fine structure of Trypanosoma cyclops in noncellular cultures. J. Protozool. 21: 232-238.
- Hilenski, L.L. and P.L. Walne. 1985a. Ultrastructure of the flagella of the colorless phagotroph Peranema trichophorum (Euglenophyceae). I. Flagellar mastigonemes. J. Phycol. 21: 114-125.
- Hilenski, L.L. and P.L. Walne. 1985b. Ultrastructure of the flagella of the colorless phagotroph Peranema trichophorum (Euglenophyceae) II. Flagellar roots. J. Phycol. 21: 125-134.

- Hohfeld, I., J. Otten and M. Melkonian. 1988. Contractile eukaryotic flagella: centrin is involved. Protoplasma 147: 16-24.
- Hogan, J.C. and C.L. Patton. 1976. Variation in intramembrane components of Trypanosoma brucei from intact and X-irradiated rats: a freeze-cleave study. J. Protozool. 23: 205-215.
- Hollande, A. 1974. Etude comparee de la mitose Syndinienne et de celle des Peridiniens libres et des Hypermastigines. Infrastructure et cycle evolutif des Syndinides parasites de Radiolaires. Protistologica 10: 413-451.
- Hyams, J.S. 1982. The Euglena paraflagellar rod: structure, relationship to other flagellar components and preliminary biochemical characterization. J. Cell Sci. 55: 199-210.
- Jahn, T.L. and E.C. Bovee. 1968. Locomotive and motile response in Euglena. In Buetow, D.E. (ed.), The Biology of Euglena, Academic Press, N.Y., Vol. 1, pp. 45-108.
- Joyon, L. and J. Lom. 1969. Etude cytologique, systematique, et pathologique d'Ichthybodo necator (Henneguy 1883) Pinto 1928 (Zooflagelle). J. Protozool. 16: 703-719.
- Keysselitz, G. 1908. Studien uber protozoen. Archiv. Protistenk. 11: 334-350.
- Killen, R.P., R.L. Willey, and F.A. Durum. 1984. Docking behavior of Colacium libellae (Euglenophyceae): cell-substrate adhesion and flagellar resorption. Trans. Am. Microsc. Soc. 103: 67-73.
- Kivic, P.A. and M. Vesik. 1972. Structure and function in the euglenoid eyespot apparatus: the fine structure, and response to environmental changes. Planta (Berl.) 105: 1-14.
- Kivic, P.A. and P.L. Walne. 1983. Algal photosensory apparatus probably represent multiple parallel evolutions. BioSystems 16: 31-38.
- Kivic, P.A. and P.L. Walne. 1984. An evaluation of a possible phylogenetic relationship between the Euglenophyta and Kinetoplastida. Origins of Life 13: 269-288.
- Kofoed, C.A. and O. Swezy. 1921. The Free-living Unarmoured Dinoflagellata. Univ. of Calif. Press.
- Leadbeater, B. and J.D. Dodge. 1967a. Fine structure of the dinoflagellate transverse flagellum. Nature 213: 421-422.
- Leadbeater, B. and J.D. Dodge. 1967b. An electron microscope study of dinoflagellate flagella. J. Gen. Microbiol. 46: 305-314.

- Leblond, P.H. and F.J.R. Taylor. 1976. The propulsive mechanism of the dinoflagellate transverse flagellum reconsidered. Biosystems 8: 33-39.
- Lee R.E. 1980. Phycology. Cambridge University Press, Cambridge, England.
- Leedale, G.F. 1967a. Euglenoid Flagellates. Englewood Cliffs, New Jersey, Prentice-Hall.
- Leedale, G.F. 1967b. Euglenida/Euglenophyta. Ann. Rev. Microbiol. 21: 31-48.
- Leedale, G.F. 1978. Phylogenetic criteria in euglenoid flagellates. BioSystems 10: 183-187.
- Leedale, G.F., B.S.C. Leadbeater and A. Massliski. 1970. The intracellular origin of flagellar hairs in the Chrysophyceae and Xanthophyceae. J. Cell Sci. 6: 701-719.
- Loeblich, A.R. 1976. Dinoflagellate evolution: speculation and evidence. J. Protozool. 23: 13-28.
- Loeblich, A.R., J.L. Sherley and R.J. Schmidt. 1979. The correct position of flagellar insertion in Prorocentrum and description of Prorocentrum rhathymum sp. nov. (Pyrrhophyta). J. Plankt. Res. 1: 113-120.
- Maruyama, T. 1981. Motion of the longitudinal flagellum in Ceratium tripos (Dinoflagellida): a retractile flagellar motion. J. Protozool. 28: 328-336.
- Maruyama, T. 1982. Fine structure of the longitudinal flagellum in Ceratium tripos, a marine dinoflagellate. J. Cell Sci. 58: 109-123.
- McGhee, R.B. and W.B. Cosgrove. 1980. Biology and physiology of the lower Trypanosomatidae. Microbiol. Rev. 44: 140-173.
- McQuade, A.B. 1983. Origins of the nucleate organisms. II. BioSystems 16: 39-55.
- Melkonian, M. and H.R. Preisig. 1982. Twist of central pair microtubules in the flagellum of the green flagellate Scourfieldia caeca. Cell Biol. Internat. Rep. 6: 269-277.
- Melkonian, H. Robenek and J. Rassat. 1982. Flagellar membrane specializations and their relationship to mastigonemes and microtubules in Euglena gracilis. J. Cell Sci. 55: 115-136.
- Mignot, J. 1966. Structure et ultrastructure de quelques Euglenomonadines. Protistologica 2: 51-117.

- Moestrup, O. 1982. Flagellar structure in algae: a review, with new observations particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae and Reckertia. Phycologia 21: 427-528.
- Parke, M. and P.S. Dixon. 1968. Check list of British marine algae, 2nd revision. J. Mar. Biol. Ass. U. K. 48: 783-832.
- Pitelka, D.R. 1963. Electron-microscopic structure of protozoa. Macmillan Co., New York.
- Pitelka, D.R. and C.N. Schooley. 1955. Comparative morphology of some protistan flagella. Univ. Calif. Publ. Zool. 61: 79.
- Postell, F.J. & R.B. McGhee. 1981. An ultrastructural study of Phytomonas davidi Lafont (Trypanosomatidae). J. Protozool. 28: 78-83.
- Rees, A.J.J. and G.F. Leedale. 1980. The dinoflagellate transverse flagellum: three-dimensional reconstructions from serial sections. J. Phycol. 16: 73-80.
- Robenek, H. and M. Melkonian. 1983. Structural specialization of the paraflagellar body membrane of Euglena. Protoplasma 117: 154-157.
- Rowton, E.D., W.B. Lushbaugh & R.B. McGhee. 1981. Ultrastructure of the flagellar apparatus and attachment of Herpetomonas ampelophilae in the gut and Malpighian tubules of Drosophila melanogaster. J. Protozool. 28: 297-301.
- Russell, D.G., R.J. Newsam, G.C.N. Palmer and K. Gull. 1983. Structural and biochemical characterisation of the paraflagellar rod of Crithidia fasciculata. Eur. J. Cell Biol. 30: 137-143.
- Scagel, R.D., R.J. Bandoni, G.E. Rouse, W.B. Schofield, J.R. Stein, T.M.C. Taylor. 1965. Plant Diversity: An Evolutionary Approach. Belmont, California: Wadsworth Publishing Co.
- Scagel, R.D., R.J. Bandoni, J.R. Maze, G.E. Rouse, W.B. Schofield, J.R. Stein. 1982. Nonvascular Plants: An Evolutionary Survey of the Plant Kingdom. Belmont, California: Wadsworth Publishing Co.
- Schneider, A., H. Lutz, R. Marugg, P. Gehr and T. Seebeck. 1988. Spectrin-like proteins in the paraflagellar rod structure of Trypanosoma brucei. J. Cell Sci. 90: 307-315.
- Senn, G. 1911. Oxyrrhis, Nephroselmis und einige euflagellaten. Etschr. Wiss. Zool. 97: 605-672.
- Sleigh, M.A. 1981. Flagellar beat patterns and their possible evolution. BioSystems 14: 423-431.

- Solomon, J.A., P.L. Walne, P.A. Kivic. 1987. Entosiphon sulcatum (Euglenophyceae): flagellar roots of the basal body complex and reservoir region. J. Phycol. 23: 85-98.
- Soyer, M.O. 1970. Les ultrastructures liees aux fonctions de relation chez Noctiluca miliaris S. Z. Zellforsch. 104: 29-55.
- Steidinger, K.A., E.W. Truby and C.J. Dawes. 1978. Ultrastructure of the red tide dinoflagellate Gymnodinium breve. I. General description. J. Phycol. 14: 72-79.
- Soyer, M.O. 1970. Les ultrastructrues liees aux fonctions de relation chez Noctiluca miliaris S. Z. Zellforsch. 104: 29-55.
- Spero, H.J. 1982. Phagotrophy in Gymnodinium fungiforme (Pyrrhophyta): the peduncle as an organelle of ingestion. J. Phycol. 18: 356-60.
- Surek, B. and M. Melkonian. 1986. A cryptic cytostome is present in Euglena. Protoplasma 133: 39-49.
- Swale, E.M.F. 1973. A study of the colorless flagellate Rhynchomonas nastuta (Stokes) Klebs. Biol. J. Linn. Soc. 5: 255-264.
- Taylor, A.E.R. and D.G. Godfrey. 1969. A new organelle of bloodstream salivarian trypanosomes. J. Protozool. 16: 466-470.
- Taylor, F.J.R. 1975. Non-helical transverse flagella in dinoflagellates. Phycologia 14: 45-47.
- Taylor, F.J.R.. 1976. Flagellate phylogeny: a study in conflicts. J. Protozool. 23: 28-40.
- Triemer, R.E. 1982. A unique mitotic variation in the marine dinoflagellate Oxyrrhis marina (Pyrrophyta). J. Phycol. 18: 399-411.
- Triemer, R.E. and L. Fritz. 1987. Structure and operation of the feeding apparatus in a colorless euglenoid, Entosiphon sulcatum. J. Protozool. 34: 39-47.
- Vickerman, K. 1969. On the surface coat and flagellar adhesion in trypanosomes. J. Cell Sci. 5: 163-193.
- Vickerman, K. and T.M. Preston, 1976. Comparative Cell Biology of the Kinetoplastid flagellates. In W.H.R. Lumsden and D.A. Evans (eds.), Biology of the Kinetoplastida, Vol. 1, Academic Press, London, pp. 35-130.
- Walne, P.L. 1980. Euglenoid flagellates. In Phytoflagellates, Vol. II. (Cox, E.R., ed.), Elsevier/North Holland, New York, pp. 165-212.

- Walne, P.L. and P.A. Kivic. 1985. Euglenophyta. In: Handbook of Protoctista (Margulis, L., J.O. Corliss, D. Chapman, eds.), New York: Science Books Intl. van Nostrand. In press, 1988.
- Walne, P.L, O. Moestrup, R.E. Norris, and H. Ettl. 1986. Light and electron-microscopical studies of Eutreptiella eupharyngea sp. nov. (Euglenophyceae) from Danish and American waters. Phycologia 25: 109-126.
- West, L.K., P.L. Walne, J. Rosowski. 1980. Trachelomonas hispida var. coronata (Euglenophyceae). I. Ultrastructure of cytoskeletal and flagellar systems. J. Phycol. 16: 489-497.
- Wolken, J.J. 1977. Euglena: the photoreceptor system for phototaxis. J. Protozool. 24: 518-522.

APPENDIX

Table 1. A Partial Listing of Pertinent Literature on the Phylogeny of the Euglenoids, Dinoflagellates and Kinetoplastids.

Date	Author	Contribution
1937	Chadefaud	Postulated a phylogenetic linkage between the euglenoids and dinoflagellates based on similar mucous bodies and flagellar grooves.
1965	Scagel et al.	Reviewed plant diversity and concluded that the euglenophytes are not closely related to any other group.
1967a	Leedale	Presented nine diagnostic features of the Euglenophyta and suggested a phylogenetic dendrogram.
1968	Parke and Dixon	Classified <u>Oxyrrhis</u> as a member of the family Pronoctilucaceae, order Peridinales, class Dinophyceae.
1975	Cavalier-Smith	Proposed that the euglenoids, dinoflagellates and cryptophytes are closer to each other than to other algal groups.
1976	Hanson	Summarized the major evolutionary trends in animal protists and examined the criteria for establishing homologous relationships between organisms.
1976	Loeblich	Reviewed dinoflagellate evolution and suggested that they are an old eukaryotic group that diverged from the higher eukaryotic lineage before the evolution of eukaryotic chromatin but after the evolution of repeated DNA. Suggested an affiliation of the dinoflagellates with euglenoids and trypanosomes due to a similar "extra-axonemal structure".
1976	Taylor	Examined the ultrastructure of four organelle systems - nuclei, flagella, mitochondria and chloroplasts in the principal flagellate groups and suggested possible phylogenetic relationships. Noted that paraxial (or paraflagellar) rods are found in flagella of the euglenoids and bodonids, as well as in the single flagellum

Table 1 (continued)

Date	Author	Contribution
1976	Taylor	(continued) of trypanosomes, supporting their suggested affinity. Suggested that the presence of PARs within flagella may be a useful indication of affinity.
1978	Leedale	Reviewed euglenoid phylogeny and found no justification for linking the euglenoids with the chlorophyll a + c-containing algae; postulated that the closest living relatives of the euglenoids might be found among the dinoflagellates or hypermastigote flagellates.
1979	Cachon et al.	Noted that <u>Oxyrrhis</u> had no clear taxonomic position among the dinoflagellates, a group they considered to be an evolutionary "hinge group" between prokaryotes and eukaryotes.
1981	Bujak and Williams	Reviewed the evolution of dinoflagellates and proposed the plate fragmentation model to explain the development of the cellulosic theca in the Dinophyceae.
1981	Corliss	Examined the taxonomic and evolutionary relationships of the Protozoa to the Protista; assigned the trypanosomes and some non-pigmented "lower zooflagellates" to the Euglenid group.
1983	Kivic and Walne	Suggested that the older more evolutionarily conserved elements in algal photoresponse mechanisms are the motile elements (axoneme and flagellar roots . . .) whereas the more recently acquired components such as the photoreceptor and light processing accessories (like the stigma) show greater variation.
1983	McQuade	Reviewed the origin of nucleate organisms and assigned the euglenophytes a crucial position in protist evolution as the originally phagotrophic group ancestral to all other algae that possess flagellar mastigonemes.

Table 1 (continued)

Date	Author	Contribution
1984	Kivic and Walne	Postulated a phylogenetic affiliation between the Euglenophyta and the Kinetoplastida based on morphological homologies; suggested that the euglenophytes had their origin in the trypanosomatid/bodonid zooflagellates; suggested that the introduction of the chloroplast and a phototrophic mode of nutrition was a relatively late step in euglenoid evolution.
1986	Surek and Melkonian	Found a cryptic cytostome/reservoir pocket in <u>Euglena</u> (<u>E. mutabilis</u> , <u>E. gracilis</u> strain T, <u>E. spec.</u> (palmelloid) that was structurally and positionally homologous with the cytostome/cytopharynx of colorless Euglenophyceae and bodonids which provided the first sound evidence that the photosynthetic Euglenophycean organisms originated from phagotrophic ancestors.

A Partial Listing of Shared Characteristics of Euglenoids
and Kinetoplastids and of Euglenoids and Dinoflagellates,
in Addition to the PAR

Shared Characteristics of Euglenoids and Kinetoplastids

- 1) euglenoid movement is also described in the bodonids, Cryptobia vaginalis, Rhynchomonas metabolita and Isonema (of uncertain taxonomic affinity)
- 2) similar external and general morphology (See Fig. 7 in Kivic & Walne, 1984)
- 3) flat, rather than tubular, mitochondrial cristae with constricted bases (Kivic and Walne, 1984); mitochondrial network with discoidal cristae (Brugerolle, 1985)
- 4) chondriome in both (at least as represented by that of Euglena gracilis) is a single large reticulum which varies in shape (Kivic & Walne, 1984)
- 5) closed mitosis with an intranuclear spindle (Brugerolle, 1985)
- 6) the "multitubular structure (MTS) in Phacus and Hyalophacus (Dynesius & Walne, 1974, 1975) is similar to the "subpellicular organelle" found in Trypanosoma brucei, T. congolense and T. vivax (Taylor and Godfrey, 1969). The MTS is a group of 4 subpellicular microtubules lying near the point of attachment of the flagellum and associated with a vesicular membrane (on the same side as axonemal doublet #8; perpendicular to the plane of the central pair of microtubules of the axoneme).
- 7) two unequal heterodynamic flagella (Rhynchomonas metabolita) that emerge from an ovoid depression or flagellar pit on the dorsal side of the organism
- 8) long, unilateral mastigonemes along the length of the flagellum (only the euglenoids possess the second, shorter type of mastigoneme; Walne & Kivic, 1984)
- 9) three independent fibres at the level of the flagellar pocket (Brugerolle, 1985)
- 10) similar fibres in the cytopharyngeal complex (Brugerolle, 1985)
- 11) a long transition zone with a featureless, hollow appearing center (Walne & Kivic, 1984)

Shared Characteristics of Phagotrophic Euglenoids and Kinetoplastids

- 1) heterokont (with flagellar hairs in some cases)
- 2) secretion of surface glycoproteins
- 3) the centering of endo- and exocytosis about a flagellar canal and reservoir
- 4) sub-pellicular microtubules
- 5) a 'primitive' mitotic mechanism for partitioning genomes: a totally closed mitosis, an intranuclear spindle, and no metaphase plate formation
- 6) fusion of mitochondria into a network (a cyclical condition in euglenoids; a permanent condition in kinetoplastids) (Vickerman & Preston, 1976)
- 7) lattice-like trichocysts (reported for Entosiphon, Isonema and Rhynchobodo Lackey (Brugerolle, 1985))
- 8) cytopharynx of Rhynchomonas metabolita (Bodonidae) (Burzell, 1973) is similar to siphon of Entosiphon (Triemer & Fritz, 1987), and Peranema (and to peduncle of dinoflagellates)
- 9) Rhynchomonas metabolita is highly compressed in the dorsoventral plane; trailing flagellum is dragged passively behind the organism during locomotion, the cell glides over surfaces on its ventral side; the proboscis is an outward extension of the cytopharynx
- 10) cytopharynx-proboscis of Rhynchomonas metabolita (Bodonidae) (Burzell, 1973) is similar to siphon of Entosiphon (Triemer & Fritz, 1987) and Peranema (and to the peduncle of dinoflagellates); Rhynchomonas metabolita is highly compressed in the dorsoventral plane. Trailing flagellum is dragged passively behind the organism during locomotion, the cell glides over the surface on its ventral side, the proboscis is an outward extension of the cytopharynx (Burzell, 1973)

Shared Characteristics of Euglenoids and Dinoflagellates

- 1) Unusual forms of eukaryotic mitosis:

Mitosis in euglenoids:

- a. the nucleolus (endosome) persists and divides
- b. chromosomes are without centromeres
- c. the nuclear envelope remains fully intact throughout division (with no gaps at the poles)
- d. microtubules form in the nucleus only
- e. there are no chromosome-to-pole spindle microtubules (Lee, 1980) and no cytoplasmic microtubules

Shared Characteristics of Euglenoids and Dinoflagellates (continued)

- f. there is no relationship between the dividing nucleus and the flagellar basal bodies
- g. chromosome numbers are generally high and polyploidy probably occurs in some genera (Leedale, 1967b)

Mitosis in dinoflagellates:

- a. chromosomes are without centromeres
- b. the nuclear envelope remains intact through division
- c. microtubules form outside the nucleus only (in cytoplasmic channels which form parallel to the future division axis)
- d. there is no contact of channel microtubules with the flagellar bases
- e. the nucleus constricts perpendicular to the cytoplasmic channels (Leedale, 1967b)

"Euglenoid mitosis" in a dinoflagellate (Oxyrrhis) (Triemer, 1982):

- a. the nucleus is deeply hollowed by a furrow but no microtubules are present in the channel
- b. longitudinal fibrils of the rod-shaped chromosomes are attached by one end to the nuclear envelope (no kinetochore)
- c. attachment sites for the chromosomes to the nuclear envelope (Cachon et al., 1979) are similar to that seen in Trachelomonas hispida var. coronata (West et al., 1980)
- d. separation and segregation of the daughter chromosomes are effected by elongation of the nuclear envelope

- 2) unilateral array of fine flagellar hairs in the Euglenophyceae and on the transverse flagellum in the Dinophyceae (Leedale et al., 1970; Lee, 1980)
- 3) predominately asexual reproduction: sexual reproduction has been reported in one putative euglenoid, Scytomonas and observed in a few dinoflagellates (see Bujak & Williams, 1981)
- 4) flagellar "grooves" are found in representatives of both groups: Anisonema and Peranema (Hilenski & Walne, 1985a) in euglenoids and in many dinoflagellate
- 5) presence of ejectile organelles in both groups: trichocyst development in Entosiphon and Peranema is similar to trichocyst ontogeny in marine dinoflagellates (Walne, 1980)
- 6) similar kind of pellicle/thecal plate articulation: in euglenoids, each pellicular strip has a thick edge that fits under the thin flange end of an adjacent pellicular strip - this construction is similar to that of the thecal plates in the Dinophyceae (Lee, 1980)

Shared Characteristics of Euglenoids and Dinoflagellates (continued)

- 7) in Woloszynskia micra (Leadbeater & Dodge, 1966) mitochondria often contain a large zone of DNA, frequently with crystalline, fibrous inclusions (Dodge, 1971); this is similar to electron-dense region in some mitochondria of Eutreptia pertyi (see Fig. 2, PART III)
- 8) in Noctiluca (Soyer, 1968) there are many microtubules present beneath the theca and a number of fibrous bands criss-cross the tentacle (Dodge, 1971); similar to pellicular cytoskeleton
- 9) the complex "microtubular basket" - composed of curved imbricate rows of microtubules in Crypthecodinium cohnii and Katodinium glandulum - runs from one end of the cell, past the flagellar bases to near the nucleus; this has obvious structural and positional similarities to the cytopharynx of ex. Isonema
- 10) the pusule of Amphidinium herdmanni - a central collecting chamber opens into the flagellar canal; numerous pusule vesicles open into the collecting chamber. "The pusule is an organelle found only in dinoflagellates but it is not present in all species" but it is found in marine and freshwater organisms. The gross morphology of the pusule is very variable, but the basic structure is the same: the main part of each pusule usually consists of a central chamber or duct which opens to the exterior by way of the flagellar canal. In the simplest pusules (like Woloszynskia micra) a number of secondary vesicles open directly into collecting chamber; in more complex pusules, like that of Gymnodinium fuscum, the collecting chamber appears to be situated at some distance from the flagellar canal but connected by a pusule canal; the wall of the canal is lined by fibrous material; The function of the pusule is thought to be osmoregulatory.
- 11) the peduncle of the non-photosynthetic phagotrophic dinoflagellate, Gymnodinium fungiforme, is an extensible structure which protrudes 8-12 μ m from the sulcal-cingular vicinity during feeding. In cross section, the peduncle has a circular-oval ring of overlapping microtubules; this "microtubular basket" looks like the supporting microtubular structure around the cytopharynx of Isonema (Spero, 1982). A similar microtubular structure was found in the cytopharynx of Kofooidinium and Oxyrrhis marina (Cachon & Cachon, 1974); "a similar and possibly homologous organelle in both structure and function is found in the tenacle of the suctorian ciliates; the tenacle has an outer ring of microtubules surrounding an inner microtubular basket

{(Note: in the opinion of NAD, the peduncle "microtubular basket" is very similar to the proboscis of Rhynchomonas metabolita, an outward extension of the cytopharynx (Burzell, 1973).}

Table 2. A Partial Listing of Pertinent Literature on Euglenoid, Dinoflagellate and Kinetoplastid Ultrastructure, with Emphasis on the PAR and Associated Structures of the Flagellum.

Date	Author	Contribution
General		
1981	Dentler	Summarized the structural and biochemical information about the interactions between flagellar/ciliary microtubules and flagellar/ciliary membranes and correlated this with their possible functions.
1982	Melkonian & Preisig	Found central pair microtubule twist in the straight region and in the flagellar tip of the green flagellate, <u>Scourfieldia caeca</u> .
1982	Moestrup	Reviewed flagellar structure in algae, including the types of paraxial inclusions. Suggested that their functions are as diverse as their morphologies.
Euglenoids		
1966	Mignot	Detailed the structure of the flagella at different levels in representative euglenoids (<u>Entosiphon</u> , <u>Anisonema</u> , <u>Peranema</u> , <u>E. acus</u> , <u>Menoidium</u> and <u>Scytomonas</u>), and noted the structural variations of the PAR in the biflagellates.
1968	Jahn & Bovee	Suggested that the function of the non-tubular mastigonemes of <u>Euglena</u> is to amplify the movement generated by flagellar beating.
1972	Kivic & Vesik	Reported the ordered substructure of the paraflagellar body (PFB) and the paraflagellar rod (PAR) in <u>E. gracilis</u> .
1975	Dodge	Described flagellar structure for <u>Trachelomonas volvocina</u> . Emergent flagellum has a row of fine hairs and the tip region has tufts of hairs that attach to the

Table 2 (continued)

Date	Author	Contribution
1975	Dodge	(continued) flagellar sheath. PAR in the free portion of the flagellum. Non-emergent flagellum lacks a PAR.
1975	Dynesius & Walne	Described the ultrastructure of the reservoir and flagellar regions of <u>Phacus pleuronectes</u> including a PAR in the emergent flagellum.
1976	Checcucci et al.	Showed that the paraflagellar swelling contains the putative primary photoreceptor pigments, flavin chromophores bound to protein macromolecules, that are reportedly responsible for phototaxis in <u>Euglena</u> .
1977	Wolken	Investigated the structure and flavin composition of the PFB, the supposed photoreceptor component in <u>Euglena</u> phototaxis.
1978	Bouck et al.	Studied the surface organization and composition of flagellar mastigonemes in <u>Euglena</u> and found that the longer of the non-tubular mastigonemes (~3 μ m) appear to attach (singly or in clusters of 3 to 4) to the PAR whereas the shorter non-tubular mastigoneme (~1.5 μ m) units attach parallel to, but outside of, the flagellar membrane.
1980	West et al.	Studied the ultrastructure of the flagella and cytoskeleton in <u>Trachelomonas hispida</u> var. <u>coronata</u> and reported cross-striated ribbon-like structures that linked the PAR to striated filaments that extend from the paraflagellar body.
1982	Hyams	Investigated the microarchitecture of the PAR in <u>Euglena gracilis</u> ; reported that the PAR does not assume a consistent orientation with respect to the central pair microtubules (Bouck et al., 1978 also).
1982	Melkonian et al.	Reported that the long non-tubular mastigonemes (previously thought to originate at the PAR - Leedale, 1967a and

Table 2 (continued)

Date	Author	Contribution
1982	Melkonian et al.	(continued)
		Bouck et al., 1978) link indirectly to both the PAR and one axonemal doublet.
1982	Moestrup	Described long non-tubular mastigonemes in <u>Eutreptiella gymnastica</u> , 4-5 μ m long, assembled into bundles. Bases of individual hairs are arranged in a row and form clusters of 3-4 hairs per bundle.
1983	Robenek and Melkonian	Reported a structural specialization of the flagellar membrane overlying the PFB and suggested that this also reflects a functional specialization.
1986	Surek and Melkonian	Found structural and positional homology between a cryptic cytostome in <u>Euglena</u> (<u>E. mutabilis</u> , <u>E. gracilis</u> strain T., and <u>Euglena spec.</u>) with the cytostome of colourless Euglenophyceae and the bodonids (Kinetoplastida). They describe the flagellar root system 3-3-5 (DR, IR and VR) in <u>Euglena</u> from basal body level to canal and define reservoir symmetry (left, right, dorsal, ventral) based on inherent features of flagellar apparatus: ventral flagellum (F2)/basal body is associated with IR and VR; dorsal flagellum (F1)/basal body is associated with the DR. They report both DPMB (from DR) and VPMB (from IR). They identify the ventral root as the flagellar root associated with the reservoir pocket/cryptic cytostome.
1986	Walne et al.	Described <u>Eutreptiella eupharyngea</u> . The two flagella show heteromorphy: the slightly thinner, short flagellum and the thicker long flagellum. PAR in both but regular striated periodicity in long flagellum only. Unilateral acuminate hair bundles occur on both flagella but those on the long flagellum appear to be thicker and larger. Both flagella are capable of vigorous movement; the longer flagellum moves around the anterior part of the cell or just in front of it with rapid, irregular

Table 2 (continued)

Date	Author	Contribution
1986	Walne et al.	(continued) movements. The shorter flagellum sometimes moves rapidly and irregularly in front of the cell; it may also appear more passive and directed anteriorly. The long flagellum can suddenly stretch out to its full length in front of the cell; on occasion, both flagella may do so concurrently, resulting in a cessation of swimming, followed immediately by a resumption of swimming in a different direction. That the flagella are unequal in length is obvious, though whether they should be considered distinctly heterodynamic is less clear.
1987	Solomon et al.	Described the flagellar root system of <u>Entosiphon sulcatum</u> and compared it to other euglenoid and kinetoplastid systems. DR is associated with the basal body of the F1 (anterior flagellum), the VR originates at basal body of F2 (trailing flagellum) and the IR originates between F1 and F2 nearer the F2 basal body. Described the spatial relationship between the two flagella predictable, with the PAR's being adjacent to one another and the two flagellar membranes linked by fine fibrils; in the reservoir, the trailing flagellum is always located opposite a ridge containing the IR which projects into the reservoir. The lattice-like array of the PAR of the ventral F2 flagellum is oriented towards the root. Fine fibrils (100-150 nm long) interconnect to the F2 flagellar membrane next to the PAR2 with the ridge of the reservoir membrane in the reservoir-canal transition region.
1987	Triemer and Fritz	Elucidated the ultrastructure of the tubular ingestion apparatus (siphon) of the phagotrophic euglenoid, <u>Entosiphon sulcatum</u> and proposed a mechanism for the operation of the organelle. This mechanism is compared with that of <u>Peranema</u> where the cytostome opens into the reservoir canal instead of towards the cell apex.

Table 2 (continued)

Date	Author	Contribution
Dinoflagellates		
1908	Keyssselitz	Reported the flagella of <u>Oxyrrhis</u> were similar and contained a uniformly heavy axial filament which lay towards one side of the sheath.
1911	Senn	Observed the axial filament of <u>Oxyrrhis</u> but was unable to determine whether it was axial or lateral in position.
1921	Kofoid and Swezy	Found that the longitudinal flagellum was thread-like and more rigid than the transverse flagellum which was ribbon-like and consisted of a stout fibril bordered on one side by a fin-like sheet of rippled or folded membrane.
1925	Hall	Studied the flagellar "neuromotor apparatus" of <u>Oxyrrhis</u> .
1934	Deflandre	Noted that the longitudinal flagellum of <u>Glenodinium uliginosum</u> was cylindrical with a long pointed tip and that the transverse flagellum had a distinct outline on one side but not on the other.
1952	Dragesco	First to study dinoflagellate ultrastructure in the electron microscope; reported the two flagella of <u>Oxyrrhis</u> appeared identical and that they were composed of a central axis of complex structure enclosed in a tunic strengthened by marginal fibrils.
1955	Pitelka and Schooley	Studied the flagella of a <u>Gyrodinium</u> sp.; the longitudinal flagellum was surrounded by a sheath that surrounded a single thick fibre from the axoneme; the transverse flagellum consisted of a dense axoneme and a unilaterally expanded sheath with a unilateral array of fine mastigonemes. [The "accessory fibril" in the micrographs of the transverse flagellum by Pitelka and Schooley was later termed the "striated strand" by Leadbeater and Dodge, 1967a.]

Table 2 (continued)

Date	Author	Contribution
1967a	Leadbeater and Dodge	Reported that the transverse flagellum in <u>Woloszynskia micra</u> had normal axoneme plus a shorter, "striated strand" plus organized packing material which occupied the space not filled by the axoneme and the striated strand; also reported that the longitudinal flagellum had a normal axoneme plus organized packing material in the proximal two-thirds; suggested that the transverse flagellum has a helical form that dictates (helical) movement.
1967b	Leadbeater and Dodge	Examined a wide range of dinoflagellates to observe flagellar structure, especially that in <u>Woloszynskia micra</u> , <u>Gymnodinium vitiligo</u> and <u>Gymnodinium veneficum</u> . Noted the structure of the packing material in the longitudinal and transverse flagella and suggested a helical model for the course of the transverse flagellum whereby the striated strand passes through the coils of the axoneme.
1970	Dodge and Crawford	Studied the morphology and fine structure of <u>Ceratium hirundinella</u> --a large, chloroplast-containing phagotrophic dinoflagellate.
1971a	Dodge and Crawford	Described the general ultrastructure of the phagotrophic, unarmoured, marine dinoflagellate, <u>Oxyrrhis marina</u> .
1971b	Dodge and Crawford	Described the fine structure of the flagella of <u>Oxyrrhis marina</u> , including flagellar length, insertion point, shape, and external (mastigoneme) and internal (PM ⁺ and PAR [*]) morphology. Based on flagellar structure, they suggested that <u>Oxyrrhis</u> may provide a link between the dinoflagellates and the Zoomastigophora. ⁺ "packing material" in the ventral and dorsal flagella in this paper is amorphous [*] "PAR" in this paper appears to be the same as the "packing material" in Leadbeater and Dodge, 1967b.

Table 2 (continued)

Date	Author	Contribution
1973	Dodge and Bibby	Described and illustrated the flagella of <u>Prorocentrum</u> as dissimilar in function and structure.
1975	Taylor	Called the 'striated strand' (term of Leadbeater and Dodge) a 'paraflagellar rod'. Proposed a hemi-helical model, instead of the helical model of Leadbeater and Dodge (1967), for the course of the beat of the transverse flagellum (i.e., hemi-helical rather than planar or helical).
1977	Berdach	Studied the 3-diminsional structure of the transverse flagellum of <u>Peridinium cinctum</u> by SEM.
1977	Herman and Sweeney	Found that the structure of the transverse flagellum in <u>Gymnodinium splendens</u> , an unarmoured dinoflagellate, agreed with Taylor's "hemi-helical model".
1978	Steidinger et al.	Described the general ultra-structure of <u>Gymnodinium breve</u> - reported that "packing material" in the longitudinal or transverse flagellum was not observed (However, micrographs of the transverse flagellum in longitudinal section do appear to indicate an axoneme, a striated strand and packing material (or PAR?) present).
1979	Berman and Roth	Studied the longitudinal flagellum of <u>Peridinium cinctum</u> fa. <u>westii</u> by SEM and suggested that the ridged appearance of the flagellum for most of its length was probably due to the internal packing material around the axoneme.
1979	Loeblich et al.	Found that for three <u>Prorocentrum</u> species, <u>P. minimum</u> , <u>P. rathymum</u> and <u>P. triestinum</u> , both flagella emerge from a single large pore (dinstinct from the auxiliary pore). Noted that the flagellum that undulates and contains a paraxonemal striated strand has been interpreted as the homolog of the transverse flagellum which is found in dinoflagellates possessing cingulum and sulcus. The other flagellum that tends

Table 2 (continued)

Date	Author	Contribution
1979	Loeblich et al.	(continued) to run along the cell away from the flagellar region is thought to be equivalent to the longitudinal flagellum.
1980	Rees and Leedale	Reconstructed the transverse flagellum of <u>Peridinium cinctum</u> by serial sections and proposed that the axoneme is helically coiled, this differs from the hemi-helical configuration of the axoneme in Taylor, 1975; Leblond and Taylor, 1976; Herman and Sweeney, 1977.
1982	Maruyama	Described the fine structure of the longitudinal flagellum in <u>Ceratium</u> in both retracted and relaxed states. The packing material (=PAR?) consisted of a lattice-like central core with <u>tubules</u> and radiating fin-like structures that link the PM to the axoneme in the proximal region of the flagellum. PM links also to the R-fibre i.e., connecting two motile systems (the axoneme for undulation and the R-fibre for retraction).
1982	Triemer	Described euglenoid mitosis in <u>Oxyrrhis marina</u> and discussed its possible taxonomic relationship with other dinoflagellates and protists.
1983	Bradbury et al.	Studied the ultrastructure of the dinoflagellate, <u>Polykrikos kofoidi</u> , including the flagellar apparatus; the longitudinal flagellum contains periodic material associated with the axoneme but the period and appearance differs from that of the strand in the transverse flagellum.
1985	Gaines and Taylor	Studied the form and function of the dinoflagellate transverse flagellum. The form of the transverse axonemal wave is helical; the flagellar beat always proceeds from base to tip of the flagellum (similar to the unidirectional, base-to-tip flagellar beat found in the majority of flagellates - Sleight 1981) and travels clockwise when the

Table 2 (continued)

Date	Author	Contribution
1985	Gaines and Taylor	(continued) cell is viewed from the cell posterior (counterclockwise if see from the cell apex). There is assymetry in the beat because there is a greater velocity of the beat on the outer edges which undulate in a helical fashion (on the axonemal side) compared to the inner edge which does not beat and is curvilinear (on the striated strand side); the usual wavelength of the beat is 3-6 μm . All cell rotation in the dinoflagellates is leiotropic (i.e. in the direction of the transverse flagellar beat-- i.e. from the organism's right towards its left). Contraction of the fibrous strand causes the transverse flagellum to pull inwards, associated with stoppage of the flagellar beat. With the exception of a few genera (for example, in <u>Ceratium</u> , where the cell is capable of sustained backward swimming using the longitudinal flagellum), the longitudinal flagellum appears to act primarily as a rudder; however, this includes not only directional change of travel of the cell but also the stabilizing of the cell during transverse flagellar beat and some forward propulsion of its own.
1988a	Cachon et al.	Characterized the structure of the PAR in the longitudinal flagellum of <u>Oxyrrhis marina</u> .
1988b	Cachon et al.	Reviewed the known ultrastructural and biochemical composition of the PAR in euglenoids, dinoflagellates and kinetoplastids and called the PAR "a structure in search of a function."
1988	Cosson et al.	Described the swimming behavior of <u>Oxyrrhis marina</u> and the movement of the flagella.
1988	Hohfeld et al.	Found centrin in the transverse flagellum of the dinoflagellate, <u>Peridinium inconspicuum</u> which they suggest confers contractility to the flagellum.

Table 2 (continued)

Date	Author	Contribution
Kinetoplastids		
1963	Pitelka	First to report an intraflagellar structure in <u>Bodo saltans</u> .
1965	Anderson and Ellis	Studied the flagellar ultra-structure of <u>T. lewisi</u> and noted the PAR-peripheral axonemal tubule connections; suggested that the PAR played a role in maintaining the rigidity of the flagellum.
1965	Boisson et al.	Suggested that the intraflagellar structure (IFS) in trypanosomes had a muscular (i.e. contractile) function.
1969	Fuge	Studied the intraflagellar structure (IFS; also called "lattice-like structure", "bande striee du flagelle" or "accessory filament") of <u>Trypanosoma brucei</u> flagella on the basis of ultra-thin sections in various planes, and documented both PAR-axoneme and PAR-flagellar membrane connections as well as the microarchitecture. Suggested that the IFS, in itself, is not contractile but rather a kind of abutment for the contractile fibers of the axoneme.
1969	Joyon and Lom	Studied <u>Ichtyobodo necator</u> (Henneguy) Pinto (Zooflagelle) Bodonidae, cell is quadrangular, flattened dorso-ventrally, has longitudinal groove on 2/3rd of right-ventral surface that forms a short canal in the anterior depression. A cytostome with a ring of reinforcing pairs of microtubules originates near the anterior part of the canal and curves in an arch into the anterior end of the cell (see Fig. 10). The sessile cell attaches to a host cell by a plate and a penetrating sucking organelle that contains the cytostome. Two flagella (dorsal and ventral) originate near the anterior end of the canal and extend toward the cell posterior in a grooved region on the ventral surface. Both flagella have "unruban paracristullin", "soit cylindrique, soit parallelepipedique in dorsal and the

Table 2 (continued)

Date	Author	Contribution
1969	Joyon and Lom	(continued) membrane is dialated. (My comments: dorsal flagellar PAR looks like it has multiple plates like <u>Eutreptia</u> ventral flagellum; PAR in ventral flagellum looks like it has the electron-dense core more typical of <u>Eutreptia</u> dorsal flagellum).
1969	Taylor and Godfrey	Found a "subpellicular organelle", a group of four microtubules lying near the attachment of the flagellum, in the trypanosomes, <u>T. brucei</u> , <u>T. con-</u> <u>golense</u> and <u>T. vivax</u> .
1969	Vickerman	Noted, while studying the surface coat and flagella in trypanosomes, the membrane adhesion process and the paraxial rod- axonemal linkages.
1973	Swale	Studied the ultrastructure of the colorless flagellate, <u>Rhynchomonas nasuta</u> which has accessory material ("paraflagellar mater- ial") in both the short and long flagella. Noted that the paraxial flagellar material in <u>Rhynchomonas</u> is similar to that found in the euglenoid, <u>Entosiphon</u> .
1974	Heywood et al.	Studied the ultrastructure of <u>Trypanosoma cyclops</u> . The PAR of the flagellum contains longitudinal elements which are parallel to each other and to the microtubules of the flagellar axoneme. The longitudinal elements have a periodicity of 18-19.5 nm. There is specialization of the cell plasma membrane where it makes contact with the flagellar membrane: there are several ~60 nm long projections that extend from the cell membrane into the cytoplasm at regular intervals of ~55 nm. These projections may help to maintain contact between the cell and the flagellum when the latter is beating. A cytostome is present at the level of the flagellar pocket.
1975	Burzell	Reported on the fine structure of <u>Bodo</u> <u>curvifilus</u> . Two heterodynamic flagella arise from a subapical flagellar pocket and there

Table 2 (continued)

Date	Author	Contribution
1975	Burzell	(continued) are PAR's in each flagellum. The most active flagellum is the anterior flagellum (as in most species) and has an unusual permanent curvature which occupies $\sim 1/2$ of its free segment. It has a very rapid type of "paddling stroke." As is characteristic of most bodonids investigated, the posterior flagellum usually trails passively behind the organism. It may serve to keep <u>Bodo</u> in contact with detritus of substrate and with bacterial populations on which it feeds. Both flagella have terminal hairs--this accounts for $\sim 10\%$ of the emergent length in the trailing flagellum. The PARs are similar to those found in other species of <u>Bodo</u> and in <u>Rhynchomonas</u>, some trypanosomes and certain euglenoids. The PAR in c.s. is crescent-shaped, lying against 4 of the outer doublets of the axoneme. There is some periodically ordered substructure in the PAR but the precise nature of the pattern cannot be elucidated. In <u>B. curvifilus</u>, the PAR begins at a point distal to the emergence of the flagellum, originating in an electron-dense plate, the "paraxial plate" which is slightly distal to the basal plate of the flagellum. "As in <u>B. saltans</u> and in <u>Rhynchomonas metabolita</u>, the paraxial rods of the two flagella are characteristically oriented facing one another near their points of origin. <u>Bodo curvifilus</u> possesses a highly ordered bundle of 19 cross-linked microtubules in a "microtubular prism."
1976	Hogan and Patton	Studied the intramembrane particle arrangements of isolated cells and flagellar membranes of <u>T. brucei</u> .
1976	Vickerman and Preston	Reviewed the flagellar apparatus of the kinetoplastid flagellates, including the PAR complex. Noted that two axoneme-plus-PAR complexes inside a common flagellar membrane are frequently encountered in sections of trypanosomatids

Table (continued)

Date	Author	Contribution
1976	Vickerman and Preston	(continued) and that one such complex lying intracellularly had also been reported.
1979	Brugerolle et al.	Observed the progressive transformation and evolution of cellular structures in bodonids and cryptobiids (<u>Bodo</u> , <u>Cryptobia</u> , and <u>Trypanoplasma</u>).
1980	De Souza and Souto-Padron	Investigated PAR structure in trypanosomatids (<u>Trypanosoma cruzi</u> , <u>Herpetomonas samuelpessoai</u> , <u>Leptomonas samueli</u> , <u>H. megaseliae</u> and <u>Crithidia hamosa</u>) and found that it is more developed in some species than in others; found that the PAR and the Ax maintain a fixed position.
1980	McGhee and Cosgrove	Reviewed the general cellular organization of the lower trypanosomatids, including flagellar structure and activity. Noted that the paraxial rod is absent from the flagella of <u>Crithidia oncopelti</u> , <u>Blastocrithidia culicis</u> and probably <u>Crithidia deanei</u> .
1981	Postell and McGhee	Studied the typanosomatid, <u>Phytomonas davidi</u> by electron microscopy. Found a paraxial rod in the single emergent flagellum. It originates above the distal basal plate of the kinetosome and accounts for the sudden increase in the diameter of the flagellum above this point. The PAR apparently extends the entire length of the axoneme. They also reference reports of the PAR in the trypanosomatids, <u>Phytomonas elmassiani</u> and <u>Phytomonas staheli</u> .
1981	Rowton et al.	Studied ultrastructure of the flagellar apparatus of <u>Herpetomonas ampelophilae</u> . Malpighian tubule promastigotes are biflagellated but do not have a PAR. Endoperitrophic flagellates have a single flagellum, although a pair of kinetosomes is found between the flagellar pocket and kinetoplast; this flagellum also has a well developed PAR. The PAR is always on the side of the flagellum toward the

Table 2 (continued)

Date	Author	Contribution
1981	Rowton et al.	(continued) host (<u>Drosophila</u>) intestinal lumen; the parasites secure themselves by weaving their flagella through microvilli (without hemidesmosome attachment).
1983	Russell et al.	Described the structural and biochemical characteristics of the PAR in the trypanosomatid, <u>Crithidia fasciculata</u> .
1985	Brugerolle	Studied the rod-like trichocysts of the bodonid, <u>Rhynchobodo</u> , which has an anterior rostrum that acts as a cytostome-cytopharynx and two flagella--one anteriorly and one posteriorly--both the paraxial rods, although the one in the anterior flagellum may not continue to the tip of the flagellum. Rod-like trichocysts have a cylinder of 24 longitudinal fibres around which helicoidal fibres are tightly twisted thus giving a lattice pattern; these are similar in morphology to those reported for <u>Entosiphon</u> and <u>Isonema</u> .
1988	Schneider et al.	Found spectrin I-like proteins in the PAR of <u>Trypanosoma brucei</u> .

PART II: FINE STRUCTURE OF EUTREPTIA PERTYI

I. ABSTRACT

The biflagellated cells of the photosynthetic marine euglenoid, Eutreptia pertyi (Prings.), 60-100 μm long and 20-25 μm wide, have nearly equal-length, heterodynamic flagella that emerge from the subapical opening of a tubular canal/reservoir cavity which extends 12-15 μm into the cell. In addition to the "9 + 2" axonemal microtubules, both flagella contain lattice-like filaments that form a paraxial rod, although the substructure varies in each flagellum; externally, the arrangement of long and short non-tubular mastigonemes is different on each flagellum. An evagination of the ventral/ventral-right reservoir wall forms a presumptive cytopharyngeal pocket in the reservoir-canal transition zone and is delimited by a ventral row of microtubules; these microtubules are associated, in the upper canal region, with a striated fibril, reported for the first time in the euglenoids. A pyrenoid center, surrounded by paramylon granules, occupies an antero-median position in the cell; 25-30 ribbon-shaped chloroplasts radiate from the periphery of this complex. A relatively smooth pellicle, composed of ca. 100 S-shaped strips, imparts helical symmetry to the cell; portions of the upper canal cytoskeleton are contiguous with the cortical pellicular cytoskeletal-epiplasmic complex. Certain cytoplasmic vesicles, termed here "mastigoneme vesicles," are reported for the first time in the Euglenophyta and may contain mastigoneme precursors or fully formed mastigonemes; the fibrillar contents are extruded by exocytosis into the lumen of the reservoir where they are presumably assembled and deposited onto the flagellar membrane.

II. INTRODUCTION

Flagellates of the genus Eutreptia are characterized by so-called "primitive" green euglenoid features, being naked (i.e., no lorica), solitary, chloroplast-containing cells, with two equal/subequal-length flagella, an elastic pellicle and no cell flattening. Thus, they are considered the "basic" green euglenoid in several phylogenetic schemes (Leedale, 1967, 1978, 1983). In this regard, Eutreptia species may occupy a pivotal position in euglenoid phylogeny, presumably having a close association with both phagotrophic ancestors and more advanced green relatives.

The members of the genera Eutreptia and Eutreptiella have traditionally been separated by flagellar features, with species of the former genus having flagella of approximately equal length and those in the latter genus having flagella that are more noticeably unequal in length; however, this taxonomic criterion has been recognized as a highly variable feature. In Pringsheim's review of Eutreptia (1953), he noted that in no case are the flagella of Eutreptia the **same** length, and since intermediate flagellar lengths, from almost equal to very unequal, could be found in different species, he concluded that all species with two flagella should be included in the genus Eutreptia. Pringsheim's major reservation in forming a consolidated group was that few species of either genus had been critically evaluated to determine if, excluding flagellar length, the taxa could be distinguished.

This investigation of Eutreptia pertyi Pringsheim provides the first ultrastructural study of the genus and compares its fine structural

features with those known for various other members of the order and phylum. These data should be useful in future taxonomic and phylogenetic reevaluations of the order Eutreptides and the phylum Euglenophyta.

III. MATERIALS AND METHODS

Cultures

Cultures of Eutreptia pertyi Prings. (LB 1290) were obtained from the Culture Collection of Algae, University of Texas, Austin, and grown in several media: a) a 1:1 mixture of biphasic soil water enriched with a garden pea, and pasteurized seawater enriched with Alga-Gro (Carolina Biological Supply Co., Burlington, NC) (SWAgSW), pH 8.0; b) pasteurized F/2 seawater (Guillard & Rhyther 1962) enriched with a garden pea (F/2SW), pH 8.0; c) a 1:1 mixture of F/2SW and SWAgSW enriched with ammonium-magnesium phosphate (West 1977), pH 7.8; d) a 1:1 mixture of biphasic soil water and pasteurized seawater enriched with a pea and ammonium-magnesium phosphate (SWSW + PO₄), pH 8.0; or, e) a 1:1 mixture of soil water and F/2SW enriched with a garden pea (SWF/2SW), pH 8.0. The cultures were maintained at 19°C under ca. 1500 lx (~27 $\mu\text{E} \cdot \text{M}^{-2} \cdot 5'$) provided by coolwhite fluorescent lights, on a 16:8 hr LD cycle.

Light Microscopy (LM)

Actively swimming cells were observed with a Nikon Optiphot Biological Microscope outfitted with differential interference contrast (DIC) optics. Images were recorded on Kodak Technical Pan 2415 B/W film with a Nikon Microflex UFX-II photomicrographic system attached to the microscope.

Scanning Electron Microscopy (SEM)

After fixation with 1.0% OsO₄/1.0% Triton X-100 (final concentrations) in F/2SW for 1 hr at 23°C, cells were pelleted gently and washed in 90% F/2SW - 10% H₂O. After a drop of concentrated cells

settled onto glow-discharged, 12-mm round glass coverslips coated with 0.1% (w/v) poly-L-lysine, they were dehydrated in a graded acetone series and critical point dried in a Ladd Critical Point Dryer. Coverslips were attached to aluminum SEM stubs with graphite paint and coated with gold-palladium in a Denton DV 515 vacuum evaporator. The cells were examined with an ETEC Autoscan Scanning Electron Microscope operating at 20 keV, and secondary electron images were recorded on Polaroid Type 55 P/N film.

Negative Staining

After a drop of concentrated cells, pelleted in a clinical centrifuge, adhered to a formvar-coated (0.5 or 0.75%), carbon-coated, glow-discharged 400-mesh copper grid, excess growth medium was carefully drawn off from one side, while a drop of 0.1% Triton X-100 was added to the opposite side of the grid. After 30 s, excess Triton X-100 was removed with filter paper, and a drop of 0.5% or 1.0% neutralized potassium phosphotungstic acid (KPTA) was added. Excess stain was removed after 10-15 s, and grids were air dried briefly before examination in a Hitachi H-600 electron microscope operating at 75 or 100 keV.

Shadowcasting

A drop of concentrated unfixed cells was allowed to settle onto formvar-coated (0.5 or 0.75%), carbon-coated, glow-discharged, 400-mesh grids for 1 min. After a 30 s treatment with 0.1% Triton X-100, cells were washed with dH_2O , air dried, shadowed with gold-palladium at an angle of 20° in a Denton DV-515 vacuum evaporator and examined in a Hitachi H-600 electron microscope.

Transmission Electron Microscopy (TEM)

After pelleting in a clinical centrifuge, cells were fixed in a mixture of 1.0% glutaraldehyde (final concentration), saponin (0.7 mg/ml), tannic acid (2 mg/ml) in F/2SW - SWAgSW culture medium, pH 7.2, for 30 min at 23°C. After a brief rinse in the culture medium, the cells were post-fixed in 1.0% OsO₄ in SWF/2SW medium, pH 6.0, for 45 min, 23°C, and then dehydrated in a graded acetone series. Infiltration of the cells overnight in an equal mixture of 100% acetone and Spurr's low-viscosity resin (Spurr 1969) was followed by embedding in 100% Spurr's resin the next day. Cells were densely pelleted into BEEM capsules by centrifugation in an Eppendorf 5414 microfuge for 10 min at 15,000 x G. After polymerization in a 60°C oven for 48 hr, thick (0.2-0.3 µm) and thin (90-100 nm) sections were cut with glass and diamond knives using a Reichert OM U3 ultramicrotome. Sections were stained sequentially with methanolic uranyl acetate and lead citrate, then examined in a Hitachi H-600 electron microscope operating at 75 or 100 keV.

Computer Image Enhancement

Transmission electron images recorded on Dupont Cronar Ortho S Litho COS-7 film were digitized at 256 x 256 pixel and 8 bit gray level resolution and manipulated with a Perceptics 9200 Real Time Image Processor.

IV. RESULTS

The morphological characteristics that distinguish Eutreptia pertyi Prings. include cellular dimensions of 60 - 100 μm in length and 20 - 30 μm in width, a delicately striated pellicle, a pyrenoid complex surrounded by paramylon granules, a canal and reservoir that extend approximately 15 μm into the cell anterior, and two subequal-length emergent flagella (Figs 1-3¹).

Orientation

Dorsoventral and right-left orientation for the organism have been determined by the relatively fixed location of major organelles in the anterior region that can be observed at both the light- and electron-microscopic levels. The flagellar apparatus, stigma, contractile vacuole, and vestigial cytopharynx occupy fixed positions in the cell relative to the reservoir-canal and serve as positional markers for reservoir/cell symmetry. Figures and verbal descriptions of longitudinal views of the cell are presented as if one is viewing the anteriorly flagellated organism from its right side with the anterior end of the cell at the top of the micrograph; cross-sectional views of the cell are presented as if one is viewing the organism from its posterior end, with the flagellar dynein arms projecting clockwise (imbrication of the basal bodies is counterclockwise). With respect to rotational orientation, cross sections through the upper reservoir-canal

¹All figures referenced in Part II are found in the appendix at the end of Part II.

region are arranged in the figures such that the dorsal (F1) flagellum is located toward the top of the micrograph and the ventral (F2) flagellum is located toward the bottom of the micrograph. An axis that separates the two flagella/basal bodies distinguishes the dorsal and ventral halves of the reservoir/cell which correspond, respectively, to the top and bottom halves of the micrograph, (i.e., a horizontal axis). Perpendicular to the horizontal axis, the vertical axis bisects the flagella/basal bodies and separates left and right halves of the cell/reservoir and correspond, respectively, to the left and right halves of the micrograph. Thus, the location of the major organelles in the anterior region are flagellar insertion and stigma on the dorsal side of the organism, contractile vacuole on the right side, and presumptive cytopharyngeal pocket on the ventral/right-ventral side of the reservoir-canal transition zone.

Cell Motion, Encystment and Flagellar Autotomy

During swimming, cells are usually 60-80 μm in length and exhibit a preferential shape that is typically conical, bluntly rounded anteriorly and tapered posteriorly (Fig. 5). During euglenoid movement or "metaboly," a wide range of cell lengths (50-110 μm) and shapes (rounded to elongated) are demonstrated during the peristaltic movements of the cell (Figs 1, 2, 4, 6-10).

After two months in culture, actively growing and dividing cells usually cease swimming and other movements and form cysts. Observations with LM show that prior to encystment, cells lose their flagella; this process occurs within seconds as the emergent portions of the flagella are shed. The point of flagellar breakage appears to be within the

upper canal, a region that is structurally rigid (see below) and perhaps functionally specialized (Bouck, 1982). Encysting cells conform to a ball shape and rotate in a fixed position during secretion of an extensive extracellular matrix, eventually forming a thickened sheath, approximately 1-5 μm in diameter, around the cell (Fig. 3).

Cortical Cytoplasm and Associated Organelles

Pyrenoid Center, Paramylon, Chloroplasts and Accumulation Bodies.

A prominent pyrenoid center typically occupies an anterior-median position in the cell (Figs 1, 6) and is surrounded by 6-8 large, ellipsoidal paramylon granules (Fig. 1); a few smaller granules also lie free in the cytoplasm. Ribbon-shaped chloroplasts, typically ~25-35 per cell, extend radially from the periphery of the pyrenoid center (Figs 9-10). Within the chloroplast envelope, photosynthetic lamellae form grana-like stacks of variable numbers of thylakoids (Figs 26, 33), occasionally separated by lipid globules. Healthy non-degenerate cells contain accumulation bodies, with contents that resemble digested food, waste or storage material; these single-membrane-bounded vesicles are observed throughout the cytoplasm and are especially common in the anterior region of the cell (Figs 11, 35). The mitochondria are typical except for occasional prominent electron-dense inclusions (Fig. 12).

Nucleus and Mucocysts. The interphase nucleus typically occupies a posterior position in the cell (Figs 1-2, 6-7, 9-10, 14). Condensed chromosomes are distributed in the granular nucleoplasm around a large endosome (nucleolus), and chromatin is associated with the inner nuclear membrane (Fig. 14).

Exclusive of the area occupied by the nucleus, numerous mucocyst-like structures fill the remaining cytoplasmic region of the cell posterior (Fig. 14). They closely underlie the posterior pellicular strips but are distinct from the small muciferous channels associated with the subpellicular system of ER (Fig. 14, small arrow). The amorphous mucocysts do not appear to be ejectile organelles.

Mastigoneme Vesicles. All Eutreptia pertyi cells examined contain numerous large single-membrane-bounded vesicles with fibrillar contents (Figs 11, 29). These mastigoneme vesicles or "fibrous bodies," the term used for similar structures in dinoflagellates (Dodge, 1971; Dodge & Bibby, 1973), are located throughout the cytoplasm; they have a variable diameter (0.1-1.2 μm) but are typically $\sim 0.7 \mu\text{m}$ in width. The contents of the vesicles are usually arranged in either loose whorls or stiff parallel fibrillar bundles. The individual fibrils are $\sim 1-3 \text{ nm}$ in diameter; their intravesicular length varies but fibrils measuring up to 1.3 μm have been recorded. The general morphology and dimensions of the fibrils are comparable to those of the non-tubular mastigonemes on the flagellar surface (see below).

It is highly probable that these vesicles are intracellular reservoirs of mastigonemes, although their presence has not been demonstrated in other euglenacean taxa. The preponderance of mastigoneme vesicles in the cytoplasm of Eutreptia pertyi is consistent with the large numbers of mastigonemes that form a thick hairy covering on both flagella (Figs 48-54); Bouck and co-workers (1978) estimated that there are >30,000 mastigonemes on the single emergent flagellum of Euglena gracilis.

It is unclear whether the fibrillar contents represent mastigoneme precursors or fully formed mastigonemal units; in either case, we suggest that the term mastigoneme vesicles be used for these cytoplasmic inclusions.

The site of mastigoneme synthesis is unknown, but an intracellular origin is suspected and has been suggested in other organisms (Bouck, 1972). For example, fibrillar material is found in dilated regions of the ER near the nucleus (Fig. 32) and the perichloroplastidic space (Fig. 33). Moreover, processing of presumptive mastigoneme precursors through the Golgi apparatus is also suggested by the close proximity of the vesicles to the dilated arms of the dictyosomes (Fig. 11) and the presence of similar fibrillar material in the dilated lobes of the cisternae (Fig. 34).

In the cell anterior, mastigoneme vesicles are commonly observed in the reservoir region near the contractile vacuole (Figs 39-41); fusion of the mastigoneme vesicular membrane with the reservoir membrane during exocytosis releases the fibrillar contents into the reservoir lumen (Fig. 41). The liberated contents probably constitute mastigonemal precursors that require subsequent assembly and processing. Eventual deposition of the mastigonemal units on the flagellar membranes presumably occurs within the intrareservoir space; fibrillar material is commonly observed in the reservoir cavity and is especially concentrated around the flagella in the restricted canal region (Figs 42-44).

Pellicle and Associated Structures

The surface of the Eutreptia cell is typically covered by a thin extracellular matrix of mucilage (Figs 24-25, 28); the thickness of this

layer may increase during cell encystment to form an extensive sheath, 1-5 μm in diameter (Fig. 3). Beneath this matrix, delicate striations of the pellicular complex are evident (Figs 5, 7, 17).

During intussusceptive growth of the pellicular complex, new strips originate by intercalation between old strips (Hofmann & Bouck, 1976), and thus the width of the strip is an indicator of strip age (Leedale, 1982; see Fig. 5 in Dawson et al. 1988). Computer-enhanced image processing (Fig. 18) of a shadowcast preparation of the pellicle of a 'mature' cell surface (Fig. 17) reveals the concavity of wide (older) pellicular ridges, a feature usually visible in images of thin sectioned material. Successive growth of ~ 24 initial strips in the upper canal region (Fig. 15) results in articulation of ~ 100 pellicular strips in the peripheral cytoskeletal complex (Figs 4, 7); the number of cortical pellicular strips is reduced to ~ 22 in the bluntly attenuated cell posterior (Fig. 16).

The outermost component of the pellicular complex, the plasma membrane (Figs 25, 28), overlies the ridgegroove articulation of the overlapping pellicular strips. Each stria or strip unit is composed of a notch, an arched (i.e., young strips) or slightly concave ridge (i.e., older strips), a convex groove and a heel region where adjacent strips interlock (Fig. 25); these regions are underlain by a dense and semi-continuous epiplasmic layer. The relatively thin pellicular complex seen in cross section and the lack of an extensive region of overlap between adjacent striae (Figs 24-28) may contribute to the flexible nature of the Eutreptia periplast during metaboly.

There is a species-specific number and arrangement of microtubules associated with each pellicular unit. In Eutreptia, two cortical microtubules, m_1 and m_2 , are characteristically associated with each ridge-notch region, and two subcortical microtubules, m_3 and m_4 , are associated with each groove-heel region (Figs 25-26). During intussusceptive pellicular growth in the upper canal, one of the microtubules (possibly m_4) of the complex is missing (Fig. 24). Interstrip linkers (traversing fibers) in the vicinity of cortical microtubules m_1 and m_2 connect the dense layers of adjacent striae (i.e., the dense ridge layer of one to the dense heel region of the adjacent strip) (Fig. 25). This point of interconnection between adjacent striae is characterized by a) m_2 , partially embedded in the epiplasmic layer of the heel, b) a thinner layer of extracellular mucilage in the groove than in the layer that overlies the ridge, and c) absence of an epiplasmic dense layer. Muciferous channels, part of an extensive subpellicular system of ER, run parallel to each ridge subjacent to the subcortical cytoskeleton (Figs 24-28). Various precursors or products of presumed fusion and membrane recycling are commonly observed in the pellicular region (Figs 20-22).

The ridge-groove pattern of pellicular strip articulation, typical of the cortical regions, also continues for a short distance ($> 2 \mu\text{m}$) into the subapical canal opening on the ventral side of the organism (Fig. 12). In negative-stain preparations, the pellicular groove regions, which selectively take up the KPTA stain, appear to terminate as the pellicular complex flattens into the smooth cortical region of the canal (Fig. 15). The trumpet-shaped canal opening attenuates

strongly into a highly constricted multi-lobed luminal space near the cell anterior and subsequently forms a left-right compressed aperture with an oval profile in more proximal regions near the reservoir-canal transition zone. Some of the cortical pellicular microtubules and subjacent epiplasm extend longitudinally into the canal (Fig. 12). The two-over-one triplet arrangement of longitudinally directed microtubules in this upper canal region (Fig. 30) suggests that three of the four pellicular microtubules extend into the constricted canal region. These microtubules and associated epiplasmic material are thus contiguous with at least some of the longitudinally directed microtubules and electron-dense material in the upper canal wall. The canal cytoskeletal complex is, in turn, encircled by transversely directed microtubules and fibrillar material that form a collar around the constricted canal (Figs 29-30). Delimiting the collar are extensions of the cortical endoplasmic reticulum (Fig. 29, arrows).

Canal and Reservoir Region

Contractile Vacuole. This organelle typically occupies the right/right-ventral portion of the cytoplasm in the mid-to-upper reservoir, adjacent to the VR (Figs 40-41). The discharge of vacuolar contents occurs every 1-2 mins by the fusion of the contractile vacuolar and reservoir membranes; endo/exocytotic vesicles and elements of endoplasmic reticulum are often juxtaposed to the reservoir membrane in this region (Fig. 40). "Coated" vesicles are commonly observed in the contractile vacuole region and may represent Golgi-derived secretory, auto- or phagotrophic products since organelles presumably involved in these functions, are often located in the nearby cytoplasm (Figs 11, 29,

35). The dynamics of membrane recognition, fusion and reclamation during systolic-diastolic stages of vacuolar contraction are facilitated by the absence of microtubular bands in the right/ventral-right wall of the reservoir (Figs 40-41). The three groups of microtubular bands (DR, IR and VR) present at this level delimit the other relatively stable (dorsal, left and ventral) sides of the cavity. Membrane-limited vesicles, commonly observed in the reservoir pocket (Figs 41, 43-44), may represent residual products of exocytosis.

Stigma. The extrachloroplastidic stigma, a compact assemblage of numerous reddish, membrane-bounded granules, lies in the cytoplasm closely appressed to parallel, evenly spaced singlet microtubules (Fig. 38). These stigma-associated microtubules are the DMT microtubules (or DPMB of Surek & Melkonian 1986) (see below) and are closely associated with the plasmamembrane that delimits the dorsal reservoir wall (Figs 38, 42-44). The stigma granules are typically ovoid, 0.2 to 0.6 μm in diameter, and are arranged in staggered moniliform layers that form a slightly cup- or trapezoid-shaped complex that follows the curvature of the reservoir wall (Figs 6, 8, 35, 38-39). Distention (Fig. 8), compression and/or "super-twisting" of the anterior portion of the organism during metaboly can result in lateral displacement of the reservoir pocket and even retroflexion of the reservoir cavity toward the anterior end of the cell. The movements of the stigma complex appear to be coordinated with the restricted temporal and spatial displacements of the swelling (PFB) on the dorsal flagellum during metaboly.

Flagella and Related Structures

At the light microscopic (LM) level both flagella are approximately as long as the cell (Figs 2, 10) and are distinguishable by heterodynamic flagellar motion. The more active flagellum, designated here as the dorsal (F1) flagellum, $\sim 1.5 \mu\text{m}$ wide and $80 \mu\text{m}$ long, projects anteriorly from the canal opening (Figs 2, 6). The slightly wider and longer flagellum, designated here as the ventral (F2) flagellum, $\sim 2 \mu\text{m}$ wide and $90 \mu\text{m}$ long, usually projects laterally (Figs 2, 6-7) or trails posteriorly adjacent to the cell body.

Flagellar Insertion. Originating from parabasal sites in the dorsal reservoir wall (Figs 6, 37), the flagella curve slightly in the upper reservoir region, pass anteriorly through an obliquely angled, constricted canal and finally emerge from the subapical canal opening on the ventral side of the organism (Figs 2, 6, 10, 35). This curvature restricts any extreme lateral rotation of the flagella in the reservoir-canal transition zone and effectuates the spatial alignment of the swelling (PFB) (the assumed photoreceptor) on the dorsal flagellum to the stigma complex in the nearby dorsal cytoplasm (Figs 6, 10, 35, 38-39).

Internal Structure. In addition to their heterodynamic motion, the flagella can be distinguished from one another by differences in diameter and paraxial rod (PAR) complexity. The PAR is enclosed by the flagellar membrane, lies parallel to the "9 + 2" axoneme, and extends the length of each flagellum (Fig. 44). The dorsal flagellum, $\sim 1.2-1.5 \mu\text{m}$ in diameter, has a PAR with a circinate electron-dense core in cross section and lattice-like filaments visible in oblique and longitudinal

sections. The PAR of the slightly wider ventral flagellum, 1.5-2.0 μm in diameter, has a plate-like substructure in cross-section and a multi-periodic lattice-like structure in longitudinal section (Figs 42-45, 53). The typical positional relationship between the flagella in the upper reservoir and canal regions is such that the PARs oppose one another, and the interflagellar canal space is small and filled with fine fibrillar material (Figs 42-44, arrows). A detailed investigation of the PAR microstructure in the flagella of Eutreptia is the subject of Part III.

External Structure. Both long and short nontubular mastigonemes attach beneath and extend through the flagellar membranes but the mastigonemal arrangements differ. The dorsal flagellum has continuous and interlocking tiers of short mastigonemes that form two crescent-shaped mats (best seen in cross sectional views, e.g., Fig. 52), each up to 1.3 μm wide (Fig. 54), around the respective axonemal and PAR sides of the flagellum. In longitudinal views (e.g., Figs 49, 51), the tiers of short mastigonemes have a 0.11 μm periodicity and are arranged at $\sim 75^\circ$ to the long axis of the dorsal flagellum (Fig. 49, top arrow-head), results suggesting that the short mastigoneme mats have a slight helical arrangement from flagellar base to tip. On the dorsal flagellum, long mastigonemes, $\sim 4 \mu\text{m}$ in length, are arranged in periodic bundles (>10 individual mastigonemes per bundle) that extend unilaterally from the flagellum between the mats of short mastigoneme (Figs 52, 54). The ventral flagellum has a single mat of short mastigonemes, ~ 0.25 - $0.28 \mu\text{m}$ long, which are arranged in tiers, with a periodicity of 0.08 μm , that extend only from the PAR side of the

flagellum. Bundles of long mastigonemes, up to 5.5 μm in length and with a periodicity of 0.09 μm , extend only from the axonemal side of the flagellum (Figs 48, 50, 53).

Reservoir-Associated Hairs. The reservoir-associated hairs appear to be of three types: 1) bundles of wavy, bristly hairs that emanate from the basal reservoir membrane into the interflagellar space (Fig. 37); 2) stiff hairs, extending into the reservoir near the IR, are in close association with the ventral flagellum (Fig. 40); 3) long hairs occur in the expanded portion of the reservoir, seemingly unattached to the flagellar membrane(s) (Fig. 37).

Flagellar Roots and Cytopharynx

To eliminate further confusion concerning flagellar root and flagellar terminology in the euglenoid literature, these general suggestions are offered: 1) that the flagellum which originates from the dorsal-root-associated kinetosome be, hereafter, referred to as the **dorsal flagellum**, and the flagellum which originates from the intermediate and ventral root-associated kinetosome be referred to as the **ventral flagellum**; and 2) that cross-sectional views of the cell, whenever possible, be presented in the plates with the dorsal side of the organism toward the top of the micrograph, the ventral side of the organism toward the bottom of the micrograph, and the left and right sides of the organism corresponding to those same respective sides of the micrograph. This arrangement would effectively convey the in vivo spatial disposition of the cellular organelles and facilitate analyses of flagellar roots and other cytological features in particular interest.

Three groups of microtubules delimit the reservoir and canal membrane and constitute extensions of the dorsal (DR), intermediate (IR) and ventral (VR) flagellar roots that originate at the basal body complex (Fig. 37). The spatial arrangement of the three rows of microtubules with other reservoir constituents is shown in transverse and oblique sections through the reservoir/canal region (Figs 39-43). The DR is associated with the dorsal flagellum and the PFB on the dorsal side of the reservoir; the IR in the left reservoir wall is typically aligned with the ventral flagellum. There are lateral fibrillar projections from the DR and IR, fine fibrillar interconnections between evenly spaced DMT and VR microtubules, and fine connections which tether bundles of linked microtubules to adjacent bands, plates and overlying membrane.

In addition to a preferential reservoir shape ("normal" shape), very limited intrareservoir flagellar motion is suggested by the consistent alignment of the DR and DMT with the dorsal flagellum and the IR with the ventral flagellum. Specifically, in Eutreptia, the IR is aligned with the axoneme of the ventral flagellum and occasionally shapes a small convexity or concavity in the left reservoir wall. The pocket originates as an evagination of the ventral reservoir wall, accompanied by a "9 + 3" arrangement of longitudinally directed VR microtubules. The vestigial pocket extends $\sim 2-3 \mu\text{m}$ (Solomon et al., submitted) into the right-ventral portion of the reservoir between the VR and the DMT and terminates within the mid-canal region near a striated fibril (with a periodicity of $\sim 20 \text{ nm}$, Fig. 45), proximal to a

band of transversely directed microtubules that encircles the mid and upper canal region.

Summary of the Spatial Progression of Roots.

What is known of the spatial progression of the microtubular reservoir cytoskeleton from the origin at basal body level in the dorsal reservoir wall to the point of integration with the pellicular cytoskeleton in the canal region of Eutreptia pertyi can be summarized as follows:

Basal body level: 5 DR - 6 IR - 8 (6 + 2?) VR

Lower reservoir: 5 DR nucleates 20+ DMT - 10 IR - 20 (18+2) VR with underlying plate

Upper reservoir near stigma and PFB: 5 DR with plate toward DMT - 6⁺ IR - 15 VR which may begin to split into two parts

Reservoir-Canal transition: 6 (4 + 2) DR with plate toward DMT - 9 IR - 11 (9 + 3) VR: large band of evenly spaced microtubules follows cytopharyngeal pocket, and small band of closely grouped microtubules may split into a separate band between the large VR band on the ventral-right side and the DMT on the dorsal-right side; cytopharyngeal pocket is most extensive at this level

Lower canal: DR and IR begin to fuse on dorsal-left side and DMT microtubules begin to double, beginning on dorsal-left (DR) side and progressing toward dorsal right (VR) side; VR band(s) begins to fuse/link with DMT on right side and IR on left-ventral side

Upper canal: DR - IR - VR fused and increased number of doublet microtubules; cytopharyngeal pocket terminates at striated fiber

Near ventral canal opening: "bottlenecked" canal with doublet and triplet arrangement of canal and pellicular microtubules

Presumptive Cytopharynx. The ventral row of microtubules (VR) in the ventral-right reservoir wall demarcates a narrow evagination of the ventral reservoir-canal transition region. The outpocketing appears to be a vestigial cytopharynx (Figs 35, 43), as noted by Solomon et al. (1987), and is similar to structures reported in other photosynthetic euglenoids (Willey & Wibel, 1985 a,b; Surek & Melkonian, 1986).

V. DISCUSSION

Cell Motility

The strong metabolic movements of Eutreptia are similar to those of Distigma proteus (Gallo & Schrével, 1982; Gallo et al., 1982), Eutreptiella eupharyngea, E. gymnastica, E. marina da Cunha (Walne et al., 1986) and many Euglena species. During swimming, modifications of cell length and shape allow for maximum propulsive thrust and minimum body drag, while the bluntly attenuated "tail" may effect subtle changes in swimming speed and direction. The data suggest that ultrastructural differences in the two flagella with respect to PAR substructure and arrangement of mastigonemes may account for, or contribute to, the heterodynamic motion. During swimming, modifications of the cell to a slightly shortened length, persistent conical shape, and clockwise "screw" rotation of the cell about the anterior-posterior axis allow for maximum propulsive thrust and minimum body drag. The bluntly attenuated posterior 'tail' of E. pertyi, more pronounced and elongated in E. viridis (Pringsheim, 1953), may effect subtle changes in swimming speed and direction.

Encysting Cells

The encystment response in cultured Eutreptia pertyi cells may be initiated by the absence of biotin, vitamin B₁₂ or thiamin nutritional requirements for this organism (Round, 1965); or it may represent a normal adaptive feature of this species, since it is naturally found in brackish environments where there are frequent low and high tides (Pringsheim, 1953). Flagella are shed during the initial stages of

encystment at a "breakage point" within the upper canal; a phenomenon reported also for the single emergent flagellum of Euglena gracilis (Bouck, 1982). In Eutreptia, autotomy at this preferential juncture preserves the PFB on the dorsal flagellar stub and hence the potential for photoreception. The extracellular mucilaginous matrix appears to be uniformly distributed on the cell surface by circumferential rotation of the Eutreptia cells. The ensheathing matrix is probably derived from polysaccharide material extruded into the reservoir region, in a process similar to stalk formation in Colacium (Willey et al., 1977) and cyst formation in Euglena gracilis (Triemer, 1980). Additionally, our data suggest that the right reservoir wall is structurally flexible and may be a preferential site for extrusion of sheath material and other extracellular glycoproteins (see below). The mucocyst-like vacuoles in the posterior cytoplasm of Eutreptia may also be involved in the secretion of the extensive mucilaginous sheath.

Cytoplasmic Organelles of Interest

Differences in organelle structure and location may be useful taxonomic criteria for Eutreptia species. For example, the single, large pyrenoid center in Eutreptia pertyi, referred to in the older literature as the "paramylon-initiation center" because of 6-10 encircling paramylon granules (Pringsheim, 1953), is morphologically similar in all the cells examined. In comparison, an aggregated or compound type of pyrenoid, surrounded by paramylon plates, is described for several Eutreptiella species (Throndsen, 1973; Walne et al., 1986). The ribbon-shaped chloroplasts of healthy Eutreptia pertyi cells become

discoidal or ellipsoidal during senescence, a phenomenon also observed by Pringsheim (1953).

The nucleus occupies a posterior-to-median position in Eutreptia, whereas it is more centrally located near the basal body complex in Eutreptiella. These observations indicate that, excluding differences in flagellar length, the genera Eutreptia and Eutreptiella may possibly be differentiated on cytological attributes, such as structure and distribution of the pyrenoid, chloroplasts and nucleus. Nonetheless, some cytological features of the two genera are similar: both possess mucocyst-like vacuoles in the posterior end of the cell, although they are smaller and more numerous in Eutreptia; and both genera have autophagosome-like vacuoles surrounding the canal (cf. Fig. 35 with Fig. 13 of Walne et al., 1986). The autophagosome-like vacuoles are not restricted to the cell anterior in Eutreptia pertyi (Fig. 11); they have morphological and spatial similarities to accumulation bodies commonly found in a variety of dinoflagellates (see below).

Unusual electron-dense inclusions, occasionally observed in mitochondria of Eutreptia are also found in dinoflagellate mitochondria where they reportedly contain "large patches of DNA" (Leadbeater & Dodge, 1966; Dodge, 1971). This structural feature in the euglenoid chondriome is also reminiscent of the dense mitochondrial matrix that contains bundles of kinetoplast DNA fibrils (kDNA) in the kinetoplastid zooflagellates (Brugerolle et al., 1979). No fibrils were distinguished within the electron-dense mitochondrial regions of Eutreptia, but this superficial resemblance may be significant since a phylogenetic affiliation is proposed for the Euglenida with the Kinetoplastida (Leedale,

1978; Leedale, 1983; Kivic & Walne, 1984) and perhaps with the dinoflagellates. Further biochemical and immunological investigations are necessary to determine if non-fibrillar, kinetoplast-like or dinoflagellate-like mitochondrial DNA is localized within the Eutreptia condriome.

The accumulation bodies of Eutreptia cells have structural and perhaps functional similarities to the accumulation bodies reported in symbiotic and free-living dinoflagellates (Kevin et al., 1969; Tomas & Cox, 1973; Dodge & Crawford, 1974; Wilcox et al., 1982; Wilcox & Wedemayer, 1984). The euglenoid accumulation bodies resemble food vacuoles but, the contents are not the recognizable remains of large endosymbiotic or ingested prey organisms (see below for discussion of possible "passive" phagotrophy in this photosynthetic organism). The occurrence of the bodies throughout the cytoplasm in healthy, non-senescent cells suggests that they may function in normal lysosomal or autolytic processes as "intracellular waste depositories" (Wilcox et al., 1982); hence, the contents may represent stored or recycled products in various states of catabolism.

Mastigoneme Vesicles and Mastigonemes

Mastigoneme vesicles, containing precursors or preformed flagellar mastigonemes, are a significant finding of this investigation. This is the first report of an intracellular localization of mastigonemal components in the euglanoid flagellates. These findings are consistent with the general supposition of an intracellular origin for algal flagellatar hairs (Leadbeater, 1971; Bouck, 1972).

Presumptive non-tubular mastigonemes in intracellular vacuolar compartments, similar in dimension and appearance to those found in Eutreptia pertyi, are also reported in marine and freshwater dinoflagellates, including Amphidinium carteri (Dodge & Crawford, 1968), Symbiodinium microadriaticum (Kevin et al., 1969), Peridinium westii (Messer & Ben-shaul, 1969), Oxyrrhis marina (Dodge & Crawford, 1971), Wolosynskia micra Leadbeater & Dodge (Dodge, 1971), several Prorocentrum species (see Table 1 in Dodge & Bibby, 1973) and Gymnodinium breve (Steidinger et al., 1978). A concentration of mastigoneme vesicles in the subreservoir cytoplasm of Eutreptia pertyi suggests that they may be translocated from the site of synthesis to the site of attachment on the flagellar surface.

Based upon their general appearance in the vacuolar compartments, two hair types can be distinguished: a) long and wavy (Fig. 33) and b) shorter and straight (Fig. 32). Both are found in vesicles throughout the cytoplasm, but those with wavy hairs appear to be more abundant, findings suggesting several possibilities: 1) two distinct types of extraflagellar mastigonemes are processed in separate intracellular compartments (see below); 2) for a single type of mastigoneme, the (rigid) shaft and the (flexible) filament portions of the "mastigoneme unit," are processed separately (as defined by Bouck et al., 1978); 3) the hair types represent mastigonemes in different stages of development, for example, from an immature precursor to a fully formed mastigonemal unit; 4) mastigonemes and other reservoir-associated hairs below are processed separately.

From biochemical studies of xylosylation of mastigonemes in Euglena gracilis, Rogalski and Bouck (1980, 1982) also suggested an intracellular origin for euglenoid flagellar hairs but the exact sequence of biosynthetic processing for nontubular mastigonemes remains unknown.

Our ultrastructural data suggest these possibilities: 1) the site of synthesis may be the perichloroplastidic space or the perinuclear continuum (Figs 32-33); 2) there is probable involvement of the Golgi complex in mastigonemal morphogenesis and secretion: a. dilated cisternal arms contain fibrils similar to those in the mastigoneme vesicles (Fig. 34), b. Golgi complexes are concentrated in the cell anterior near the presumed site of external liberation, c. mastigonemes are composed of xylosylated glycoproteins (Rogalski & Bouck, 1980) and the Golgi complex has a recognized role in the biosynthetic trafficking of glycoproteins (Farquhar, 1983), and d. there is a single report of glycoproteinaceous filaments within the Golgi complex of a euglenoid (Mignot et al., 1972); 3) translocation of the vesicular contents from the mastigoneme vesicles to the flagellar membrane probably involves membrane fusion, exocytosis and possibly retrieval of the mastigoneme vesicular membrane near the reservoir (Fig. 41).

Bouck (1972, 1982) proposed that during cytoplasmic processing, tubular mastigonemes remain constantly attached to a portion of the original vesicular membrane, and that after extrusion into the reservoir, the vesicular membrane becomes incorporated into new flagellar membrane. A similar process of flagellar membrane growth and nontubular mastigoneme attachment can be envisaged for Eutreptia, whereby mastigoneme units and associated "mastigoneme vesicle" membrane

are extruded into the reservoir and form "reservoir membrane mastigoneme pools" that follow the basal addition of flagellar membrane components (Rogalski & Bouck, 1980; Bouck 1982). Future biochemical studies of Eutreptia pertyi could substantiate this proposed mechanism; such studies could also determine the membrane composition of the mastigoneme vesicles and whether xyloglycorien, the major flagellar membrane glycoprotein (Rogalski & Bouck, 1980; Bouck & Rogalski, 1982), is, in fact, a major constituent of the mastigoneme vesicle membrane.

The mastigoneme vesicles of Eutreptia also have some structural and functional similarities to the scale-containing vesicles of chrysomonad (Mignot & Brugerolle, 1982) and prasinophycean (Moestrup & Thomsen, 1974) flagellates. The latter authors proposed that liberation of the scale and subtending membrane results in growth of **both** the flagellar membrane and the cortical plasmalemma of the cell. A similar "moving membrane" process, initiated during liberation of euglenoid mastigonemes, would be consistent with the process of pellicular strip morphogenesis and duplication in the flagellar canal (Mignot et al., 1987) and could account for the characteristic "directed" molecular constituents of the flagellar and plasmamembranes (Rogalski & Bouck, 1980; Bouck & Chen, 1984).

Flagellar Roots and Associated Structures

The pattern of flagellar roots and pellicular microtubular bands within the reservoir and canal strongly suggests their integral involvement in maintenance of reservoir and canal shape. The distribution of the roots imparts asymmetrical support to the reservoir/canal. Resilience of the root microtubules in this type of

cytoskeletal pattern could minimize and counteract the disruptive forces in the right reservoir wall generated during contractile vacuole discharge and mastigoneme vesicle liberation. Clustering of microtubules into discrete bands, interconnection of adjacent microtubules by fine fibrils, and reinforcement of some microtubular bands by underlying electron-dense material, all suggest that the component microtubules of a single band move as a coherent group and that the cytoskeleton provides integrated structural and functional support to the reservoir. Lateral fibrillar projections from the root microtubules superficially resemble the dynein arm ATPases of the axonemal doublet microtubules, and may themselves constitute actively contractile elements. Microtubular interconnections of constitutive dynein-like proteins are implicated in force-generation for microtubular rod movement in the ingestion apparatus of Entosiphon sulcatum Triemer & Fritz, 1987). A "microtubule-dynein system" is also proposed in the sliding strip theory of euglenoid movement (Suzaki & Williamson, 1986). A similar microtubule-dynein system could effect coordinated movement of root microtubules as well. The links may also resist elastic stretching of the associated microtubules and provide a restoring force so that the band returns to the original resting configuration, functionally similar to the microtubular bands and associated fibers in the feeding tentacle of the suctorian ciliate, Tokophrya (Tucker, 1974). These various types of interactions could account for coordinated movement of the roots around the reservoir, including also spatial displacement of the reservoir and closely associated organelles, like the contractile vacuole and stigma.

Root Associations within the Reservoir/Canal

Associations of the roots with other structures in the canal/reservoir region may, collectively or singly, have functional and phylogenetic significance in the Euglenophyta. For example, an IR-associated ridge is not as pronounced in Eutreptia as it is in some euglenoids and bodonids (e.g., Entosiphon sulcatum, Solomon et al., 1987). Moreover, the striated fiber at the VR terminus has some structural and spatial similarities to the striated plaque (P_S) of Bodo sp. and Cryptobia intestinalis (see Figs 12, 16, 32-33 in Brugerolle et al., 1979).

Functional Significance of Root Cytoskeleton

In the canal the flagella are surrounded by intrareservoir/canal hairs in the confined, ellipsoidal canal space. Conformity of the flagellar membranes to one another and to the contour of the right-left flattened canal wall suggest that flagellar root and pellicular microtubular bands lend restrictive support to a relatively rigid canal. The encircling sheath of longitudinally and transversely oriented microtubules seems to provide extra stability and rigidity to the canal; this observation is consistent with Nisbet's assumption (1974) that the absence of encircling microtubules in the canal wall of Peranema trichophorum reflects the highly extensible and expandable nature of the canal in this organism. Flagellar motion is more restricted in the mid- and upper-canal regions where the canal has a fluted cross-sectional shape.

Arrangement of reservoir and canal microtubules can be correlated with the relative plasticity of the structural domains. Within the flexible reservoir, at the level of the contractile vacuole, the cyto-

skeletal system is arranged in discrete but discontinuous bands. In the less flexible, reservoir-canal transition region, doubling of the DMT singlet microtubules and lateral fusion of the microtubular bands forms a continuous collar around the ellipsoidal canal space. Infolding of the doublet microtubular collar forms an irregularly invaginated luminal space. In the most distal canal region, the microtubular doublets are embedded in an electron-dense matrix; an electron-dense spot, possibly a microtubular organizing center or morphogenetic center, near each microtubular doublet appears to nucleate a third microtubule; subsequent rearrangement of the microtubules into a triangular pattern within the dense matrix forms a thick and rigid collar around the highly fluted lumen. The two-over-one triplet arrangement of upper canal microtubules and attenuation of material from each of 24 pellicular strips in the canal opening (Fig. 15) strongly suggest integration and continuity of the upper-canal microtubules with at least three of the cortical pellicular strip microtubules.

Derivation of the Pellicular Cytoskeleton

Continuity of the flagellar root-derived cytoskeleton and the external pellicular cytoskeleton has been suggested for many euglenoids (Leedale & Hibberd, 1974 for Rhabdomonas; Guttman & Ziegler, 1974 and Surek & Melkonian, 1986 for Euglena gracilis; West et al., 1980 for Trachelomonas hispida var. coronata; Leedale, 1982 for Euglena viridis; Willey & Wibel, 1987 for Colacium libellae; Mignot et al., 1987 for Cyclidiopsis acus. A plausible trajectory for the canal microtubules of Eutreptia pertyi would be that the doublet microtubules nearest the lumen become microtubules 1 and 2 of the cortical pellicular ridge, and

the singlet microtubule nearest the cytoplasm becomes one of the cortical heel microtubules (i.e., m_3 or m_4), the other heel microtubule presumably nucleated outside of the canal space; the electron-dense collar around the upper canal could form part of the epiplasmic dense layer of the pellicle (Figs 24, 30, 47).

Such spatial progression would be consistent with intercalary strip development and vectorial growth of the epiplasmic layer in the canal of Cyclidiopsis acus, documented by Mignot and co-workers (1987). Thus, the peripheral cytoskeletal system, pellicle and epiplasmic layers, seems to be an extension and embellishment of the flagellar-apparatus-derived cytoskeletal complex, such that the species-specific number and arrangement of microtubules in the pellicle is directly related to a species-specific number and arrangement of microtubules in the reservoir, canal and basal body complex. Future investigations using serial sectioning techniques could document the exact internal-to-external progression of the cytoskeleton in Eutreptia.

Cytopharynx and Associated Structures

This study confirms the presence of a small, presumptive cytopharyngeal pocket in the reservoir-canal transition zone of Eutreptia pertyi, as reported earlier by Solomon et al. (1987), and reports for the first time in euglenoids a striated fibril associated with the distal portion of the VR. The staggered "9 + 3" arrangement of VR microtubules in E. pertyi is similar in its disjunction to the "5 + 4" arrangement of MTR microtubules in the bodonid, Cryptobia (Nohynková, 1984). It is uncertain whether all of the "9 + 3" VR microtubules in Eutreptia pertyi are of basal body (root) origin or if some of the

twelve microtubules are nucleated near the "cytopharyngeal" opening. The striated fibril near the VR terminus in E. pertyi is structurally similar to a fibril found near the cytostomal opening in the bodonids (Nohynková, 1984) and may be similar to the dense plaque situated near the cytostome in some trypanosomes where it is proposed to have a nucleating function for some of the subpellicular and/or cytopharyngeal microtubules (Lom et al., 1980).

The occurrence and structural features of a vestigial cytopharynx in a wide variety of euglenoids could be significant to our understanding and assessment of phylogenetic trends and relationships among euglenoids and other protistan groups.

It is difficult to postulate a function for the cytopharynx based on ultrastructural evidence alone; however, the vestigial nature of the cytopharynx suggests that it is not an ingestion organelle that actively engulfs prey, as does for example, the protrusive siphon of Peranema and Entosiphon (Heteronematales). Several structural observations provide tentative evidence that the cytopharynx may function as a rudimentary phagotrophic organelle in Eutreptia. For example, unusual membrane-bounded vesicles with reticulated, tubular contents are occasionally observed in the cytopharyngeal region (Fig. 36) and strikingly resemble the vacuoles associated with food digestion in the phagotrophic dinoflagellate, Oxyrrhis marina (Dodge & Crawford, 1974). Moreover, the occurrence of numerous, small "coated" vesicles in the lumen of the cytopharynx and subjacent cytoplasm (Fig. 44) suggest the possibility of active pinocytotic exocytotic events. Similar "coated" vesicles are well known in some systems to be involved in transport operations,

specifically in receptor-mediated endocytosis and recovery of membrane from the cell surface and recycling to the Golgi complex (Farquhar, 1983). Pinocytotic vesicles are reported alongside the cytopharyngeal membrane in Trypanosoma danilewskyi, T. cruzi and Crithidia fasciculata and are suggested to reflect nutrient uptake activity in these kinetoplastid zooflagellates (Lom et al., 1980). The reservoir-associated hair could possibly filter particulate food from water brought in during swimming; small particulate food could be "passively" digested in the nearby vestigial cytopharynx. Further investigations are needed to determine the function of this presumptive cytopharyngeal organelle in the photosynthetic euglenoids which retain this vestige.

LIST OF REFERENCES

- Bouck, G.B. 1972. Architecture and assembly of mastigonemes. Adv. Cell Mol. Biol. 2: 237-271.
- Bouck, G.B. 1982. Flagella and the cell surface. In: The Biology of Euglena. Vol. 3 (Ed. by D.E. Buetow) pp. 29-51. Academic Press, N.Y.
- Bouck, G.B. and S. Chen. 1984. Synthesis and assembly of the flagellar surface. J. Protozool. 31: 21-24.
- Bouck, G.B. and A.A. Rogalski. 1982. Surface properties of the euglenoid flagellum. In: Prokaryotic and Eukaryotic Flagella (Ed. by W.B. Amos and J.G. Duckett) pp. 381-397. Cambridge University Press, Great Britain.
- Bouck, G.B., A. Rogalski and A. Valaitis. 1978. Surface organization and composition of Euglena. II. Flagellar mastigonemes. J. Cell Biol. 77: 805-826.
- Brugerolle, G., J. Lom, E. Nohynková and L. Joyon. 1979. Comparison et évolution des structures cellulaires chez plusieurs espèces de Bodonides et Cryptobiidés appartenant aux genres Bodo, Cryptobia et Trypanoplasma (Kinetoplastida, Mastigophora). Protistologica 15: 197-221.
- Dawson, N.S., J.R. Dunlap and P.L. Walne. 1988. Structure and elemental composition of pellicular warts of Euglena spirogyra (Euglenophyceae). Br. phycol. J. 23: 61-69.
- Dodge, J.D. 1971. Fine structure of the Pyrrophyta. Bot. Rev. 37: 481-508.
- Dodge, J.D. and B.T. Bibby. 1973. The Prorocentrales (Dinophyceae). I. A comparative account of fine structure in the genera Prorocentrum and Exuviaella. Bot. J. Linn. Soc. 67: 175-187.
- Dodge, J.D. and R.M. Crawford. 1968. Fine structure of the dino-flagellate Amphidinium carteri Hulbert. Protistologica 4: 231-248.
- Dodge, J.D. and R.M. Crawford. 1971. Fine structure of the dino-flagellate Oxyrrhis marina II. The flagellar system. Protistologica 7: 399-409.
- Dodge, J.D. and R.M. Crawford. 1974. Fine structure of the dino-flagellate Oxyrrhis marina. III. Phagotrophy. Protistologica 10: 239-244.

- Farquhar, M.G. 1983. Multiple pathways of exocytosis, endocytosis, and membrane recycling: validation of a Golgi route. Fed. Proc. 42: 2407-2413.
- Gallo, J.M. and J. Schrével. 1982. Euglenoid movement in Distigma proteus. I. Cortical rotational motion. Biol. Cell. 44: 139-148.
- Gallo, J.M., E. Karenti, M. Bornens, A. Delacoute and J. Schrével. 1982. Euglenoid movement in Distigma proteus. II. Presence and localization of an actin-like protein. Biol. Cell. 44: 149-156.
- Guillard, R.R.L. and J.H. Rhyther. 1962. Studies on marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula conferracea (Cleve) Gran. Can. J. Microbiol. 8: 229-239.
- Guttman, H.N. and H. Ziegler. 1974. Clarification of structures related to function in E. gracilis. Cytobiol. 9: 10-22.
- Hofmann, C. and G.B. Bouck. 1976. Immunological and structural evidence for patterned intussusceptive surface growth in a unicellular organism (A postulated role for submembranous proteins and microtubules). J. Cell Biol. 69: 693-715.
- Kevin, M.J., W.T. Hall, J.J.A. McLaughlin and P.A. Zahl. 1969. Symbiodinium microadriaticum Freudenthal, a revised taxonomic description, ultrastructure. J. Phycol. 5: 341-350.
- Kivic, P.A. and P.L. Walne. 1984. An evaluation of a possible phylogenetic relationship between the Euglenophyta and Kinetoplastida. Origins of Life 13: 269-288.
- Leadbeater, B.S.C. 1971. The intracellular origin of flagellar hairs in the dinoflagellate Woloszynskia micra Leadbeater & Dodge. J. Cell Sci. 9: 443-451.
- Leadbeater, B. and J.D. Dodge. 1966. The fine structure of Woloszynskia micra sp. nov., a new marine dinoflagellate. Br. phycol. Bull. 3: 1-17.
- Leedale, G.F. 1967. Euglenoid Flagellates. Prentice-Hall, Englewood Cliffs, N.J.
- Leedale, G.F. 1978. Phylogenetic criteria in euglenoid flagellates. BioSystems 10: 183-187.
- Leedale, G.F. 1982. Ultrastructure. In: The Biology of Euglena. Vol. 3 (Ed. by D.E. Buetow) pp. 1-27. Academic Press, N.Y.
- Leedale, G.F. 1983. Phytomastigophorea. In: Illustrated Guide to the Protozoa (Ed. by J.J. Lee, S.H. Hutner, E.C. Bovee) Allen Press, Lawrence, KA.

- Leedale, G.F. and D.J. Hibberd. 1974. Observations on the cytology and fine structure of the euglenoid genera Menoidium Perty and Rhabdomonas resenius. Arch. Protistenk. Bd. 116: 319-345.
- Lom J., J.J. Paulin and E. Nohynková. 1980. The fine structure of the fish trypanosome, Trypanosoma danilewskyi: I. Presence of a cytopharyngeal complex in bloodstream trypomastigotes. Protistologica 16: 365-373.
- Messer, G. and Y. Ben-Shaul. 1969. Fine structure of Peridinium westii Lemm., a freshwater dinoflagellate. J. Protozool. 16: 272-280.
- Mignot, J.P. and G. Brugerolle. 1982. Scale formation in Chrysomonad flagellates. J. Ultrastruc. Res. 81: 13-26.
- Mignot, J.P., G. Brugerolle and G. Bricheux. 1987. Intercalary strip development and dividing cell morphogenesis in the euglenid Cyclidiopsis acus. Protoplasma 139: 51-65.
- Mignot, J.P., G. Brugerolle and G. Metenier. 1972. Compléments a l'étude des mastigonèmes des protistes flagellés utilisation de la technique de Thiéry pour la mise en evidence des polysaccharides sur doupes fines. J. Microsc. (Paris) 14: 327-342.
- Moestrup, Ø. and H.A. Thomsen. 1974. An ultrastructural study of the flagellate Pyramimonas orientalis with particular emphasis on Golgi apparatus activity and the flagellar apparatus. Protoplasma 81: 247-269.
- Nisbet, B. 1974. An ultrastructural study of the feeding apparatus of Peranema trichophorum. J. Protozool. 21: 39-48.
- Nohynková, E. 1984. A new pathogenic Cryptobia from freshwater fishes: a light and electron microscopic study. Protistologica 20: 181-195.
- Pringsheim, E.G. 1953. Salzwasser-Eugleninen. Arch. Mikrobiol. 18: 149-164.
- Rogalski, A.A. and G.B. Bouck. 1980. Characterization and localization of a flagellar-specific membrane glycoprotein in Euglena. J. Cell Biol. 86: 424-435.
- Rogalski, A.A. and G.B. Bouck. 1982. Flagellar surface antigens in Euglena: immunological evidence for an external glycoprotein pool and its transfer to the regenerating flagellum. J. Cell Biol. 93: 758-766.
- Round, F.E. 1965. The Biology of the Algae. Pitman Press, Bath, pg. 156.
- Solomon, J.A., P.L. Walne and P.A. Kivic. 1987. Entosiphon sulcatum (Euglenophyceae): flagellar roots of the basal body complex and reservoir region. J. Phycol. 23: 85-98.

- Solomon, J.A., P.L. Walne, N.S. Dawson and R.L. Willey. The ultrastructure of Eutreptia pertyi (Euglenophyceae). II. The flagellar root system and cytopharynx. Submitted.
- Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res. 26: 31-43.
- Steidinger, K.A., E.W. Truby and C.J. Dawes. 1978. Ultrastructure of the red tide dinoflagellate Gymnodinium breve. I. General description. J. Phycol. 14: 72-79.
- Surek, B. and M. Melkonian. 1986. A cryptic cytostome is present in Euglena. Protoplasma 133: 39-49.
- Suzaki, T. and R.E. Williamson. 1986. 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.
- Thronsdon, J. 1973. Fine structure of Eutreptiella gymnastica (Euglenophyceae). Norw. J. Bot. 20: 271-280.
- Tomas, R.N. and E.R. Cox. 1973. Observations on the symbiosis of Peridinium balticum and its intracellular alga. I. Ultrastructure. J. Phycol. 9: 304-323.
- 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. and L. Fritz. 1987. Structure and operation of the feeding apparatus in a colorless euglenoid, Entosiphon sulcatum. J. Protozool. 34: 39-47.
- Tucker, J.B. 1974. Microtubule arms and cytoplasmic streaming and microtubule bending and stretching of intertubule links in the feeding tentacle of the suctorian ciliate Tokophrya. J. Cell Biol. 62: 424-437.
- Walne, P.L., Ø. Moestrup, R.E. Norris and H. Ettl. 1986. Light and electron-microscopical studies of Eutreptiella eupharyngea sp. nov. (Euglenophyceae) from Danish and American waters. Phycologia 25: 109-126.
- West, L.K. 1977. Cellular organization and biomineralization of the envelope of Trachelomonas hispida var. coronata. Ph.D. dissertation, University of Tennessee, Knoxville.
- West, L.K., P.L. Walne and J. Rosowski. 1980. Trachelomonas hispida Var. coronata (Euglenophyceae). I. Ultrastructure of cytoskeletal and flagellar systems. J. Phycol. 16: 489-497.

- Wilcox, L.W. and G.J. Wedemayer. 1984. Gymnodinium acidotum Nygaard (Pyrrophyta), a dinoflagellate with an endosymbiotic cryptomonad. J. Phycol. 20: 236-242.
- Wilcox, L.W., G.J. Wedemayer and L.E. Graham. 1982. Amphidinium cryophilum sp. nov. (Dinophyceae) a new freshwater dinoflagellate. II. Ultrastructure. J. Phycol. 18: 18-30.
- Willey, R. and R.G. Wibel. 1985a. A cytostome/cytopharynx in green euglenoid flagellates (Euglenales) and its phylogenetic implications. BioSystems 18: 369-376.
- Willey, R. and R.G. Wibel. 1985b. The reservoir cytoskeleton and a possible cytostomal homologue in Colacium (Euglenophyceae). J. Phycol. 21: 570-577.
- Willey, R.L. and R.G. Wibel. 1987. Flagellar roots and the reservoir cytoskeleton of Colacium libellae (Euglenophyceae). J. Phycol. 23: 283-288.
- Willey, R.L., K. Ward, W. Russin and R.G. Wibel. 1977. Histochemical studies of the extracellular carbohydrate of Colacium mucronatum (Euglenophyceae). J. Phycol. 13: 349-353.

APPENDIX

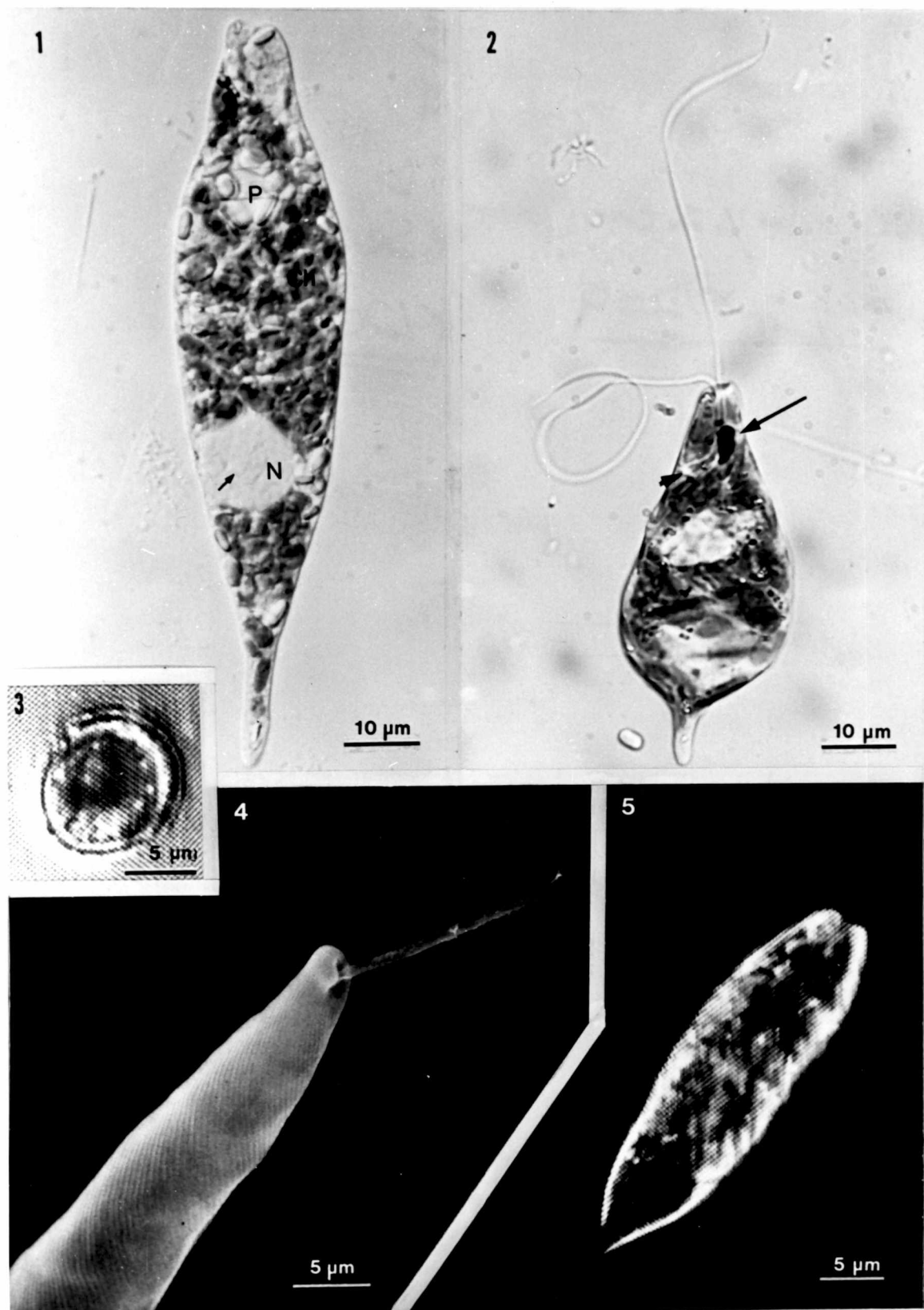
Figure 1. LM overview of Eutreptia pertyi. Elongated cell showing typical positional relationships of major organelles: chloroplasts (Ch), nucleus (N), nucleolus (arrow), pyrenoid center (P) encircled by paramylon grains. DIC.

Figure 2. LM view of typical E. pertyi with flagella. Cell has prominent stigma (arrow) and two nearly equal-length flagella that originate in cytoplasm below dorsal reservoir wall (arrow) and emerge through subapical opening of canal on ventral side of organism. Dorsal flagellum (F_1) is typically directed anteriorly; ventral flagellum (F_2) usually occupies a lateral position. DIC.

Figure 3. Encysted, nonflagellated cell with extensive mucilaginous sheath. Computer-digitized and enhanced DIC image from VHS still frame.

Figure 4. SEM of E. pertyi. Thin, evenly spaced pellicular strips impart S-shaped helical symmetry to elongated, demembranated cell. Two entwined flagella extend from subapical canal opening on ventral side of cell.

Figure 5. Computer-digitized image of swimming cell. Characteristic conical shape of undisturbed swimming cell. Computer-digitized and enhanced DIC image from VHS still frame.



Figures 6-7. Images of a living cell following cessation of metabolism but prior to flagellar detachment.

Figure 6. LM view of cell showing major cell organelles. Anterior end of cell flattened by coverslip shows positional relationship of major structures in flagellar region: stigma (S), canal (thick arrow), contractile vacuole (arrowhead), flagellar swelling (thin arrow) attached to anteriorly directed dorsal flagellum (F_1); double arrows denote sites of flagellar insertion through dorsal reservoir wall. DIC.

Figure 7. Optically defocused view of cell shown in Figure 6. Approximately 100 delicate pellicular striations are visible beneath the plasma membrane. DIC.

Figure 8. Staggered tiers of moniliform stigma granules (arrow) in cell with protruding canal. DIC.

Figure 9. Large ribbon-shaped chloroplasts radiate from pyrenoid center (P) and prominent nucleus (N) is evident. DIC.

Figure 10. Heterodynamic flagella originate from parabasal sites below dorsal reservoir wall (arrow) and emerge through reservoir and constricted canal (arrowhead). DIC.

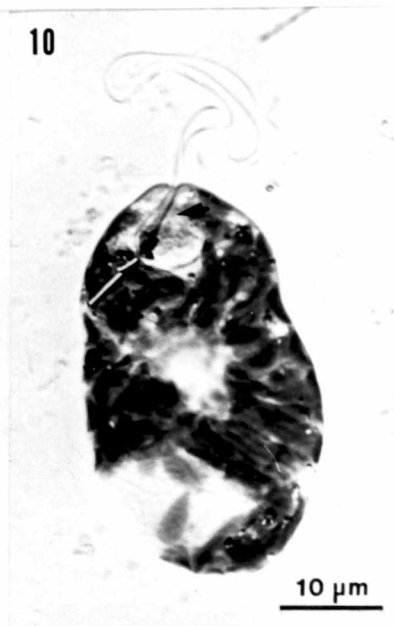
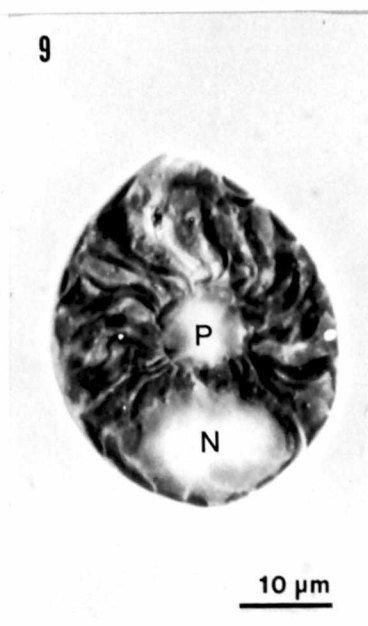


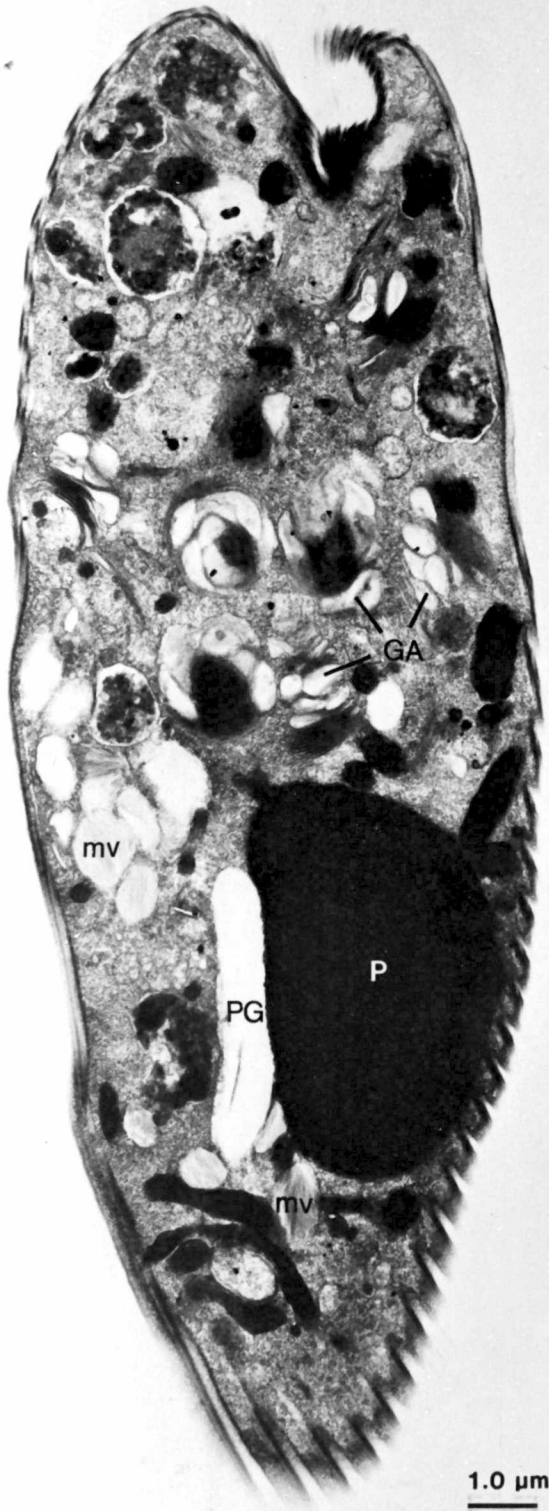
Figure 11. Oblique section through anterior two-thirds of cell. Pyrenoid center (P), paramylon grain (PG), Golgi apparatus (GA), mastigoneme vesicles (mv) and accumulation bodies (AC) in cytoplasm. TEM.

Figure 12. Glancing section through anterior region of cell. Cortical pellicular complex attenuates in the distal canal. Continuity of some pellicular and canal microtubules (arrows) is suggested. TEM.

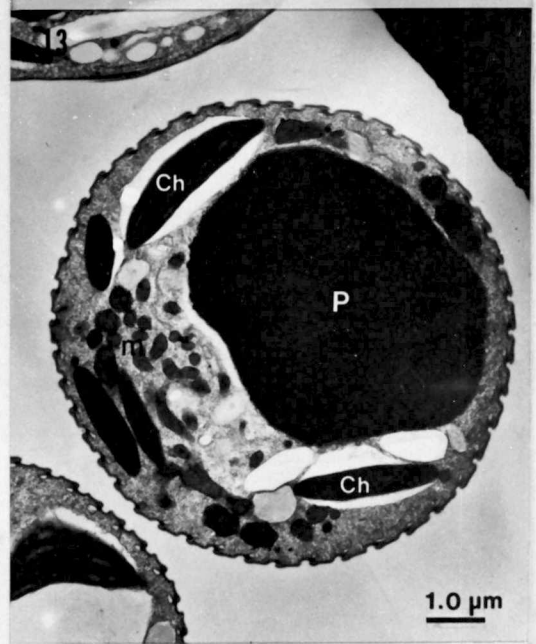
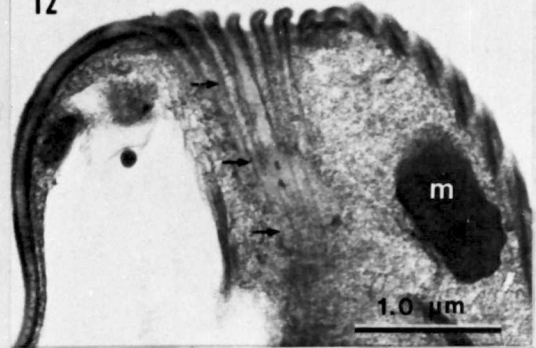
Figure 13. Section through midportion of cell showing pyrenoid center (P), chloroplasts (Ch) and mitochondria (m). TEM.

Figure 14. Transverse section near cell posterior. Numerous mucocyst-like bodies (mb) are in the cell periphery, muciferous canals (small arrows) underlie pellicular ridges, and interphase nucleus (N) has large nucleolus (Nu) and condensed chromatin material (large arrow). TEM.

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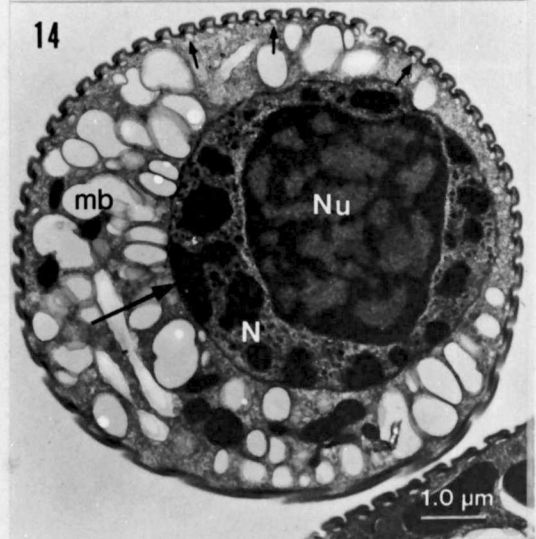


Figure 15. Whole mount of collapsed cell showing termination of pellicular grooves (arrows) in canal region. Selective staining of grooves suggests that pellicular ridges flatten, taper and integrate into the smooth cortical region of canal. Negative stain.

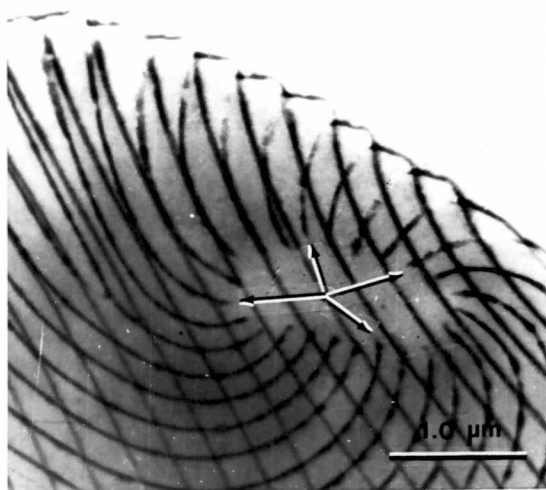
Figure 16. Successive intussusception (arrows) of ca. 22 pellicular strips at cell extremity results in ca. 100 striae in midregions of cell (cf. Figures 3, 7 & 17). Negative stain.

Figure 17. Uniform articulation of pellicular striae imparts regular surface pattern of alternating wide ridges (gray) and narrow grooves (white). Shadowcast.

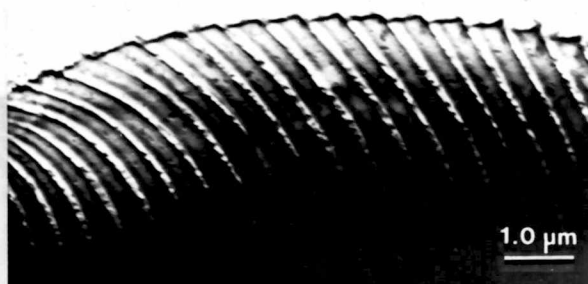
Figure 18. Computer-assisted, high-pass filtered image of image shown in Figure 17. Digital processing enhances visualization of concavity of wide pellicular ridges (arrow), a feature of older striae usually apparent in sectioned material only (see Figure 22).

Figure 19. Dorsoventral compression of pellicular grooves (arrow) accentuates bilateral, helical symmetry of cell. Negative stain.

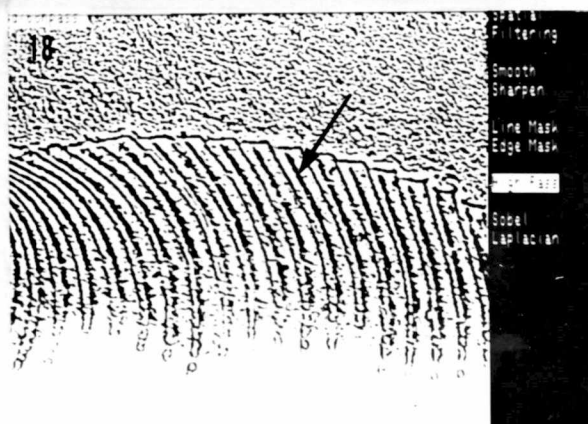
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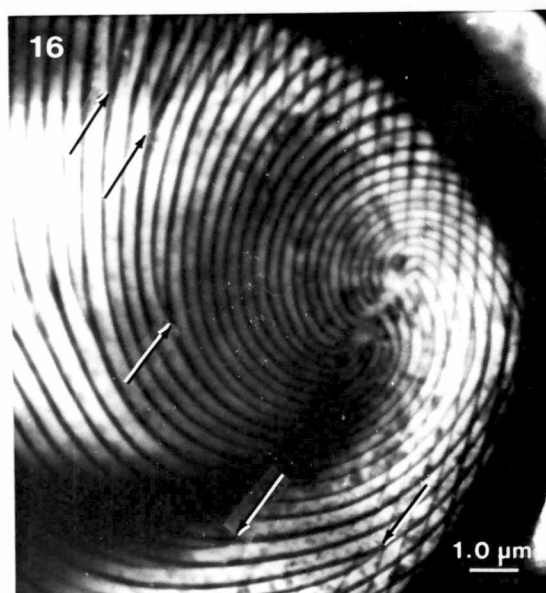
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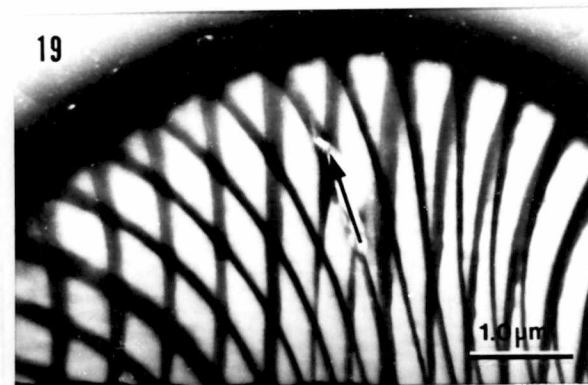


Figure 20. Electron-dense vesicle with fibrillar contents. TEM.

Figure 21. Contiguous vesicles near lobes of chloroplasts and pellicle. Vesicle nearest pellicle has concentric accumulation product with dense center. TEM.

Figure 22. Granular accumulation product in vesicle near muciferous canals underlying pellicular striae. TEM.

Figure 23. Glancing section through cell posterior shows alternating wide ridges and narrow grooves of pellicle. Microtubules, m_1 and m_2 (small arrows), subtend notched region of each ridge (cf. Figure 25); mucocyst-like bodies are visible in peripheral cytoplasm (large arrow; cf. Figure 14). TEM.

Figure 24. Spatial arrangement of canal microtubules during intussusceptive pellicular growth. Box denotes one strial unit. TEM.

Figure 25. Transverse section through pellicle showing major features of pellicular complex. Interstrip linkers (small arrows) connect adjacent striae; m_1 is located in ridge region (ri) beneath epiplasmic dense layer; m_2 is at crest of heel (h), adjacent to notch (arrowhead); m_3 and m_4 occupy lower heel region beneath groove (g). Large arrow indicates the direction toward the cell posterior. TEM.

Figure 26. Thin section through pellicle complex shows varying groove diameters between adjacent pellicular striae. $a = 0.11 \mu\text{m}$; $b = 0.1718 \mu\text{m}$; $c = 0.13 \mu\text{m}$. TEM.

Figure 27. Oblique thick section shows muciferous canals subtending each pellicular ridge. TEM.

Figure 28. Thick section shows notch-to-heel articulation of adjacent pellicular striae. A prominent interstrip linker (arrow) connects m_1 with heel region in the adjacent stria; plasma membrane (PM) and epiplasmic dense layer (EL) are overlain by an extracellular matrix of presumptive mucilage (EM). TEM.

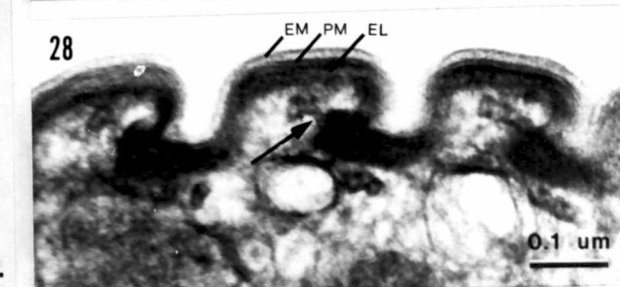
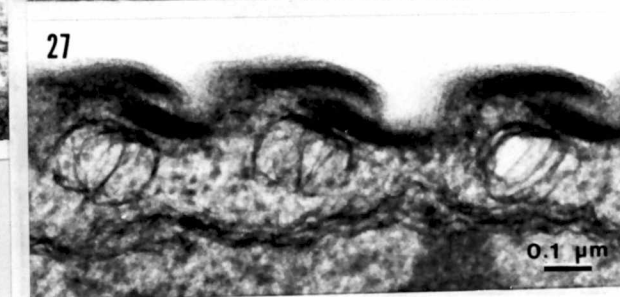
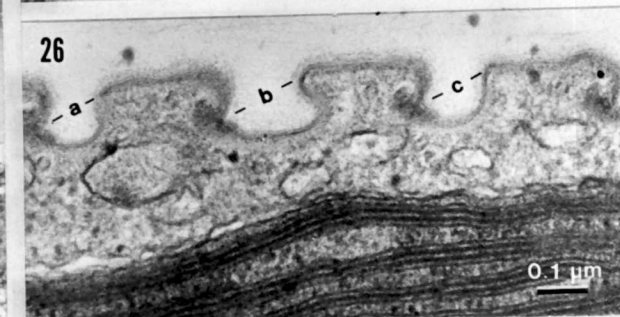
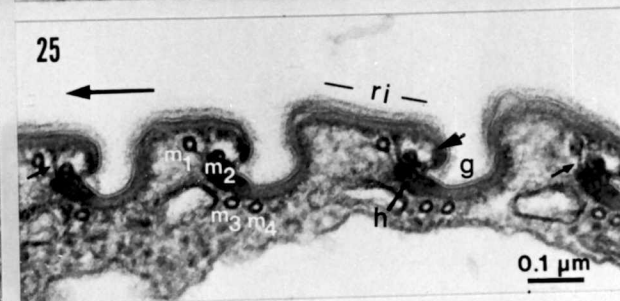
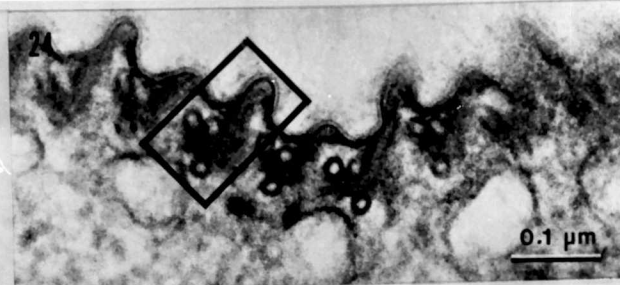
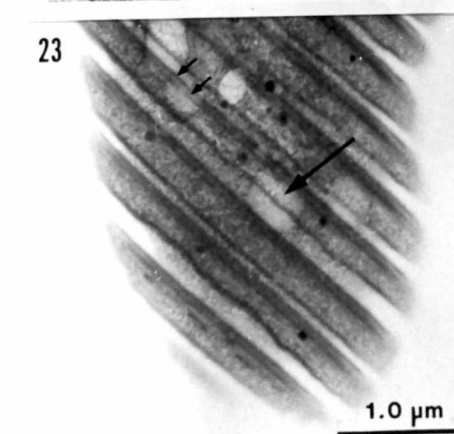
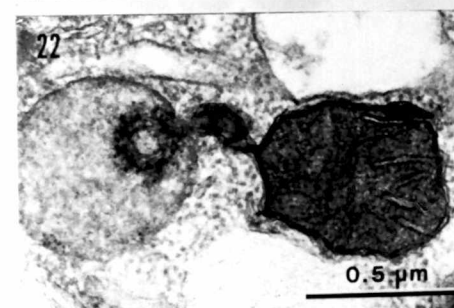
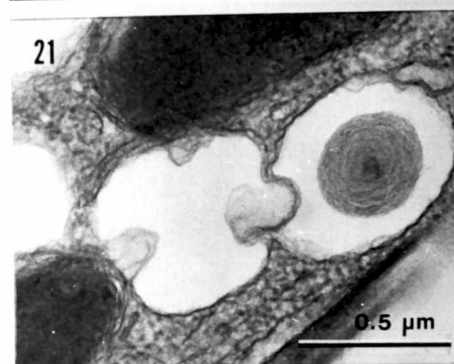
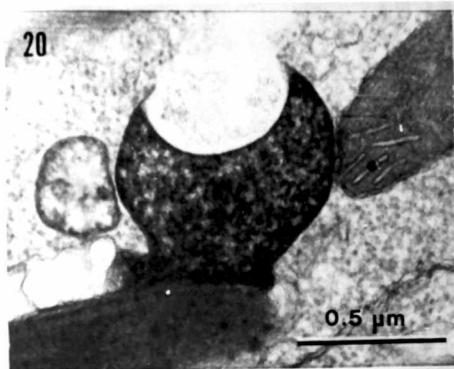


Figure 29. Transverse section through anterior end of cell. Elements of endoplasmic reticulum (arrows) and a collar of microtubules and fibrils surround lobes of constricted canal (C); numerous secretory vesicles near Golgi apparatus (GA), mastigoneme vesicles (mv) and mitochondria (m) in cytoplasm. TEM.

Figure 30. Transverse section through distal canal where convoluted canal wall is surrounded by contiguous microtubular band(s) with a two-over-one arrangement. TEM.

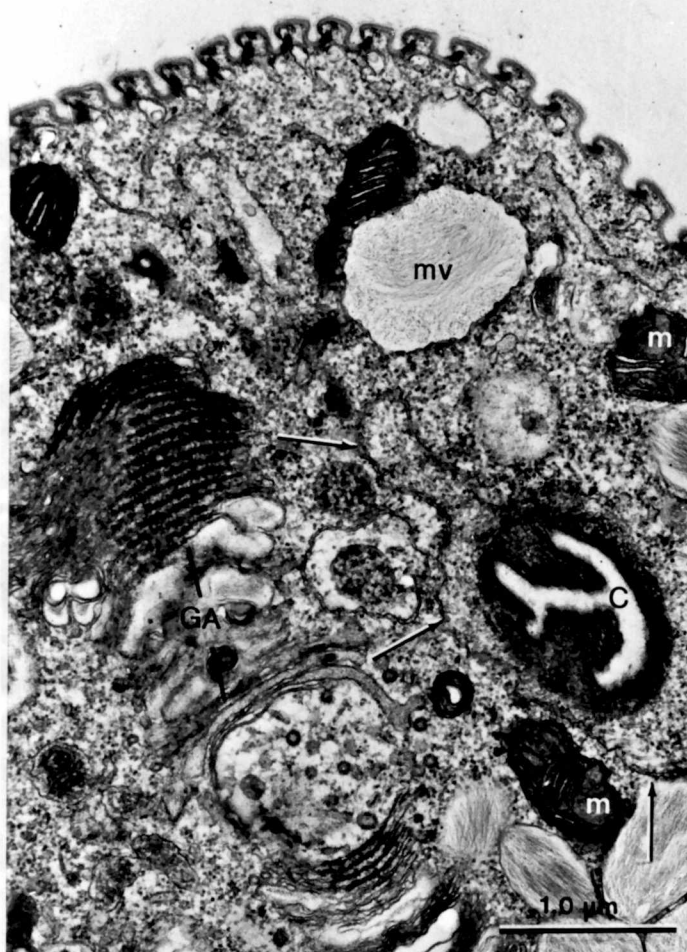
Figure 31. Lobe of ribbon-shaped chloroplast showing thylakoids and lipid droplets. TEM.

Figure 32. Fibrillar material in mastigoneme vesicle (mv) between nucleus and pellicle. TEM.

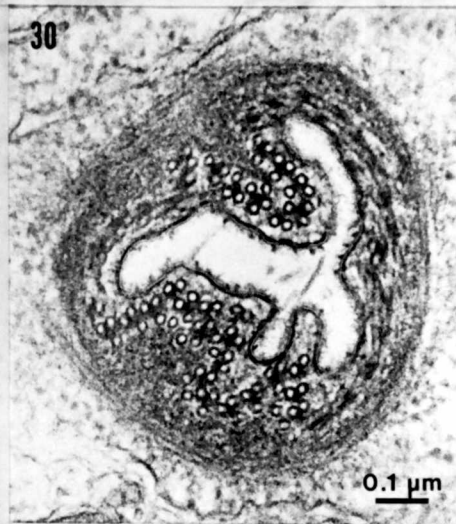
Figure 33. Membrane-bounded vesicle containing loosely arranged fibrils abuts a chloroplast and elements of endoplasmic reticulum. TEM.

Figure 34. Golgi apparatus of ca. 35 cisternae with dilated regions containing fibrillar material similar to contents of mastigoneme vesicle (mv). TEM.

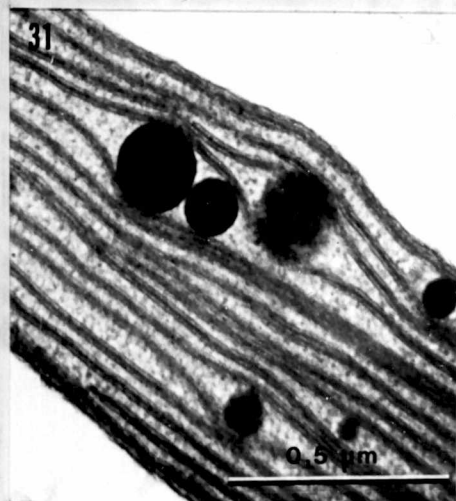
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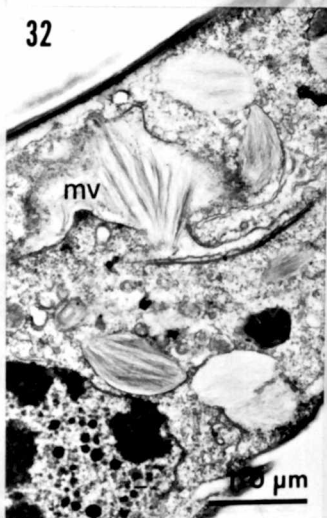
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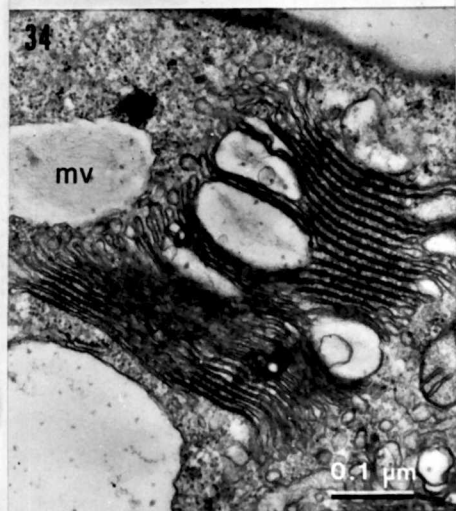


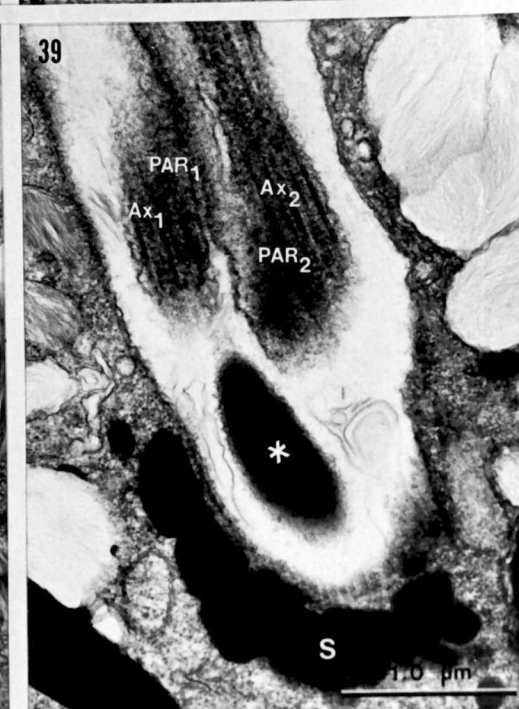
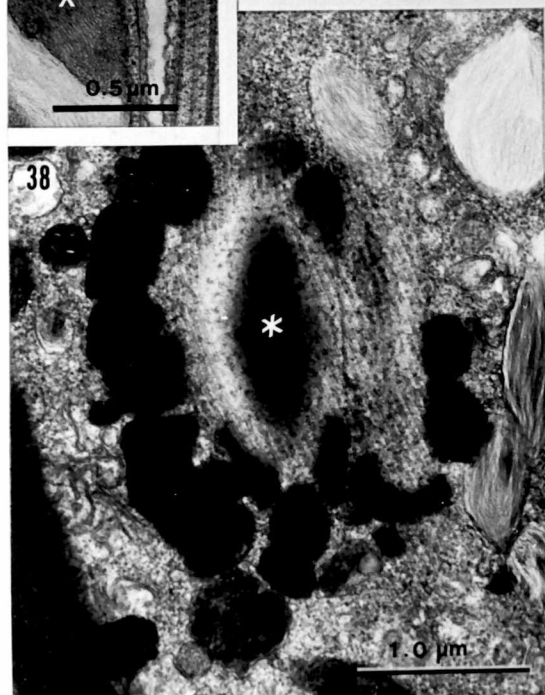
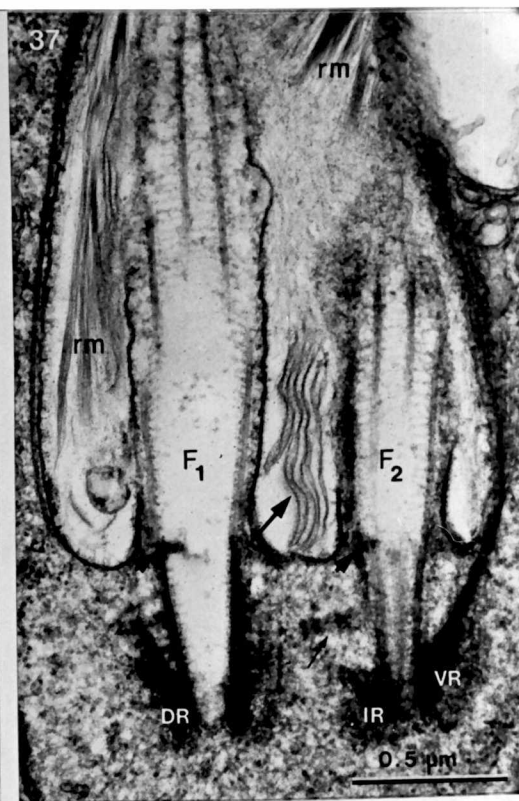
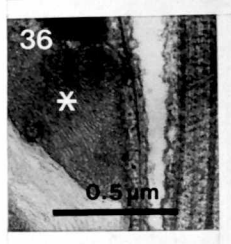
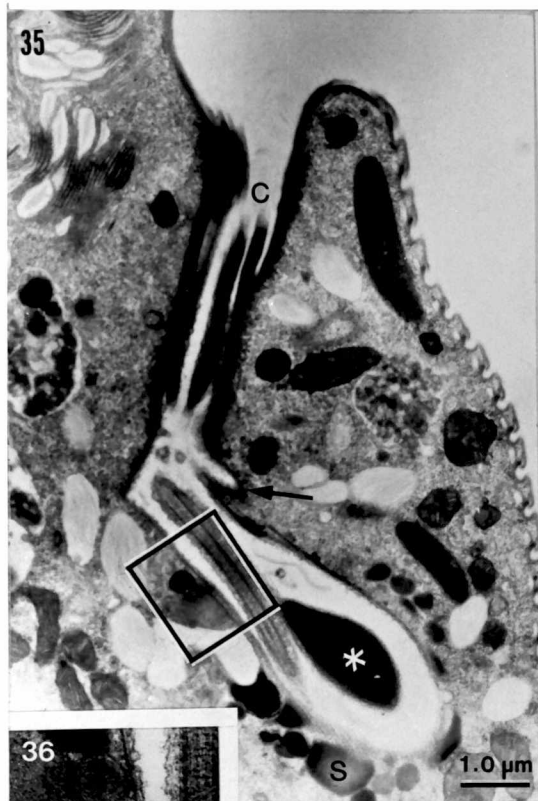
Figure 35. Oblique section in dorsoventral plane of cell anterior. The cell has a subapical, ventral canal opening (C), a presumptive vestigial cytopharyngeal pocket (thick arrow) in canal-reservoir transition zone and accumulation bodies (AC) in nearby cytoplasm. Flagellar swelling (asterisk) on dorsal (F_1) flagellum is overlain by stigma (S) granules in dorsal cytoplasm. TEM.

Figure 36. Higher magnification of inset indicated in Figure 35. Unusual cytoplasmic vesicle (asterisk) near dorsal (F_1) flagellum contains tubular reticulum. TEM.

Figure 37. Longitudinal section through dorsal reservoir wall showing emergence of flagella from functional basal bodies and positional relationship of some constituents of basal body complex. Striated fibrils of basal body connector (small arrow) and microtubular flagellar roots [dorsal root (DR) associated with dorsal (F_1) flagellum; intermediate root (IR) located between dorsal (F_1) and ventral (F_2) flagella; ventral root (VR), associated with ventral (F_2) flagellum]. V-shaped inflection (arrowheads) of flagellar membrane in transition zone marks point of emergence of each flagellum into reservoir; a bundle (large arrow) of long, wavy mastigonemes extends into the lower reservoir between the flagella; reservoir-associated hairs (rm) are in reservoir lumen. TEM.

Figure 38. Stigma granules closely associated with longitudinally oriented, evenly spaced singlet microtubules (the dorsal band of microtubules, DMT, cf. Figures 42-44) in the reservoir wall near flagellar swelling (asterisk). TEM.

Figure 39. Oblique section through dorsal reservoir wall showing stigma granules (S), flagellar swelling (asterisk), and paraxial rods. (PAR_1 of dorsal, F_1 flagellum and PAR_2 of ventral, F_2 flagellum). TEM.



Figures 40-44. Sections of reservoir and canal proceeding distally toward canal opening at anterior end of cell.

Figure 40. Oblique section of the upper reservoir region at level of contractile vacuole, just proximal to flagellar swelling and stigma (cf. Figure 6). Elements of contractile vacuole (CV) extend into ventral-right portion of cytoplasm. Ventral root (VR) delimits left-ventral side of reservoir; intermediate root (IR) delimits dorsal-left margin of reservoir near ventral (F_2) flagellum; dorsal microtubular band, near dorsal (F_1) flagellum, delimits dorsal-right side of reservoir. TEM.

Figure 41. Oblique section through reservoir at level of flagellar swelling (asterisk). A mastigoneme vesicle (mv) with fibrillar contents protrudes into ventral-right reservoir where numerous other vesicles are evident; PFB (asterisk) of dorsal (F_1) flagellum; ventral (F_2) flagellum. Note PAR-to-PAR orientation of flagella. TEM.

Figure 42. Transverse section through lower reservoir-canal transition zone at level of paraflagellar body (asterisk). The dorsal row of microtubules is composed of two microtubular groups: one of ca. 40 evenly spaced microtubules, the dorsal microtubular band (DMT) (cf. the stigma-associated microtubules shown in Figure 38), constitutes the right margin; another, the dorsal root (DR) (cf. also Figure 43), composes the extreme left margin. A 9-membered intermediate root (IR) occupies ventral-left cytoplasm near ventral (F_2) flagellum. Fine fibrils (arrows) connect axonemal doublets of ventral flagellum (F_2) to flagellar membrane. Note alignment of PAR₂ plates toward PFB on dorsal (F_1) flagellum, and oval profile of canal (cf. Figures 42-44 with Figures 40 & 41). TEM.

Figure 43. Transverse section through reservoir-canal transition zone distal to PFB. The dorsal row at this level is composed of a dorsal microtubular band (DMT) of ca. 30 microtubules (cf. Figure 42; see also the stigma-associated microtubules shown in Figure 38) and a 5-membered dorsal root (DR), underlain by an electron-dense plate. A 9-membered group of microtubules constitutes the intermediate root (IR). Exclusive of the area occupied by the flagella, the small canal is filled with fine fibrils (Figures 42-44). TEM.

Figure 44. Transverse section through upper region of reservoir-canal transition zone. The dorsal microtubular band (DMT) is composed of ca. 22 microtubules interconnected by fine fibrils (small arrows), and a dorsal root (DR), arranged in a 1-over-5 pattern. A group of 11 microtubules forms the intermediate root (IR) along ventral-left canal wall. Evagination of part of ventral-right canal wall forms presumptive cytopharyngeal pocket (CP) delimited by a 12-membered ventral root (VR) composed of 3 microtubules underlain by an electron-dense plate and 9 microtubules interconnected by fine fibrils. Coated vesicles (long arrows) are prevalent in ventral-right cytoplasm near vestigial cytopharynx (CP). TEM.

Figure 45. Transverse section through mid-canal region where microtubular bands almost completely surround opening. Note striated structure (arrow) in association with distal portion of presumptive cytopharyngeal pocket. TEM.

Figure 46. Higher magnification of the inset denoted in Figure 45 showing periodic striations of striated fiber. TEM.

Figure 47. Transverse section through distal canal region showing infolding of reservoir wall that forms a multi-lobed lumen. Paired microtubules bracket electron-dense spots (arrows) (cf. Figure 30, a more distal canal section, where the microtubules surrounding the canal have a two-over-one arrangement). TEM.

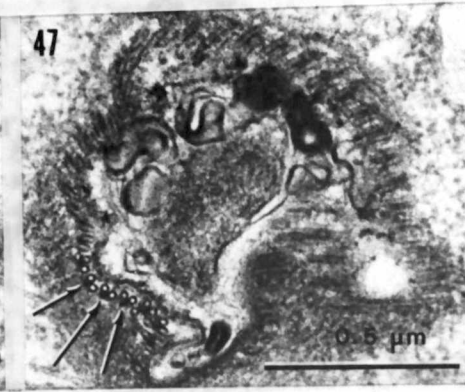
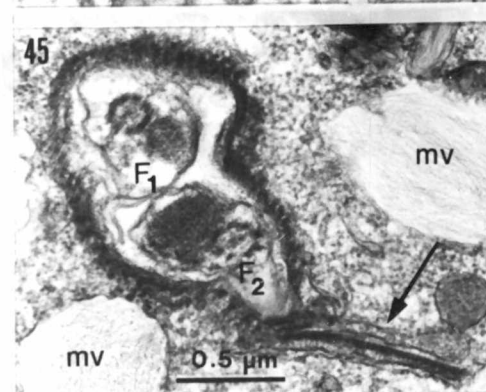
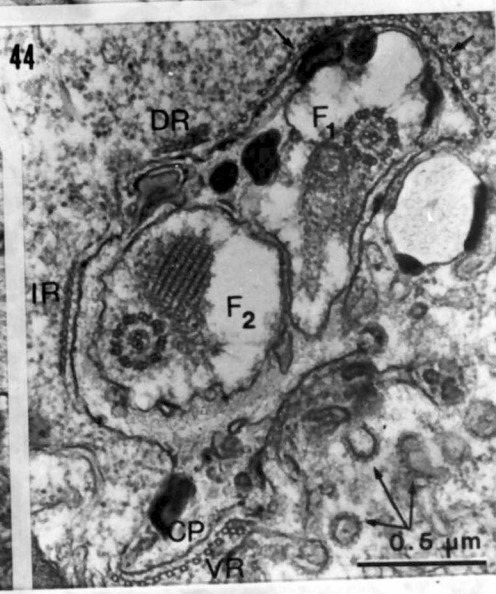
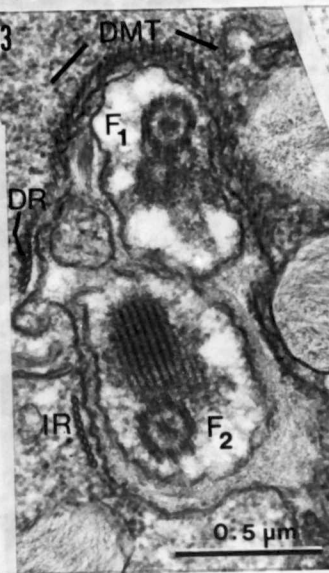
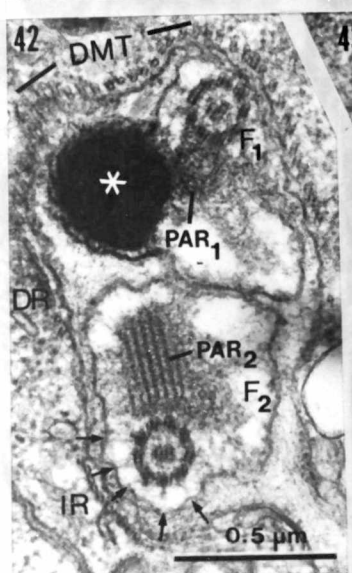
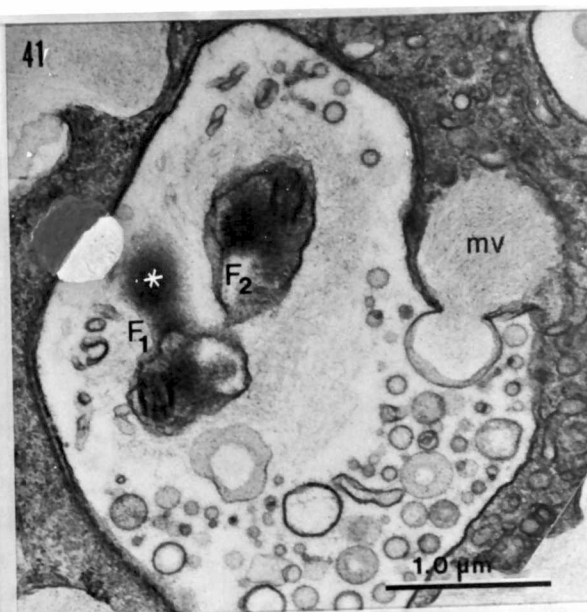
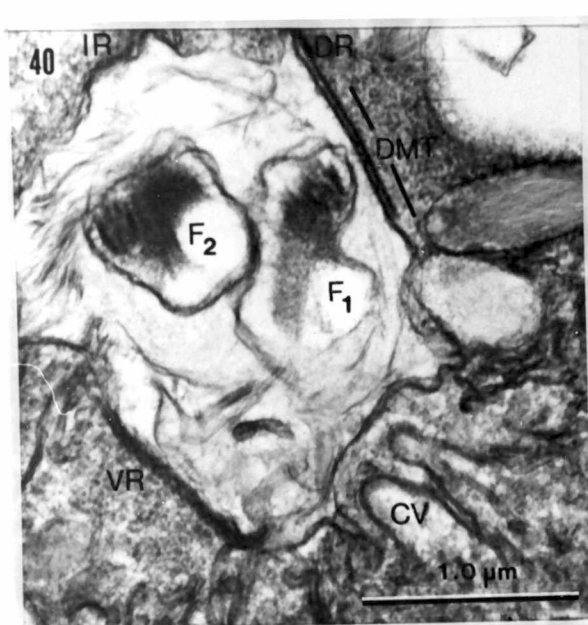


Figure 48. Negative stain image of detached ventral (F_2) flagellum. Long mastigonemes (large arrows) extend from axoneme (Ax_2 , a lightly stained narrow strand), and a mat of short mastigonemes (small arrows) extends from paraxial rod (PAR_2 , a wider and more densely stained strand). Note increasing diameter and intensity of staining of rod toward flagellar tip, and twisting of axonemal and PAR strands along flagellar length.

Figure 49. Thick section collage of dorsal (F_1) flagellum. Bundles of short mastigonemes originate from dome-shaped regions (white arrows) between PAR_1 and flagellar membrane (arrowheads). TEM.

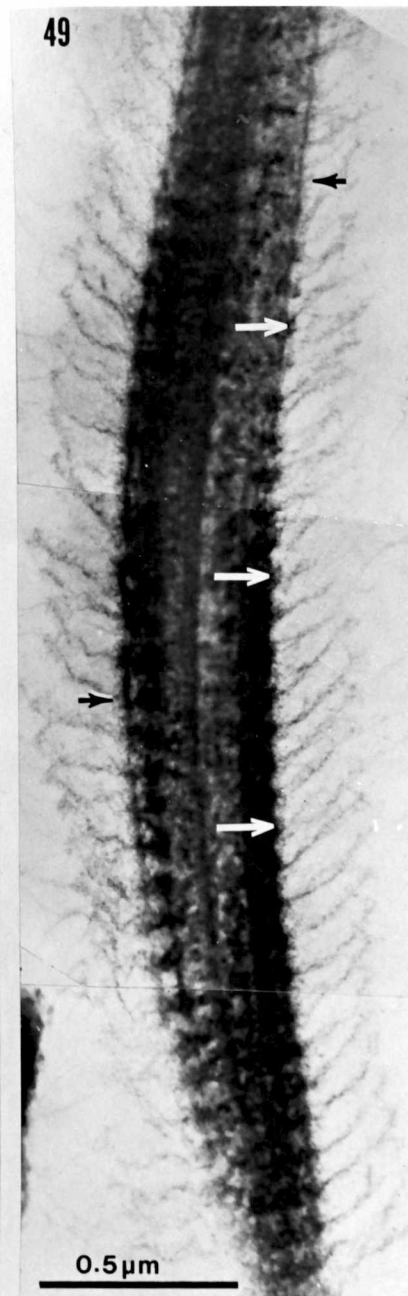
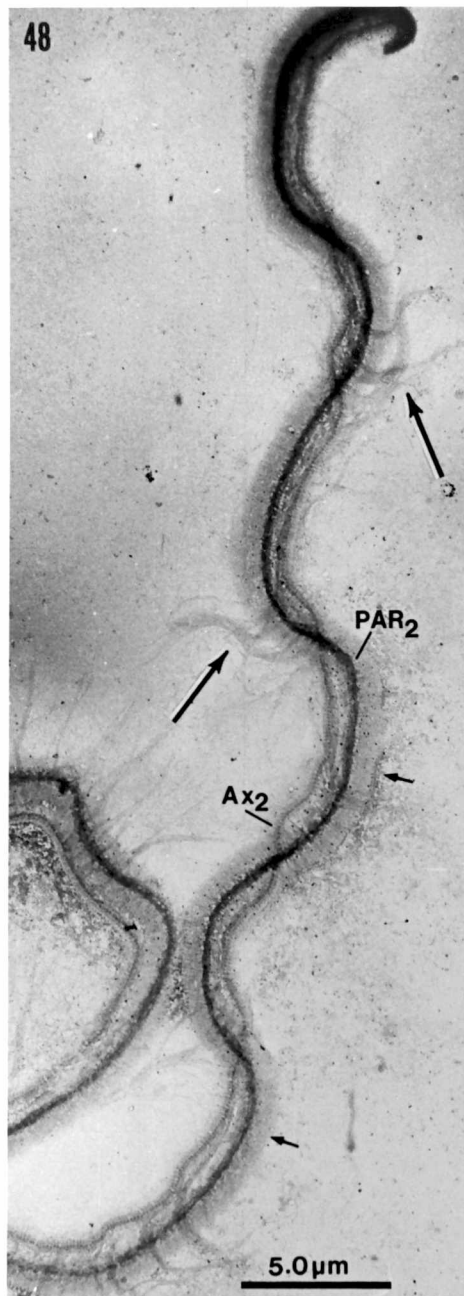


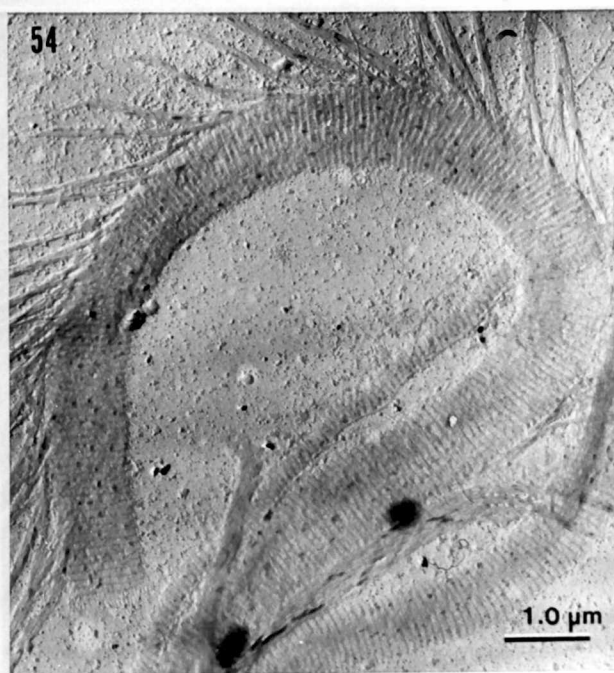
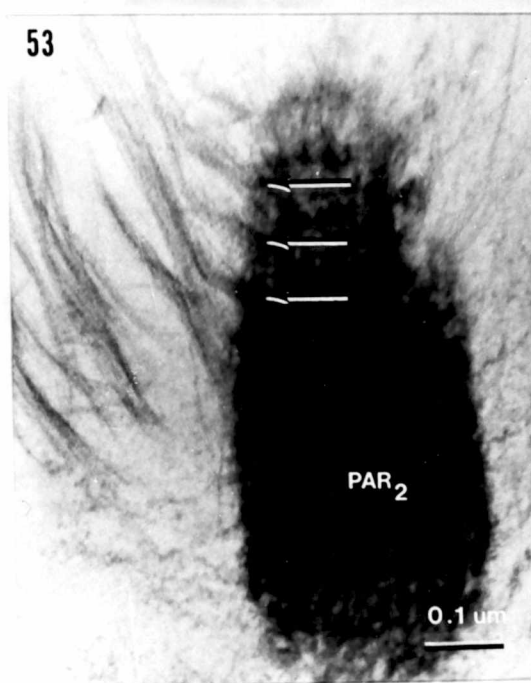
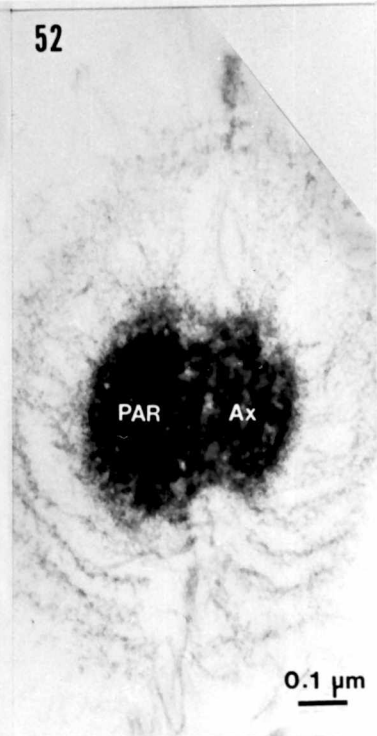
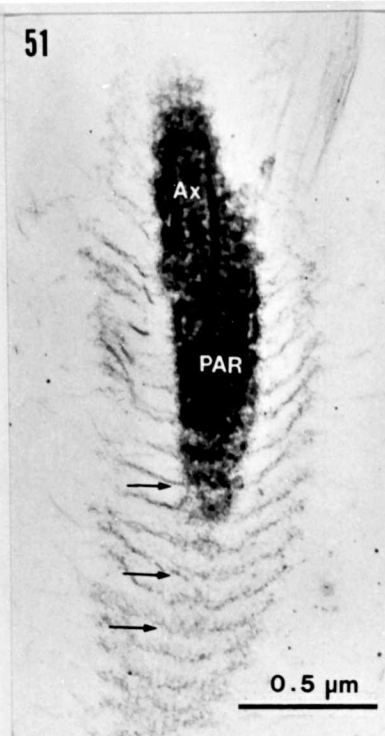
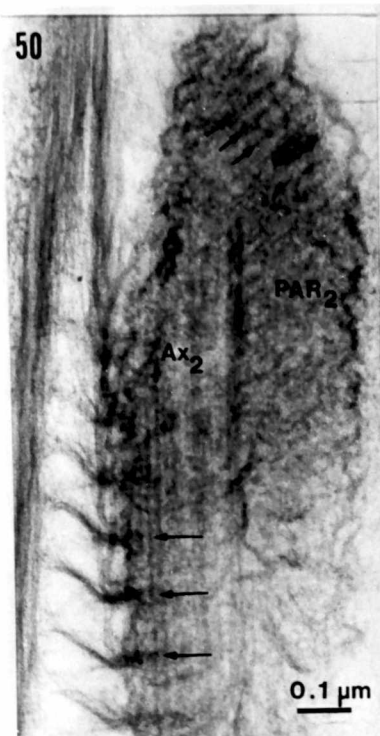
Figure 50. Glancing section through ventral (F_2) flagellum showing periodic insertion of bundles of long mastigonemes on axonemal side of flagellum. TEM.

Figure 51. Glancing section through dorsal (F_1) flagellum showing tiers of short mastigonemes (large arrows), oblique filaments of PAR_1 (small arrows), and axonemal microtubules (arrowhead). TEM.

Figure 52. Transverse section through dorsal (F_1) flagellum showing splayed mats of short mastigonemes on Ax and PAR sides. TEM.

Figure 53. Oblique thick section through ventral (F_2) flagellum. Long mastigonemes on axonemal side are arranged in periodic tiers (arrows) and are absent on the PAR_2 side of the flagellum. Note lattice-like appearance of PAR_2 filaments. TEM.

Figure 54. Unilateral array of bundles of long mastigonemes and mats of short mastigonemes on F_1 flagellum. Shadowcast preparation.



PART III: STRUCTURAL CHARACTERIZATION OF THE FLAGELLAR
PARAXIAL RODS IN EUTREPTIA PERTYI

I. ABSTRACT

This investigation has shown that the paraxial rods in the euglenoid flagellum are highly ordered and differentiated structures. The rod in the F1 flagellum is composed of 1 major substructural component, the oblique filaments, which are arranged in reciprocal axes that give PAR1 a "lattice-like" appearance. The PAR in the F2 flagellum is composed of 3 major substructural components: horizontal, longitudinal and oblique filaments which are organized into trigonal-hexagonal "unit cells" that collectively impart a "paracrystalline, plate-like" appearance to PAR2. The organization of PAR subcomponents seems to have a direct relationship to the organization of flagellar mastigonemes.

The symmetrical shape of PAR1, the reciprocal axes of the PAR1 subcomponents, and the helical arrangement of some flagellar mastigonemes are related to the rapid and helical waveform of F1. Conversely, the asymmetrical crescent shape of PAR2, the disposition of the PAR2 subcomponents in three unequal axes, the single axis of the oblique filaments and the large "plated" substructure are related to the slow, planar waveform of F2.

Analytical ultrastructural characterization of the PARs in Eutreptia pertyi has provided a basis for comparing PAR-like structures in the flagella of other protists, including the dinoflagellates and kinetoplastid zooflagellates. These findings suggest that the PAR1 in euglenoids is homologous with the PAR in the anterior flagellum of bodonids and that the PAR2 in the trailing flagellum of euglenoids is

structurally homologous with the PAR in the recurrent flagellum of bodonids and the single recurrent flagellum of trypanosomatids. The PAR (i.e., periodic packing material) in the longitudinal flagellum of some dinoflagellates has structural similarity to the PAR1 of euglenoids, but it is unclear whether these PARs are homologous. The PAR appears to be an important structural marker for establishing "homologous flagella" among species in the Euglenophyceae and for suggesting taxonomic relationships within this class based upon flagellar criteria. The PAR also seems to be a reliable structural character for suggesting phylogenetic affiliations among protists that manifest this type of flagellar accessory component.

II. INTRODUCTION

Although some euglenoid genera have only one emergent flagellum and a few genera are multiflagellated, most euglenoids are biflagellated. As a rule, the flagella have heterodynamic motion and can be distinguished at the light-microscopic level by their characteristic waveforms and their position with respect to the cell. The biflagellated phagotrophic euglenoids usually have one anteriorly directed (locomotory flagellum), and one laterally or posteriorly directed flagellum that trails next to the cell body. In the phototrophic and osmotrophic euglenoids, the trailing flagellum may be reduced to a non-emergent stub within the flagellar pocket (i.e., reservoir).

Euglenoid flagella are surrounded by a "unit" membrane that is continuous with the plasmalemma and contain a typical eukaryotic "9 + 2" axonemal structure. In addition to the axoneme, emergent flagella usually contain an accessory structure, termed the paraxial rod (PAR), which parallels the axoneme for most, if not all, of the flagellar length.

Paraxial rod-like structures, similar to those found in euglenoids, are reported also in other algal classes--dinoflagellates and chrysophytes--and in the kinetoplastid zooflagellates (bodonids and trypanosomatids). The extra-axonemal structure is called by various names--PAR and paraflagellar rod (PFR) in the euglenoid flagellates; packing material or striated strand in the dinoflagellates and

intraflagellar structure (IFS), paraxial fibre, "bande striee du flagelle" or "accessory filament" in the kinetoplastid zooflagellates.

Some ultrastructural and biochemical studies of the PAR in the classes mentioned above include the works of Cachon et al. (1988) characterizing the PAR in the longitudinal flagellum of Oxyrrhis (dinoflagellate), De Souza & Souto-Padron (1980) and Farina et al. (1986) characterizing the PFR in Trypanosoma and De Souza & Souto-Padron (1980) and Cunha et al. (1984) characterizing the PAR in Herpetomonas (kinetoplastid zooflagellate), and Hyams (1982) characterizing the PAR in the emergent flagellum of Euglena gracilis and Mignot (1966) comparing general PAR structure in several euglenoid genera.

The superficial resemblance of the PAR in the aforementioned protistan groups is that of a 3-dimensional cylindroid structure composed of filaments in a paracrystalline array. The precise substructure and degree of complexity of the PAR can, however, be markedly different from species to species of a given class and from flagellum to flagellum in heterodynamic biflagellates.

In biflagellated euglenoids, the flagella can usually be distinguished at the light-microscopic level by differences in flagellar motion, and at the ultrastructural level by differences in PAR substructure. In euglenoids with two emergent, heterodynamic flagella, the PAR structure is typically larger and more highly differentiated in one flagellum than in the other. This is the observed condition in Eutreptia pertyi, a "primitive" green biflagellate in the order Eutreptiales, with flagella of nearly equal length but differing width and motion. The PAR in the "anteriorly directed" or dorsal flagellum is

cylindrical and has a "lattice-like" appearance, whereas the PAR in the "trailing" or ventral flagellum is larger, crescent-shaped and has a "plate-like" appearance.

The objectives of this study are four-fold: 1) to characterize the PAR substructure in both flagella of Eutreptia pertyi, 2) to develop a 3-dimensional model of the PAR in the trailing flagellum, depicting its relationship to the axoneme, flagellar membrane and mastigonemes, 3) to define the "unit cell" of that PAR substructure, and 4) to attempt to relate flagellar structure to function and to suggest a possible function for the PAR.

III. MATERIALS AND METHODS

Cultures of Eutreptia pertyi Prings. were obtained from the Culture Collection of Algae (LB 1290), University of Texas, Austin. The cells were cultured and processed for light microscopy, transmission electron microscopy, negative staining, shadowcasting and computer image enhancement as described elsewhere (Part II). Fast Fourier Transform analysis was done at Perceptics, Inc., Oak Ridge, TN. using Perceptics 9200 Digital Image Processing System. Eastman Kodak VHS recordings of living cells were done with a Panasonic Color Video Digital Camera (WV-3260/8AF) with a speed of 30 frames/s.

IV. RESULTS

General Features of the Cell

Eutreptia pertyi, a unicellular, photosynthetic euglenoid that lives in marine to brackish environments, is typically 60-100 μm long and 20-30 μm wide, with two nearly equal-length flagella that extend anteriorly from the cell. Both flagella originate on the dorsal side of the organism beneath the wall of an expanded reservoir cavity, extend through a narrow canal and emerge on the ventral side of the organism through a subapical opening (Fig. 1¹).¹

Cell Shape and Motion

The cell moves in primarily two ways--euglenoid movement ("metaboly"), typically a slow type of cellular motion, and by swimming, typically a faster type of cellular motion. During "metaboly," a wide range of cell shapes can be exhibited, from rounded to elongated extremes; peristaltic movements of the cell contribute to forward cell body displacement at a slow-to-moderate rate of advancement. A more rapid type of cellular movement is displayed by the swimming cell which has a swimming velocity of $\sim 200 \mu\text{m} \cdot \text{s}^{-1}$. During swimming, the cell has a conical shape and rotates clockwise around its anteriorposterior axis. The helicoidal swimming path, whose trajectory is mainly linear, has a $\sim 1\text{-}5 \mu\text{m}$ wide helix. The S-shaped (left-handed) helical symmetry

¹All figures referenced in Part III are found in the Appendix at the end of Part III.

of the pellicle (Fig. 2) appears to minimize cell drag in the forward clockwise (right-handed) rotational vector.

General Characteristics of the Flagella

The two flagella of Eutreptia pertyi can be distinguished at the light-microscopic level by noticeable differences in flagellar motion and by subtle differences in length, width, and typical position with respect to the cell body. The more active flagellum, identified in this study as the **F1** or **dorsal flagellum** (see below), is usually directed anteriorly and is $\sim 1.5\text{-}1.7\ \mu\text{m}$ wide $\times\ \sim 80\ \mu\text{m}$ long. The slightly wider and longer flagellum, identified in this study as the **F2** or **ventral flagellum**, usually projects laterally or trails curvilinearly next to the cell body and is $\sim 1.8\text{-}2.0\ \mu\text{m}$ wide $\times\ \sim 95\ \mu\text{m}$ long.

The flagella are inserted in parabasal sites beneath the dorsal reservoir wall (Fig. 1). At the point of origin in the basal body complex, F1 flagellum is nearer the dorsal microtubular root, and hence is identified as the dorsal flagellum; similarly, the F2 flagellum, nearer the ventral microtubular root, is identified as the ventral flagellum (Fig. 5). This specific flagellum-flagellar root association is generally consistent within the reservoir and contributes to spatial alignment of the flagellar swelling (paraflagellar body, PFB) on F1, to the stigma complex in the dorsal cytoplasm (Fig. 10). In the reservoir-canal transition zone and neck of the canal, there is an extensive flagellar-root-derived cytoskeleton. In these regions, F1 is normally positioned on the dorsal/dorsal-right side of the canal adjacent to the dorsal root and the dorsal microtubular band and F2 is positioned on the ventral/ventral-left side of the canal near the intermediate root

(Figs. 3, 12; and Figs. 42-44 in Part II). A cluster of stiff reservoir hairs (Fig. 18) often extends from a prominent canal ridge (Fig. 2), containing the intermediate flagellar root, and connects to the F2 membrane (see also Fig. 40 in Part II).

There is a noticeable and consistent spatial apposition between F1 and F2 from points of origin to points of emergence, such that the structural components of one flagellum (i.e., axoneme and paraxial rod material) occupy a relatively static position with respect to similar structural components in the other flagellum. Thus, the flagella have "facing" profiles (Figs 3-7, 10-13, 18-20, 25-28; and Figs 40-44 in Chapter II), and the axonemes are diametrically opposed--Ax1 of F1 nearer the dorsal reservoir wall and Ax2 of F2 nearer the ventral reservoir wall (Figs 3, 11-13). The small interflagellar space (Figs 4, 25-28) and the contiguous and correlative outlines of the flagellar membranes (Figs 10, 12, 19) are also consistent features that indicate a close spatial and perhaps functionally significant relationship between F1 and F2.

Paraxial Rods (PARs)

Well-organized accessory material, present in both flagella, is defined and identified by its **axial** relationship to the flagellar axoneme and is therefore termed, the **paraxial rod** or **PAR**. The PARs originate near the flagellar bases and extend along the length of the flagellum, parallel to the axoneme. Different complexities of the PAR substructure in F1 and F2 can be used to identify the flagella at the ultrastructural level.

From their point of inception, the PARs appose one another and are effectively separated only by the flagellar membranes and the narrow interflagellar space. The rod-to-rod orientation is evident in both transverse and longitudinal sections through the flagella and appears to be consistent throughout the length of F1 and F2. Intracellularly, the PARs are localized toward the canal interior so that a line bisecting Ax1 and Ax2 also bisects PAR1 and PAR2. Hence, the PARs have relatively fixed lateral placement within the upper reservoir and canal and a fixed positional relationship to one another.

The major constituents of the flagella, axoneme, PAR and flagellar membrane, are structurally integrated. One side of the flagellar membrane appears to be "tethered" to the other side of the flagellar membrane by a series of bridge-like connections: flagellar membrane--axoneme--PAR--flagellar membrane. For example, filaments, 10 nm in diameter and spaced 38 nm apart, periodically connect PAR2 plates with neighboring Ax2 microtubules, maintaining a 20-nm space between the two structures (Fig. 23). Also, fine fibrils, evident in both F1 and F2, connect the peripheral axonemal doublets to the flagellar membrane (Fig. 12). Other "connectors" in F1 and F2, that are themselves extensions of major substructural components of the PARs, are discussed below.

PAR1.

The paraxial rod in F1 (PAR1), originates ~1.0 μm distal to the point of F1 emergence into the reservoir and lies adjacent to the "9 + 0" axoneme (Figs 5-6). Initially, PAR1 appears as an ill-defined electron-dense mass, adjacent to a peripheral axonemal doublet

microtubule (Figs 6-7). Spatial development in more distal regions usually results in two distinct zones within PAR1--an electron-transparent zone that is, in turn, encircled by an electron-dense zone (Fig. 7). Minute filaments interconnecting the PAR1 zones are occasionally observed; they have some structural and spatial similarities to the horizontal filaments of PAR2 (see below) (Figs. 3, 7 11-13). The fully formed PAR1 structure lies adjacent to the "9 + 2" axoneme of F1 (Ax1), throughout the intracellular length of the flagellum (Figs 3-4, 10-13, 19-20, 25-28). PAR1 and Ax1 are each $\sim 0.18 \mu\text{m}$ wide. At the level of the cytoplasmic stigma complex and the PFB on F1, PAR1 is interposed between the PFB and Ax1, such that the presumptive photoreceptor (i.e., PFB) is actually apposed to PAR2 of F2 and the dorsal reservoir membrane (Figs 10-12); moreover, substructural components of PAR2 are "in-line" with one of the paracrystalline lattices of the PFB (Fig. 12).

The microarchitectural components of PAR1 are most apparent in longitudinal perspective. Intersection of prominent oblique filaments, oriented in reciprocal axes at $\pm 40/45^\circ$ to the long axis of PAR1, gives an overall "lattice-like" appearance to PAR1 (Figs 4, 10, 19-20, 25-28). Oblique filaments inclined in the $-40/50^\circ$ axis in PAR1, have an almost identical counterpart in the substructure of PAR2 (i.e., the oblique filaments in PAR2, see below). Oblique filaments in PAR1 are $\sim 4\text{-}6 \text{ nm}$ in diameter and have a periodicity of 30 nm (Figs 3, 7, 10-11, 13, 19, 28). Occasionally, additional substructural components are observed in PAR1, seemingly distinct from the $\pm 45^\circ$ oblique filaments of PAR1 or the longitudinally oriented microtubules of Ax1. These often

unnoticed components appear as fine filaments, with longitudinal (Figs 19, 28) or horizontal (Figs 11, 13) orientation to the long axis of PAR1; they may have some rudimentary structural similarity to the well-defined longitudinal and horizontal filaments in PAR2 (see below).

PAR2.

The paraxial rod in F2 (PAR2), originates $\sim 1.0 \mu\text{m}$ distal to the point of flagellar emergence into the reservoir (Fig. 37). Initially, it has the appearance of a finely striated electron-dense mass and is associated with a specific peripheral axonemal doublet. Proximal to the origin of the central pair of axonemal microtubules, PAR2 acquires a "plate-like" substructure. Six-to-seven parallel plates extend perpendicular to the long axis of the Ax2 microtubular cylinder and "point" toward the interflagellar space. These "plates" form the incipient microarchitectural framework of PAR2 (Fig. 7).

Spatial development of PAR2, presumably by acquisition of additional plates, produces a crescent-shaped cylindroid structure composed of 8-13 nearly parallel plates (Figs 3, 8-9). The fully formed PAR2, $\sim 0.27 \mu\text{m}$ wide, parallels the axonemal cylinder, $\sim 0.18 \mu\text{m}$ wide, and significantly increases the diameter of F2.

Three major structural domains are evident within PAR2--a proximal plate zone, $\sim 48 \text{ nm}$ wide, nearest Ax2; an intermediate electron-transparent zone, $\sim 22 \text{ nm}$ wide; and an extensive distal plate zone, $\sim 200 \text{ nm}$ wide (Fig. 8).

The "plate-like" organization of PAR2 is best visualized in transverse sections through F2. This perspective usually shows eight well-defined plates spaced $\sim 25 \text{ nm}$ apart (Figs 3, 8-9, 21). The major

subcomponents that form and interconnect the plates are defined and identified by their orientation with respect to the long axis of PAR2 and are termed longitudinal, horizontal and oblique filaments. A minor subcomponent is also identified within the PAR2 substructure.

Each plate is composed of periodically spaced longitudinal and horizontal filaments that together form an open plate meshwork. The PAR2 plates are interconnected periodically by the oblique filaments that "weave" in and out through the meshwork of the plates.

Paracrystalline disposition of these three major microarchitectural components imparts the overall diamond-shaped pattern, the characteristic "signature" of the PAR2 substructure (Figs 4, 24, 29-31, 34).

Longitudinal Filaments. Longitudinal filaments, ~6-8 nm in diameter, are localized in the distal and proximal plate zones of PAR2. They show a periodicity of 12-13 nm and are oriented at 0° to the long axis of PAR2 and the long axis of Ax2. A single plate is composed of 16-20 longitudinal filaments in the distal zone (Figs 19-20, 23) and ~2-3 longitudinal filaments in the proximal zone (Fig. 23). When there is slight curvature of F2, there is concomitant curvature of the longitudinal filaments (Figs 25-28).

Horizontal Filaments. Horizontal filaments are parallelogram shaped, ~8-10 nm wide and ~18 nm long, and are the largest of the substructural components. In transverse section of PAR2 (Figs 3, 6-9), these filaments are the noticeable constituents of the plates. At the level of the PFB in F1, the horizontal filaments of PAR2 point toward the PFB (Figs 11-12).

The horizontal filaments of PAR2 have two different appearances in longitudinal sections. In sections parallel to Ax2 (Figs 4, 22, 24-29), the horizontal filaments are visualized as electron-dense "spots" or "junction sites", spaced 30 nm apart, along the longitudinal axis of each plate, and appear as "beads-on-a-string" along the longitudinal filament of a single plate. Spatial alignment of the horizontal filaments in adjacent plates (Figs 10, 29) forms an inherent axis at $+45^\circ$ with respect to the longitudinal axis of PAR2 (i.e., inclined toward PAR1 and declined toward the reservoir wall). The horizontal filaments do not appear to be physically connected in this axis; however, very fine filaments may connect the horizontal filaments in another plane (see minor subcomponent filaments, below).

In longitudinal sections perpendicular to Ax2 (Figs 10, 19, 23), the horizontal filaments are visualized as "rungs-on-a-ladder," (the ladder constituting an individual plate), spaced $\sim 25\text{-}35$ nm apart and oriented at $\sim 90\text{-}95^\circ$ to the long axis of the Ax2 microtubules. The ends of the horizontal filaments extend to the F2 flagellar membrane (i.e., toward the interflagellar space on one side and toward Ax2 on the other side of F2) (Figs 15, 26 and unpublished data). The horizontal filaments show a change in periodicity (from ~ 30 nm to ~ 22 nm) in the distal portion of F2 where the width of PAR2 increases from ~ 0.25 μm to ~ 0.4 μm .

In oblique sections through PAR2 (Figs 11-17), the horizontal filaments of adjacent plates are spaced 30-33 nm apart and appear to be confined to the proximal and distal plate zones of PAR2 (Figs 11, 14). Yet, horizontal filaments are also observed in transverse planes of

section of the intermediate zone of PAR2 (Figs 8-9). These seemingly contradictory results can be explained in at least two ways. One interpretation is that a single horizontal filament **is continuous** throughout the zones of PAR2, but it deviates from the normal orientation ($90-95^\circ$ to the long axis Ax2) in the intermediate zone, either inclining or declining in the plane of the plate. Another possibility is that a horizontal filament in the distal plate zone is **not continuous** with its counterpart (i.e., the horizontal filament in the same plate) in the proximal zone. An oblique change in direction in the intermediate region (i.e., the apparent "untwisting" of horizontal filaments shown in Figs 8-9) could connect a horizontal filament in the distal plate zone to the horizontal filament of an adjacent plate in the proximal zone, in Y- or V-shaped fashion. Both of these interpretations are supported by ultrastructural evidence: 1) the presence of intersecting V-shaped structures in the intermediate zone (Fig. 19), 2) the apparent "untwisting" of some horizontal filaments in the intermediate and proximal zones (Figs 8-9), and 3) the slightly staggered arrangement of the horizontal filament "ends" in the proximal and distal plates zones (Figs 11, 14). Thus, the intermediate zone may form a structural, and possible functional, "hinge" zone between PAR2 and Ax2.

Oblique Filaments. Oblique filaments interconnect the plates in the proximal and distal zones of PAR2. Each oblique filament, $\sim 4-6$ nm in the widest dimension, appears to be a twist of two "subfilaments," each $\sim 2-3$ nm in diameter (Fig. 29). The oblique filaments are **consistently** oriented in a $\sim 40/50^\circ$ axis to the long axis of PAR2, such

that they are **declined** toward PAR1 and **inclined** toward the reservoir wall (Figs 22, 24, 26-27). This specific orientation for the oblique filaments explains why they are observed in some (i.e., Figs 15-16, spaced 12.5 nm apart) but not all (i.e., Figs 11, 14) planes of oblique section through PAR2. The oblique filaments oriented in $\sim 45/50^\circ$ axis are the only similar substructural components in PAR1 and PAR2.

The oblique filaments appear to "weave" through the open meshwork of the plates, periodically intersecting the horizontal and longitudinal filaments in both the vertical and horizontal dimensions of PAR2 (Figs 15-17, 22, 24-30, 32). Fast Fourier Transform analysis suggests that "weaving" of adjacent plates is accomplished by 1) "over-under" interlacing, whereby the oblique filament courses **over** one horizontal filament and **under** the nearby horizontal filament of the adjacent plate, or 2) intertwining of the oblique "subfilaments" **around** the horizontal filament, such that the narrow twist of the "subfilaments" occurs between adjacent plates, and the widest part of the twist is at the horizontal filament "junction sites"; progressive intertwining eventually interlaces all the plates (Figs 31-32).

Stereo imaging of PAR2 (Figs 25-28) reveals that the ends of the oblique filaments terminate close to the F2 membrane, and further suggests that the terminal ends of adjacent oblique filaments (i.e., spaced 30 nm apart in the vertical dimension of PAR2) extend to **opposite** sides of the flagellar membrane. This arrangement accounts for the staggered appearance of the ends of the oblique filaments on the side of F2 membrane visible in Figs 26-27. Stereoanalysis also suggests that the oblique filament ends are slightly declined or curved near the

flagellar membrane; this phenomenon is also observed in slightly oblique transverse sections of PAR2 (Fig. 18).

Minor Subcomponent Filaments. Very fine filaments, $\sim 1\text{-}2$ nm in diameter, are occasionally detected within the substructure of PAR2. In transverse sections of PAR2, they have a periodicity of ~ 13 nm and traverse the plates diagonally at $\sim 60^\circ$ to the horizontal axis of PAR2 and are aligned with one of the lattice planes in the PFB (Fig. 12). In longitudinal sections of PAR2, the very fine filaments appear to "span" the diamond-shaped spaces (i.e., the space formed by the intersection of the three major PAR2 subcomponents) (Fig. 29).

Flagellar Mastigonemes and Their Associations with PARs

Long and short non-tubular mastigonemes are found on both F1 and F2, but their arrangement differs on each flagellum. The organization of the mastigonemes, **external** to the flagellar membrane, appears to be related, in part, to the **internal** organization and periodicities of the axonemal and paraxial rod subcomponents. This relationship has been studied most closely in F2 where the inherent spacing of the PAR2 subcomponents can be correlated with the spacing of the short mastigonemes.

Mastigonemes of F1. The dorsal flagellum has two sheaths of short mastigonemes (the "half-sheaths" sensu Bouck, 1982), each ~ 1.3 μm wide (see Fig. 54, Part II), and one unilateral array of long mastigonemes.

One sheath of short mastigonemes extends from the PAR1 side of the flagellum. Bundles of (5-10) "felt-like," short mastigonemes, ~ 0.25 μm in length, are laterally aligned into tiers, spaced ~ 86 nm apart and

(presumably helically) inclined at $\sim 75^\circ$ to the long axis of the flagellum (Fig. 35; see also Figs 49, 51-52, 54 in Part II).

The other sheath of short mastigonemes extends from the Ax1 side of the flagellum. Bundles of straight, short mastigonemes, $\sim 0.125 \mu\text{m}$ in length, extend from prominent protuberances at the flagellar membrane; bundles of short mastigonemes form laterally aligned and (presumably vertically overlapping) tiers, spaced 97 nm apart along the long axis of F1.

A unilateral array of long mastigonemes ($\sim 4 \mu\text{m}$ in length) arises between the two sheaths of short mastigonemes. Bundles of long mastigonemes, $\sim 4 \mu\text{m}$ in length (each bundle containing at least 10 mastigonemes) extend from globular projections, spaced ~ 110 nm apart along the membrane (Figs 35, 38, 40).

Mastigonemes of F2. A single mat of short mastigonemes extends from the PAR2 side and a single mat of long mastigonemes extends from the Ax2 side.

Bundles of short mastigonemes (~ 0.25 - $0.28 \mu\text{m}$ in length) originate beneath the flagellar membrane in dome-shaped projections, spaced ~ 83 nm apart along the long axis of PAR2. Lateral alignment of the dome-shaped projections forms a single centrifugal tier of short mastigonemes, inclined at $\sim 90^\circ$ to the long axis of PAR2 (Fig. 33). Vertical alignment of the tiers forms a continuous, multi-tiered mat ($\sim 1.5 \mu\text{m}$ wide) of short mastigonemes on the PAR2 side of F2 (Figs 33, 39, 41). Critical examination of the tiered projections of short mastigonemes on the PAR2 side of the F2 membrane reveals that each convex domed-shaped projection is supported by converging struts that impart the domed shape. The

struts appear to be extensions of the horizontal and oblique filaments of PAR2 (Figs 37, 43-44).

Tiers of long mastigonemes, $\sim 5.5 \mu\text{m}$ in length, emanate from globular projections, spaced 90-110 nm apart, along the Ax2 side of F2 (Figs 33, 41; see also Figs 48, 50, 53 in Part II).

3-Dimensional Model and "Unit Cell" of PAR2

A diagram of a 3-dimensional model of the ventral flagellum in Eutreptia pertyi (Fig. 45) is drawn approximately to scale and shows the relationships among the major F2 constituents and reflects our current understanding of PAR2 substructure.

A diagram of the "unit cell" structure of PAR2 is shown in Fig. 46. Diamond-shaped patterns, characteristic of PAR2 substructure, are formed by the ordered arrangement of repeating subunits (i.e., the "unit cell"). Each "unit cell" is a trigonal-hexagonal "crystal" (a six-sided "solid" having three unequal axes), formed by the intersecting axes of the three major PAR2 constituents (i.e., horizontal filaments inclined in 90° axes, longitudinal filaments inclined in the 0° axes, and oblique filaments inclined in the $-40/50^\circ$ axes). This "paracrystalline" substructure was confirmed by optical diffraction studies of PAR2 (data not shown).

A plausible trajectory for the very fine filaments, 1-2 nm diameter, (i.e., the minor subcomponent) in PAR2 (Fig. 47) demonstrates how they may connect "unit cells" of adjacent PAR2 plates.

Flagellar Motility

At the light-microscopic level, the flagella can be distinguished by heterodynamic flagellar motion. The more active flagellum,

identified as the dorsal or **F1 flagellum**, has an asymmetrical, 3-dimensional helical waveform. Three to four waves are simultaneously propagated along the flagellum, giving the appearance of an "extended-retracted" lasso-type of motion. The waves are propagated clockwise (as viewed from the flagellar base) and travel unidirectionally from base to tip, showing a decreased wave amplitude toward the flagellar tip.

F1 is the primary locomotory (force-generating) flagellum in cell swimming. The propulsive beat appears to **pull** the organism in the forward direction. Clockwise rotation of the swimming cell, around the anterior-posterior axis, ostensibly compensates for the helical torque produced during the effective stroke of F1. The gyrating pitch (1-5 μ m in diameter) of the swimming cell may be attributed to the asymmetrical component in the helicoidal waveform of F1.

The drawings of Eutreptia pertyi, (Fig. 48), based upon tracings of the recorded cell in consecutive frames of VHS tape, document the anteriorly directed, asymmetrical helical waveform of F1.

The motion of the ventral or **F2 flagellum** is markedly different from the motion of F1, in that it has a planar sinusoidal waveform that is much slower and more "cilium-like." One to two waves are propagated along the flagellum at a given time and travel unidirectionally from base to tip at a given time. During swimming, F2 is usually positioned next to or near the cell body; the planar motion appears to guide the cell, in steering or rudder-like fashion, and prevents major vectorial deviations from a helicoidal swimming path. Occasionally, during reversed swimming, F2 moves in erratic and saltatory lashing motions and moves from a lateral/trailing position to a more anterior position near

F1. After cell reorientation, F2 assumes the typical trailing position near the cell body.

The drawings of Eutreptia pertyi, shown in Fig. 49, are based upon tracings of the recorded cell in consecutive frames of VHS tape and document the slow, laterally directed planar waveform of F2 (i.e., **away from** the anteriorly directed helical motion of F1).

Relating Flagellar Structure to Flagellar Function

This ultrastructural study has revealed two major structural differences between the flagella that may account for heterodynamic flagellar motion. They are 1) the different complexities of the PARs in F1 and F2, including the types and arrangement of PAR1 and PAR2 subcomponents, and 2) the different arrangement of the long and short mastigonemes on F1 and F2.

The critical analysis of PAR1 and PAR2 substructure has shown one axis of oblique filaments in PAR2 (i.e., the axis at $\sim 45^\circ$ to the long axis of PAR2 and **declined** toward PAR1), and two axes of oblique filaments in PAR1 (i.e., oriented in reciprocal axes at $\sim 45^\circ$ and $\sim +45^\circ$ to the long axis of PAR1). Also, some PAR2 substructural components extend to the flagellar membrane (i.e., ends of the horizontal filaments extending on one side to Ax2, and on the other side to the flagellar membrane, and the ends of adjacent oblique filaments extending to opposite sides of the flagellar membrane). Furthermore, short mastigonemes, found exclusively on the PAR2 side of the membrane, were shown to originate at or beneath the F2 membrane in "strutted" dome-shaped projections, the "struts" appearing to be extensions of the

PAR2 horizontal and oblique filaments on the sides of the membrane associated with PAR2.

One inference from the cumulative ultrastructural data is that the ordered PAR2 constituents have a predictable relationship with the ordered tiers of short mastigonemes, and that the PAR2 constituents, on a micro-level, convey and actually coordinate the "passive" movements of the short mastigonemes on the PAR2 side to the "active" movements on the Ax2 side. Further regulation could be effected in the structural "hinge zone" (see intermediate zone of PAR2, above).

The large and complex substructure of PAR2 appears to be directly related to the slow flagellar motion of F2. Conversely, the relatively small size of the PAR1 complex and the reciprocal arrangement of oblique filaments appears to be directly related to the anteriorly directed and rapid helical motion of F1. The axis of the oblique filaments in PAR2, declined toward PAR1, accounts for the preferential lateral bending (presumably away from the rapid and anteriorly directed motion of F1) and the slow planar motion of F2.

V. DISCUSSION

Characterization of the substructural components and degree of complexity in paraxial rods (PAR) in the two flagella of Eutreptia has shown that the PAR may be an important structural marker for systematic and phylogenetic relationships among algae and zooflagellates that possess similar types of paraxonemal inclusions. Structural characterization of the PARs has also suggested a possible functional role for these organelles in regulating heterodynamic flagellar motion.

PAR Structure as a Phyletic Marker in Heterokont Protists

Similar paraxial rod inclusions are present in at least three major groups of protistan flagellates: euglenoids and dinoflagellates (algae) and in the kinetoplastid zooflagellates (See Tables 1, 2, and 3, Appendix). Early descriptive studies of flagella in these organisms gave rise to several names for the accessory structure: paraxial rods (PAR), paraflagellar rod (PFR) or "flagellar armature" in the euglenoids, intraflagellar structure (IFS), paraxial fibre, "bande striee du flagelle" or accessory filament in the kinetoplastid zooflagellates, and "packing material" and "striated strand" in the dinoflagellates.

The **superficial** resemblance of the PAR in the aforementioned major groups is that of a 3-dimensional, paracrystalline lattice composed of filaments. Nevertheless, the precise PAR substructure can vary considerably, imparting a different appearance to the PARs in the flagella of a heterokont organism. PAR substructure can vary significantly in number, size, periodicity and arrangement of the

constituent filaments. The PARs of relatively few protists have been critically examined in order to document the precise structural composition, and this lack has made it difficult to determine the extent of structural similarity among flagella of several protistan groups.

Eutreptia was chosen for study because it is the model "primitive green" euglenoid (Leedale, 1978), with cell and flagellar features presumably **intermediate** between the phagotrophic and more advanced green euglenoids. Phagotrophic euglenoids have two flagella, one anteriorly directed (F1) and one trailing thick flagellum (F2), similar to the (respective) anterior and recurrent flagella of their presumed ancestral relatives, the bodonids and cryptobids; the (single) recurrent flagellum of the trypanosomatids is similar to the trailing flagellum of the euglenoids (Kivic & Walne, 1984). The advanced green euglenoids usually possess two flagella, an emergent locomotory flagellum (F1) and a non-emergent or highly reduced second flagellum (F2).

Based upon general PAR structure, there appear to be three types of flagella in the biflagellated euglenoids: a flagellum with a highly differentiated and complex "plate-like" PAR, a flagellum with a "lattice-like" PAR and a flagellum with an unstructured or missing PAR inclusion.

Substructural characterization of the two prominent types of PARs in the flagella of Eutreptia provides a basis for evaluating structurally similar or homologous flagella in the euglenoids and kinetoplastids and possibly the dinoflagellates. Plate 1, Appendix, summarizes the major substructural features of PAR1 and PAR2 in Eutreptia pertyi and the available information in the literature on PAR

structure in representative members of euglenoids, dinoflagellates and kinetoplastid zooflagellates.

"PAR1-like substructure," found in the anteriorly directed, dorsal flagellum of Eutreptia, has one major substructural component, the oblique filaments, which are arranged in opposing axes forming a "lattice-like" cylinder next to the axoneme. The "PAR2-like substructure," found in the trailing, ventral flagellum of Eutreptia, has three major substructural components, longitudinal, horizontal and oblique filaments, which form a crescent-shaped cylindroid with internal "plates" oriented perpendicular to the long axis of the axoneme. Studying PAR substructure with these general features in mind may aid in the determination of whether certain flagella in different species of green and phagotrophic euglenoids and in the kinetoplastids may be homologous.

For example, based upon similar "PAR2-like" rod structure, the trailing flagella of Eutreptia, Eutreptiella (Walne et al., 1986), Anisonema (Mignot, 1966) and Distigma (Mignot, 1966) are probably homologous and may also be homologous with the recurrent flagellum of the bodonids (Bodo) and cryptobids (Cryptobia), and the single flagellum of the trypanosomatids (Trypanosoma, Phytomonas, Herpetomonas, and Crithidia fasciculata).

It is less clear whether the periodic filamentous packing material in the dinoflagellates represents a primitive or derived form of a "PAR1-like" or "PAR2-like" rod of a euglenoid, like Eutreptia. For example, the rod in the longitudinal flagellum of Ceratium tripos has some structural similarities to the PAR2 rod of Eutreptia, but the rod

in the longitudinal flagellum of Oxyrrhis marina (Dodge & Crawford, 1971; Cachon et al., 1988) shows morphological similarities closer to the PAR1 rod of Eutreptia. It will be important to document paraxial rod substructure carefully in future studies and taxonomic descriptions of the euglenoids, dinoflagellates and kinetoplastids in order to make valid comparisons of structural homology. Also, the situation in the dinoflagellates is complicated by the presence of additional accessory inclusions (i.e., "banded paraxial rods" and "striated strands"; Taylor, 1975; Moestrup, 1982) in transverse and longitudinal flagella that are apparently distinct from the periodic, "packing material" that superficially resembles the euglenoid PAR.

PAR Substructure as an Indicator of Flagellar Maturation

If maturation of the euglenoid flagellum requires two cell cycles, as reported for the prasinophyte, Nephroselmis olivacea (Melkonian et al., 1987), PAR complexity may be an indicator of the stage of structural (and possibly functional) development of the flagellum. For example, if the dorsal (F1) flagellum of the first cell generation becomes the ventral (F2) flagellum in the second cell generation, (as suggested for the phagotrophic euglenoid, Ploeotia costata, Farmer & Triemer, 1988 and for the phototrophic euglenoid, Euglena mutabilis, Melkonian et al., 1987), then the PAR1-like substructure represents an (structurally and/or functionally) immature flagellum, and the PAR2-like substructure represents a similarly mature flagellum. Given the substructural constituents of PAR1 and PAR2 in Eutreptia flagella, this would mean that oblique filaments of PAR1 (inclined in two reciprocal axes at $\pm 45^\circ$ to the long axis of PAR1) would be lost in the $+45^\circ$ long

axis (since these are not observed in PAR2). In PAR2, the acquisition of longitudinal and horizontal filaments, together with oblique filaments, would collectively form the plates and might explain the rare occurrence of longitudinal and horizontal filament components in PAR1 in Eutreptia. This structural change could account for the accompanying functional change associated with the flagella, whereby an actively beating flagellum (F1) becomes a flagellum that only periodically stiffens (F2), (e.g., in Ploetia; Farmer & Triemer, 1988). This phenomenon of structurally and functionally maturing flagella may explain the correlative relationship between PAR substructural complexity and typical flagellar motion in Eutreptia pertyi (see below).

Origin and Growth of the PAR Substructure

Primordial PAR material in the euglenoids may arise from the radial fiber(s) that extend from the periphery of the axonemal doublets in the transition zone. Deposition of the PAR2 "plate" subcomponents, and possible plate-like structures at the point of origin in PAR1, seems to precede the addition of the oblique filaments, which appear in more distal regions where there is distention of the flagellar sheath.

Growth of PAR2 presumably takes place by acquisition of new plates on either side of the original plate (i.e., the plate associated with the specific axonemal doublet microtubule). This type of growth could account for the variable number of identical plates in the distal plate zone in the ventral (F2) flagella of Eutreptia, 13 plates; Eutreptiella, 16 plates (Walne et al., 1986); Entosiphon, 16 plates, (Mignot, 1966; Solomon et al., 1987), and Anisonema, 31 plates (Mignot, 1966), where they reach maximum proportion. Bidirectional growth from the point of

origin may account for the less structured plate appearance in the peripheral portions of PAR2.

The PAR2-like rod in kinetoplastid flagella has a variable number of plates: in general, a relatively small number of plates for bodonid, cryptobid and Trypanosoma species and a larger number for Herpetomonas, Phytomonas and Crithidia trypanosomatid species, although a PAR is missing in trypanosomatids like Crithidia oncopelti that have endosymbionts.

Paraxial rod inclusions in protists effectively double or triple the width of the flagellum, since the axonemal cylinder has a fairly constant diameter of 180-220 nm; flagellar width and shape (ex. elliptical vs. cylindrical) may indicate the presence, size and possibly the complexity of a paraxial rod inclusion.

PAR Substructure and Its Implications in Flagellar Motility

Structural Integration of the Flagella in the Canal Region.

In the canal region of Eutreptia pertyi, the F2 flagellum is associated with a cytoplasmic ridge (Fig. 2) which protrudes into the canal lumen and appears to "hold" the F2 flagellum in place by stiff reservoir hairs that interconnect the reservoir wall and F2, usually on the PAR2 side of the flagellum. This distinctive spatial association for a cytoplasmic ridge/reservoir hairs/F2 flagellum is also observed in Entosiphon sulcatum (see Figs 16-19 in Solomon et al., 1987). The functional significance of the association is unclear, although the ridge appears to stabilize the F2 flagellum on the left/ventral-left reservoir wall and may be important in relaying mechanical stimuli to

and from the canal cytoskeleton and F2, thereby integrating cytoskeletal and F2 flagellar movements.

A series of "bridges" structurally integrates the flagella to one another and the entire reservoir/canal wall. Beginning on the left/ventral-left side of the reservoir and proceeding to the right/dorsal-right side of the reservoir, these interconnections are observed:

- reservoir hairs connect reservoir membrane (in the vicinity of the intermediate root) to the F2 membrane
- F2 membrane is connected to Ax2 cylinder by fine filaments (Fig. 12)
- rod-like filaments, 10-nm in diameter and spaced 38 nm apart along the long axis of PAR2, connect peripheral Ax2 doublets to filaments in the proximal plate zone of PAR2
- proximal plate zone of PAR2 is connected to distal plate zone by the structurally "hinged" filaments in the intermediate zone (see results section)
- horizontal and oblique filaments of PAR2 extend to the F2 membrane nearest the intracanal space
- fine hairs (possibly flagellar mastigonemes) connect F2 and F1 membranes
- fine fibrillar links connect F1 membrane to PAR1 (see Fig. 44, Part II)
- PAR connects to peripheral axonemal doublets (Fig. 3)
- Ax1 connect to F1 membrane (see Fig. 44, Part II)

These data show how impulses (motory, tactile, photosensory, chemosensory), originating in any part of the canal cytoskeleton or the

flagella, could be relayed to spatially separated organelles or "microdomains" of a given organelle, and strongly suggest that the flagella are functionally, as well as structurally, integrated.

PAR - PFB Associations.

The flagellar swelling or paraflagellar body (PFB) is the presumed photoreceptor in euglenoid phototaxis and is a single monoclinic crystal (characterized by three axes of unequal length, two of which intersect obliquely and are perpendicular to the third), with axes of $a = 8.9 \text{ nm}$, $b = 7.7 \text{ nm}$, $c = 8.3 \text{ nm}$, $B = 110^\circ$ (Piccinni & Mammi, 1978); the a axis points to the axoneme, b axis points to "the lesser flagellum" (F2) in Euglena gracilis, and the c axis is parallel to the axoneme. This crystalline configuration of the PFB has some similarity to the "lattice-like" arrangement of the PAR1 constituents (oblique filaments, oriented at 90 - 110° to each other, and perhaps oriented at 90° to ill-defined longitudinal or horizontal filaments), and to the "plate-like" arrangement of the PAR2 constituents (oblique filaments in the -45 to 55° axis; horizontal filaments oriented at 90° to the long axis of Ax2 and longitudinal filaments oriented at 0° to the long axis of PAR2). Dodge (1975) noted that the crystalline nature of the PFB in the emergent flagellum of the euglenoid, Trachelomonas, contained a regular periodicity of 11 - 12 nm . Mignot (1966) suggested a similarity between the crystalline constituents of the PFB (on F1) and the paracrystalline constituents of PAR2. Two subcomponents of the PAR2 substructure seem to have a consistent orientation to the PFB on F1 in Eutreptia: a) the fine filaments (minor subcomponents), oriented in approximately the horizontal plane of PAR2 and inclined at 60° to the axis of the

horizontal filaments (Fig. 12), and b) the ends of the horizontal filaments, oriented in an "inherent axis" at $+45^\circ$ to the long axis of PAR2, usually "point" to the PFB in F1 (Figs 11-12). It is unclear what functional associations may exist among the semi-crystalline lattice planes of the PFB, PAR1 and/or PAR2 constituents during phototaxis and whether, during a two-cycle flagellar maturation process, it is possible that some of PFB constituents on F1 could become some of the PAR2 constituents on F2.

PAR - Axonemal Associations.

From their points of origin, PAR1 and PAR2 are closely associated with a **specific** peripheral axonemal doublet in the respective flagella. In the more distal regions of PAR development, the PAR1 cylinder and the PAR2 crescent-shaped hemicylinder are closely associated with the nearest group of 3-4 axonemal doublets. Throughout the length of the flagellum, PARs and the axonemes maintain a parallel and sometimes physically connected relationship.

The PARs in trypanosomatids have a **fixed** relationship to the central pair of axonemal microtubules and are usually associated with peripheral axonemal doublets 4-7 (De Souza & Souto-Padron, 1980). The PAR1 in the emergent flagellum of Euglena gracilis does not have a fixed orientation with respect to the central pair of microtubules (Bouck et al., 1978; Hyams, 1982). The orientation of PAR1 and PAR2 with respect to the central pair of axonemal microtubules was not precisely determined in Eutreptia, but several data suggest that a **random** arrangement of the PARs with respect to the peripheral axonemal doublet microtubules is unlikely: 1) during formation, the PARs are associated

with a specific axonemal doublet such that they have a consistent opposing relationship from the point of inception to the point of emergence; 2) there is a relatively "fixed" lateral placement of the PARs and the flagella within the canal; 3) there are multiple bridge-like linkages that structurally integrate the flagella to one another and to the reservoir wall; and 4) there are prominent rod-like connections (10 nm in diameter) between Ax2 and PAR2 that link the proximal plate region to a specific axonemal doublet(s) and maintain a 20-nm space between the two structures (Fig. 23).

These findings suggest that the rods are **permanently** fixed to a specific peripheral axonemal doublet during assembly and that the PARs do not move successively from doublet to doublet during the beat of the locomotory flagellum. Rather, the variable relationship between the PARs and the central pair of singlet microtubules suggests that they twist along the length of the flagellum or that they alter position during the beat of the flagella. Twisting of the central pair of axonemal microtubules has been documented in the green alga, Scourfieldia caeca (Melkonian & Preisig, 1982), an organism with a helical beat.

Consistent PAR-Ax associations may aid in the determination of the planes of flagellar beat in the euglenoid flagellum. The PARs may be inherent regulatory structures that are an integral part of the flagella and specify the doublet or groups of doublets which are differentially activated in the mechanism of axonemal sliding. This regulation may be through the direct PAR-Ax linkages (i.e., the 10-nm filaments, spaced 38

nm apart along PAR2) or by indirect connections of PAR subcomponents to specific peripheral axonemal microtubules.

PAR- Mastigoneme Associations.

Long and short nontubular mastigonemes occur on both flagella of Eutreptia, but their arrangement and association with either the axonemal or PAR sides of the flagellum vary. In F1, the mat of short mastigonemes that extends from the PAR1 side has a presumed **helical** arrangement, inclined at $\sim 75^\circ$ to the long axis of the flagellum in Eutreptia (Fig. 35), similar to the helically arranged short mastigonemes on the dorsal (F1) flagellum of Anisonema (Mignot, 1966) and to the single emergent flagellum (F1) of Euglena gracilis (Bouck et al., 1978). The mat of short mastigonemes on the Ax1 side of F1 in Eutreptia is probably not helically inclined (Fig. 35). The unilateral array of long mastigonemes in the F1 of Eutreptia appears to arise between the two mats of short mastigonemes, possibly between the axonemal and PAR sides of the flagellum. The oblique filaments of PAR1 are organized in reciprocal axes in a "lattice-like" or **helical** arrangement, as shown in the 3-dimensional model of PAR1 in the emergent flagellum of Euglena gracilis (Hyams, 1982).

In F2 of Eutreptia, laterally aligned and longitudinally overlapping transverse tiers (i.e., non-helical) of short mastigonemes form a single mat on the PAR2 side of the flagellum. Transverse tiers of long mastigonemes arise on the Ax2 side of F2 in Eutreptia.

The collective data suggest that mastigoneme arrangement on the PAR1 and PAR2 sides of the flagella in Eutreptia may be related to the substructural configuration of the PAR constituents. The helical

arrangement of PAR1 oblique filaments may account for the helical arrangement of the mat of short mastigonemes on that side of F1. Conversely, the arrangement of the PAR2 subcomponents, presumably by lateral extensions of the horizontal or oblique filaments to the F2 membrane, may account for the transverse orientation of the tiers of short mastigonemes on F2.

Preliminary analysis of F2 suggests that the short mastigonemes in a single tier are spaced 25 nm apart. This spacing may be correlated with the periodicity of the oblique filaments in PAR2 (spaced 12.5-13 nm apart), such that the ends of alternate oblique filaments in the **horizontal** dimension extend to one side of the F2 membrane (i.e., $2 \times 12.5 = 25$ nm) and form an intraflagellar attachment for a single short mastigoneme in a single tier. Other data suggest that the ends of adjacent oblique filaments in the **vertical** dimension of PAR2 extend to **opposite** sides of the membrane (Fig. 26). The vertical spacing of the oblique filaments (29-30 nm) along the long axis of F2 (i.e., on the two lateral "flattened" sides of F2) and possibly the spacing (30.5 nm apart) and orientation (in an inherent "+45°" axis to the long axis of PAR2) of the horizontal filaments (i.e., on the side of the flagellum opposite to the PAR2-Ax2 connection) may be related to the vertical spacing (83-89 nm, i.e., $29 \text{ nm} \times 3$) of the tiers of short mastigonemes and related to the degree of overlap between mastigonemes in adjacent tiers. For example, if the short mastigoneme units are ~180 nm long, the tiers and therefore the mastigonemes, could be one-half overlapping in the vertical dimension). When long or short mastigonemes arise on

the axonemal side of the flagella, they appear to be arranged in transverse tiers.

In Euglena gracilis, the organization of the long and short mastigonemes was reportedly related to the pattern of the intramembrane particles (IMP) of the emergent flagellum (F1). The IMPs were suggested to act as templates for mastigoneme attachment (Melkonian et al., 1982). Our results suggest that some PAR2 (and possibly PAR1) subcomponents extend to and through the flagellar membrane and form transmembrane structures (via the IMPs?). The PAR subcomponents form internal/external flagellar membrane connections and act as anchorage sites for the extraflagellar mastigonemes. The PAR links the "passive" movements and sensory responses of the mastigonemes to the "active" movements (e.g., axonemal sliding, beating) of the axoneme. Thus, the PAR coordinates the external and internal changes of the flagellum. Additionally, the periodic arrangement of the PAR2 subcomponents, especially the oblique and horizontal filaments, may be directly related to the periodic arrangement of the short mastigonemes into laterally aligned and vertically overlapping tiers on the PAR2 side of F2. Further studies are necessary to confirm this postulated relationship between the PAR substructure and the arrangement of the mastigonemes.

PAR Substructure and Flagellar Beat Patterns.

The cylindrical shape of PAR1, the symmetrical "lattice-like" arrangement of the PAR1 oblique filaments in $\pm 45^\circ$ axes, and the helical arrangement of at least one mat of short mastigonemes may, individually or collectively, account for or contribute to, the rapid, helical motion of F1. The presumed transverse orientation of the other

mat of short mastigonemes (on the Ax1 side of F1) and the unilateral array of long mastigonemes may contribute to the asymmetrical aspect of the F1 waveform.

The "flattened" elliptical shape of F2, the asymmetrical crescent-shape of PAR2, the paracrystalline "plate-like" arrangement of the PAR2 subcomponents, the transverse orientation of the tiers of short mastigonemes (on the PAR2 side of F2) and the tiers of long mastigoneme (on the Ax2 side of F2) may, individually or collectively, account for or contribute to the slow, non-helical planar waveform of F2. The preferential plane of flagellar bending seems to be directly related to the single -45° axis of the oblique filaments in PAR2. Since adjacent oblique filaments in PAR2 extend to opposite sides of the flagellar membrane, they could transmit the movements of the short mastigonemes on **both** sides of the flagellum to the axoneme. In this way, localized distortion of specific PAR2 subcomponents could be transferred via the paracrystalline substructure to more extensive sites in PAR2 and to localized sites along the axoneme. These mechanical impulses could initiate axonemal doublet sliding which would, in turn, result in localized bending of the flagellum. By this proposed mechanism, PAR2 may modulate the flagellar beat in the F2 flagellum.

In the euglenoids, slow (planar) motion is usually associated with F2 (the ventral flagellum, usually laterally directed or trailing next to the body), (e.g., Anisonema, Mignot, 1966; and Eutreptia) and helical motions are associated with F1 (the dorsal and usually anteriorly directed flagellum) in euglenoids (e.g., Euglena gracilis, Eutreptia, Menoidium, Eutreptiella, Entosiphon).

In the dinoflagellates, the transverse flagellum typically has a helical (hemi-helical) beat, and the longitudinal flagellum can have planar (e.g., Ceratium tripos, Maruyama, 1982) or helical movement (e.g., Oxyrrhis marina, Cosson et al., 1988).

In the bodonids, the anterior flagellum has a helical type of waveform, and the recurrent flagellum has a planar waveform (Rhynchomonas, Bodo) (Burzell, 1973, 1975). The single recurrent flagellum of trypanosomatids usually adheres permanently to the cell body.

These findings suggest the characteristic waveform of a flagellum in these protists may be directly related to the type of PAR substructure in the flagellum. The evolutionary "solution" to homodynamic flagellar movement appears to be the development of intraflagellar, heteromorphic PARs which provide an "internal" mechanism for coordination and heterodynamic operation of the flagella in biflagellated organisms.

PAR Substructure and Its Relationship to the Biochemical Composition of the PAR

This study has characterized the microarchitecture of the PARs in the flagella of Eutreptia. The current understanding of the substructure of PAR1 and PAR2 may be considered and addressed in light of the preliminary biochemical data obtained for these structures in a few organisms.

Table 4 (Appendix) summarizes the information from the literature about the protein constitution of PARs in euglenoids and

trypanosomatids. No information is available on the biochemical composition of the PARs in the dinoflagellates.

The PAR subcomponents with the most variable orientation are the oblique filaments, inclined in the 40-50° axes, specifically in the +/- axes in PAR1 and only in the - axis in PAR2. Mignot (1966) suggested that contractile elements may be present in PAR2 in the trailing flagellum of Anisonema and in the recurrent flagellum in certain bodonids, based on the "jump-on-a-stick" motion of this flagellum. If contractile elements are present in the PARs of the euglenoids, the most likely filaments to undergo spatial or temporal contraction are the oblique filaments. In PAR2 these filaments are 4-6 nm and seem to be a twist of two "nanofilaments," each 2-3 nm in diameter, that are structurally entwined with the longitudinal and horizontal filaments. The ends of the oblique filaments appear to extend to and possibly through the F2 membrane and connect the mastigonemes to the internal PAR2 framework. Mastigoneme movements could effectively "pull" the oblique filaments or alternatively, the oblique filaments may themselves be contractile proteins; in either case, this situation could account for the somewhat variable orientation of the oblique filaments with respect to the long axis of PAR2.

Gallo and Schrével (1985) found a common epitope in the PAR proteins from trypanosomes (Trypanosoma brucei, a PAR2-like rod, see Table 4, Appendix) and euglenoids (Euglena gracilis, with a PAR1-like rod and Distigma proteus, with a PAR1-like rod in F1 and a PAR2-like rod in F2, see Mignot, 1966), results suggesting that the PARs are homologous structures in these protists. Gallo and Schrével also

reported the presence of a PAR-like "precursor" protein in Crithidia oncopelti, a trypanosomatid with no apparent PAR structure. Recently, Schneider et al. (1988) found spectrin-like proteins (M.W. 180/200) in the PAR of Trypanosoma brucei that show a homology with spectrin band 1. (Spectrin is the important building block in the erythrocyte membrane skeleton and the spectrin-like proteins have these properties: a rod-like structure, antigenetic cross-reactivity, the ability to form polymers, a tertiary structure that is a triple-helical structure, and spectrin interacts with intermediate filaments.

The most likely structural components in PAR1 or PAR2 that may have spectrin-like properties are the horizontal filaments of PAR2. Relatively large and rod-like, 8-10 nm wide and 18 nm long, these filaments may be composed of subfilaments or bundles of intermediate filaments. The two major lower molecular weight proteins (68 - 80,000 M.W.) found in the PAR in trypanosomatids and euglenoids (Table 4, Appendix) may correspond to the oblique and longitudinal filaments, 4-6 nm and 6-7 nm in diameter, respectively, whose sizes suggest they may be (non-actin) microfilaments.

The PARs are sensitive to trypsin (Hyams, 1982). Russell et al. (1983) suggest that the cross-linkages between the PAR and the Ax are the sensitive sites in the PAR complex where PAR-Ax dissociation occurs.

LIST OF REFERENCES

- Anderson, W.A. and R.A. Ellis. 1965. Ultrastructure of Trypanosoma lewisi: flagellum, microtubules, and the kinetoplast. J. Protozool. 12: 483-499.
- Attias, M. and W. De Souza. 1985. Fine structure and cytochemistry of Phytomonas davidi. J. Submicrosc. Cytol. 17: 205-212.
- Baker, J.T. and R.G. Bird. 1968. Trypanosoma avium: Fine structure of all developmental stages. J. Protozool. 15: 298-308.
- Berdach, J.T. 1977. In situ preservation of the transverse flagellum of Peridinium cinctum (Dinophyceae) for scanning electron microscopy. J. Phycol. 13: 243-251.
- Berman, T. and I.L. Roth. 1979. The flagella of Peridinium cinctum fa. westii: in situ fixation and observation by scanning electron microscopy. Phycologia 18: 307-311.
- Boisson, C., X. Mattei, M.E. Boisson. 1965. Le flagelle de Trypanosoma gambiense etudie au microscope electronique. C. R. Soc. Biol. 159: 228-230.
- Bouck, G.B. 1982. Flagella and the cell surface. In: The Biology of Euglena, Vol. 3, (D.E. Buetow, ed.), Academic Press, New York, pp. 29-51.
- Bouck, G.B., A. Rogalski and A. Valaitis. 1978. Surface organization and composition of Euglena. II. Flagellar mastigonemes. J. Cell Biol. 77: 805-826.
- Bradbury, P., J.A. Westfall and J.W. Townsend. 1983. Ultrastructure of the dinoflagellate Polykrikos II. The nucleus and its connections to the flagellar apparatus. J. Ultrast. Res. 85: 24-32.
- Brugerolle, G., J. Lom, E. Nohynková and L. Joyon. 1979. Comparison et evolution des structures cellulaires chez plusieurs especes de bodonides et cryptobiides appartenant aux genres Bodo, Cryptobia et Trypanoplasma (Kinetoplastida, Mastigophora). Protistologica 15: 197-221.
- Burzell, L.A. 1973. Observations on the proboscis-cytopharynx complex and flagella of Rhynchomonas metabolita Pshenin, 1964 (Zoomastigophorea: Bodonidae). J. Protozool 20: 385.
- Burzell, L.A. 1975. Fine structure of Bodo curvifilus Griessmann (Kinetoplastida: Bodonidae). J. Protozool. 22: 35-39.

- Cachon, J., M. Cachon and M. Charnier. 1972. Ultrastructure de bodonide Trypanophis grobbeni Pache, parasite de siphonophores. Protistologica 8: 223-236.
- Cachon, J., J. Cosson, M. P. Cosson, P. Huitorel and J. Cachon. 1988. Ultrastructure of the flagellar apparatus of Oxyrrhis marina. Biol. Cell 63: 151-158.
- Cosson J., M. Cachon, J. Cachon and M.P. Cosson. 1988. The swimming behaviour of the unicellular biflagellate O. marina: in vivo and in vitro movement of the two flagella. Biol. Cell 63: 117-126.
- Cunha, N.L., W. DeSouza and A. Hasson-Voloch. 1984. Isolation of the flagellum and characterization of the paraxial structure of Herpetomonas megaseliae. J. Submicrosc. Cytol. 16: 705-713.
- De Souza, W. and T. Souto-Padron. 1980. The paraxial structure of the flagellum of trypanosomatidae. J. Parasitol. 66: 229-235.
- Dodge, J.D. 1975. The fine structure of Trachelomonas (Euglenophyceae). Arch. Protistenkd. 117: 65-77.
- Dodge, J.D. and B.T. Bibby. 1973. The Prorocentrales (Dinophyceae). I. A comparative account of fine structure in the genera Prorocentrum and Exuviella. Bot. J. Linn. Soc. 67: 175-187.
- Dodge, J.D. and R.M. Crawford. 1970. The morphology and fine structure of Ceratium hirundinella (Dinophyceae). J. Phycol. 6: 137-149.
- Dodge, J.D. and R.M. Crawford. 1971. Fine structure of the dinoflagellate Oxyrrhis marina II. The flagellar system. Protistologica 7: 399-409.
- Dynesius, R.A. and P.L. Walne. 1975. Ultrastructure of the reservoir and flagella in Phacus pleuronectus (Euglenophyceae). J. Phycol. 11: 125-130.
- Farina M., M. Attias, T. Souto-Padron and W. DeSouza. 1986. Further studies on the organization of the paraxial rod of trypanosomatids. J. Protozool. 33: 552-557.
- Farmer, M.A. and R.E. Treimer. 1988. A redescription of the genus Ploetia Duj. (Euglenophyceae). Taxon 37: 319.
- Fize, A. and R. Michel. 1972. Ultrastructure d'Astasia fennica Michajlow (Euglenida) parasite du tube digestif de Copepodes Cyclopidae. Protistologica 8: 143-153.
- Freytmuller, E., E.P. Camargo. 1981. Ultrastructural differences between species of trypanosomatids with and without endosymbionts. J. Protozool. 28: 175-181.

- Fuge, H. 1969. Electron microscopic studies on the intra-flagellar structures of trypanosomes. J. Protozool. 16: 460-466.
- Gallo, J.M. & J. Schrével. 1984. La fibre paraflagellaire chez les Kinetoplastides et les Eugleniens; etude a l'aide d'un anticorps monoclonal. J. Protozool. 31: 72A.
- Gallo, J. and J. Schrével. 1985. Homologies between paraflagellar rod proteins from trypanosomes and euglenoids revealed by a monoclonal antibody. Eur. J. Cell Biol. 36: 163-168.
- Herman, E.M. and B.M. Sweeney. 1977. Scanning electron microscopic observations of the flagellar structure of Gymnodinium spendens (Pyrrophyta, Dinophyceae). Phycologia 16: 115-118.
- Heywood, P. D. Weinman & M. Lipman. 1974. Fine structure of Trypanosoma cyclops in noncellular cultures. J. Protozool. 21: 232-238.
- Hilenski, L.L. and P.L. Walne. 1985. Ultrastructure of the flagella of the colorless phagotroph Peranema trichophorum (Euglenophyceae). I. Flagellar mastigonemes. J. Phycol. 21: 114-125.
- Hogan, J.C. and C.L. Patton. 1976. Variation in intramembrane components of Trypanosoma brucei from intact and X-irradiated rats: a freeze- cleave study. J. Protozool. 23: 205-215.
- Hollande, A. 1974. Etude comparee de la mitose Syndinienne et de celle des Peridiniens libres et des Hypermastigines. Infrastructure et cycle evolutif des Syndinides parasites de Radiolaires. Protistologica 10: 413-451.
- Hyams, J.S. 1982. The Euglena paraflagellar rod: structure, relationship to other flagellar components and preliminary biochemical characterization. J. Cell Sci. 55: 199-210.
- Kivic, P.A. and P.L. Walne. 1984. An evaluation of a possible phylogenetic relationship between the Euglenophyta and Kinetoplastida. Origins of Life 13: 269-288.
- Leadbeater, B. and J.D. Dodge. 1967. An electron microscope study of dinoflagellate flagella. J. Gen. Microbiol. 46: 305-314.
- Lee, R.E. 1977. Saprophytic and phagocytic isolates of the colourless heterotrophic dinoflagellate Gyrodinium lebouriae Herdman. J. Mar. Biol. Assoc. U.K. 57: 303-315.
- Leedale, G.F. 1967. Euglenoid Flagellates. Englewood Cliffs, New Jersey, Prentice-Hall.
- Leedale, G.F. and D.J. Hibberd. 1974. Observations on the cytology and fine structure of the euglenoid genera Menoidium Perty and Rhabdomonas Fresenius. Arch. Protistenkd. 116: 319-345.

- Lumsden, W.H.R. and D.A. Evans. 1979. Biology of Kinetoplastida, Vol. 2, Academic Press, London.
- Maruyama, T. 1982. Fine structure of the longitudinal flagellum in Ceratium tripos, a marine dinoflagellate. J. Cell Sci. 58: 109-123.
- McGhee, R.B. and W.B. Cosgrove. 1980. Biology and physiology of the lower Trypanosomatidae. Microbiol. Rev. 44: 140-173.
- Melkonian, M. and H.R. Preisig. 1982. Twist of central pair microtubules in the flagellum of the green flagellate Scourfieldia caeca. Cell Biol. Internat. Rep. 6: 269-277.
- Melkonian, M., H. Robenek and J. Rassat. 1982. Flagellar membrane specializations and their relationship to mastigonemes and microtubules in Euglena gracilis. J. Cell Sci. 55: 115-136.
- Mignot, J. 1966. Structure et ultrastructure de quelques Euglenomonadines. Protistologica 2: 51-117.
- Moestrup, Ø. 1982. Flagellar structure in algae: a review, with new observations particularly on the Chrysophyceae, Phaeophyceae (Fucophyceae), Euglenophyceae and Reckertia. Phycologia 21: 427-528.
- Pereira, N.M., W. De Souza, R.D. Machado and F.T. DeCastro. 1977. Isolation and properties of flagella of Trypanosomatids. J. Protozool. 24: 511-514.
- Piccinni, E. and M. Mammi. 1978. Motor apparatus of Euglena gracilis: ultrastructure of the basal portion of the flagellum and the paraflagellar body. Boll. Zool. 45: 405-444.
- Pitelka, D.R. 1963. Electron Microscopic Structure of Protozoa. Macmillan Co., New York.
- Postell, F.J. & R.B. McGhee. 1981. An ultrastructural study of Phytomonas davidi Lafont (Trypanosomatidae). J. Protozool. 28: 78-83.
- Rowton, E.D., W.B. Lushbaugh & R.B. McGhee. 1981. Ultrastructure of the flagellar apparatus and attachment of Herpetomonas ampelophilae in the gut and Malpighian tubules of Drosophila melanogaster. J. Protozool. 28: 297-301.
- Russell, D.G., R.J. Newsam, G.C.N. Palmer and K. Gull. 1983. Structural and biochemical characterisation of the paraflagellar rod of Crithidia fasciculata. Eur. J. Cell Biol. 30: 137-143.
- Sanabria, A. 1966. Ultrastructure of Trypanosoma cruzi in the rectum of Rhodnius prolixus. Exp. Parasit. 19: 276-299.

- Schneider, A., H. Lutz, R. Marugg, P. Gehr and T. Seebeck. 1988. Spectrin-like proteins in the paraflagellar rod structure of Trypanosoma brucei. J. Cell Sci. 90: 307-315.
- Schuster, F.L. and B. Hershenov. 1974. Khawkinea quartana, a colorless euglenoid flagellate. I. Ultrastructure. J. Protozool. 21: 33-39.
- Solomon, J.A., P.L. Walne and P.A. Kivic. 1987. Entosiphon sulcatum (Euglenophyceae): flagellar roots of the basal body complex and reservoir region. J. Phycol. 23: 85-98.
- Souto-Padron, T. and W. De Souza. 1978. Ultrastructural localization of basic proteins in Trypanosoma cruzi. J. Histochem. Cytochem. 26: 349-358.
- Soyer, M.O. 1970. Les ultrastructures liees aux fonctions de relation chez Noctiluca miliaris S. Z. Zellforsch. 104: 29-55.
- Steidinger, K.A., E.W. Truby and C.J. Dawes. 1978. Ultrastructure of the red tide dinoflagellate Gymnodinium breve. I. General description. J. Phycol. 14: 72-79.
- Surek, B. and M. Melkonian. 1986. A cryptic cytostome is present in Euglena Protoplasma 133: 39-49.
- Swale, E.M.F. 1973. A study of the colourless flagellate Rhynchomonas nasuta (Stokes) Klebs. Biol. J. Linn. Soc. 5: 255-264.
- Taylor, A.E.R. and D.G. Godfrey. 1969. A new organelle of bloodstream salivarian trypanosomes. J. Protozool. 16: 466-470.
- Taylor, F.J.R. 1975. Non-helical transverse flagella in dinoflagellates. Phycologia 14: 45-47.
- Thronsdon, J. 1973. Fine structure of Eutreptiella gymnastica (Euglenophyceae). Norw. J. Bot. 20: 271-280.
- Triemer, R.E. and L. Fritz. 1987. Structure and operation of the feeding apparatus in a colorless euglenoid, Entosiphon sulcatum. J. Protozool. 34: 39.
- Vickerman, K. 1962. The mechanism of cyclical development in trypanosomes of the Trypanosoma brucei subgroup: an hypothesis based on ultrastructural observations. Trans. Roy. Soc. Trop. Med. Hyg. 56: 487-495.
- Vickerman, K. 1969. On the surface coat and flagellar adhesion in trypanosomes. J. Cell Sci. 5: 163-193.
- Vickerman, K. and T.M. Preston. 1976. Comparative Cell Biology of the Kinetoplastid flagellates. In: Biology of the Kinetoplastida,

Vol. 1, (W.H.R. Lumsden and D.A. Evans, eds.), Academic Press, London, pp. 35-130.

- Walkosz, R.A. 1982. Protein analysis of the paraxial rod of selected trypanosomatids. J. Protozool. 29: 3 (Abst.).
- Walne, P.L. 1971. Comparative ultrastructure of eyespots in selected euglenoid flagellates. In: Contributions in Phycology (B.C. Parker and R.M. Brown, eds.), Allen Press, Lawrence, Kansas pp. 107-120.
- Walne, P.L., Ø. Moestrup, R.E. Norris, and H. Ettl. 1986. Light and electron-microscopical studies of Eutreptiella eupharyngea sp. nov. (Euglenophyceae) from Danish and American waters. Phycologia 25: 109-126.
- West, L.K., P.L. Walne, J. Rosowski. 1980. Trachelomonas hispida var. coronata (Euglenophyceae). I. Ultrastructure of cytoskeletal and flagellar systems. J. Phycol. 16: 489-497.

APPENDIX

Table 1. Occurrence of the PAR in the Class Euglenophyceae.**

Organism	Information	Reference
Order Eutreptiales		
<u>Distigma proteus</u>	F2**, remnant in F1**	Mignot, 1966 Leedale, 1967
<u>Eutreptia pertyi</u>	well dev. in F2(ventral) less dev. in F1(dorsal)	Dawson, unpublished
<u>Eutreptia viridis</u>	F2, remnant in F1	Mignot, 1966
<u>Eutreptiella eupharyngea</u>	F1 & F2	Walne et al., 1986
<u>Eutreptiella gymnastica</u>	F1 & F2	Trondsen, 1973
Order Euglenales		
<u>Astasia</u> sp.	F1 and/or F2	Leedale, 1967
<u>Astasia fennica</u>	F1 and/or F2	Fize & Michel, 1972
<u>Colacium</u>	F1 and/or F2	Leedale, 1967
<u>Cyclidiopsis</u>	F1	Mignot, 1966
<u>Euglena acus</u>	F1 and/or F2	Mignot, 1966
<u>Euglena gracilis</u>	F1 only ⁺	Mignot, 1966 Leedale, 1967 Piccinni et al., 1975 Bouck et al., 1978 Hyams, 1982 Melkonian et al., 1982
<u>Euglena granulata</u>	F1	Walne, 1971 Walne & Arnott, 1967
<u>Euglena gymnastica</u>	F1 & F2	Thronsdon, 1973
<u>Euglena mutabilis</u>	electron-dense core in (dorsal) F1 only	Surek & Melkonian, 1986
<u>Euglena sanguinea</u>	F1	Walne, 1971
<u>Euglena stellata</u>	F1 and/or F2	Mignot, 1966

Table 1 (continued)

Organism	Information	References
<u>Khawkinea</u>	F1 only	Leedale, 1967 Schuster & Hershenov, 1974
<u>Lepocinclis buetschlii</u>	F1 and/or F2	Walne, 1971
<u>Phacus pleuronectes</u>	F1 only	Leedale, 1967 Dynesius & Walne, 1975
<u>Trachelomonas</u> sp.	F1 only	Leedale, 1967 Dodge, 1975
<u>Trachelomonas hispida</u> var. <u>coronata</u>	F1 (emergent) only (electron-dense core)	West et al., 1980
<u>Trachelomonas volvocina</u>	F1 only	Dodge, 1975
Order Rhabdomonadales		
<u>Menoidium bibacillatum</u>	F1; possibly in F2	Mignot, 1966 Leedale, 1967 Leedale & Hibberd, 1974
<u>Rhabdomonas costata</u>	remnant in F1 only	Leedale, 1967 Leedale & Hibberd, 1974
Order Sphenomonadales		
<u>Anisonema costatum</u>	F2 ⁺⁺ , remnant in F1	Mignot, 1966
Order Heteronematales		
<u>Entosiphon sulcatum</u>	amorphous(electron-dense core) in F1; well developed plates in F2 ⁺⁺⁺	Mignot, 1966 Leedale, 1967 West et al., 1980 Solomon et al., 1987 Triemer & Fritz, 1987
<u>Peranema trichophorum</u>	F2, remnant in F1	Pitelka, 1963 Leedale, 1967 Hilenski & Walne, 1985

Table 1 (continued)

Organism	Information	Reference
<u>Of Uncertain Affinity</u>		
<u>Scytomonas pusilla</u>	remnant in F1 (the sole flagellum)	Mignot, 1966

** F1 = the principal flagellum F2 = the accessory flagellum
 = anterior flagellum = recurrent flagellum
 = emergent flagellum = non-emergent flagellum
 = leading flagellum = trailing flagellum

+ the short (non-emergent) flagellum (F2) of Euglena gracilis
 = the long flagellum of Eutreptiella

++ thick trailing flagellum of Anisonema (F2) = long, thick
 flagellum of Eutreptiella

+++ long thick trailing flagellum of Entosiphon (F2) = long,
 thick flagellum of Eutreptiella and laterally projected
 trailing ventral flagellum of Eutreptia

Table 2. Occurrence of a PAR-like structure in the Dinoflagellates.*

Organism	Information	Reference
Class Syndiniophyceae		
<u>Solenodinium fallax</u>	PAR absent	Hollande, 1974
<u>Syndinium globiforme</u>	PAR absent	Hollande, 1974
Class Dinophyceae		
Order Prorocentrales		
<u>Prorocentrum</u> sp.	PAR in both flagella	Dodge & Bibby, 1973
Order Gymnodiniales		
<u>Ceratium hirundinella</u>	PAR absent; only a striated collar is present around the canal	Dodge & Crawford, 1970
<u>Ceratium tripos</u>	lf (pm) and a lateral fin	Maruyama, 1982
<u>Gymnodinium breve</u>	tf - ?; lf - absent (pm)	Steidinger et al., 1978
<u>Gymnodinium spendens</u>	lf	Herman & Sweeney, 1977
<u>Gyrodinium lebouriae</u>	lf	Lee, 1977
<u>Noctiluca miliaris</u>	sporocyte (gamete) single flagellum has a "cordon spirale" (pm) similar to the pm of the ventral flagellum (tf) of <u>Oxyrrhis</u>	Soyer, 1970 Dodge & Crawford, 1971b
<u>Oxyrrhis marina</u>	PAR in ventral flagellum (tf); PAR absent in dorsal flagellum (lf) but pm is present in both flagella	Dodge & Crawford, 1971b

Table 2 (continued)

Organism	Information	Reference
<u>Polykrikos</u> <u>kofoidi</u>	PAR periodicity differs in tf & lf	Bradbury et al., 1983
<u>Woloszynskia</u> <u>micra</u> = <u>Gymnodinium</u> <u>micrum</u>	pm in tf pm in lf	Leadbeater & Dodge, 1967
Class Peridinales		
<u>Peridinium</u> <u>cinctum</u>	lf tf - accessory strand?	Berdach, 1977
<u>Peridinium</u> <u>cinctum</u> fa. <u>westii</u>	lf	Berman & Roth, 1979

- * The morphologically similar PAR structure in dinoflagellates is called "packing material" (pm) or "cordon spirale"; this structure is morphologically different from either the striated strand or the "R-fibre" (banded structures) which is found in the flagella of many dinoflagellates.

Table 3. Occurrence of the PAR Structure in Bodonids and Trypanosomatids (the Kinetoplastidae). * ** ***

Organism	Information	Reference
Lower Bodonids		
<u>Bodo caudatus</u>	"une fibre paraxiale" in both flagella	Brugerolle et al., 1979
<u>Bodo saltans</u>	"intraflagellar structure" in both flagella	Vickerman, 1962 Pitelka, 1963
<u>Rhynchomonas nasuta</u>	in both flagella	Swale, 1973
<u>Rhynchomonas metabolita</u>	in both flagella	Burzell, 1973
<u>Rhynchomonas agilis</u> (=Rhynchobodo)	in trailing flagellum; probably absent in anterior flagellum	Burzell, 1973
Advanced Bodonids		
<u>Cryptobia heliciis</u>	"une fibre paraxiale" in both flagella	Brugerolle et al., 1979 Pitelka, 1963
<u>Cryptobia intestinalis</u>	"une fibre paraxiale" in both flagella	Brugerolle et al., 1979
<u>Cryptobia vaginalis</u>	in both flagella but absent in tip(s)	Vickerman, 1977
<u>Trypanophis brobbeni</u>	vestigial	Cachon et al., 1972 Vickerman & Preston, 1976
<u>Trypanoplasma borreli</u>	"une fibre paraxiale" in both flagella	Brugerolle et al., 1979
Trypanosomatids		
<u>Blastocrithidia culicis</u>	absent	Walkosz, 1982
<u>Crithidia deanei</u>	probably absent	McGhee & Cosgrove, 1980 De Souza & Souto- Padron, 1980

Table 3 (continued)

Organism	Information	Reference
<u>Crithidia fasciculata</u>	poorly developed	Vickerman & Preston, 1976 Pereira et al., 1977 Walkosz, 1982 Russell et al., 1983
<u>Crithidia harmona</u>	+	De Souza & Souto- Padron, 1982
<u>Crithidia oncopelti</u>	absent	Vickerman & Preston, 1976 De Souza & Souto-Padron, 1980 McGhee & Cosgrove, 1980 Freymuller & Camargo, 1981 Walkosz, 1982
<u>Herpetomonas ampelophilae</u>	well-developed in single flagellum of gut flagellates; PAR missing in promastigotes	Rowton et al., 1981
<u>Herpetomonas megaseliae</u>	well-developed	De Souza & Souto- Padron, 1980
<u>Herpetomonas muscarum</u>	+	De Souza & Souto- Padron, 1980 Walkosz, 1982
<u>Herpetomonas samuelpessoai</u>	+	De Souza & Souto-Padron, 1980
<u>Leishmania tarentolae</u>	+	Pereira et al., 1977
<u>Leptomonas samueli</u>	+	De Souza & Souto-Padron, 1980
<u>Phytomonas davidi</u>	well-developed in single flagellum	Attias & De Souza, 1985 Postell & McGhee, 1981
<u>Trypanosoma avium</u>	+	Baker & Bird, 1968

Table 3 (continued)

Organism	Information	Reference
<u>Trypanosoma brucei</u>	+	Vickerman, 1962 Fuge, 1969 Hogan & Patton, 1976
<u>Trypanosoma congolense</u>	+	Vickerman, 1969 Taylor & Godfrey, 1969
<u>Trypanosoma cruzi</u>	++	Sanabria, 1966 Pereira et al., 1977 Souto-Padron & De Souza, 1978 De Souza & Souto-Padron, 1980
<u>Trypanosoma cyclops</u>	well developed, Heywood et al., 1974 18-19.5 nm spacing between parallel longitudinal filaments	
<u>Trypanosoma danilewskyi</u>	+	Lumsden & Evans, 1979
<u>Trypanosoma evansi</u>	+	Vickerman, 1969
<u>Trypanosoma gambiense</u>	+	Boisson et al., 1965
<u>Trypanosoma lewisi</u>	+	Anderson & Ellis, 1965 Vickerman, 1969
<u>Trypanosoma rhodesiense</u>	+	Vickerman, 1969
<u>Trypanosoma vivax</u>	+	Taylor & Godfrey, 1969

* The anterior flagellum of bodonids is homologous to the single flagellum of trypanosomatids (Vickerman & Preston, 1976) (My comment: based upon PAR structure, the trailing flagellum of bodonids is homologous to the single flagellum of trypanosomatids).

** In Blastocrithidia and Trypanosoma, the flagellum is attached to the cell body for part or all of its length.

*** The PAR is also called "intraflagellar structure" in trypanosomes and bodonids and sometimes "une fibre paraxiale" in bodonids.

Table 3 (continued)

- + Occurrence of the PAR structure is reported but no further information is given
- ++ The PAR appears in epi- and trypomastigote forms as the spheromastigote forms and the flagellum elongates; the PAR is absent in the spheromastigote (also called amastigote) stage.

Table 4. Comparative Molecular Weight Determinations of the Protein Constituents of the Paraxial Rods in Euglenoids and Kinetoplastids.

Species	Molecular Weights	Reference
Euglenoids		
<u>Euglena gracilis</u>	80 kD	Hyams, 1982
	69 kD	Hyams, 1982
	76 kD	Gallo & Schrével, 1985
	67 kD	Gallo & Schrével, 1985
Kinetoplastids		
<u>Trypanosoma brucei</u>	75 kD	Gallo & Schrével, 1985
	72 kD	Gallo & Schrével, 1985
	180 kD	Schneider et al., 1988
	200 kD	Schneider et al., 1988
<u>Herpetomonas megaseliae</u>	78 kD	Cunha et al., 1984
	73 kD	Cunha et al., 1984
<u>Herpetomonas mus.</u>	71 kD	Gallo & Schrével, 1985
	65 kD	Gallo & Schrével, 1985
<u>Crithidia fasciculata</u>	76 kD	Russell et al., 1983
	68 kD	Russell et al., 1983
	71 kD	Walkosz, 1982
<u>Crithidia oncopelti</u> (no apparent PAR)	87 kD	Gallo & Schrével, 1985

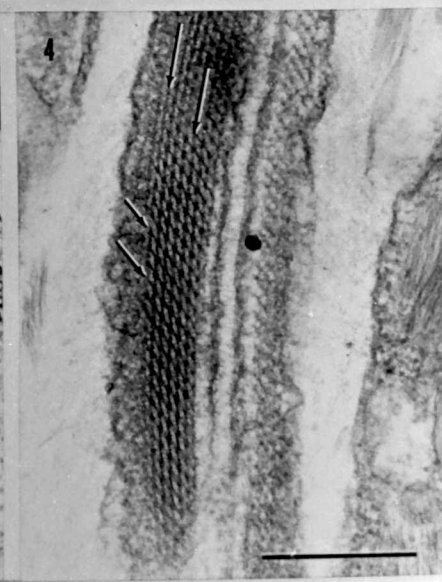
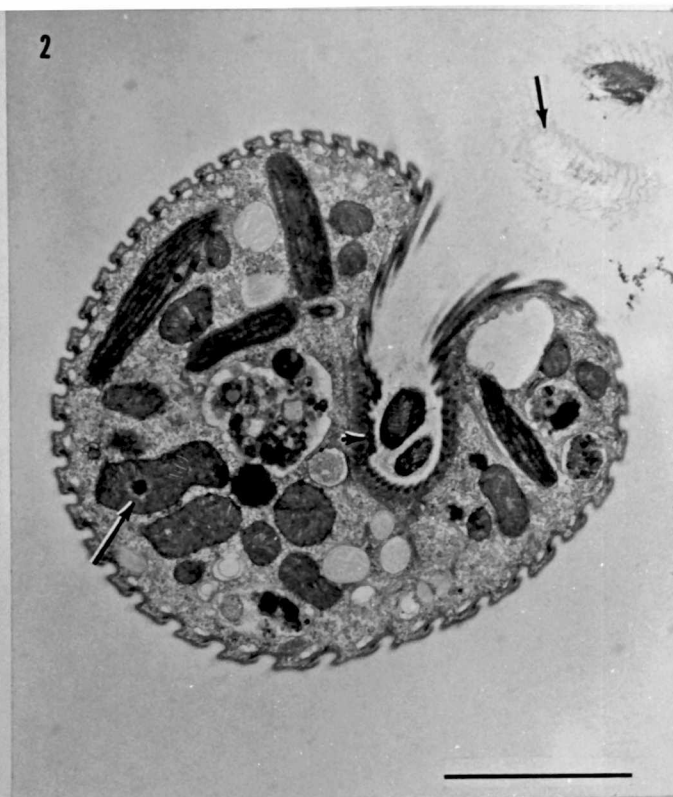
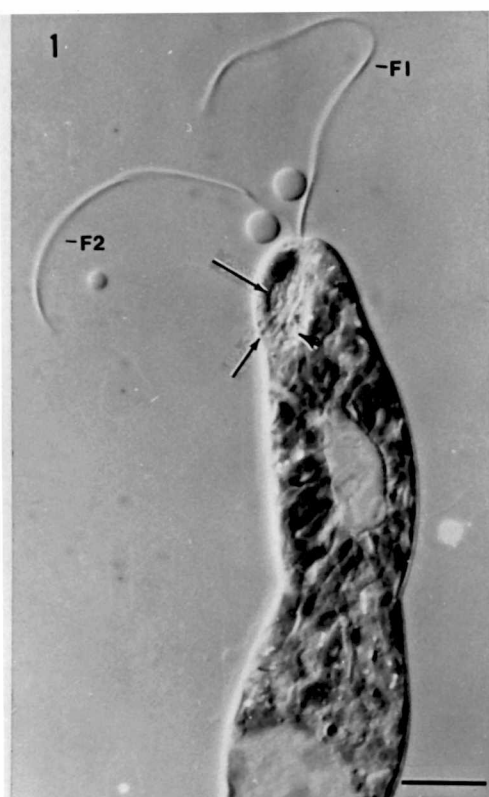
Plate 1. Comparison of the Known Structural Features of the PAR
in Representative Euglenoids, Kinetoplastids and Trypanosomatids.

Figure 1. LM image of Eutreptia pertyi. Anteriorly directed dorsal (F1) and laterally directed ventral (F2) flagella originate beneath dorsal reservoir wall (short arrow) near stigma (long arrow) and contractile vacuole (arrowhead) and emerge through subapical canal opening. LM. Bar = 10 μ m.

Figure 2. Glancing profiles of flagella in canal and external to cell showing short (short arrow) and long (thick arrow) mastigonemes. Note canal ridge (arrowhead) next to F2 and unusual electron dense inclusion (long arrow) in mitochondrion. TEM. Bar = 0.1 μ m.

Figure 3. Transverse section through reservoir-canal transition region showing substructure of F1 and F2. Lattice-like filaments that form an electron-dense region around a less electron-dense core constitute PAR1; fine fibrils connect PAR1 filaments to axonemal doublet microtubules. Perpendicular to the long axis of F2, multiple parallel "plates" (long arrows), interconnected by fine filaments, form PAR2. Note consistent spatial apposition of PARs within the canal (cf. Figures 4-7, 10, 19, 25-28). TEM. Bar = 0.5 μ m.

Figure. 4. Longitudinal section through PARs in canal. PAR2 "plates" and interlacing fine filaments are shown in plane of section parallel to the long axis of Ax1 and Ax2. Nine PAR2 "plates" are shown "edge-on." Each plate consists of longitudinal filaments (long arrows), $\sim$ 6 nm in diameter, and horizontal filaments shown "end-on" and visualized as electron-dense "spots," spaced $\sim$ 30 nm apart along the long axis of the plate. Oblique filaments (short arrows), 4-6 nm in diameter and oriented at $\sim$ 40 $^{\circ}$ to the long axis of the longitudinal filaments, regularly intersect the "plates" at the electron-dense "spots" (i.e., the horizontal filaments). The periodic intersection of the longitudinal and oblique filaments at the junction sites forms the repetitive diamond-shaped pattern characteristic of the paracrystalline substructure of PAR2. Note the similar size, inclination and periodicity of oblique filaments in PAR1 and PAR2. TEM. Bar = 0.5 μ m.



Figures 5-7. Series of transverse sections through F1 and F2 flagella near base of reservoir showing progressive development of PARs.

Figure 5. At level of flagellar emergence into the reservoir, no paraxial rod material is present in either flagellum. Note dorsal microtubular root (arrowhead) near F1 axoneme and ventral microtubular root (arrow) near F2 axoneme. TEM. Bar = 0.5 μ m.

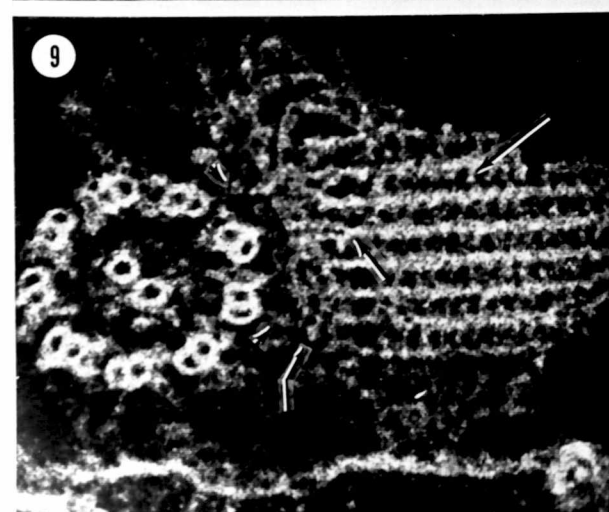
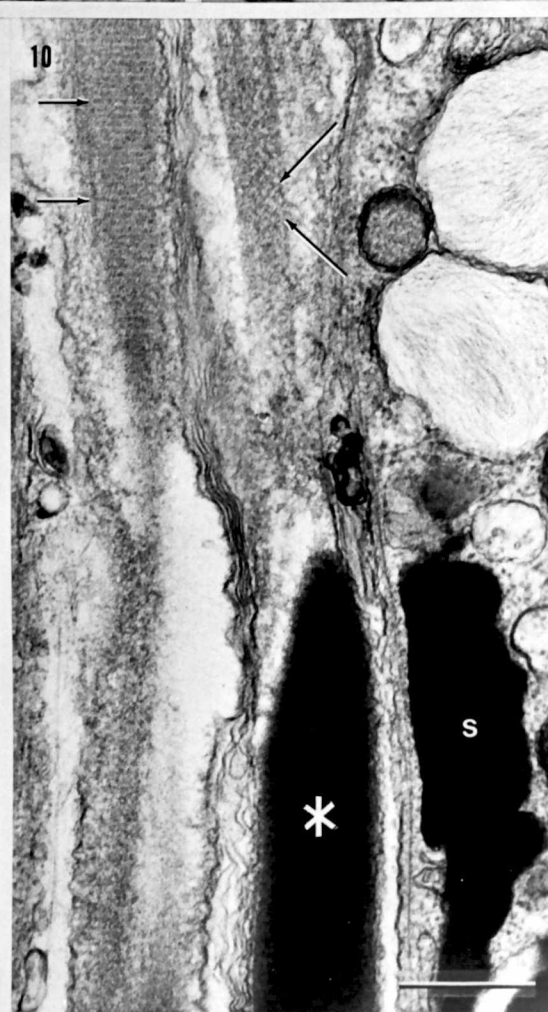
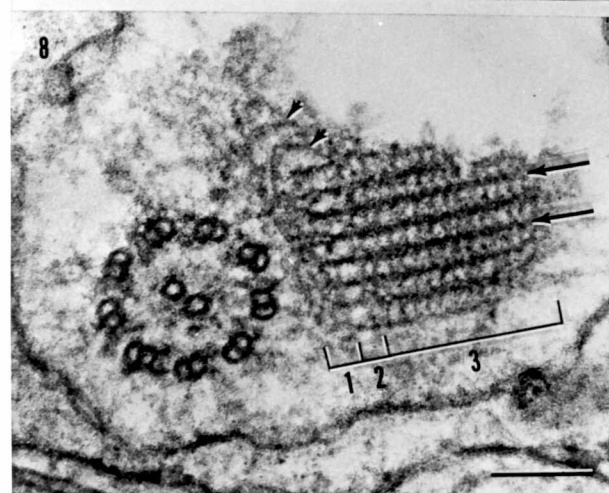
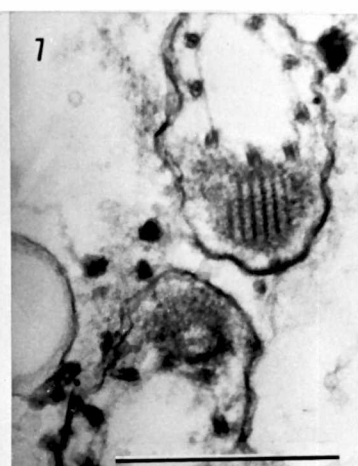
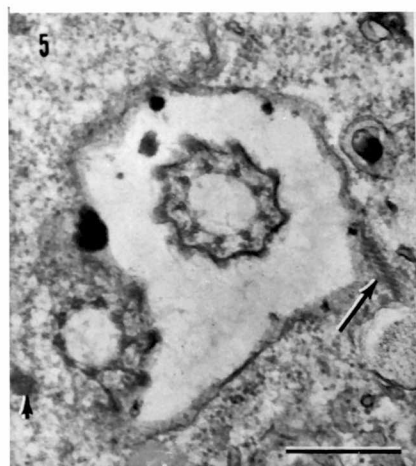
Figure 6. Origin of PARs adjacent to a peripheral microtubular doublet in each "9 + 0" axoneme. "Plate-like" substructure (arrow) is discernable in PAR₂. Note apposing orientation of the PARs. TEM. Bar = 0.5 μ m.

Figure 7. Section distal to Fig. 6 and proximal to origin of the central pair of axonemal microtubules. Well-defined "plate" organization in PAR₂ and ill-defined electron-dense material in PAR₁. TEM. Bar = 0.5 μ m.

Figure 8. Higher-magnification image of portion of Figure 3 showing three major structural domains of PAR₂. Proximal "plate" zone (1), ~48 nm wide, nearest axonemal doublets; intermediate electrontransparent zone (2), ~22 nm wide; and extensive distal "plate" zone (3), ~200 nm wide. Eight well-defined "plates" (arrows), spaced ~25 nm apart, and 3 less organized "plates" (arrowheads), arranged parallel to one another and perpendicular to longitudinal axis of Ax₂, form the irregularly shaped PAR₂ cylinder, ~270 nm wide. Ax₂ is 180 nm wide. TEM. Bar = 0.1 μ m.

Figure 9. Computer-assisted, digital-image processing of Figure 8 enhances microarchitecture of PAR₂. PAR₂ shows a) interconnection of "plates" in distal zone by fine filaments (long arrow), spaced ~13 nm apart along the horizontal axis of the plate (there are no interlacing filaments in the intermediate zone); b) presumed bifurcation or reorientation (arrowhead) of "plate" subcomponents in intermediate and proximal zones; c) obliquity (bent arrow) of "plate" subcomponents in proximal zone and d) interconnections (arrowheads) of PAR₂ subcomponents with peripheral axonemal doublet microtubules. Background subtraction and gray-value scaling of original digitized image. Negative image. Perceptics 9200 Digital Image Processing System.

Figure 10. Longitudinal section of PARs in plane perpendicular to that shown in Figure 4. Horizontal filaments (short arrows) of PAR₂ are shown in slightly oblique section. Lattice-like oblique filaments (long arrows) of PAR₁ are oriented at $\sim \pm 45^\circ$ to the long axis of F1. PFB (asterisk) on F1 is apposed to cytoplasmic stigma (S). TEM. Bar = 0.5 μ m.



Figures 11-17. Oblique sections of the PARs.

Figure 11. F1 and F2 at level of PFB (asterisk) in canal. The horizontal filaments (short arrows) of ~ 9 PAR2 plates, spaced ~33 nm apart, are oriented in line with the PFB on F1. Less prominent horizontal filaments (long arrows) of similar size, spacing and orientation to the axoneme, are evident in PAR1. TEM. Bar = 0.5 μ m.

Figure 12. Substructure of PARs in flagella at level of PFB. Very fine filaments (arrow), ~1-2 nm in diameter and spaced ~12 nm apart, traverse the PAR2 plates diagonally at ~60° to the horizontal axis of the plates and are aligned with one of the lattice planes in the PFB (see lightened disc). Note interconnections (arrowheads) between Ax2 and F2 membrane. TEM. Bar = 0.5 μ m.

Figure 13. Horizontal filaments of PAR1 (short arrows) and PAR2 (long arrows) are spaced ~31 nm apart. TEM. Bar = 0.5 μ m.

Figure 14. PAR2 in ventral flagellum. Individual horizontal filaments, spaced ~30 nm apart and not visualized in the intermediate plate zone, show staggered arrangement (arrows) between distal and proximal plate zones. TEM. Bar = 0.1 μ m.

Figure 15. Plane of section through PAR2 perpendicular to that shown in Figure 14. Horizontal filaments (thick arrows), with ~30 nm periodicity, are intersected at 90° by oblique filaments (thin arrows), with ~13 nm periodicity, in the distal and proximal plate zones. TEM. Bar = 0.1 μ m.

Figure 16. Weft of oblique and horizontal filaments in distal plate zone of PAR2. TEM. Bar = 0.1 μ m.

Figure 17. PAR2 of ventral flagellum showing intersection of subcomponent filaments. Slightly different plane of section from Figure 13 shows junction sites (electron-dense "dots," arrow) of the oblique and longitudinal filaments, spaced every ~16 nm along individual horizontal filaments in the distal plate zone of PAR2. TEM. Bar = 0.1 μ m.

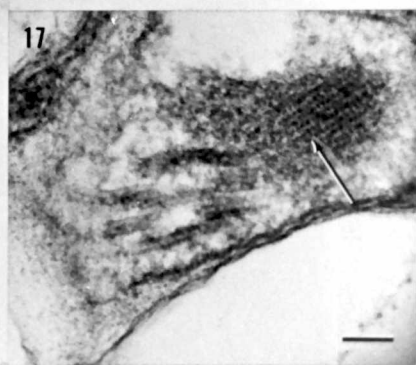
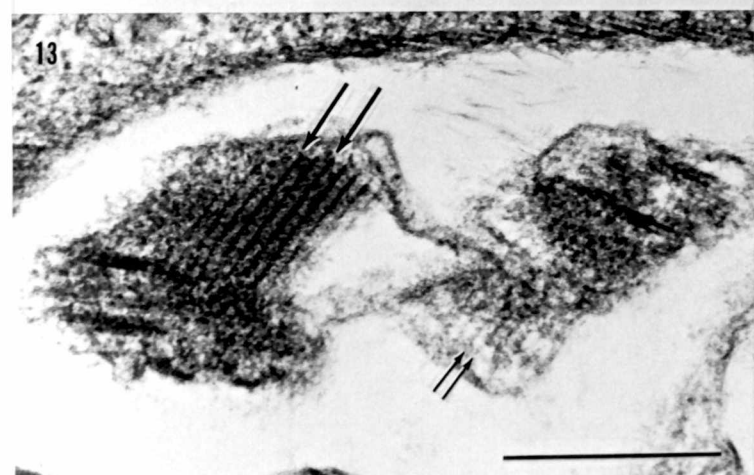
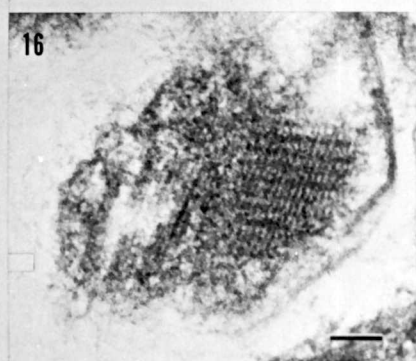
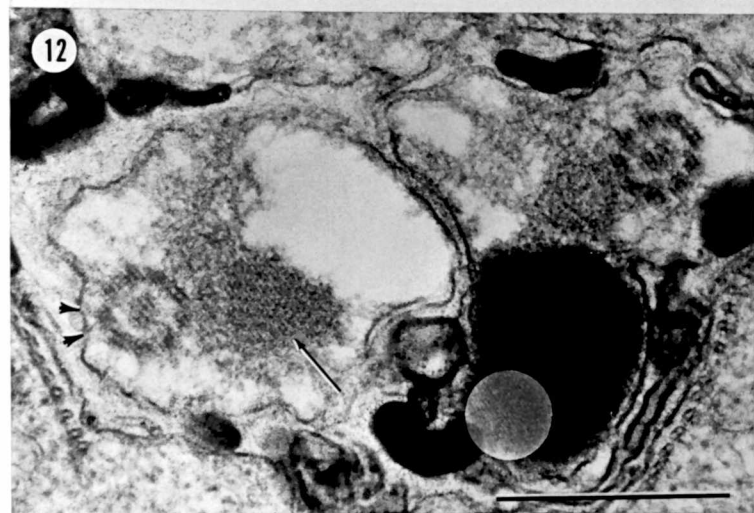
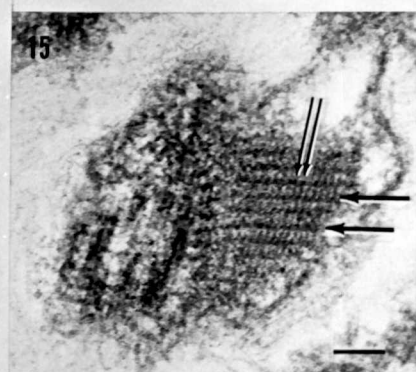
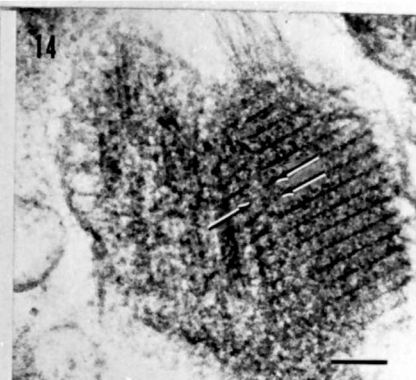
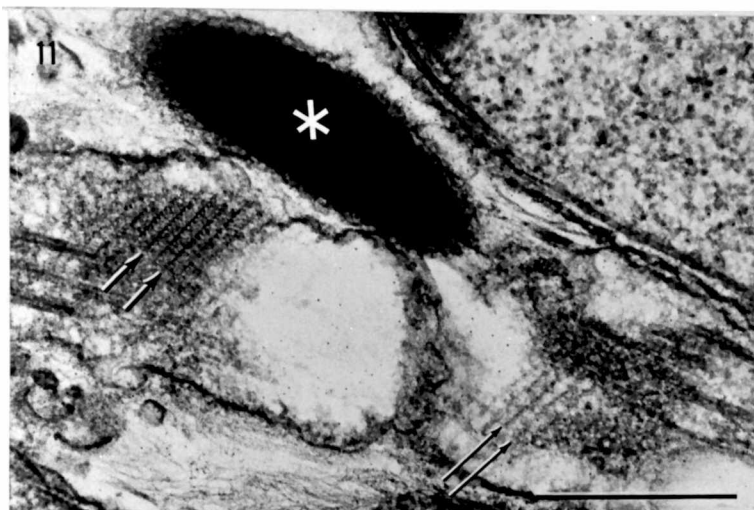


Figure 18. Slightly oblique transverse section through PARs in canal. Horizontal filaments (long arrows) of several plates are spaced ~ 25 nm apart, and obliquely oriented filaments (short arrows) extend to the flagellar membrane. TEM. Bar = $0.5 \mu\text{m}$.

Figures 19-24. Longitudinal views of PARs.

Figure 19. Higher-magnification image of PARs shown in Figure 10. Horizontal filaments (short arrows), spaced ~ 25 nm apart, appear as "rungs on a ladder" and are intersected by longitudinal (long arrow) and oblique (arrowheads) filaments that collectively impart a herringbone appearance to the PAR2 plate viewed "face-on." Change of direction of some horizontal filaments (intersecting arrows) is evident in the intermediate plate zone of PAR2. Note longitudinal filaments and lattice arrangement of oblique filaments ($\pm 45^\circ$ to long axis of F1) in PAR1, $\sim 0.14 \mu\text{m}$ wide (cf. Figures 27-28). TEM. Bar = $0.1 \mu\text{m}$.

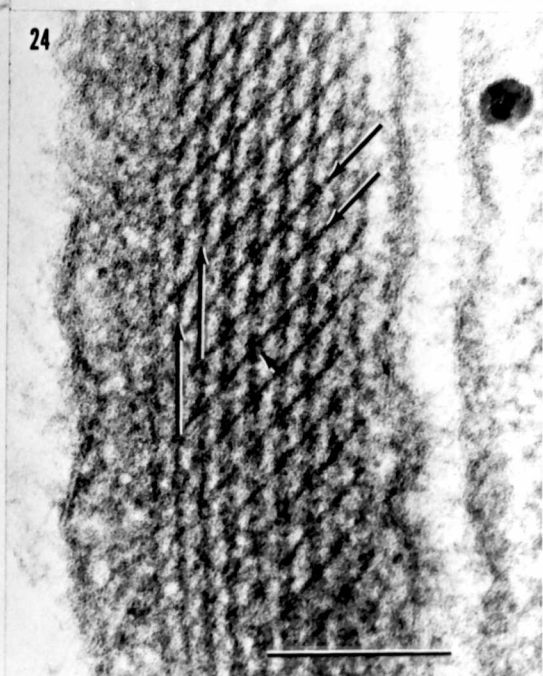
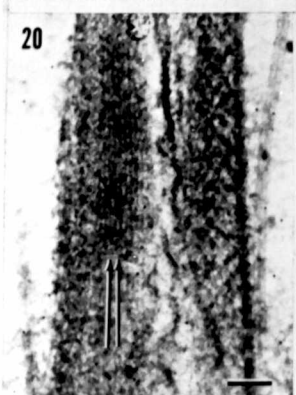
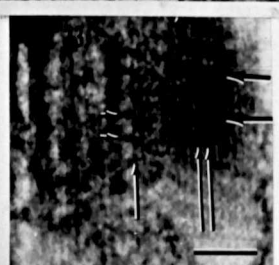
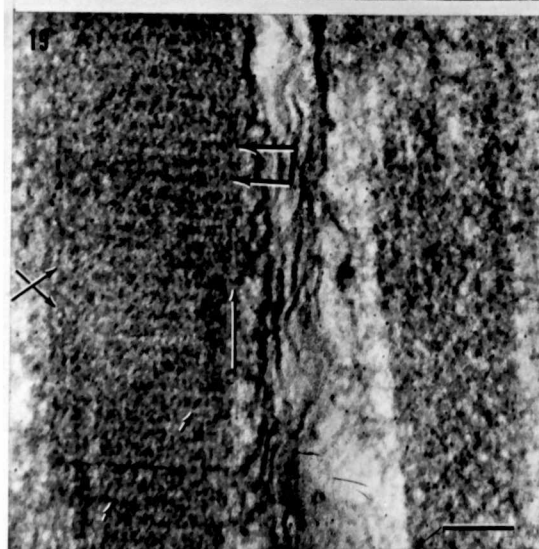
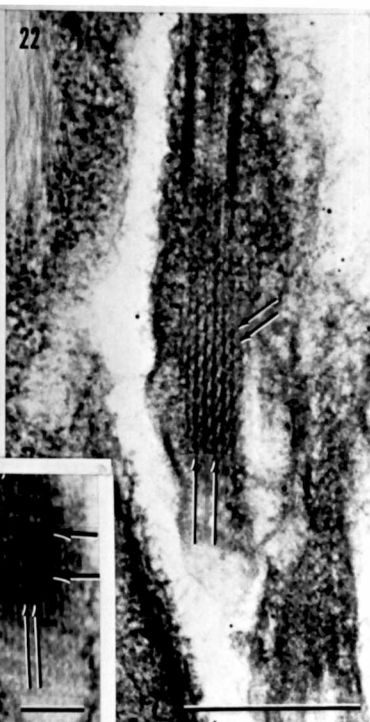
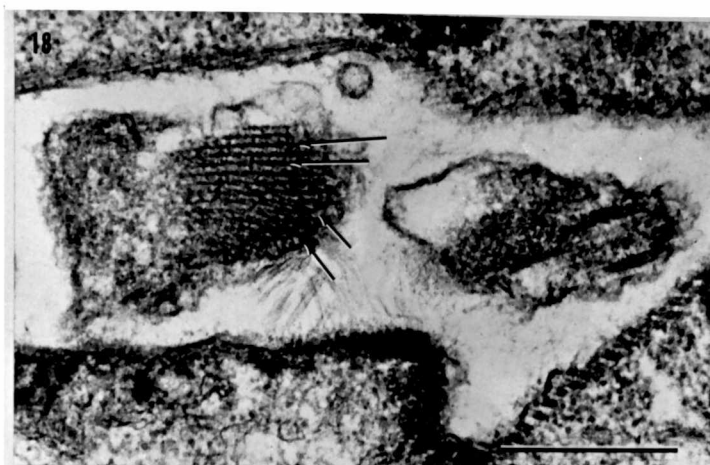
Figure 20. Longitudinal filaments, spaced ~ 12.5 nm apart, in a single PAR2 plate, viewed "face-on." Lattice-like arrangement of oblique filaments is evident in PAR1. TEM. Bar = $0.1 \mu\text{m}$.

Figure 21. Section of PAR2 perpendicular to that shown in Figure 20. Longitudinal filaments (arrows) of ~ 6 plates are spaced ~ 25 nm apart. TEM. Bar = $0.1 \mu\text{m}$.

Figure 22. Section through PAR2 parallel to long axis of Ax2. Periodic intersection of longitudinal filaments (long arrows), spaced ~ 27 nm apart, and oblique filaments (short arrows), spaced 35 nm apart and inclined at $\sim 45^\circ$, forms a repetitive diamondshaped pattern. TEM. Bar = $0.5 \mu\text{m}$.

Figure 23. Section showing bridge-like filaments between Ax2 and PAR2. Filaments are 10 nm in diameter and form periodic connections at 38.5 nm intervals (arrowheads) and maintain a 20 nm space between longitudinal filaments (single thin arrow) of the proximal plate zone and a peripheral axonemal doublet. A weft is formed in the distal plate zone by the intersection of longitudinal filaments (double thin arrows), spaced 12.5 nm apart, and slightly inclined ($\sim 95^\circ$) horizontal filaments (thick arrows), spaced 35 nm apart. TEM. Bar = $0.1 \mu\text{m}$.

Figure 24. Higher-magnification image of portion of PAR2 shown in Figure 4. Section through PAR2 plates shows diamond-shaped pattern formed by intersection of longitudinal filaments (long arrows), spaced ~ 25 nm apart, and oblique filaments (short arrows), spaced ~ 30 nm apart, at electron-dense juncture sites (arrowhead) (i.e., horizontal filaments seen "end-on"). A $\sim 90^\circ$ is formed by the reciprocal axes of the horizontal filaments (spatially aligned at $\sim -45/-50^\circ$ to the vertical axis) and oblique filaments (oriented at $+45^\circ$ to the vertical axis). TEM. Bar = $0.1 \mu\text{m}$.



Figures 25-28. Stereo images of PAR2 in longitudinal section parallel to the long axis of the axoneme, at -10° , 0° , $+10^\circ$ and $+25^\circ$, respectively. (PAR images are the same as those shown in Figures 4 & 24). Tilting is primarily around the longitudinal axis, with slight tilting also from flagellar base to tip. TEM. Bar = $0.5\ \mu\text{m}$.

Figure 25. Plane of tilt at -10° showing faint meshwork of horizontal and oblique filaments in PAR2. Fine filaments (short arrows), spaced $\sim 25\ \text{nm}$ apart, are inclined at $\sim 100^\circ$ to the long axis of F2. Oblique filaments (long arrows) are spaced $\sim 30\ \text{nm}$ apart. The image generated from this plane of tilt is similar to that of PAR2 in Figures 10 & 19.

Figure 26. Plane of tilt at 0° showing regular arrangement of PAR2 subcomponents. Fine filaments (small arrows) extend to flagellar membrane. Longitudinal filaments (long arrow) are spaced $\sim 25\ \text{nm}$ apart. Adjacent oblique filaments, spaced $\sim 32\ \text{nm}$ apart, appear to extend to opposite sides of the F2 membrane, such that along one side of the PAR, the ends of the filaments are staggered (arrowheads). Note the slight difference in spacing between oblique filaments of the PAR2 mid-region compared to their spacing nearer the flagellar membrane, a result suggesting that there is slight convex curvature and/or declination of the oblique filaments at the F2 membrane.

Figure 27. Plane of tilt at $+10^\circ$ showing paracrystalline arrangement of PAR2 subcomponents. Longitudinal filaments (long arrow), spaced $\sim 25\ \text{nm}$ apart, follow the longitudinal curvature of F2. Oblique filaments (short arrows), spaced $\sim 30\ \text{nm}$ apart and oriented at $\sim -45^\circ$ to the long axis of F2, are declined toward F1 and inclined in the opposite direction toward the reservoir wall.

Figure 28. Plane of tilt at $+25^\circ$ showing spatial alignment of electron-dense junction sites. These electron-dense junctions (i.e., horizontal filaments viewed "end-on") form a diagonal plane (thick arrows) at $\sim +45^\circ$ to the long axis and oblique filaments (thin arrows) oriented at $\sim -45^\circ$ to the long axis of F2.

Figure 29. High-magnification image of PAR2 (cf. Figures 4, 24, & 27). Oblique filaments (short arrow), spaced $\sim 30\ \text{nm}$ apart and oriented at $\sim -45^\circ$ to the long axis of the flagellum are $\sim 4\text{-}6\ \text{nm}$ in the widest dimension and appear to be a twist of two filaments. Longitudinal filaments (long arrow), $\sim 5\text{-}6\ \text{nm}$ in diameter, are spaced $\sim 25\ \text{nm}$ apart and oriented at 0° to the long axis of the flagellum. Horizontal filaments (visualized "end-on" as electron-dense spots) (arrowheads), $\sim 8\text{-}10\ \text{nm}$ wide and $\sim 18\ \text{nm}$ long, are spaced $\sim 30\ \text{nm}$ apart along the vertical axis of the longitudinal filaments. Note the very fine filaments (curved arrow) that occasionally span the diamond-shaped spaces. TEM. Bar = $0.1\ \mu\text{m}$.

Figure 30. Computer-generated image of PAR2 emphasizes the diamond-shaped subunits (in "relief") and the longitudinal and oblique filaments ("recessed") that form the PAR microarchitecture. Spatial domain, digital image processing employing edge mask algorithms of an original PAR2 image similar to Figure 29. Perceptics 9200 Digital Image Processing System.

Figure 31. FFT power spectrum of PAR2, generated from an original image similar to Figure 29. There are four major frequency vectors, $F-45^\circ$, $F-20^\circ$, $F0^\circ$ and $F+50^\circ$ with respect to the vertical axis. The dots along each frequency vector correspond to the repetitious information (first order frequency information nearest the origin, and higher order frequency information further away from the origin) contained within the PAR2 microstructure (i.e., oblique filaments inclined in -40° axes, longitudinal filaments in vertical (0°) axes, primary alignment of horizontal filaments (presented "end-on") in $+45^\circ$ axes, and secondary alignment of the horizontal filaments (seen "end-on") in -20° axes (see aligned arrowheads, Fig. 29). Fast Fourier Transform frequency domain analysis using Perceptics 9200 Digital Image Processing System.

Figure 32. Computer-generated image of PAR2, resulting from selective addition of the frequency information in the $F0^\circ$ and $F-50^\circ$ vectors of the FFT power spectrum (see Figure 31). This image suggests that oblique filaments (arrows) are probably a twist of two filaments (see Fig. 29) and "weave" over and under or intertwine with the horizontal and longitudinal filaments of adjacent plates. Inverse transformed image. Perceptics 9200 Digital Image Processing System.

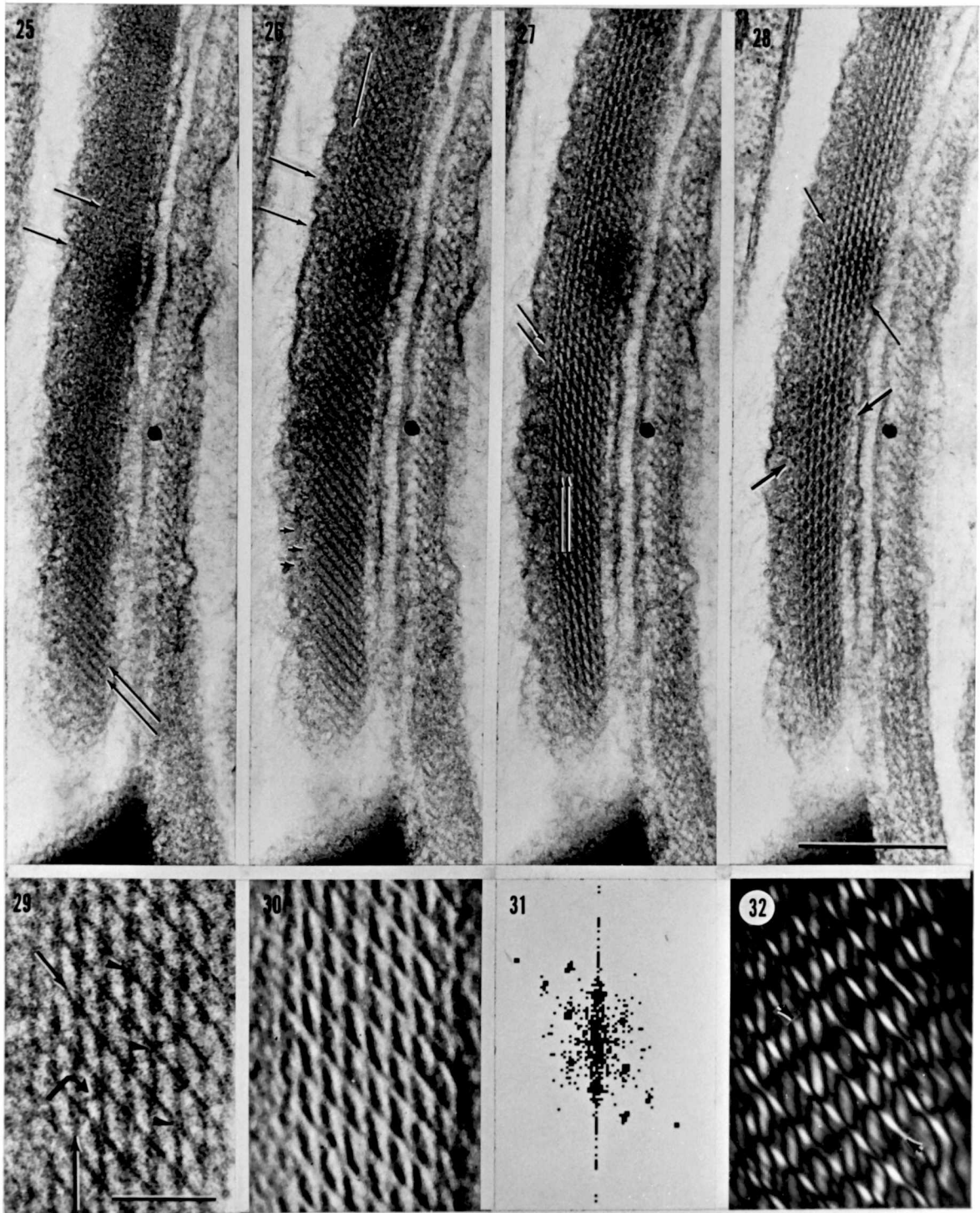


Figure 33. Collage of F2 in longitudinal thick section showing spatial relationship of flagellar mastigonemes to Ax2 and PAR2 substructural components. Bundles of short non-tubular mastigonemes (short arrows) (~ 0.25 - $0.28 \mu\text{m}$ in length and vertically spaced 86 nm apart) originate beneath the flagellar membrane (arrowhead) in dome-shaped projections, which when laterally aligned (double arrowheads) form a single centrifugal tier; vertically aligned tiers form a continuous, multi-tiered mat on the PAR side of the flagellum (seen in middle and lower portions of the micrograph). Bundles of long non-tubular mastigonemes (long arrows) ($> 0.83 \mu\text{m}$ in length in this image) are arranged in tiers, spaced 88 - 91 nm apart, and extend from the Ax2 side of the flagellum (seen in top portion of micrograph). TEM. Bar = $0.5 \mu\text{m}$.

Figure 34. Computer-generated image showing the microarchitectural "skeleton" of PAR2. A weft of filaments (intersecting arrows), oriented at 0° and $\pm 50/55^\circ$ to the long axis of PAR2, is shown along with tiered attachment sites (long arrow) for the short mastigonemes. Spatial domain processing employing background subtraction of the original image (part of the image shown in Figure 33). Perceptics 9200 Digital Image Processing System.

Figure 35. Longitudinal section through F1 showing short and long non-tubular mastigonemes extending from the flagellum. Short mastigonemes are arranged in two separate sheaths. Bundles of short mastigonemes that extend from small projections (small arrowheads) on the PAR1 side of the flagellum, are $\sim 0.25 \mu\text{m}$ in length, have a felt-like appearance and are arranged in tiers (thin arrows), spaced 86 nm apart and inclined at $\sim 75^\circ$ to the long axis of the flagellum. The bundles of short mastigonemes that extend from prominent protuberances (large arrowheads) on the Ax1 side are $\sim 0.125 \mu\text{m}$ in length, have a straight appearance and are arranged in tiers, spaced $\sim 97 \text{ nm}$ apart. Long mastigonemes (thick arrow) ($> 0.82 \mu\text{m}$ in length in this image) arise between the two short mastigoneme sheaths. TEM. Bar = $0.5 \mu\text{m}$.

Figure 36. High-magnification image of F1 showing bundles of short mastigonemes extending from the membrane. Each bundle is composed of ~ 5 - 10 short mastigonemes. TEM. Bar = $0.1 \mu\text{m}$.

Figure 37. Thick section of PAR2 showing spatial relationship between PAR2 subcomponents and tiers of short mastigonemes. Longitudinal filaments (long arrows) of PAR2 are spaced $\sim 25 \text{ nm}$ apart. Slightly curved oblique filaments (short arrows) (spaced $\sim 28 \text{ nm}$ apart and oriented at $\sim 50^\circ$ to the long axis of PAR2) and thick horizontal filaments (large arrowheads) (seen tangentially) appear to converge at the F2 membrane. Bundles of short mastigonemes, extend from dome-shaped projections (small arrowheads), spaced 76 nm apart, along each side of PAR2. TEM. Bar = $0.1 \mu\text{m}$.

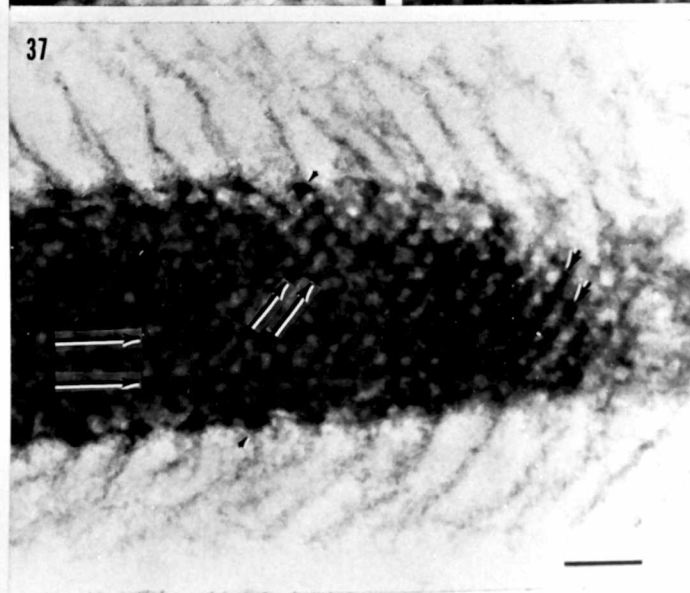
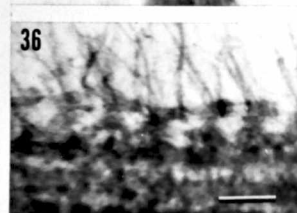
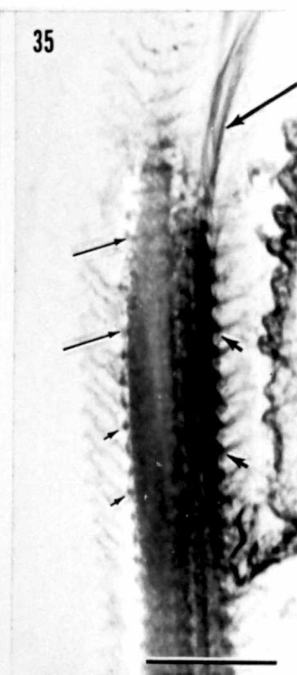
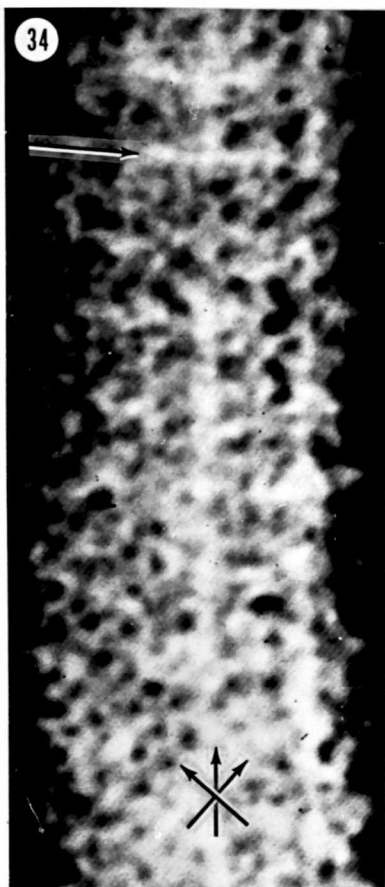
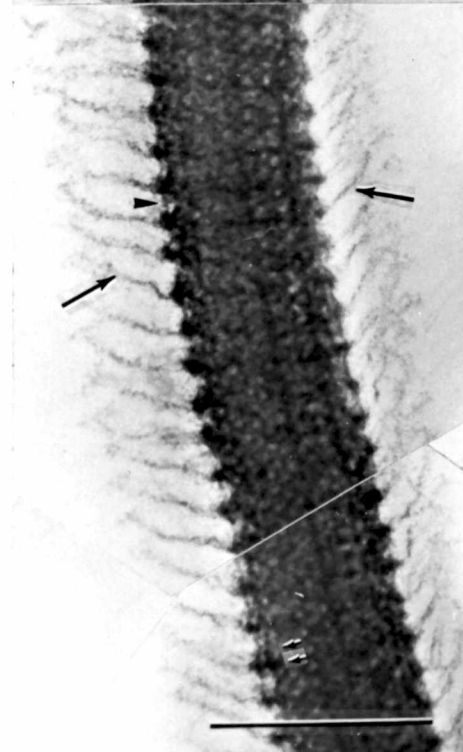
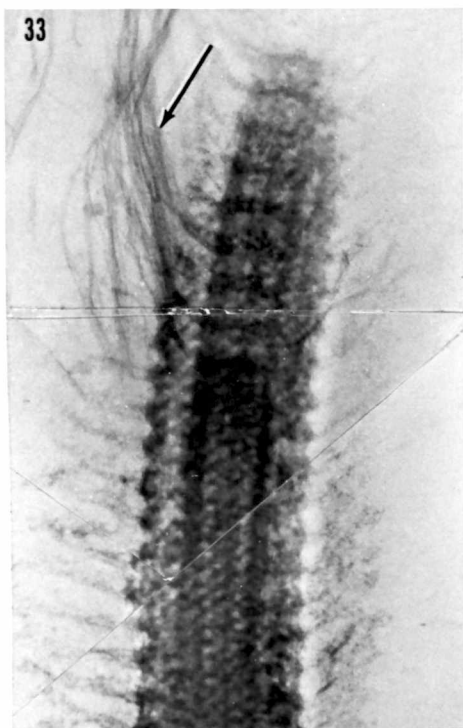


Figure 38. Surface features of F1. A unilateral array of bundles of long mastigonemes (large arrow); short mastigonemes, arranged in tiers, spaced 115 nm apart, form a sheath, 1.3 μm wide; individual short mastigonemes (short arrows) of a single tier are spaced 47 nm apart. Shadowcast. Bar = 1 μm .

Figure 39. Surface features of F2, $\sim 1 - 1.5 \mu\text{m}$ wide. Oblique filaments (long arrows) on the PAR2 side of the flagellum have a periodicity of $\sim 46 \text{ nm}$ and are oriented at $\sim 60^\circ$ to the long axis of F2; globular projections (arrowheads) on the Ax2 side of the flagellum have a periodicity of $\sim 120 \text{ nm}$. A splayed mat of short mastigonemes (asterisk), arranged in tiers with a periodicity of $\sim 110 \text{ nm}$, overlies the PAR2 ($\sim 0.33 \mu\text{m}$ wide) and Ax2 sides of the flagellum. Shadowcast. Bar = 1 μm .

Figure 40. Bundles of long mastigonemes (arrow) extend unilaterally from F1, subjacent to a splayed mat of short mastigonemes (asterisk) ($\sim 0.33 \mu\text{m}$ wide), and appear to originate from globular projections (white arrows), spaced 110 nm apart along the membrane. Shadowcast. Bar = 0.5 μm .

Figure 41. Detached, demembrated F2, $\sim 2 \mu\text{m}$ wide x 95 μm long. Long mastigonemes (long arrows), $\sim 5.5 \mu\text{m}$ in length, emanate from a strand (Ax2), $\sim 0.22 \mu\text{m}$ wide. A mat of short mastigonemes (short arrows), $\sim 1.5 \mu\text{m}$ wide, extends from an adjacent "strand" (PAR2), $\sim 0.29 \mu\text{m}$ wide. Note increased diameter (0.22 to 0.43 μm) of PAR2 toward flagellar tip and presumable twisting (arrowheads) of axonemal and PAR2 strands along the flagellar length. Negative stain. Bar = 5 μm .

Figure 42. Negative stain image of tip of F2. Image shows Ax2 microtubules (arrow) and parallel arrangement of PAR2 subcomponents (presumably the horizontal filaments) (arrowheads), spaced 22 nm apart and oriented perpendicularly to the long flagellar axis. Bar = 0.5 μm .

Figure 43. Tangential thick section through F2 showing longitudinal filaments (long arrows) of several PAR2 plates and bundles of short mastigonemes extending from projections at the flagellar membrane (short arrow). Lateral alignment of adjacent dome-shaped projections (arrowheads), spaced 25 nm apart, forms a single tier of short mastigonemes; vertical alignment of adjacent tiers, spaced 82 nm apart, forms a continuous mat of short mastigonemes along the PAR2 side of F2. TEM. Bar = 0.5 μm .

Figure 44. Longitudinal thick section of PAR2, $\sim 0.33 \mu\text{m}$ wide. Tiers (arrows) of short mastigonemes extending from dome-shaped projections, spaced 83 nm apart, along both sides of PAR2. Struts (arrowheads) within the dome-shaped projections appear to be extensions of some of the PAR2 subcomponents. TEM. Bar = 0.5 μm .

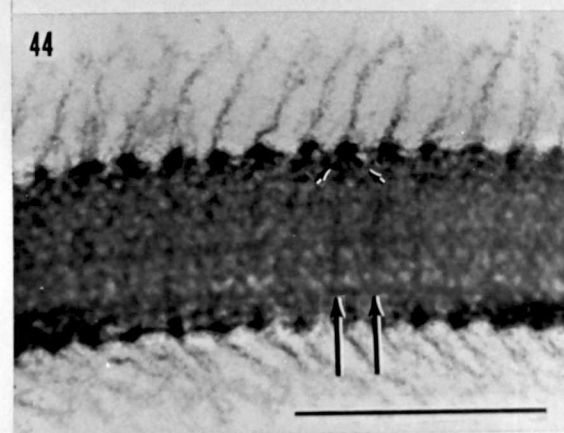
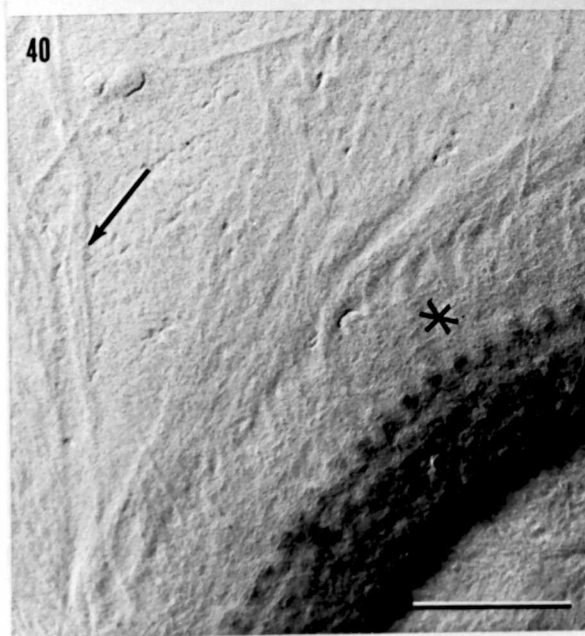
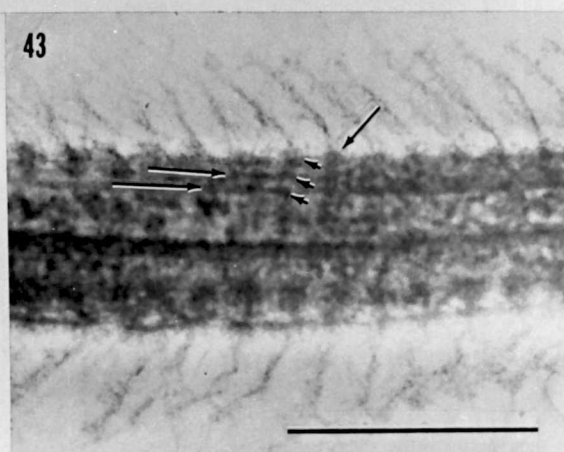
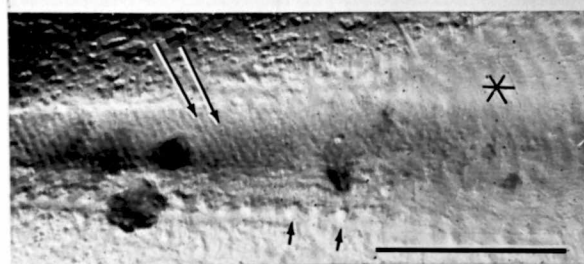
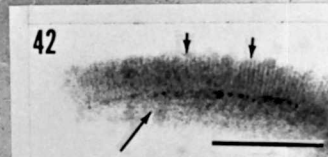
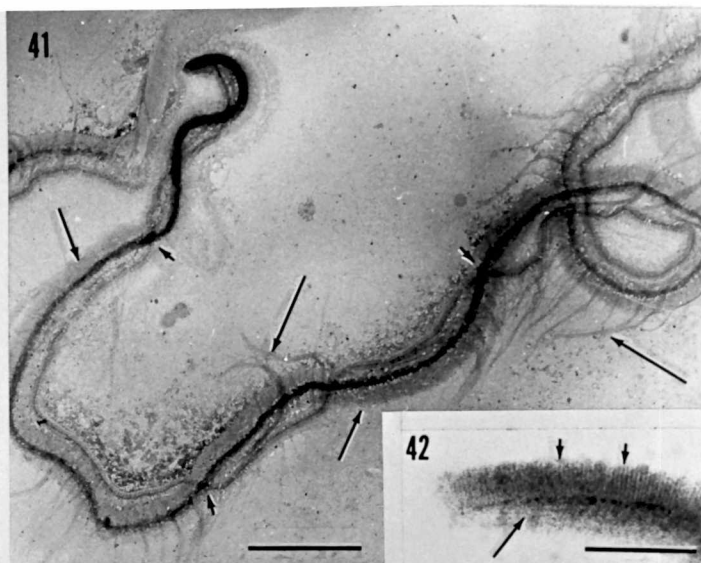
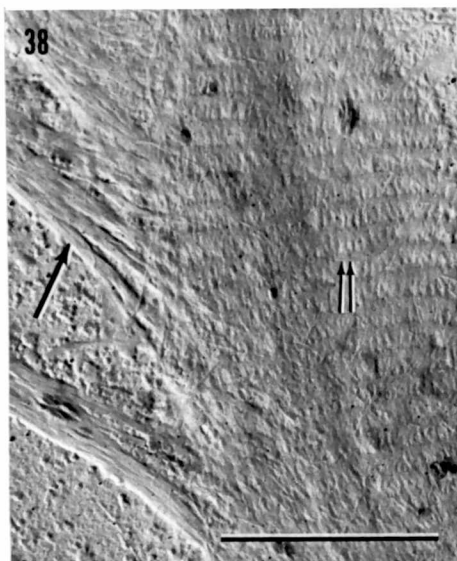


Figure 45. Working 3-dimensional model of F2 drawn approximately to scale. Plate-like substructure of PAR2 is composed of horizontal, longitudinal and oblique filaments arranged in a repeating diamond-shaped pattern. Laterally aligned and vertically overlapping bundles of short mastigonemes form tiers that extend from the PAR2 side; bundles of long mastigonemes extend from the Ax2 side.

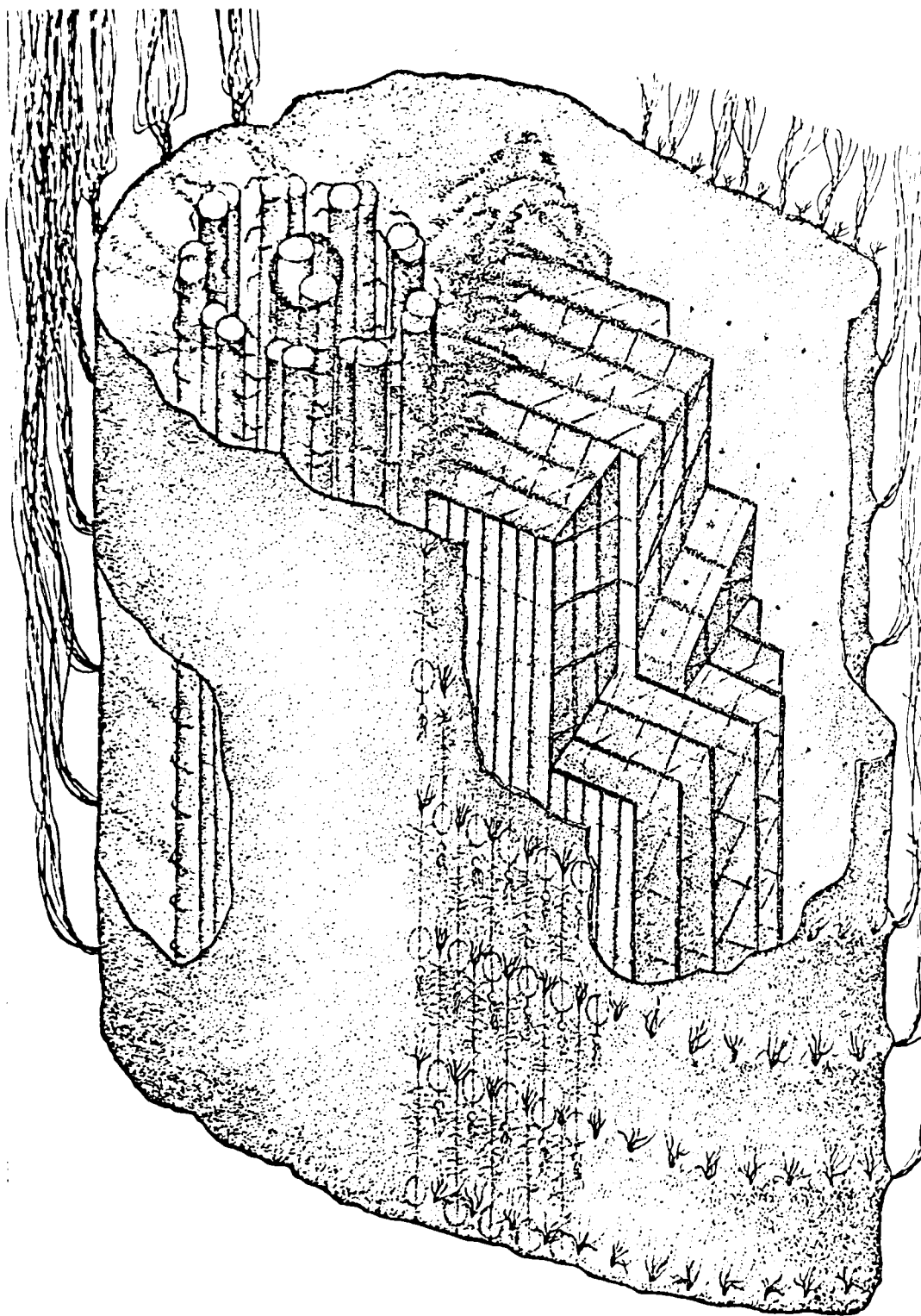
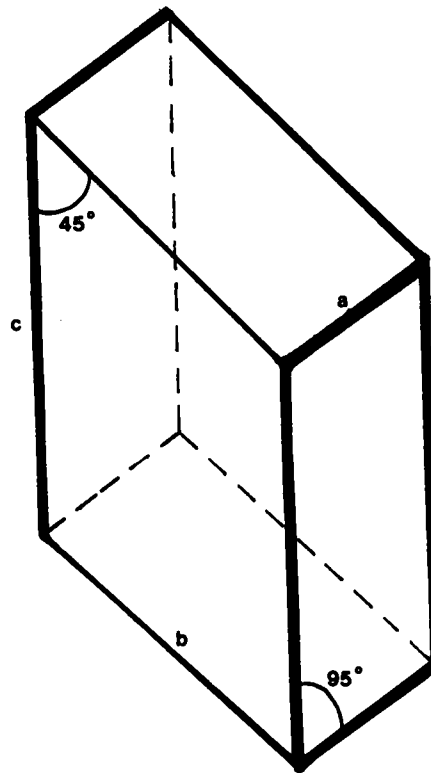


Fig. 45

Figure 46. The "unit cell" of PAR2. A trigonal-hexagonal "crystal"--a six-sided "solid" with three unequal axes--formed by the axes of the three major subcomponents: horizontal filaments in the 90° axis, longitudinal filaments in the 0° axis and oblique filaments in the ~ -40 to -50° axis.

"Unit Cell" of PAR2
Trigonal - Hexagonal "Crystal"



$a = 12.5 - 13 \text{ nm}$; horizontal filaments oriented at $95-100^\circ$ to long axis
of Ax2

$b = 25 \text{ nm}$; oblique filaments oriented -40 to -50° to long axis of Ax2

$c = 30 \text{ nm}$; longitudinal filaments oriented 0° to long axis of Ax2

Fig. 46

Figure 47. A plausible trajectory for the very fine filaments (the minor subcomponents) in the PAR2 substructure. The filaments may diagonally connect "unit cells" of adjacent plates.

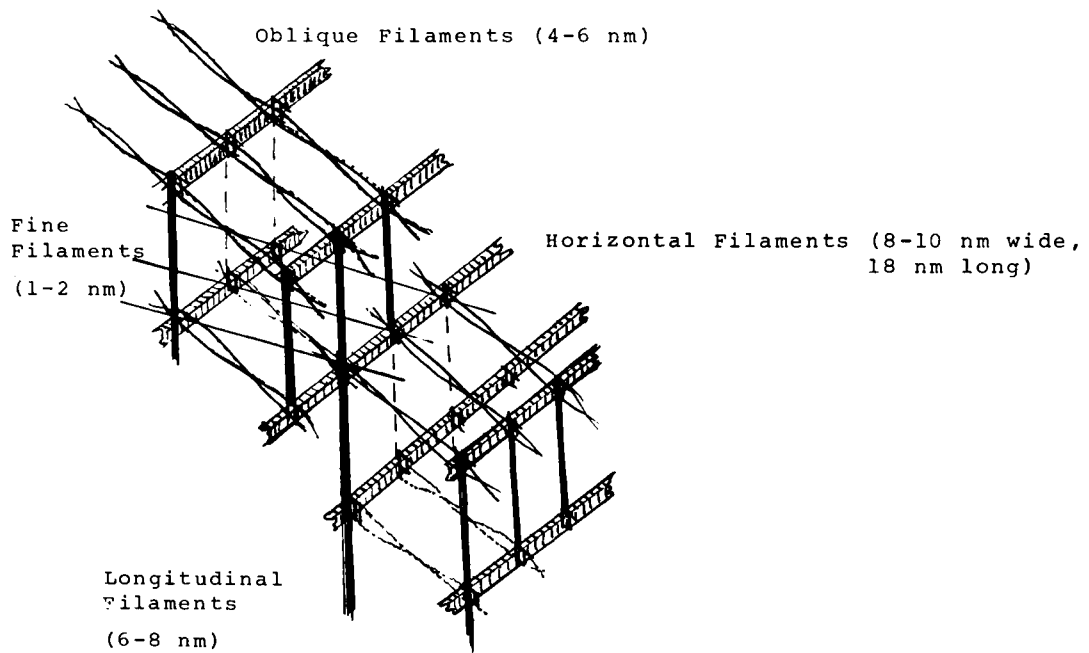


Fig. 47

Figure 48. Drawings of Eutreptia pertyi, based upon tracings of the cell in consecutive frames of VHS tape showing the anteriorly directed, asymmetrical helical waveform of F1 (the thinner flagellum). Positional changes of F1 in adjacent drawings represent flagellar motion that occurs during 1/30th s of recorded VHS tape.

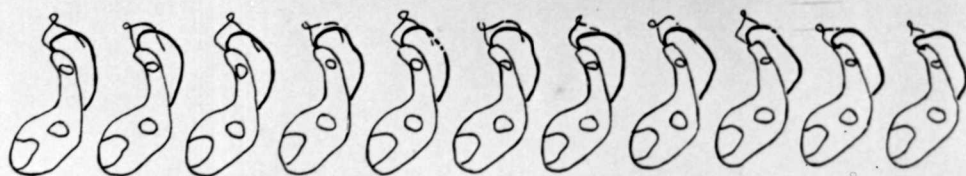
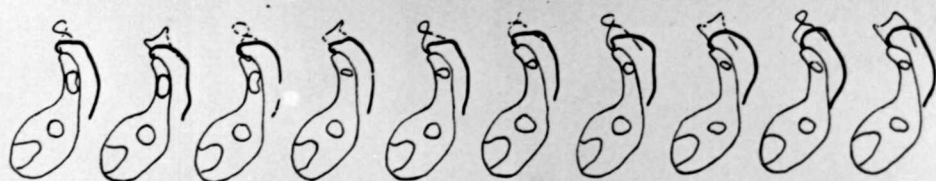
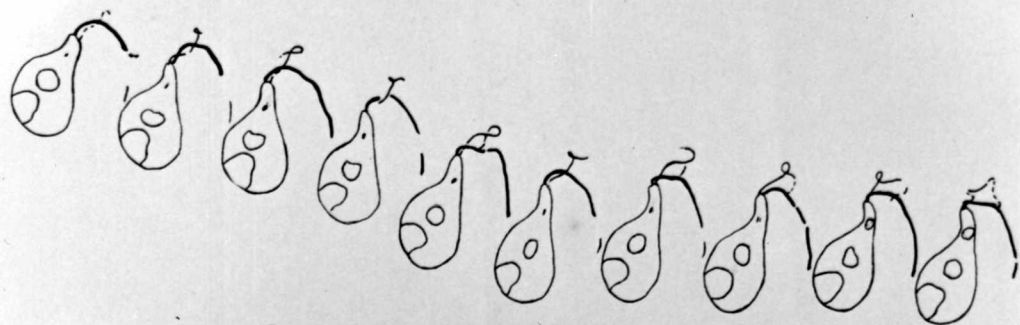


Fig. 48

Figure 49. Drawings of Eutreptia pertyi, based upon tracings of the cell in consecutive frames of VHS tape, show the laterally directed, planar waveform of F2 (the thicker flagellum). Positional changes of F2 in adjacent drawings represent flagellar motion that occurs during 1/30th s of recorded VHS tape.

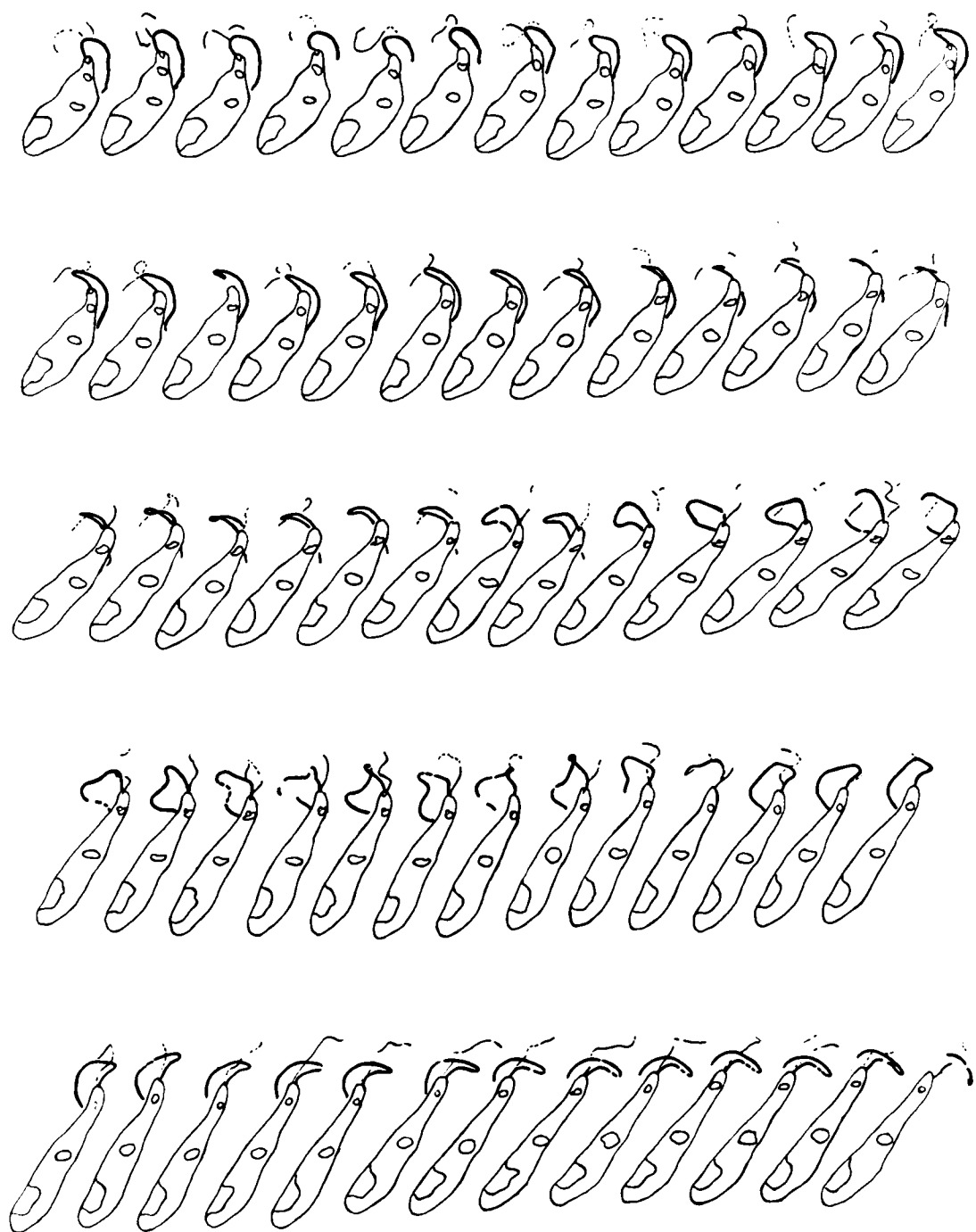


Fig. 49

VITA

Nancy Adams Dawson, daughter of Dale H. and Wilma E. Adams, was born in McAlester, Oklahoma, on November 14, 1954. She was educated in the McAlester City Schools and graduated from McAlester High School in 1973. She attended Oklahoma Baptist University, Shawnee, Oklahoma, and earned a Bachelor of Science degree with a major in biology in May, 1977. She completed a Master of Science degree at Northeast Louisiana University, Monroe, Louisiana, in August, 1978. Her thesis topic was a "Floristic Survey of the Vascular Flora of Richland Parrish, Louisiana."

From 1978-81 she worked as a research assistant in the Cell Biology Division at Southwest Foundation for Research and Education, San Antonio, Texas. From 1982-83, she worked as a research associate in the Department of Earth and Physical Sciences and taught laboratory classes in the Department of Biological Sciences at University of Texas, San Antonio.

She entered the doctoral program in the Department of Botany, The University of Tennessee, Knoxville, in August, 1983. She held the positions of Graduate Teaching Assistant in the Department of Botany from 1983-84 and Graduate Research Assistant on a NSF Grant to Dr. P.L. Walne, Department of Botany, from 1985-1988. She was the recipient of a Science Alliance Graduate Research Award from The University of Tennessee, Knoxville in 1986, 1987 and 1988. She completed the Ph.D. degree in Botany with major emphases in experimental phycology and cell biology in May, 1989. She is a member of several professional and honorary societies including Phi Kappa Phi, Mortar Board, Society of

Sigma Xi, Electron Microscopy Society and Phycological Society of America.

She is married to Dr. William David Dawson, originally of Meeker, Oklahoma, and has two children, William Zachariah and Kathryn Meghan.

	EUGLENOIDS							KINETOPLASTID ZOOFLAGELLATES						DINOFLAGELLATES		
	PAR1 in F1			PAR2 IN F2				PAR2 in F2 (recurrent flagellum)						PAR in Longitudinal Flagellum		
Characters of Flagellum and PARs	<i>Eutreptia pertyi</i> (Dawson, unpublished)	<i>Euglena gracilis</i> (Dawson, unpublished)	<i>Euglena gracilis</i> (Hyams, 1982)	<i>Eutreptia pertyi</i> (Dawson, unpublished)	<i>Eutreptiella eupharyngea</i> (Walne et al., 1986)	<i>Anisonema costatum</i> (Mignot, 1966)	<i>Entosiphon sulcatum</i> (Solomon et al., 1987)	<i>Trypanosoma brucei</i> (Fuge, 1969)	<i>Trypanosoma congolense</i> (Vickerman, 1969)	<i>Phytomonas davidi</i> (Attias & DeSouza, 1985; Farina et al., 1986)	<i>Herpetomonas megaseliae</i> (DeSouza & Souto-Padron, 1980; Cunha et al., 1984)	<i>Crithidia fasciculata</i> (Russell et al., 1983)	<i>Cryptobia heliciis</i> (Brugerolle et al., 1979)	<i>Woloszynskia micra</i> (Leadbeater & Dodge, 1967)	<i>Ceratium tripos</i> (Maruyama, 1982)	<i>Oxyrrhis marina</i> (Cachon et al., 1988)
Length of Flagellum	80 µm			95 µm												
Width of Flagellum	1.5 µm			2 µm												
Diameter of Ax	180 nm		265 nm	180 nm		210 nm		170 nm			220 nm		200 nm	164 nm		
Diameter of PAR	180 nm		160 nm	180-270 nm ^{aa}		455-930 nm		160 nm			520-600 nm	250 x 150 nm	195 nm	207 nm		200-300 nm
General Appearance of PAR Substructure	"lattice-like"	"lattice-like"	"hollow", "lattice-like"	"plate-like"	"plate-like"	"plate-like"	"plate-like"	"plate-like"	"plate-like"	"plate-like"	"plate-like"	"plate-like"	"plate-like"	somewhat "lattice-like"	somewhat "plate-like"	somewhat "lattice-like"
# of PAR2-like Plates	(-)	(-)	(-)	9-13	14-16	31	13	7-8	8	10	13	8	8			
PAR Subcomponents																
Oblique Filaments	(+)	some "double images"	(+)	(+)	(+)	(+)	2 internal microtubules (+)	(+)	(+)	(+)	(+)	(+)	(+)	tubules in core		
Size	4-6 nm	7	10-20 nm	4-6 nm ^{aaa}		7 nm		5 nm		7 nm	6 nm	7-10 nm	38 nm			8-10 nm (coiled 2-3 nm filaments)
Spacing in long PAR axis	30 nm	25 nm	35 nm	30 nm	36 nm	33 nm		23-26 nm		60 nm	27-28 nm	29-30 nm		29.5 nm	42.5 nm	37 nm
Spacing in horizontal PAR axis				12-13 nm												
Orientation to long axis	+/- 40-45°	+/- 45°		-40 to -50°*		-45°	17 nm	30 to 50°		+/- 45 to 50°			**			+45 to 55°***
Horizontal Filaments	? ill-defined	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)			
Size				8-10 nm w; 18 nm l		16 nm w; 25 nm l				25 nm = two filaments				10 nm w; 17-18 nm l		
Spacing in long axis				30 nm	26 nm	42-48 nm										
Orientation to long axis of PAR				" ⁺ 45°" ⁺		" ⁺ 55 to 60°" ⁺⁺		"30 to 50°"		+/- 45 to 50°**	+/- 45°***	+/- 45°***				
Orientation to long axis of Ax				95-100°												
Longitudinal Filaments	? ill-defined	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)			
# of long filaments in proximal zone/distal zone																
Size				3/16-20		7 nm		5 nm			6 nm					
Spacing in plane perpendicular to Ax cylinder				6-7 nm		14-15 nm										
Spacing in plane parallel to Ax cylinder				12-13 nm												
Orientation to long PAR axis				25 nm 0°	28 nm	30 nm 0°	33 nm	18-23 nm	30 nm	29-35 nm	27-29 nm 0°	25-30 nm 0°	33 nm 0°	34 nm	34.5 nm	
PAR-Ax Connectors																
Size/shape		triangular	"goblet"	10 nm; rod-like				V-shaped		Y- or V-shaped		5 nm				
Space between PAR & Ax		34 nm	56 nm	20 nm				43 nm		60 nm		10-20 nm				
Periodicity				38 nm												
PAR relationship to central pair of Ax microtubules			variable							fixed: doublets 4-7	fixed: doublets 4-7	fixed: doublets 5-6				
PAR-Flagellar Membrane Connections: anchoring periodicity								240 nm								
Other PAR Associations															PAR connects to R-fibre every 5-8 µm	
±minus (-) sign indicates the structure is missing; (+) sign indicates the structure is present; empty space indicates information is not available. ^{aa}400 nm is flagellar tip. ^{aaa}a twist of two filaments, each 2-3 nm in diameter. *axis of oblique filaments is declines toward PAR, and inclined toward reservoir wall. **axis of oblique filaments inclined away from cell; declined toward ventral side of cell body. ***axis of oblique filaments is declined to Ax, inclined to flagellar membrane; minor filaments (2-4 nm in diameter, spaced 16 nm apart are oriented -40° to long axis of PAR. +no physical attachment, just spatial alignment. ++may be only one axis (-°axis) for oblique filaments.																