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Morphogenesis of the feeding apparatus of *Entosiphon sulcatum*

An immunofluorescence and ultrastructural study

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Summary. The disruption and development of the siphon during division of *Entosiphon* have been followed by immunofluoresence with both an anti-cement MAb (IIID12) and an anti-tubulin MAb. (IVA10), by nuclear DNA labelling and by electron microscopy of serial section. The disruption of the parental siphon begins at the reservoir level where two new transversely orientated daughter siphons arise. In the degenerating bundles the cement disappears, first liberating the microtubules which then depolymerize. The first structure which surrounds the anterior part of the two young siphons is a loop of 5 microtubules linked to the reservoir membrane. From around this loop a row of perpendicular microtubules sink in the cytosplasm; they will form the primary row of microtubules in the definitive bundles. Inside the loop, reinforced microtubules are seen beneath the membrane, they will generate the future vanes, and also penetrate into the cytosplasm. Amorphous material surrounds the young siphons and may correspond to cement material. The growth of the siphons proceeds as they adopt a central longitudinal position in the cell. The cement material progressively condenses on structures such as the primary row of microtubules. The bundles, the supplementary plaque, and the scaffold. After flagellar partition each of the canals becomes distinct and cytokinesis occurs from the anterior end. These observations indicate that the microtubular loop could be the source of microtubule-organizing centre (MTOC) proteins initiating the assembly of the primary row of microtubules. Bundle microtubules start to assemble at the anterior end and extend backwards. The microtubules of the loop could be linked to roots associated with the basal bodies which double in number before division. The cement later condenses, linking and stabilizing the structures. Microfibrils play an important role in basal body and siphon separation and positioning.

Keywords: Morphogenesis; Phagotrophic; Euglenoids; Immunocytochemistry; Ultrastructure.

Abbreviations: BSA bovine serum albumin; EGTA ethylene glycol-bis(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid; GTP guanosine 5′-triphosphate; PBS phosphate buffered saline; PEG polyethylene glycol; PIPES piperazine-N,N′-bis(2-ethanesulfonic acid).

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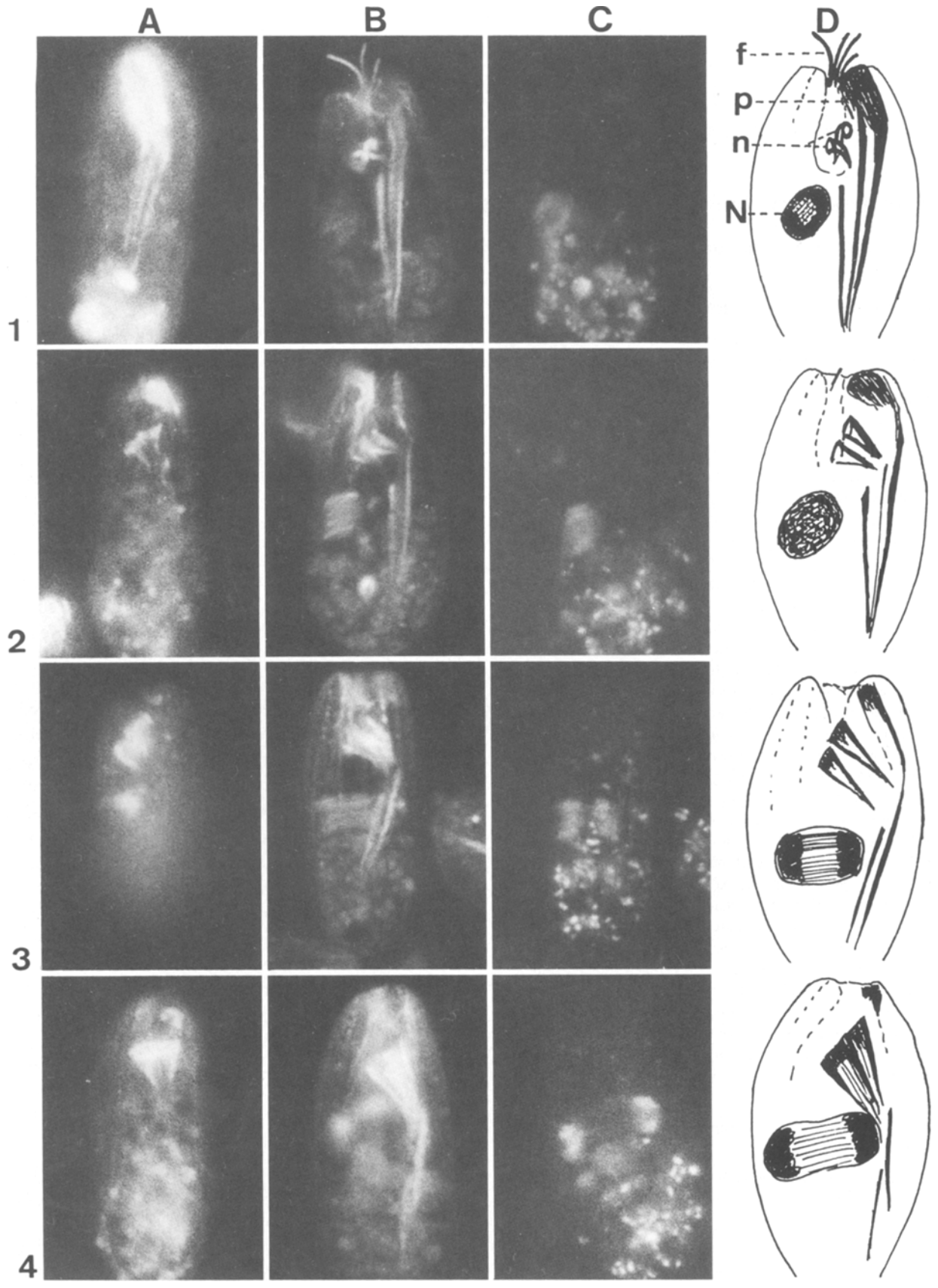
Introduction

Among the distinctive characteristics of euglenoid flagellates are the presence of permanently condensed chromosomes and a special mitotic mechanism. Hence many studies, at both the light and electron microscopic level have been concerned with nuclear division in this group (Leedale 1958, 1968; Sommer and Blum 1965; Chaly et al. 1977; Pickett-Heaps and Weik 1977; Gillot and Triemer 1978; reviewed in Bertaux et al. 1989; Triemer 1985, 1989; Triemer and Farmer 1991 a, b). Furthermore the unique feature of the cortical skeleton being comprised of longitudinal strips, has posed an interesting morphogenetic problem of strip replication during division. Morphological studies using light and electron microscopy (Pochmann 1953, Hofmann and Bouck 1976, Mignot et al. 1987) have shown that new strips develop between parental ones: thus replication is intussusceptive and semi-conservative. The origin of cortical microtubules which form the strips, and their relationship to microtubular roots arising from the basal bodies have also been clarified by electron microscopic studies of cells of *Cyclidiopsis* (Mignot et al. 1987), *Euglena mutabilis* (Surek and Melkonian 1986) and *Colacium* (Willey and Wibel 1985).

As phagotrophic euglenoids possess a huge feeding apparatus the further question as to how this complex structure is replicated during cell division arises. Early light microscopic studies, using haematoxylin staining (Dangeard 1901, Lackey 1929) showed most of the morphological events: the parental siphon breaks and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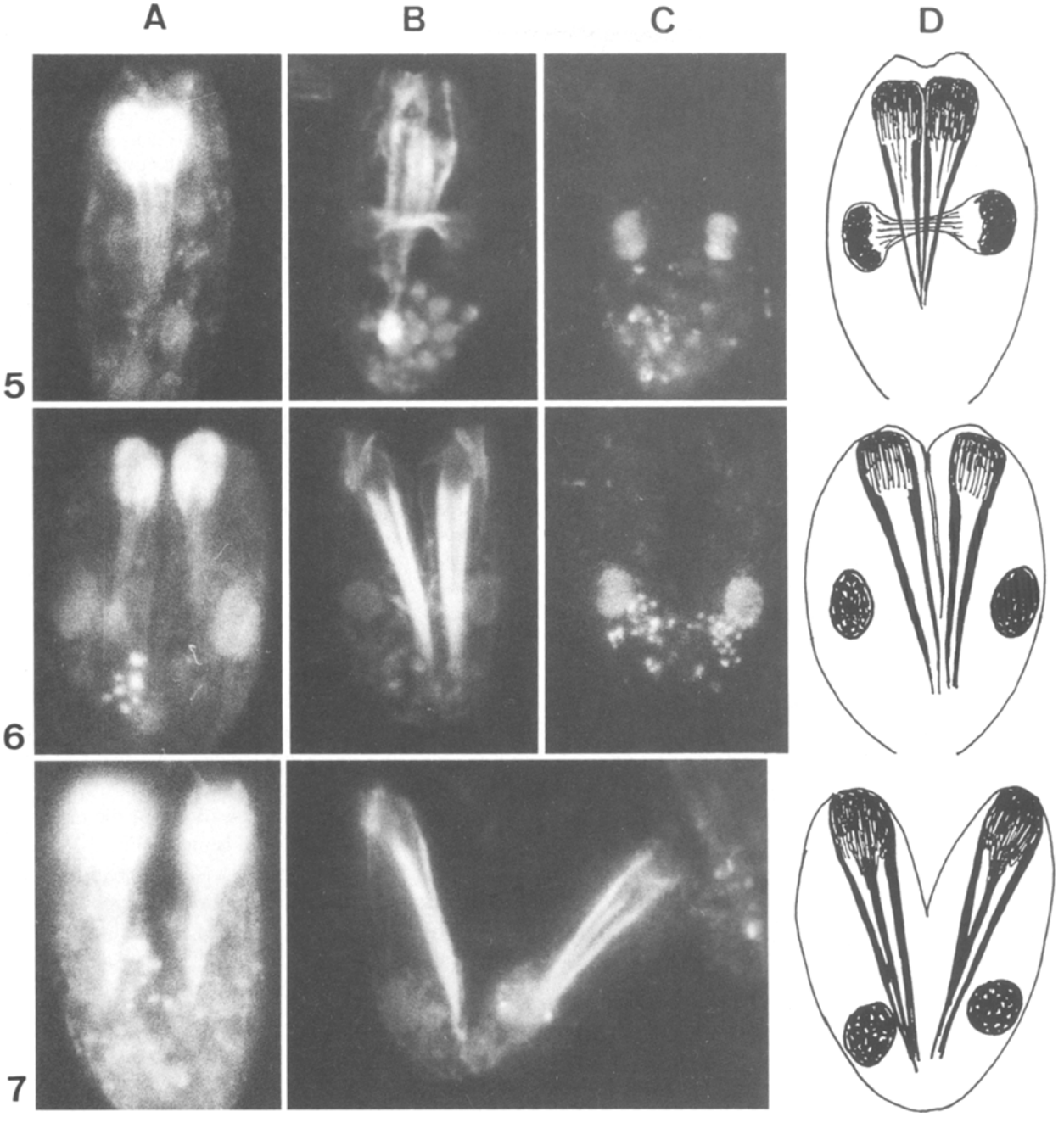

Fig. 1. Stages of development of the feeding apparatus or siphon (*1–7*) during division of *Entosiphon sulcatum*. *A* Immunofluorescence labelling with the anti-cement MAb IIID12, *B* anti-tubulin MAb IVA10 labelling of other cells, *C* nuclear DNA labelling (of the cells treated in *B*) with the fluorochrome Hoechst 33342, *D* diagrammatic representation of each stage. Stage 1: Breakage of the anterior part of the parental siphon (*p*) and development of two new siphons (*n*) at the reservoir level; four flagella (*f*) have already developed in this cell; chromatin around a central endosome in the nucleus (*N*). Stages 2 and 3: The parental siphon gradually disappears and the two newly formed siphons grow downwards. The mitotic spindle appears and elongates in the nucleus. Stages 4 and 5: The parental siphon is depolymerized and the two new siphons rotate, adopt a longitudinal position and continue their growth downwards; note the dumb-bell shape of the dividing nucleus and the interzonal microtubules (Fig. 5 B). Stages 6 and 7: Completion of siphon development in the two separating daughter cells; note the extreme posterior positions of the nuclei

disappears while two new siphons appear at the reservoir level; they grow progressively and acquire a longitudinal position before cytokinesis. Later electron microscopic studies by Mignot (1966), and more recently by Triemer (1988) provided more detail on siphon replacement and the progressive organization of developing siphons. However, several questions remain unanswered. How does the old siphon degenerate? What initiates the development and position of the new siphons? What is the temporal order of protein production and structure assembly? What is the relationship of the new siphon structure to the microtubular roots arising from the basal bodies? Is the replication of the basal bodies conservative or semi-conservative? What is the relationship of the basal bodies to the dividing nucleus?

While our present study does not pretend to answer all these questions, it has made progress on several points. By using immunofluorescence staining with the anti-cement MAb IIID12 and anti-tubulin MAb IVA10, we followed the assembly and development of the cement and the microtubular structures. Thus the timing of the development and positioning of the new siphons is established. An electron microscopic study based on serial sections at different stages of cell division has revealed new information on the construction of the siphon and its positioning. Our results on the segregation of the basal bodies and their associated fibres will be published later.

Materials and methods

Cell culture

Entosiphon sulcatum (from D. J. Patterson, Bristol, U.K.) was grown on a medium made with 1 wheat grain boiled in 10 ml of mineral water (Volvic).

Preparation of monoclonal antibodies

The anti-cement MAb IIID12 was obtained as described in Belhadri et al. (1992) and the anti-tubulin MAb IVA10 was obtained against the *Tetrahymena* tubulin, kindly provided by B. Viguès.

Indirect immunofluorescence and DNA staining

Entosiphon cells in division were permeabilized with 0.2% Triton X-100 in a stabilization buffer: 0.1 M PIPES, 2.5 mM GTP, 1 mM EGTA, 4% PEG (Kodak 1450), then fixed with 3% paraformaldehyde in the same buffer. After two washes in the above buffer, and one wash in PBS, the cells were spread on slides precoated with polylysine. Fixed cells were saturated with 1% BSA in PBS, incubated for 2 h at 37 °C with primary MAb, then washed three times with PBS and incubated for 1 h with a secondary fluorescein-conjugated antibody (Nordic Immunology) at 1/200 dilution. Following two additional washes in PBS, the slides were incubated for 2 min in 5 µg/ml Hoechst 33342 and washed in PBS prior to viewing under an epifluorescence microscope.

Electron microscopy

Cells in division were fixed in a glutaraldehyde-osmium solution containing 1% (v/v) glutaraldehyde, 1% (w/v) osmium tetroxide, and 0.05 M phosphate buffer pH 7 for 1 h at room temperature. After dehydration in a graded series of ethanol-water mixtures, cells were embedded in epoxy resin. Ultrathin sections collected on copper grids were post-stained with uranyl acetate and lead citrate prior to examination in a Jeol EX 1200 transmission electron microscope.

Results

The use of immunofluorescence staining with anti-cement and anti-tubulin MAbs allowed us to follow the different morphogenetic stages of the feeding apparatus during cell division in *Entosiphon*. Figure 1 recapitulates these stages.

First the parental siphon breaks down at the reservoir level, as shown by both anti-cement (Fig. 1 A1) and anti-tubulin labelling (Fig. 1 B1). Near the region where the parental siphon is broken, two new siphons arise in the cytoplasm and are labelled by the anti-tubulin MAb only (Fig. 1 B1). Four flagella have already developed and in the nucleus peripheral chromatin around a condensed central endosome is shown by DNA labelling (Fig. 1 C1). Disruption of the parental siphon then occurs along its major axis: the cement seems to disappear rapidly except at the head

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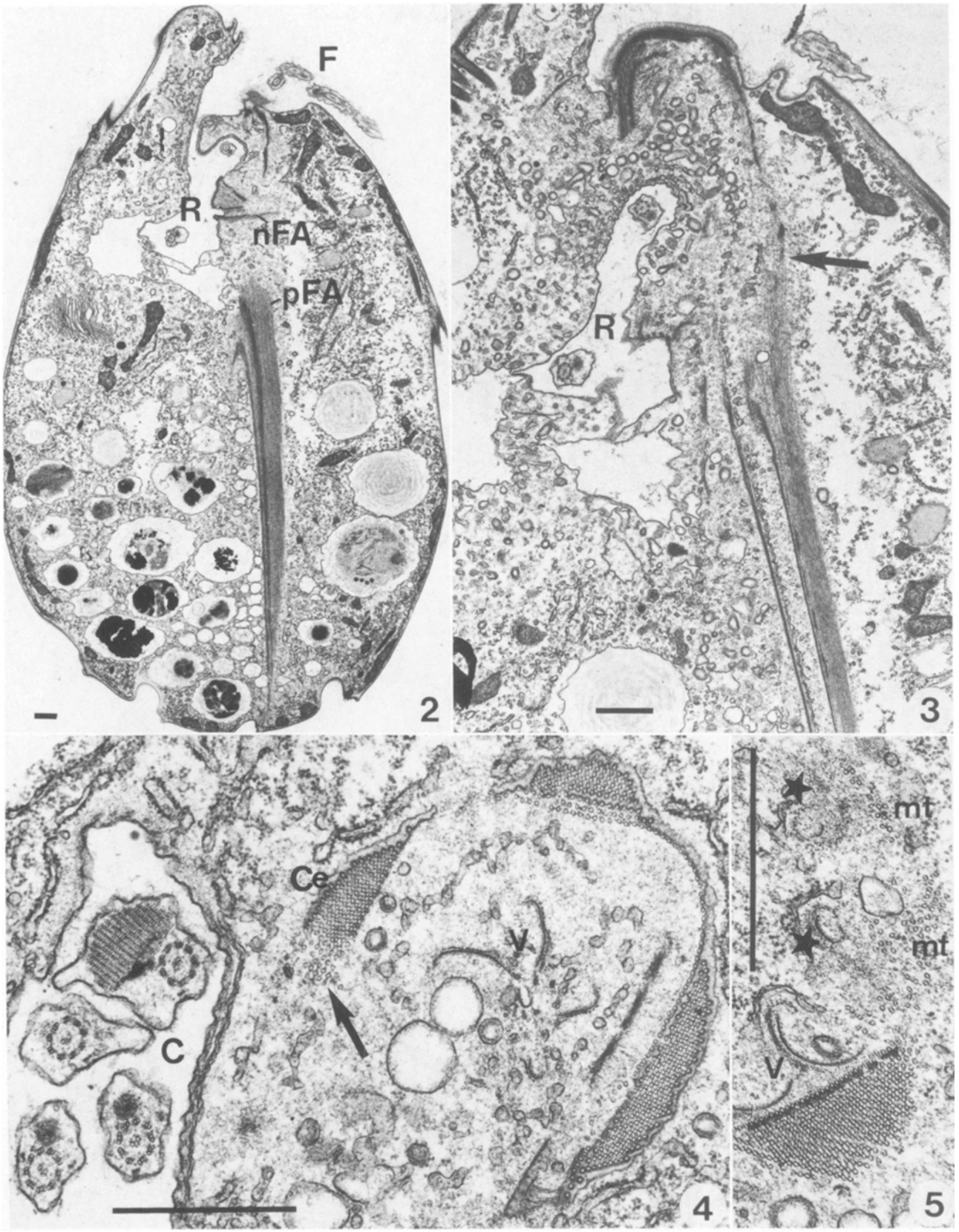
Figs. 2–5. Electron microscopy thin sections revealing depolymerization of the parental feeding apparatus and early development of the new ones. Bars: 1 µm

Fig. 2. The newly formed siphons (*nFA*) are transversely positioned at the reservoir level (*R*) near the region where the parental siphon (*pFA*) is broken in two. Four flagella (*F*) are present at the top of this cell

Fig. 3. Longitudinal section showing the zone of depolymerization of the parental siphon (arrow) in front of the reservoir (*R*)

Fig. 4 and **5.** Transverse sections at the canal level just above the newly formed siphons. In the parental siphon the microtubular bundle nearest the canal (*C*) begins to disorganize, the disappearance of cement (*Ce*) is followed by the release of microtubules (arrow). Note the presence of four flagella in the canal. At a more advanced stage (Fig. 5) two of the three microtubular bundles have become totally disorganized leaving independent microtubules (*mt*) and amorphous material (★). *V* Vanes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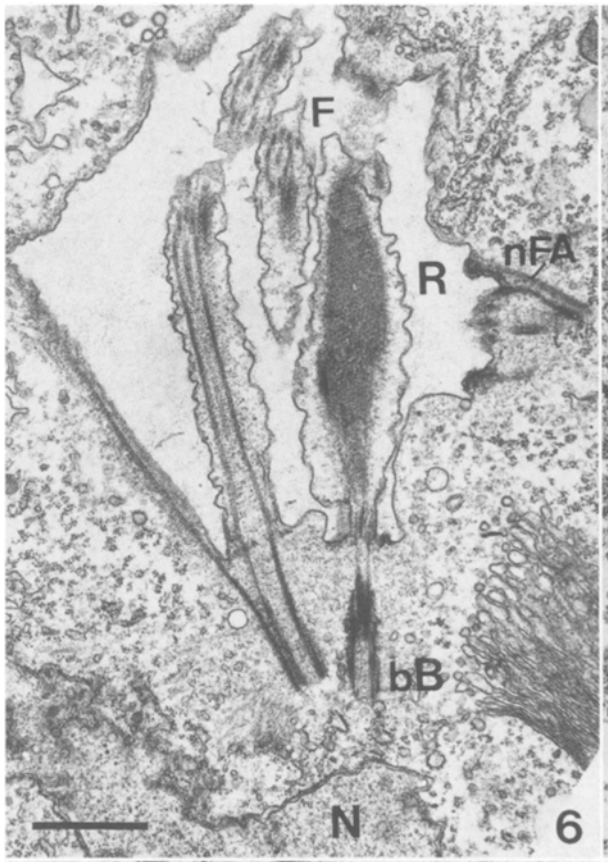
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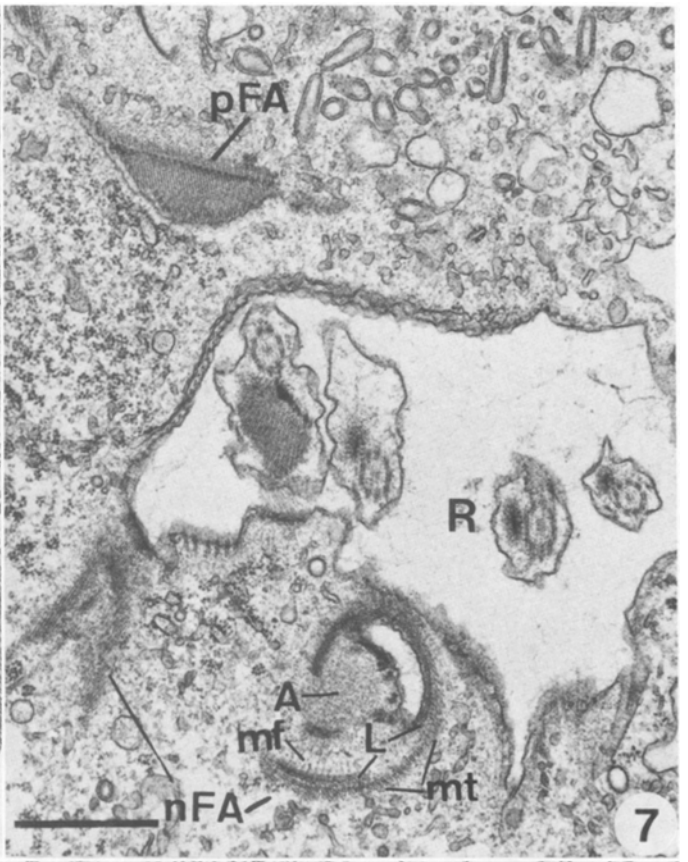
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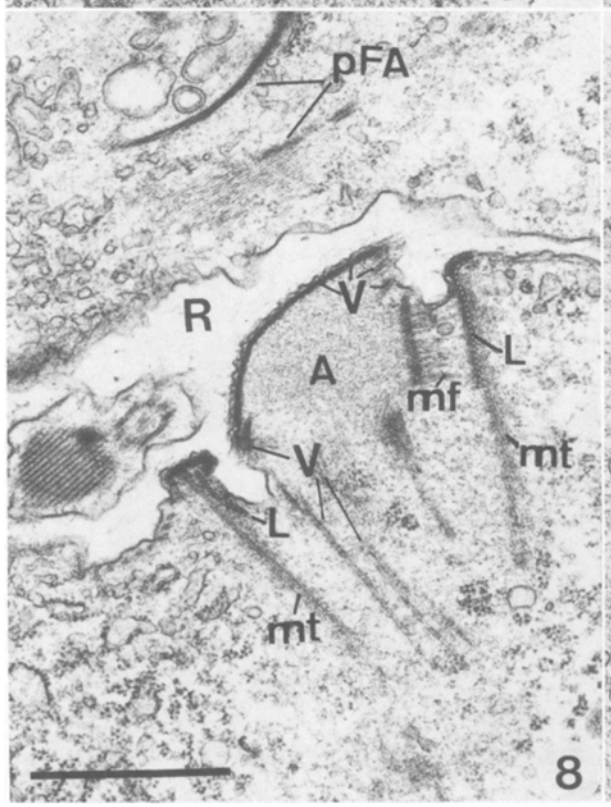
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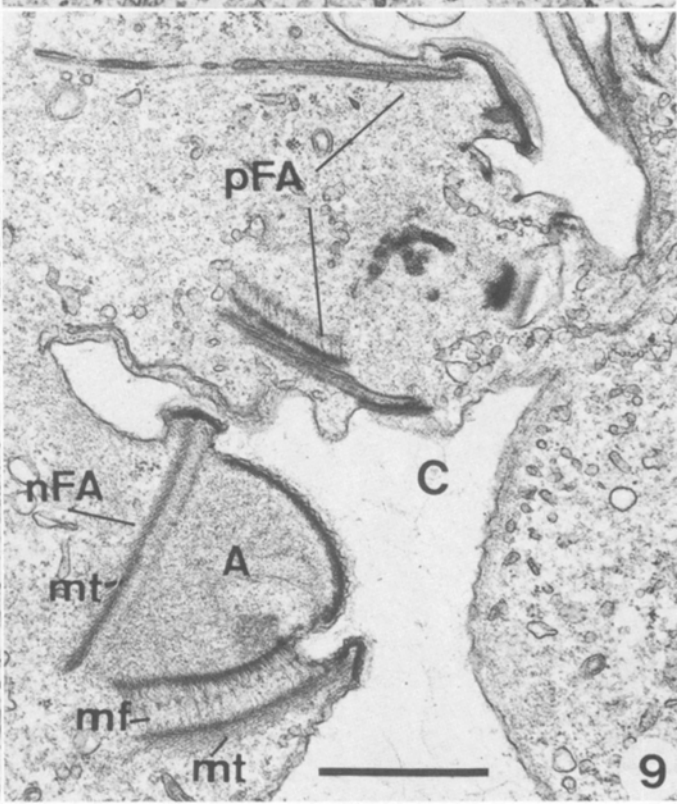
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(Fig. 1 A2); the posterior half vanishes progressively (Fig. 1 B2–4). The cement appears in the two new siphons (Fig. 1 A2 and A3) as the mitotic spindle emerges in the dividing nucleus (Fig. 1 B2 and B3) where two chromatin masses separate by late metaphase (Fig. 1 C3).

The newly developed siphons rotate to a vertical position and continue their downward growth (Fig 1 A4, B4, A5, and B5). In the dumb-bell shaped nucleus the central spindle elongates and the two chromatin masses occupy opposite poles (Fig. 1 C4 and C5). The median constriction containing the central spindle (Fig. 1 B5) then breaks, giving two daughter nuclei (Fig. 1 C6). The heads of the new siphons are intensely labelled by the anti-cement antibody (Fig. 1 A5 and A6) while the siphon trunk is strongly highlighted by the anti-tubulin antibody (Fig. 1 B6).

The development of the new siphons is almost complete (Fig. 1 B6 and C6) before separation of the two daughter cells has occurred. This is accomplished by cleavage which begins at the anterior end of the cell and progresses gradually downward, separating the two thin daughter cells each with a posterior nucleus (Fig. 1 A7 and B7).

Electron microscopy

Different stages of siphon development were examined ultrastructurally. At an early stage, as the parental siphon begins to disorganize, two new siphons develop transversely at the reservoir level (Fig. 2). Note that the two new flagella have already developed (Figs. 2, 4, and 6). Depolymerization of the parental siphon begins at the reservoir level, in front of the region where the two new siphons form (Fig. 3). The microtubular bundle nearest the canal disorganizes first, the cement disappears allowing the release of microtubules which subsequently depolymerize (Fig. 4). Later the three microtubular bundles disappear and a few isolated microtubules in an amorphous matrix (Fig. 5) represent an intermediate stage of disassembly.

The two new siphons are docked at the reservoir membrane opposite the parental siphon in a transverse position (Figs. 6 and 7). The first fixed structure is a semicircular loop of microtubules (Fig. 7) surrounded by a perpendicular row of microtubules. This encircles central amorphous material and the reinforced microtubules at the origin of the vanes. The microfibrillar structure is also linked to the loop (Fig. 7). A transverse section of the loop shows it to consist of five microtubules on one side and about 20 microtubules on the other (Fig. 8). The external row of microtubules arises close to the microtubules of the loop (Fig. 8). These will form the primary row of microtubules in the anterior part or "head" of the mature siphon. The reinforced microtubules at the origin of the vanes beneath the membrane project into the cytoplasm (Fig. 8). As the new siphons grow, more amorphous material is supplied at the centre of the loop (Figs. 8 and 9).

At a more advanced stage, when the two new siphons are orientated vertically, the microtubules have formed three bundles with the four vanes in the centre (Fig. 10). The supplementary plaques have been developed and there is no other condensed cement matrix, but amorphous material is abundant around the microtubules (Fig. 10). At a late stage preceding cytokinesis (Figs. 11 and 12), the major components of a mature feeding apparatus have been acquired by the two new ones. The cement material has encircled the microtubular bundles, but the layer is thinner than in the mature structure. This is also apparent in the supplementary

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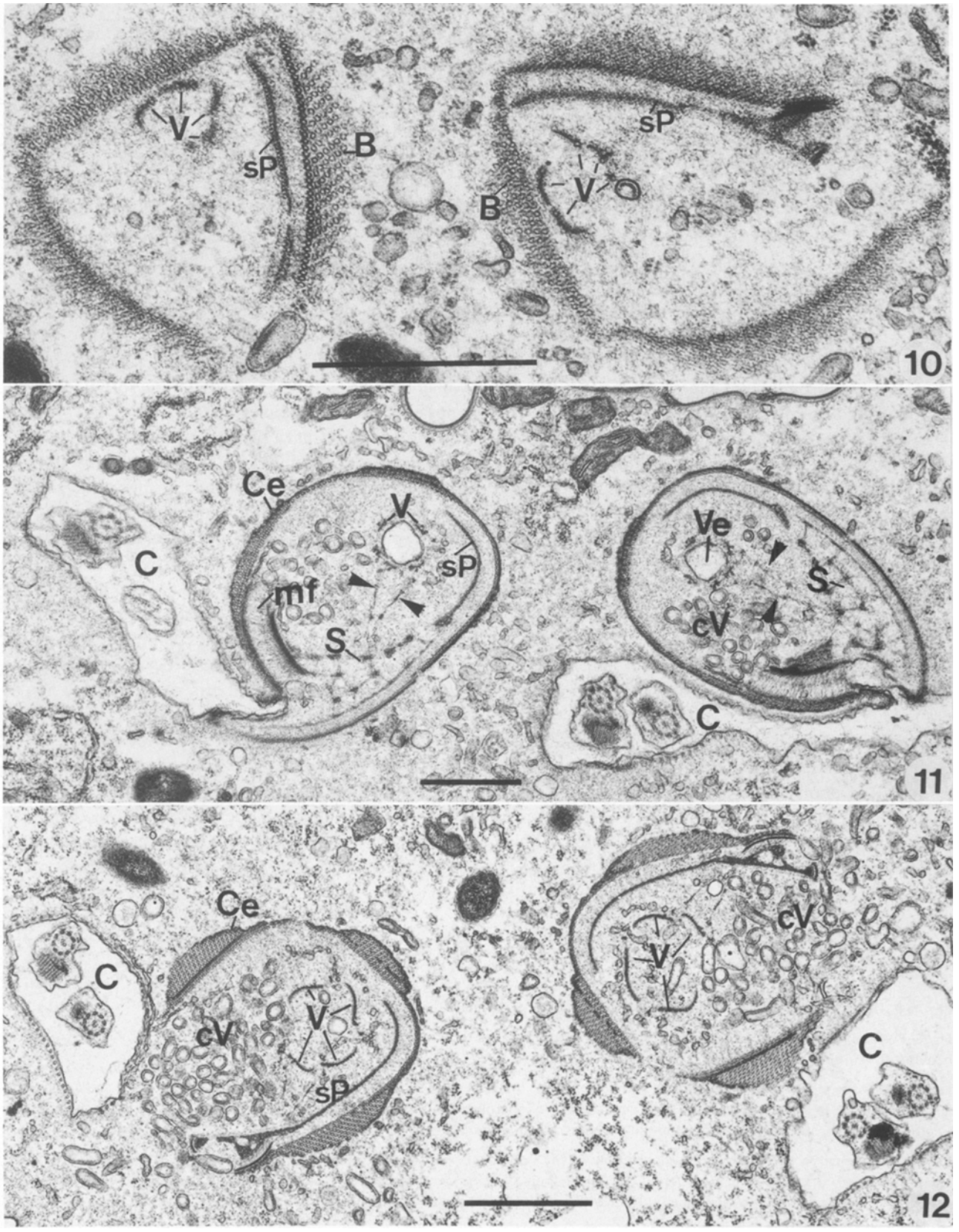
Figs. 6–9. Developmental stages of the new feeding apparatus. Bars: 1 μm

Fig. 6. Position of one new feeding apparatus (*nFA*) which is attached to the reservoir (*R*) membrane. Note the nucleus (*N*) under the basal bodies (*bB*) and the four flagella (*F*)

Fig. 7. Transverse section showing the young feeding apparatus (*nFA*), four flagella in the reservoir (*R*) and one bundle of the parental feeding apparatus (*pFA*). In the young siphon a semi-circular loop of microtubules (*L*) is surrounded by perpendicular rows of microtubules (*mt*). Central amorphous material (*A*) and a microfilamentous zone (*mf*) are also present

Fig. 8. Longitudinal section of a more developed siphon. The semi-circular microtubular loop (*L*) is sectioned at opposite sides, perpendicular microtubules (*mt*) originate from the external face of the loop. Note the central amorphous material (*A*), the dense structure of the future vanes (*V*) and the microfilamentous zone (*mf*) in relation to the microtubules of the loop. *pFA* Parental feeding apparatus, *R* reservoir

Fig. 9. At a more advanced stage the amorphous material (*A*) is present in addition to the microfilamentous zone (*mf*) and the external layer of microtubules (*mt*). The anterior end of the depolymerizing parental siphon (*pFA*) is recognizable at the top of the figure. *nFA* Newly formed siphon, *C* canal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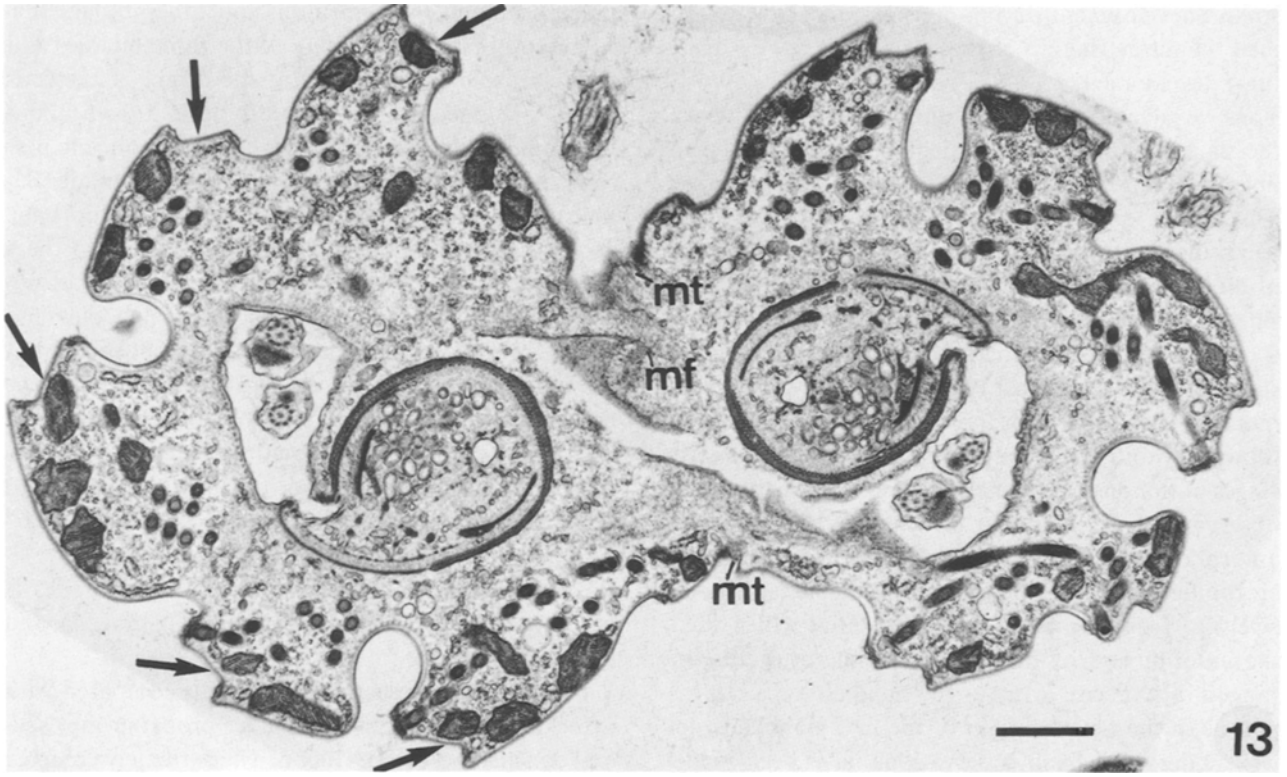

Fig. 13. Anterior cross-section of a cell undergoing cytoskinesis. The constriction between the two feeding apparatuses is marked by a few sub-membranar microtubules (*mt*) and by microfibrils (*mf*) which also surround the canals and siphons. New smaller cortical strips are intercalated between the parental ones (arrows)

plaque and particularly in the scaffold (Figs. 11 and 12). Lumen-coated vesicles are abundant at the centre of the new siphons suggesting that they are already functioning. The basal bodies and flagella have been partitioned and two flagella are present in each of the independent reservoirs and canals (Figs. 11 and 12). The membrane cytoskeleton forming the strips has duplicated in an alternating semi-conservative manner, as in other euglenoids (Fig. 13). Each new siphon is anchored to the pellicle by microfibrillar bundles and rootlets (not shown) before cytokinesis. Ultimately the cleavage furrow cuts the cell vertically into two daughter cells, beginning at the anterior end (Fig. 13).

Discussion

Early light microscopic studies (Dangeard 1901, Prowazek 1903, Lackey 1929) and the later ultrastructural studies (Mignot 1966, Triemer 1988) into the division of *Entosiphon* provided a basic outline of siphon morphogenesis and nuclear division. All these studies reported that the conspicuous feeding apparatus of *Entosiphon* disappeared during division and that two new siphons developed de novo. Nevertheless the complete morphogenetic process was not described in detail. We used an immunological approach with anti-cement and anti-tubulin MAbs to follow the different stages of

Figs. 10–12. Transverse sections of two longitudinally orientated siphons at more advanced stages. Bars: 1 μm

Fig. 10. Section of the anterior level showing three microtubular bundles (*B*), the supplementary plaque (*sP*) and the four central vanes (*V*). The definitive cement material is not yet recognizable but seems to be represented by amorphous material

Figs. 11 and **12.** Sections of the same cell at two levels with two separate canals (*C*) and flagellar pairs at a final stage before cell partition. Cement (*Ce*) is apparent around the microtubular bundles but amorphous material is still present around the structures. The scaffold structure (*S*) is in place but thinner than normal (Fig. 11). Note the two links (arrowheads) between the scaffold and the vanes (*V*) (Fig. 11). One large vesicle (*Ve*) is present between the four vanes (*V*) (Fig. 11) and lumen coated vesicles (*cV*) are abundant in the central area (Fig. 12) suggesting the new feeding apparatus is functional

siphon development during division, and have examined the ultrastructure of these stages to clarify structural development.

Thus we have observed that, during disorganization of the parental siphon, the cement begins to disappear liberating the microtubules which then depolymerize. This agrees well with the presumed role of the cement in linking and stabilizing microtubules. The breakdown of the cement is probably accomplished enzymatically since it is composed of tightly-bound proteins forming a condensed structure resistant to chaotropic agents such as KI (Belhadri et al. 1992). Conversely the cement material accumulates progressively and very late, although the anti-cement antibody is reactive in the early stages of siphon development. From the ultrastructural observations it seems that cement proteins form the amorphous material on the head and around the microtubular bundles. This material condenses progressively to form compact cement material which links the anterior end of the assembled microtubules, arranged in a precise array in the bundles.

Although the precise origin of the new siphons is not well understood fresh observations have been made. Initially a microtubular loop appears close to the reservoir membrane that circumscribes the head of the new siphons. This microtubular loop has not been observed previously, although a remnant is present at the head of the mature siphon always associated with the microfilamentous zone. This loop of microtubules could constitute the source of a MTOC, since the primary row of the bundle microtubules arises in close proximity with it. The successive rows assemble progressively on the external side of this primary row. Three bundles then become distinct and grow by assembly and addition of new microtubules. MTOCs must also be linked to the primary row of microtubules to nucleate the additional rows of microtubules. Observations on early stages suggest that the bundle microtubules begin to assemble on the head of the siphon. This is presumably the (−) microtubule end. They grow distally towards the posterior extremity which is therefore the (+) end. This type of assembly is inconsistent with that suggested by Mignot (1966), where new microtubules are presumed to assemble in contact with the vanes. Our observations of initial stage have never shown vanes in contact with forming microtubules of the future bundles. Since cement proteins are deposited after the microtubular assembly a MTOC function is very unlikely for them.

In the earliest stages observed, it seems that the major structures of the mature siphon (the loop microtubules linked to the microfilamentous zone, the primary row of microtubules at the origin of the three bundles and the reinforced microtubules at the origin of the four vanes) are already present. Nothing is known about the origin or determination of the location of the new siphons, however several hypotheses can be made. Before the cell enters division the basal bodies duplicate, as do the three microtubular roots attached to them (Solomon et al. 1987, Farmer and Triemer 1988; Brugerolle unpubl. obs.). Two pairs of basal bodies and flagella with their roots have already been formed but are not partitioned when the new siphons arise. Since the microtubular roots maintain their position on the reservoir membrane they could be linked to the new siphon structures and initiate them. We are trying to test this hypothesis and to determine the mode of partitioning of the basal bodies and flagella.

Positioning of the siphons at their definitive sites is accomplished gradually in concert with flagella and canal partition.

When morphogenesis in *Entosiphon* is compared with cytoskeleton development in other protist groups several conclusions can be drawn. The extremely complex siphon has to disintegrate or disassemble at division. Division of this organelle nor conservation in one of the daughter cells is possible, whereas the development of cortical strips is semi-conservative (Mignot et al. 1987). This is relatively unusual in protists, for example in ciliates, the parental oral apparatus is generally conserved in the proter while a new one is formed in the opisthe (Tuffrau 1984, Lynn and Corliss 1991). However, oral morphogenesis in Ciliates is very complex and there are many differences between the various subgroups. For example in *Tetrahymena* oral filaments and some microtubule elements are resorbed in the anterior (old) oral apparatus and reformed in synchrony with the posterior (new) oral apparatus (Bakowska et al. 1982, Williams et al. 1986, Williams and Honts 1987). In *Nassula*, where the nemadesm units of the cytopharyngeal basket closely resemble those of *Entosiphon* (Tucker 1968, 1970 a), the proter basket breaks down completely and disassembles while a new one is reformed in the same region, in synchrony with the opisthe basket (Tucker 1970 b). Among the flagellates, the Euglenozoa and particularly the Bodonids (Brugerolle et al. 1979) and *Stephanopogon* (Patterson and Brugerolle 1988) each have a feeding apparatus but complete studies on division and cytoskeleton development are not available. In the trichomonad flagellates the microtubules of the axostyle depolymerize at division although the striated roots such as the costa

are retained in one sister cell (Grassé 1952). Several studies on the development of the flagellar apparatus during the cell cycle in chlorophytes and chromophytes have been reviewed by Beech et al. (1991) and indicate variation in the behaviour of microtubular and fibrous roots during mitosis and flagellar transformation.

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References

Bakowska J, Nelson E, Frankel J (1982) Development of the ciliary pattern of the oral apparatus of *Tetrahymena thermophila*. J Protozool 29: 366–382

Beech PL, Heimann K, Melkonian M (1991) Development of the flagellar apparatus during the cell cycle in unicellular algae. Protoplasma 164: 23–37

Belhadri A, Bayle D, Brugerolle G (1992) Biochemical and immunological characterization of intermicrotubular cement in the feeding apparatus of phagotrophic euglenoids: *Entosiphon, Peranema,* and *Ploeotia*. Protoplasma 168: 113–124

Bertaux O, Valencia R, Magnaval R (1989) The nucleus. In: Buetow DE (ed) The biology of *Euglena*, vol 4. Academic Press, pp 137–245

Brugerolle G, Lom J, Nohynkova E, Joyon L (1979) Comparaison et évolution des structures cellulaire chez plusieurs espèces de Bodonidés et Cryptobiidés appartenant aux genres *Bodo, Cryptobia* et *Trypanoplasma* (Kinetoplastida, Mastigophora). Protistologica 15: 197–221

Chaly N, Lord A, Lafontaine JG (1977) A light and electron microscopic study of nuclear structure throughout the cell cycle in the euglenoid *Astasia longa*. J Cell Sci 27: 23–45

Dangeard PA (1901) Recherches sur les Eugléniens. Botaniste 8: 97–357

Gillot MA, Triemer RE (1978) Ultrastructure of cell division in *Euglena*. J Cell Sci 31: 25–36

Grassé PP (1952) Ordre des Trichomonadines. In: Masson (eds) Traité de zoologie, tome I, flagellés. Masson, Paris, pp 705–779

Farmer M, Triemer TE (1988) Flagellar systems in the euglenoid flagellates. Biosystems 21: 17–31

Hofmann C, Bouck BG (1976) Immunological and structural evidence for patterned intussusceptive surface growth in a unicellular organism. J Cell Biol 69: 693–715

Lackey JB (1929) Studies in the life histories of Euglenida. I. The cytology of *Entosiphon sulcatum* (Duj.) Stein. Arch Protistenkd 66: 175–200

Leedale GF (1958) Nuclear structure and mitosis in the Euglenineae (Flagellata). Nature 181: 502–503

– (1968) The nucleus in *Euglena*. In: Buetow DE (ed) The biology of *Euglena*, vol 3. Academic Press, New York, pp 17–22

Lynn DH, Corliss JO (1991) Ciliophora. In: Harrison FW, Corliss JO (eds) Microscopic anatomy of invertebrates, vol 1. Wiley-Liss, New York, pp 333–467

Mignot JP (1966) Structure et ultrastructure de quelques euglènomonadines. Protistologica II: 51–117

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

Patterson DJ, Brugerolle G (1988) The ultrastructure identity of *Stephanopogon* and the relatedness of the genus to other kinds of protists. Eur J Protistol 23: 279–290

Pickett-Heaps JD, Weik KL (1977) Cell division in *Euglena* and *Phacus* I. Mitosis. In: Rost TL, Gifford EM Jr (eds) Mechanisms and control of cell division. Academic Press, New York, pp 308–336

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

Prowazek SV (1903) Die Kernteilung von *Entosiphon*. Arch Protistenkd 2: 325–328

Solomon JA, Walne PL, Kivic PA (1987) *Entosiphon sulcatum* (Euglenophyceae): flagellar roots of the basal body complex and reservoir region. J Phycol 23: 85–98

Sommer JR, Blum JJ (1965) Cell division in *Astasia longa*. Exp Cell Res 3: 504–527

Surek B, Melkonian M (1986) A cryptic cytostome is present in *Euglena*. Protoplasma 133: 39–49

Triemer RE (1985) Ultrastructural features of mitosis in *Anisonema* sp. (Euglenida). J Protozool 32: 683–690

– (1988) Ultrastructure of mitosis in *Entosiphon sulcatum* (Euglenida). J Protozool 35: 231–237

– Farmer MA (1991 a) An ultrastructural comparison of the mitotic apparatus, feeding apparatus, flagellar apparatus and cytoskeleton in euglenoids and kinetoplastids. Protoplasma 164: 91–104

– – (1991 b) The ultrastructural organization of the heterotrophic euglenoids and its evolutionary implications. In: Patterson DJ, Larsen J (eds) The biology of free-living heterotrophic flagellates. Clarendon Press, Oxford, pp 185–203 (The Systematic Association, vol 45)

Tucker JB (1968) Fine structure and function of the cytopharyngeal basket in the ciliate *Nassula*. J Cell Sci 3: 493–514

– (1970 a) Initiation and differenciation of microtubule patterns in the ciliate *Nassula*. J Cell Sci 7: 793–821

– (1970 b) Morphogenesis of a large microtubular organelle and its association with basal bodies in the ciliate *Nassula*. J Cell Sci 6: 385–429

Tuffrau M (1984) Les aspects généraux de la morphogénèse chez les ciliés. In: Grassé PP (ed) Traité de zoologie, tome I, infusoires ciliés. Masson, Paris, pp 537–580

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

Williams NE, Honts JE (1987) The assembly and positioning of cytoskeletal elements in *Tetrahymena*. Development 100: 23–30

– – Graeff RW (1986) Oral filament proteins and their regulation in *Tetrahymena pyriformis*. Exp Cell Res 164: 295–310