Tissue & Cell, 2000 **32** (2) 178–187

DOI: 10.1054/tice.2000.0102, available online at http://www.idealibrary.com IDEAL®

Tissue&Cell

The fine structure of the axostyle and its associations with organelles in *Trichomonads*

M. Benchimol,[1,2] J.A.P. Diniz,[3] K. Ribeiro[2,4]

Abstract The fine structure of the axostyle in the protists *Tritrichomonas foetus* and *Monocercomonas sp* is described using transmission electron microscopy after quick-freezing techniques and immunocytochemistry. The axostyle microtubules presents a lateral projection formed by two protofilaments in addition to the 13 protofilaments normally found in microtubules. The axostyle is associated with other cell structures such as hydrogenosomes, endoplasmic reticulum, sigmoid filaments and glycogen particles. The microtubules of the pelta-axostylar system are connected to each other by bridges regularly spaced with an interval of 9 nm. Labeling of the axostyle was observed after cell incubation with monoclonal antibodies recognizing α-tubulin and acetylated-tubulin.

Keywords: axostyle, pelta, microtubules, trichomonads, hydrogenosome

Introduction

Among the numerous examples in the literature of highly ordered arrays of microtubules the axostyle is one of the less studied. *Tritrichomonas foetus* and *Monocercomonas sp* are protists which present an axostyle. They are used by our group as interesting models for ultrastructural studies of single cell-organisms. They lack mitochondria and peroxisomes but contain hydrogenosomes, unusual organelles that participate in the overall metabolism of the cell to a significant extent. They also present uncommon structures such as parabasal apparatus, striated roots (costa in *T. foetus* and parabasal filaments), and a microtubular structure named pelta-axostylar system (Honigberg et al., 1971). In these protists the axostyle consists of a single ribbon of microtubules in a linear array. The pelta-axostylar system consists of two microtubular sheets that overlap anterior to the nucleus, forming the pelta-axostylar junction. The pelta supports the wall of the periflagellar canal from which emerge the flagella. The anterior part of the axostyle is wider forming the capitulum and posteriorly to the nucleus, it turns upon itself forming a tube; the axostylar trunk. The axostyle appears to narrow progressively until its terminal segment, protruding from the posterior cell region, covered by the cell membrane (Honigberg & Brugerolle, 1990).

In trichomonads the axostyle seems not to present any contractility and its function is still under investigation. It was suggested that it could be a supportive entity, while the pelta could reinforce the wall of the flagellar canal (Honigberg & Brugerolle, 1990). We have shown recently that this structure participates in the cell division process, providing constriction of the nucleus during the karyokinesis (Ribeiro & Benchimol, 1999).

In the present work we report new data concerning the fine structure of the axostyle microtubules and its association with other cell components in the protists *T. foetus* and *Monocercomonas sp*. The observations were made using living cells processed by quick-freezing techniques, such as high-pressure and slam-freezing, followed by freeze-fracture and deepetching. Some samples were also submitted to freeze-substitution in acetone-osmium tetroxide. Immunocytochemistry was done using anti-tubulin antibodies.

[1]Universidade Santa Ursula, Rio de Janeiro, [2]Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, [3]Instituto Evandro Chagas-Belém-Pará, [4]Programa de Pós-Graduação em Ciências Morfológicas-Universidade Federal do Rio de Janeiro, Brazil

Received 15 December 1999
Accepted 15 February 2000

Correspondence to: M. Benchimol, Universidade Santa Ursula, Rua Jornalista Orlando Dantas, 59, CEP 222–31–010, Botafogo, Rio de Janeiro, Brazil. Tel./Fax: +55 21 553 1615; E-mail: marleneb@domain.com.br

Materials and methods

Parasites

Dr H. Guida (EMBRAPA, Rio de Janeiro, Brazil) isolated the K strain of *Tritrichomonas foetus* from the urogenital tract of a bull from the state of Rio de Janeiro, Brazil. The parasites were cultivated in Diamond's TYM medium (Diamond, 1957) for 24 h at 36.5°C. The strain of *Monocercomonas sp*. was obtained from Dr Jaroslav Kulda (Charles University, Czech Republic). The protozoon was isolated from the large intestine and cloaca of the wood-snake *Tropidophis melanurus* in La Sal, Bayamo, Cuba, and were grown in Diamond's TYM medium (Diamond, 1957) for 28 h at 28°C. The cells were collected by low speed centrifugation, washed three times in 0.1 M phosphate-buffered saline (PBS, pH 7.2) and processed using one of the following procedures:

Electron microscopy (TEM-transmission electron microscopy)

Cells were fixed overnight at room temperature in 2.5% (v/v) glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2), and post-fixed in 1% OsO_4 in cacodylate buffer plus 5 mM $CaCl_2$ and 0.8% potassium ferricyanide. Cells were washed, dehydrated in acetone and embedded in Epon. Thin sections were stained and observed in a Zeiss 900 electron microscope.

Glucose-6-phosphatase

For localization of glucose-6-phosphatase, the cells were processed according to Robinson and Karnovsky's (1983) method. Briefly, the cells were fixed in 0.5% (v/v) glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2) for 5 min and incubated for 60 min at 37°C in a medium containing 3.6 mM glucose-6-phosphatase, 10 mM $MgCl_2$, 220 mM sucrose, and 3 mM $CeCl_3$ in 50 mM Tris-maleate buffer, pH 7.2. After successive washings, the samples were re-fixed as described above and processed for TEM.

Immunocytochemistry

After overnight fixation (4% paraformaldehyde, 0.1% glutaraldehyde), samples were dehydrated in ethanol and infused in Unicryl (BB International, Cardiff, UK). For immunolabeling, sections were washed in PBS/albumin 3%, quenched in 50 mM NH_4Cl for 30 min, and subsequently incubated for 3 h in the presence of monoclonal antibodies recognizing α-tubulin and acetylated α-tubulin (Sigma Chemical Co., USA). After washing with Tween-PBS, sections were treated for 1 h at room temperature with colloidal gold (10 nm in diameter) conjugated anti-mouse IgG antibodies (BioCell, UK). Controls were performed by omission of the primary antibody.

Quick-freezing

Slam-freezing freeze-etching

Previously fixed or unfixed specimens were fast-frozen by 'slam-freezing' (Cryopress Med, St Louis, MO, USA). A polished copper block was cooled with liquid nitrogen and the specimen was projected in the free-fall against the block. Subsequently, specimens were freeze-fractured at –115°C in a Balzers BAF 300 freeze-etching machine and deep-etching was attained (10 min at – 100°C) and submitted to rotatory shadowing with platinum/carbon at 2×10^{-6} Torr at an angle of 15°. Replicas were recovered in distilled water, cleaned, and examined in a Zeiss 900 transmission electron microscope.

High-pressure and freeze-substitution

Living cells were frozen by high pressure in the Balzers high-pressure machine (HPM 010; Balzers Union, Balzers, FL, USA), transferred to liquid nitrogen and then to a mixture of anhydrous acetone containing 2% osmium tetroxide (v/v) at –90°C in a freeze-substitution unit (Balzers FSU 010). The material was gradually rewarmed during 24 h until the room temperature was attained and then embedded in Epon.

High voltage electron microscopy (HVEM)

T. foetus were allowed to adhere on Formvar coated gold grids and treated with 0.1% Triton X-100 dissolved in the microtubule stabilizing buffer as previously described (Benchimol & De Souza, 1987) for 5 min. Thereafter, the cells were fixed with glutaradehyde and osmium tetroxide as for routine TEM, dehydrated and critical point dried in liquid CO_2 with the Sorvall apparatus (Ivan Sorvall Inc., Newton, CT, USA). The grids were examined in the JEM-100 electron microscope operating at 1000 KV.

Cytoskeleton replicas

For observation of replicas of the cytoskeleton, axostyle included, the parasites were allowed to adhere to glass coverslips previously coated with poly-L-lisine (MW 70 000; Sigma). The cells were exposed to 1% Triton X-100 for 5 min and then fixed in a 0.1% glutaraldehyde – 4% paraformaldehyde solution. Next, the samples were incubated in anti-α-tubulin monoclonal antibody (Sigma, USA) as described above, and thereafter incubated in goat anti-mouse IgG-20 nm gold conjugated, washed three times in PBS, dehydrated with ethanol, and critical point dried with CO_2. Unidirectional platinum-carbon shadowing at 45°C, and carbon-replication were carried out in a Balzers BAF 300 freeze-etching machine. Replicas were released in 70% hydrofluoric acid, cleaned in 70% sulfuric acid, rinsed in distilled water, and collected on 200 mesh copper grids. They were then observed in a Zeiss 900 electron microscope.

Results

In trichomonads the axostyle is a ribbon-shaped and prominent structure in the cell. It originates anteriorly, close to the point of origin of the flagella, where the basal bodies and associated structures are localized (Figs 1, 2, 4) and runs backwards through the cell to the posterior end, appearing to

narrow slightly but progressively until its terminal segment, and turns upon itself forming a tube, which protrudes at the cell posterior tip (Fig. 2). Cells in process of division present two axostyles throughout the mitosis and they were never seen depolymerized (not shown). The axostyle is always in close association with another set of microtubular structure, forming the pelta-axostylar system (Figs 1,2, 15). The pelta is found at the anterior-most region of the cell, supporting the periflagellar canal and it overlaps with the axostyle anteriorly to the nucleus, in the pelta-axostylar junction (Fig. 1).

The total number of microtubules present in the trichomonad axostyle is about 130 as it was counted using whole axostyles seen under high voltage electron microscope. Whatever the number of microtubules, they are always organized into a single ribbon, wider at the anterior region of the cell, which is named capitulum (Figs 2, 15, 16). In cross-sections of cells after high-pressure pressure freezing of living cells it was possible to see the microtubule wall presenting 13 protofilaments plus a lateral projection made of two additional protofilaments (Figs 9 & 10). In longitudinal views the microtubules are seen side-by-side with delicate filamentous bridges connecting them (Figs 8, 11, 15, 17, 18). the periodicity of this linkage was 9 nm.

Association of the pelta-axostylar system with cell structures

With the smooth-endoplasmic reticulum
The axostyle is closely associated with smooth-surfaced, ribosome-free membranes, lying close against its surface (Figs 5,6, 12,13). These membranes are found as vesicles, as seen by freeze-etching (Fig. 12) or very thin profiles of membranous lamina closely attached to the axostyle along the opposite region of the recurrent flagellum when studied by thin section electron microscopy (Figs 5,6, 13). By freeze-etching after slam-freezing of living cells, these vesicles were seen connected to the axostyle microtubules by delicate bridges (Fig. 12). After incubation of the cells in a medium containing glucose-6-phosphate, positive reaction was seen in profiles of the endoplasmic reticulum, including the membranous profiles that follow the axostyle (Fig. 13).

With hydrogenosomes
The hydrogenosomes are frequently found following the axostyle in a linear arrangement (Figs 1, 4, 7). Electron-microscopical observation after quick-freezing allowed us to find connections between these structures in a form of delicate fibers (Fig. 8). The smooth endoplasmic reticulum (SER) that follow the axostyle seems to form the peripheral hydrogenosome vesicle (Fig. 6).

With glycogen
Glycogen granules are often seen in association with the axostyle, following the microtubules in all its extension, as α particles (rosettes) and also as β particles, which form the rosettes (Figs 1–3, 6).

With the nucleus
During the mitosis, the duplicated axostyles get in touch with the nucleus, pressing the nuclear envelope and thus participating in the karyokinesis (Figs 5 & 7).

With sigmoid filaments
Sigmoid filaments extend between the basal body #2 and the pelta (Figs 1, 4, 14). They terminate on the inner surface of the pelta in the region of the peltar-axostyar junction (Fig. 4). These structures do not present any reaction when anti-tubulin antibodies are used (Fig. 14).

Immunocytochemistry
The axostyle showed positive reaction for α-acetylated tubulin in Unicryl thin sections (Fig. 14) and for anti-α-tubulin (Fig. 16), in isolated cytoskeleton. Labeling was detected over the pelta-axostylar system and flagella after incubation with gold conjugated secondary antibodies (Figs 14 & 16).

Discussion

There are no specific studies concerning the fine structure of the axostyle in trichomonads and its interaction with other cell structures. It was suggested that the pelta-axostylar complex could be a supportive entity, with the pelta reinforcing the periflagellar canal (Honigberg & Brugerolle, 1990).

Routine thin sections of the trichomonads cells do not give all the information needed to conclude the role and the exact number and distribution of the microtubules of the axostyle. Cryofixation vastly improves the preservation of cell structure, giving the user confidence that the images produced are an accurate representation of the living cell. This has allowed better preservation of all trichomonads cell structures, axostyle included. So we performed our studies using complementary techniques such as quick-freezing of living cells, freeze-substitution and immunocytochemistry.

Some investigators claim that this axostyle is able to depolymerize and disappears during mitosis of *Trichomonas vaginalis* (Brugerolle, 1975; Viscogliosi & Brugerolle, 1994; Viscogliosi & Delgado-Viscogliosi, 1996; Juliano et al., 1986). The cited authors claim that the axostyle is a structure that disappears at the beginning of the division process, and could be depolymerized or ejected from the cell. It was considered that the axostyle is composed of labile microtubules stabilized by incubation of the cells in the presence of taxol (Juliano et al., 1986). However, in our studies, the axostyle was never seen depolymerized (Batista et al., 1988; Ribeiro & Benchimol, submitted), even when the cells were under drug treatment using colchicine or vinblastine as previously reported (Silva-Filho & DeSouza, 1986). In the present study, we observed that the axostyle was labeled with anti α-tubulin and anti-acetylated-tubulin antibodies. The tubulin acetylated isoform is characteristic of stable

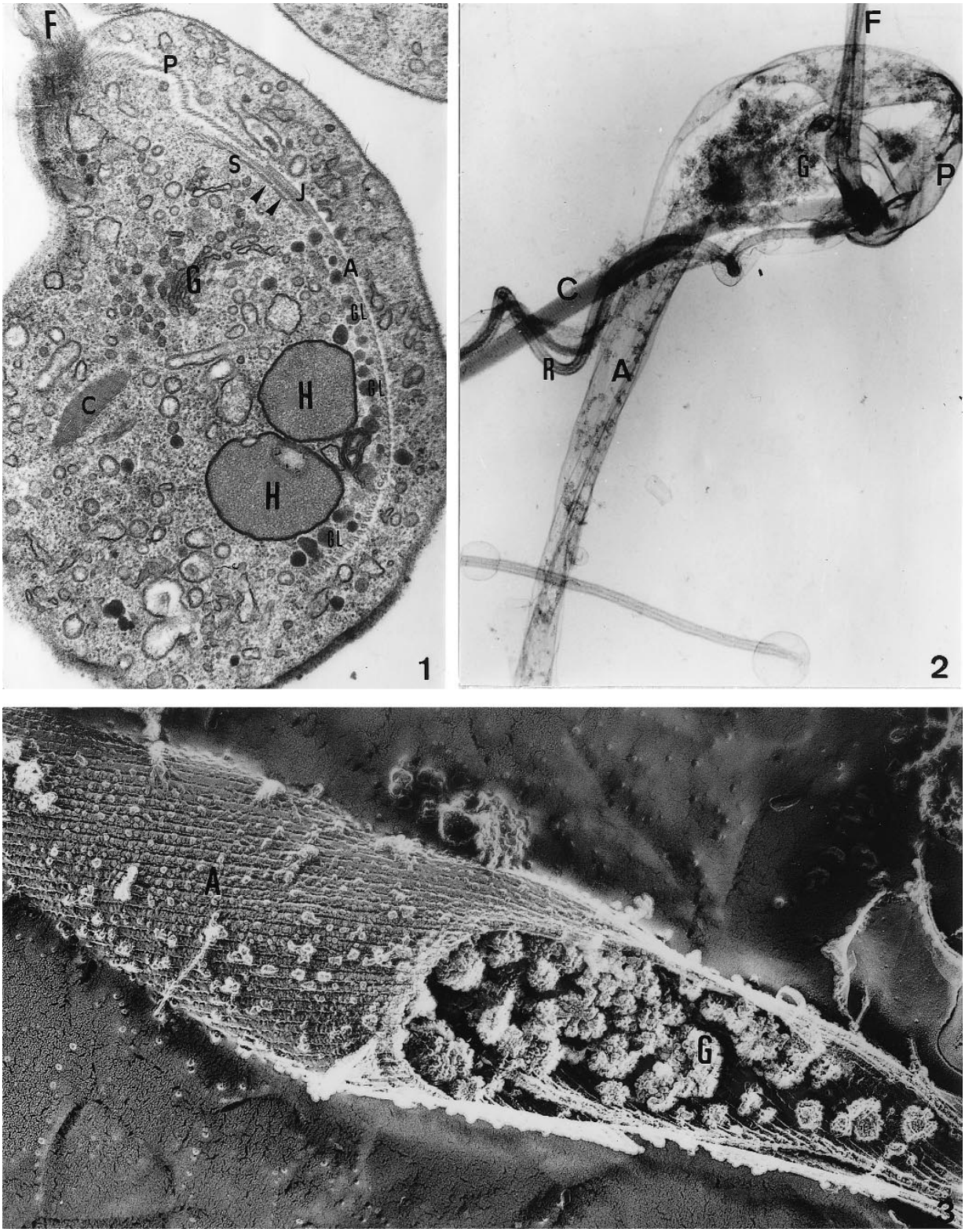

Fig. 1 General aspect of *Tritrichomonas foetus* in a routine thin section preparation. The pelta (P) and axostyle (A) are seen running along the main axis of the cell. Note the sigmoid filaments (S) and the pelta-axostylar junction (J) where the microtubules of both structures overlap (arrowheads). The hydrogenosomes (H) and glycogen rosettes (GL) are seen following the axostyle (A). G: Golgi complex; C: costa; F: flagellum. × 23 000.

Fig. 2 General view of a Triton X-100 extracted *T. foetus* as seen with the high voltage electron microscope. The microtubules which form the pelta-axostylar system are seen. The anterior part of the axostyle is wider, forming the capitulum and posteriorly it turns upon itself forming a tube, the axostylar trunk. Notice that the flagella (F) emerge from the same point of the costa and microtubules. The recurrent flagellum (R) is kept together with the costa (C), as well as the glycogen granules (G). X 16 000.

Fig. 3 Freeze-etching view of the axostyle microtubules (A) in association with glycogen (G) in quick-frozen *Tritrichomonas foetus*. × 50 000.

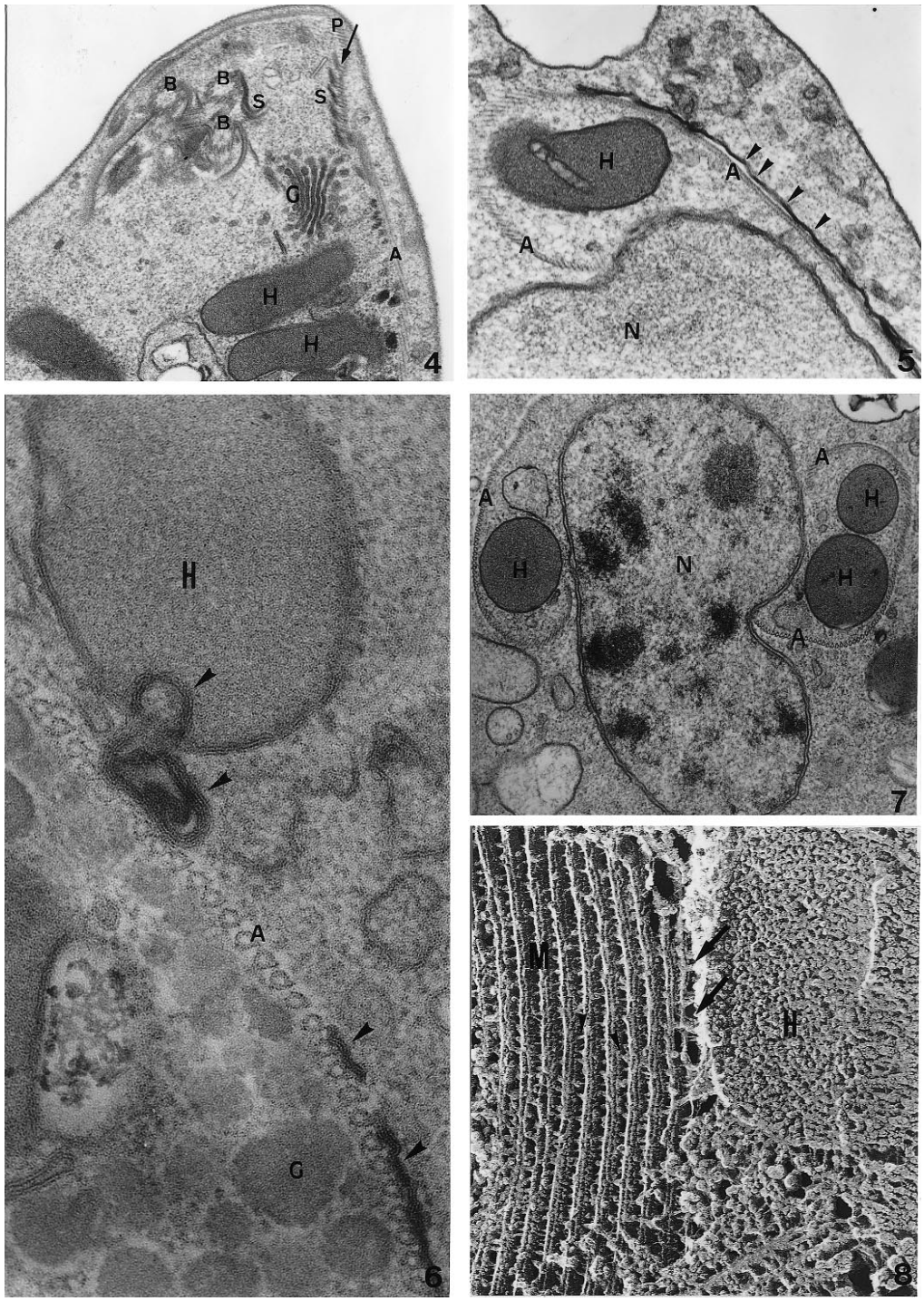

Fig. 4 Routine thin-section trough the anterior region of *T. foetus* showing the basal bodies (B), sigmoid filaments (S), hydrogenosomes (H), Golgi complex (G) and axostyle (A). The arrow points to the pelta (P) connection to the sigmoid filaments, which extend between the basal body #2 and the pelta. × 30 000.

Fig. 5 Axostyle (A) of *T. foetus* in longitudinal view, showing smooth-surfaced membrane (arrowheads) lying close against its surface. Note that the axostyle seems to constrict the nucleus (N). One hydrogenosome (H) is also seen in close association with the axostyle. × 40 000.

Fig. 6 Axostyle (A) microtubules in cross-section of routinely prepared *T. foetus*. Notice profiles of smooth endoplasmic reticulum in close association with these microtubules (arrowheads). A similar membrane is seen in continuity with the hydrogenosome (H) peripheral vesicle (arrowhead). G: glycogen. X 125 000.

Fig. 7 Association of the axostyle (A) with the nucleus (N) during karyokinesis in *T. foetus*. Hydrogenosomes (H) are also seen in this association. × 40 000.

Fig. 8 Axostyle microtubules (M) and an adjacent hydrogenosome (H) connected by filamentous structures (arrows) as seen after quick-freeze, deep-etching of *T. foetus*. X 80 000.

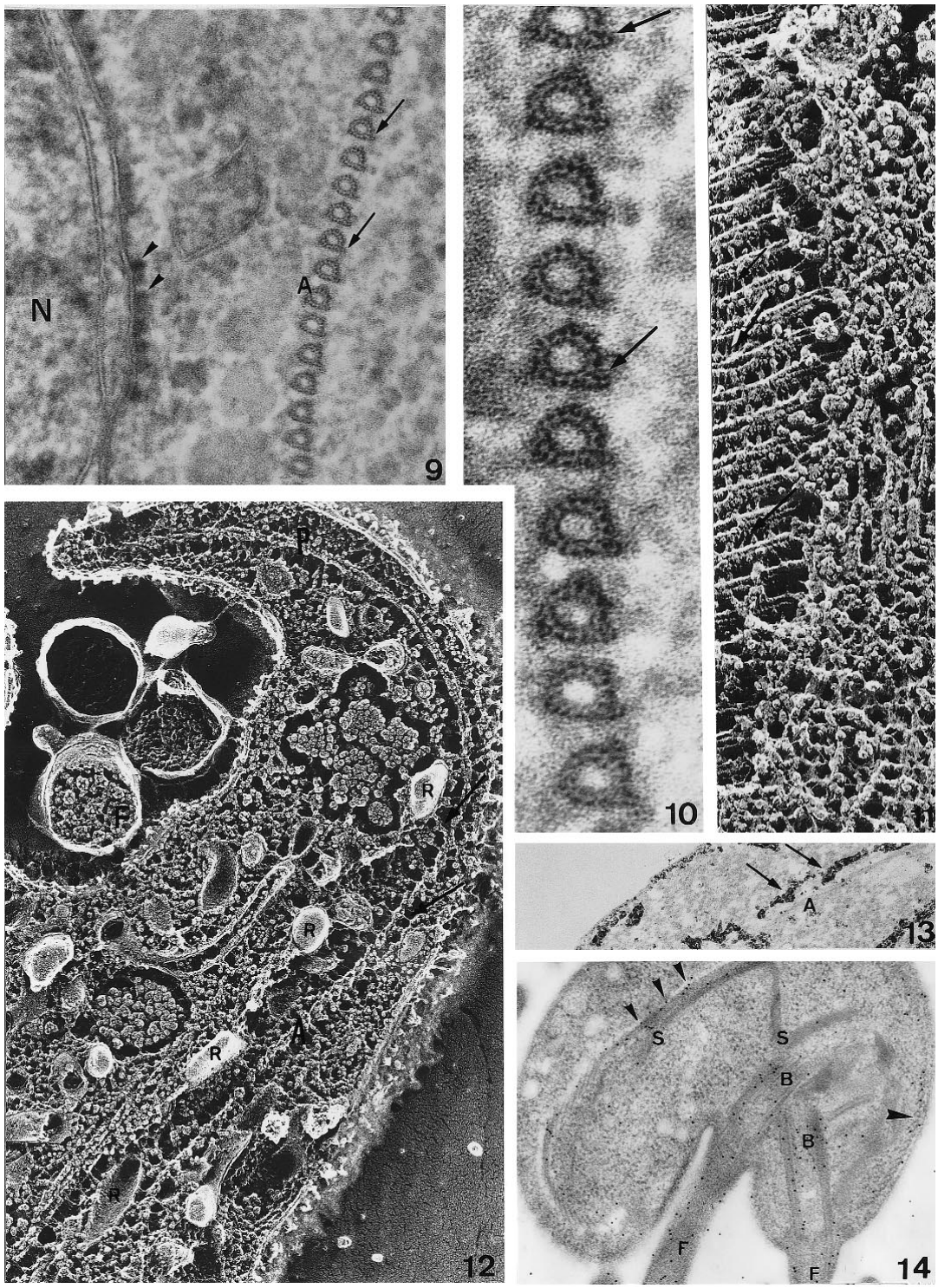

Fig. 9–10 Axostyle (A) of *Monocercomonas sp* after high-pressure freezing, followed by freeze-substitution in acetone-osmium; arrows point to microtubules lateral projection presenting two additional protofilaments. The arrowheads point to ribosomes found on the nuclear envelope. N; nucleus. Fig. 9: × 150 000; Fig. 10: × 400 000.

Fig. 11 Deep-etch view of the axostyle microtubules of *T. foetus* where filaments are seen connecting these structures (arrows). × 100 000.

Fig. 12 Anterior region of *T. foetus* after slam-freezing and freeze-etching. The row of smooth-surfaced endoplasmic reticulum like membranes (R) which lie close to the surface of the axostyle and connected by thin filaments (arrows). × 85 000.

Fig. 13 Partial view of *T. foetus* after cytochemistry for glucose-6-phosphatase. A positive reaction (arrows) is seen in the endoplasmic reticulum, including the membrane profiles close to the axostyle (A). × 40 000.

Fig. 14 Immunocytochemistry for α-acetylated tubulin using 10 nm gold-conjugated IgG in *T. foetus*. The labeling is seen over the axostyle microtubules (arrowheads), but not in the sigmoid filaments (S). The flagella (F) and the basal bodies (B) are also labeled. × 40 000.

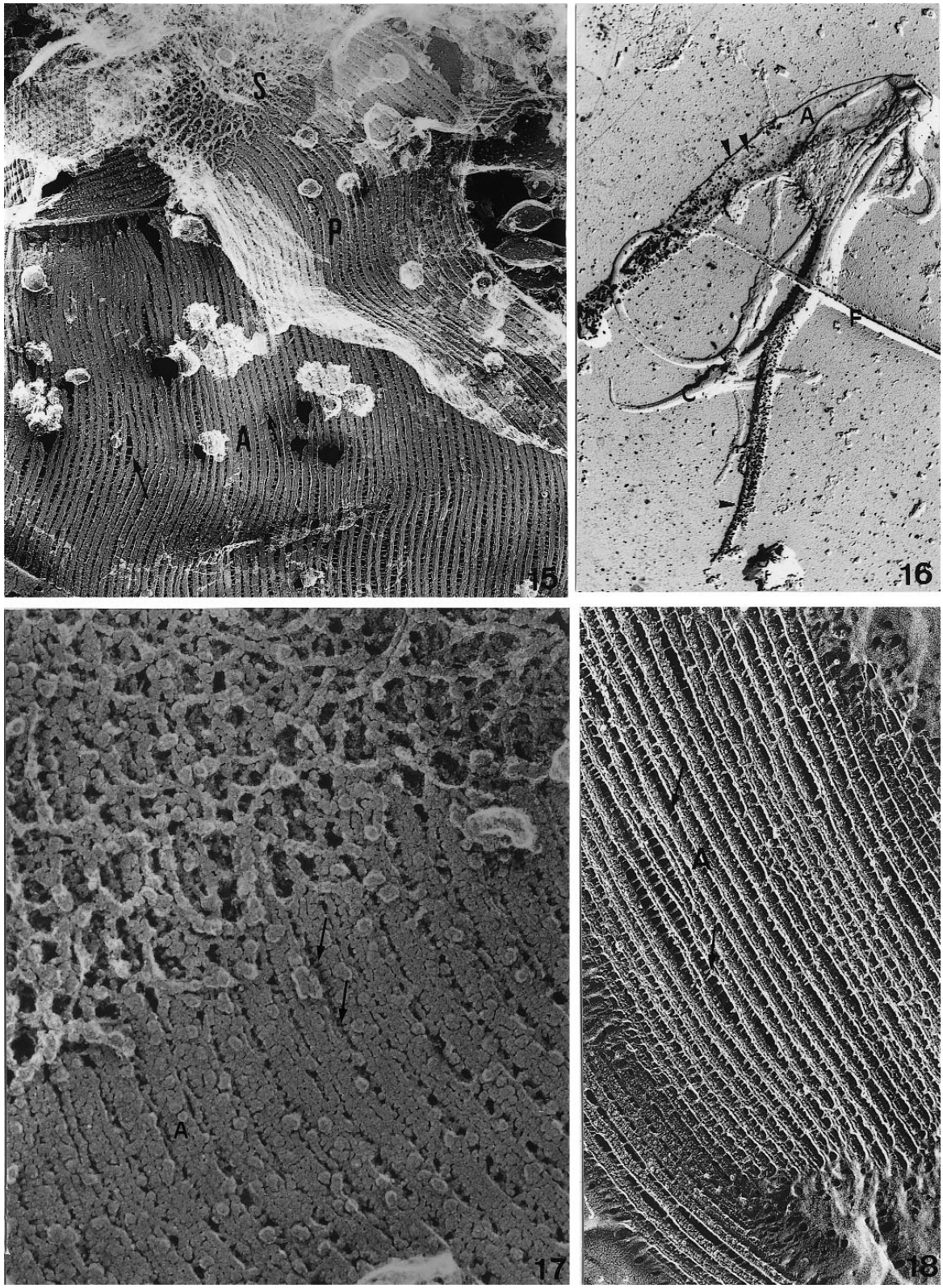

Fig. 15 General view of the pelta-axostylar system in a Triton X-100 extracted *T. foetus*. The isolated pelta-axostylar structure was processed by quick-freezing and a freeze-etching replica was obtained. The sigmoid filaments (S) are seen at the pelta (P) region as a special pattern. The axostyle (A) seen is this figure is the wider portion of the capitulum. Note the bridges connecting the microtubules which form the pelta – axostylar system (arrows). × 80 000.

Fig. 16 Replica of an isolated cytoskeleton of *T. foetus* labeled with gold conjugated anti-acetylated α-tubulin. Labeling (arrowheads) is seen over the axostyle (A) and flagella. Costa (C) and flagella (F) are still connected to the axostyle (A) which is wider at the anterior region, forming the capitulum. Note that costa (C) is unlabeled. × 10 000.

Figs 17–18 Views of freeze-etched *T. foetus* axostyle (A) microtubules connected by bridges (arrows). Fig. 17: × 160 000; Fig. 18: 90 000.

microtubules (Robson & Burgoyne, 1989), in disagreement with the findings of others who claim that this structure is not permanent (Juliano et al., 1986; Viscogliosi & Brugerolle, 1994; Delgado–Viscogliosi et al., 1996).

The axostyle in *T. foetus* and *Monocercomonas sp.* is composed of singlet microtubles appearing to narrow slightly, but progressively until its terminal segment, and turns upon itself forming a tube, which protrudes at the cell's posterior tip. Axostyle cross-bridges are seen in longitudinal images of replicas of freeze-etched cells after quick-freezing, as previously reported by our group (Benchimol et al., 1993). Similar bridges along the microtubules were described in motile axostyles, presenting a periodicity of about 16 nm (MacIntosh, 1974; Grimstone & Cleveland, 1965; Heuser, 1986). In contractile axostyles, Grimstone and Cleveland (1965) presented images of axostylar microtubules in longitudinal sections showing regular cross-connections between neighboring microtubules and showing a periodicity of about 15 nm. In the trichomonads axostyle we found a 9 nm periodicity. It does not present any contraction or movement as observed in other axostyles, such as those found in *Oxymonas*, *Saccinobaculus* and *Notila* flagellates (Grimstone & Cleveland, 1965) and also in *Cryptocercus* gut protozoa (Mooseker & Tilney, 1973). In these cells the axostyles consist of regular arrays of microtubules, the number of which varies between 100 and 5000 in different species. On the other hand, Mooseker and Tilney (1973) found ATPase activity in isolated axostyles. They also found that most of the proteins of the isolated axostyle comigrated on SDS polyacrylamide gels with dynein, tubulin, nexin and various secondary proteins of the ciliary axoneme. The axostyle in the flagellates cited above, contains bridges interacting with adjacent rows of microtubules (Mooseker & Tilney, 1973). MacIntosh (1974) reserved the term 'bridges' for connections between tubules in adjacent rows (interrow bridges) and the word 'link' for intrarow connections. As trichomonads axostyle is composed by only one row, the more appropriate term would be 'link'. We concluded that there are two sets of linkages holding the microtubules of the axostyle in trichomonads. We were not able to find any labeling after immunocytochemistry using anti-dynein antibodies (not shown).

An uncommon sub-structure of the trichomonad axostyle microtubules was found. The wall of these microtubules presents 13 protofilaments plus an additional arm consisting of two protofilaments forming a projection. We believe that this extra arm is preserved by the fast-freezing methodology used here, since it was observed after high-pressure freezing followed by freeze-substitution. This arm sub-structure is different from dynein, a MAP present in several microtubules, since it is found in a single pattern and presents two protofilaments. Although microtubules are better preserved when fixed in glutaraldehyde, and the protofilaments can be visualized in preparations where tannic acid or negative staining are used (Afzelius et al., 1990; Mizuhira & Futaesaku, 1972; Andre & Thiery, 1963; Dallai & Afzelius, 1991), we were able to examine the individual protofilaments in the axostyle microtubules without using any of these methodologies but using freeze-substitution in acetone-osmium tetroxide medium, a fixative harmful to proteins. Variations in the number of protofilaments have been described in some cell structures such as the accessory tubules of flagellar axonemes in insect spermatozoa (Afzelius & Dallai, 1994) and in developing wings of *Drosophyla melanogaster* (Mogensen et al., 1989). In this last work it was found that the microtubules in the transcellular bundles have 15 protofilaments. Although microtubules with more or less than the usual 13 protofilaments have been detected in several cell types (Eichenlaub-Ritter & Tucker, 1984), it has yet to be ascertained whether microtubules with more or less than 13 protofilaments could be hook decorated with exogenous tubulin (Mogensen et al., 1989). In the axostyle of *Saccinobaculus*, projections extending from the microtubules of one sheet to the microtubules of the next sheet were seen, although they are not formed by protofilaments (Woodrum & Linck, 1980).

The axostyle in trichomonads is associated with glycogen granules, profiles of endoplasmic reticulum, sigmoid filaments and hydrogenosomes. It is known from several other cell types that microtubules can be associated with organelles, such as mitochondria (Smith et al., 1977; Heggeness et al., 1978), Golgi complex (Kreis, 1990), endoplasmic reticulum (Terasaki et al., 1986), lysosomes (Matteoni & Kreis, 1987) and peroxisomes (Schrader et al., 1996), but there is no information available on hydrogenosomes. The association between hydrogenosomes and axostyle is well known and because of this, these organelles were, at first, named paraxostylar granules (Honigberg et al., 1971). However, why and how this association exists is an open question. The hydrogenosome is an organelle described in trichomonads, and also in organisms phylogenetically distant such as some free-living ciliates, rumen ciliates and chytrid fungi (Chytridiomycetes).

The shape, intracellular transport and distribution of most membrane-bound organelles are determined by their interaction with cytoskeleton. Studies performed by Benchimol and De Souza (1987), using the high voltage electron microscope, clearly showed the association of hydrogenosomes and the microtubules of the pelta-axostylar system. In the present study we have shown by freeze-etching that this association is made by delicate fibers. The composition of these filaments needs to be established. The association of the hydrogenosome with the axostyle could be explained-as ATP source from hydrogenosomes to the axostyle microtubules similar to what has been well established between mitochondria and microtubules (Heggeness et al., 1978). On the other hand, we observed that during the division process, the hydrogenosomes are distributed between the two daughter cells in close association to the axostyle (unpublished results). Therefore, it seems likely that the linkage between

hydrogenosomes and the axostyle could represent a way to assure the correct hydrogenosome distribution during the cytokinesis.

It was observed that several vesicles are in strict alignment with the axostyle in *Tritrichomonas foetus* and *Monocercomonas sp*. In contractile axostyles of certain flagellates the axostyle was seen associated with rough-surfaced, ribosome-bearing membranes and also small vesicles with smooth membranes in some organisms (Grimstone & Cleveland, 1965). Cytochemistry for glucose-6-phosphatase performed here, showed that structures which follow the axostyle in trichomonads are profiles of the SER. A previous study using this enzyme detection in *T. foetus* did not show this relationship (Queiroz et al., 1991). The presence of the SER near the axostyle could indicate a functional interaction between these two structures. We speculate that it could provide calcium for the axostyle microtubules. A close structural association between Ca^{2+} sequestering SER and microtubules has been shown in other cell systems (Walz, 1983; Hepler, 1980; Porter & MacNiven, 1982).

Profiles of endoplasmic reticulum in close association with the axostyle microtubules were seen to interact with the hydrogenosomal peripheral vesicle. Hydrogenosomes have been previously observed in close vicinity of ER (Benchimol et al., 1996), but the formation of the peripheral vesicle formation was not explained. Data obtained in the present study suggest that this structure could be formed by endoplasmic reticulum membranes.

the axostyle microtubules are always found in association with glycogen rosettes. It was established that the main source of energy of *Trichomonas vaginalis* consists of carbohydrate and these cells present a high content of glycogen. Endogenous glycogen reserves support the high rate of metabolism which occurs in both the cytosol and the hydrogenosomes (Honigberg & Brugerolle, 1990). The glycogen particles are seen as α and β particles. The α particles are rosettes whereas the β particles are spheroid subunits (Drochmans, 1962).

The association of the pelta microtubules with the sigmoid filaments occurs through proteinaceous bridges, which are not made of tubulin. Honigberg et al. (1971) suggested that approximately eight or nine sigmoid filaments extend between the basal body #2 terminating on the inner surface of the pelta in the region of the pelta-axostylar junction. The composition and function of these structures are unknown. They could reinforce the pelta-axostylar junction, and/or keep the basal bodies region linked to the pelta-axostylar in order to enssure the correct migration during the cell division process.

In conclusion, we have shown that the axostyle of *Tritichomonas foetus* and *Monocercomonas sp.* is a ribbon of parallel microtubules with 13 protofilaments plus a lateral projection made of two additional protofilaments. The axostyle is connected through cross-bridges to other cell structures such as hydrogenosomes, sigmoid filaments, pelta, and the endoplasmic reticulum. Further studies are necessary to clarify functional aspects of this interesting cell structure.

ACKNOWLEDGMENTS

The authors thank Dr Wanderley de Souza for critical reading of the manuscript and Rivea Cristina Rodrigues for technical assistance. This work has been supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNP_q), Fundaç~ao de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Programa de Núcleos de Excelência (PRONEX) and Associaç~ao Universitária Santa Úrsula (AUSU).

REFERENCES

Afzelius, B.A., Bellon, P.L. and Lanzavechia, S. 1990. Microtubules and their protofilaments in the flagellum of an insect spermatozoon. J. Cell Sci., 95, 207–217.

Afzelius, B.A. and Dallai, R. 1994. Characteristics of the flagellar axoneme in Neuroptera, Coleoptera, and Strepsiptera. J. Morphol., 219, 15–20.

André, J. and Thiery, J.P. 1963. Mise en evidence d'une sous-structure fibrillaire dans les filaments axonematiques des flagelles. J. Microsc. Paris, 2, 71–80.

Batista, C.M.C., Benchimol, M., Cunha e Silva, N.L. and DeSouza, W. 1988. Localization of acetylated α-tubulin in *Tritrichomonas foetus* and *Trichomonas vaginalis*. Cell Struct. Funct., 13, 445–453.

Benchimol, M. and De Souza, W. 1987. Structural analysis of the cytoskeleton of *Tritrichomonas foetus*. J. Submicrosc. Cytol., 19, 139–147.

Benchimol, M., Kachar, B. and DeSouza, W. 1993. The structural organization of the pathogenic protozoon *Tritrichomonas foetus* as seen in replicas of quick frozen, freeze-fractured and deep etched cells. Biol Cell, 77, 289–295.

Benchimol, M., Johnson, P.J. and DeSouza, W. 1996. Morphogenesis of the hydrogenosome: an ultrastructural study. Bio. Cell, 87, 197–205.

Brugerolle, G. 1975. Étude de la cryptopleuromitose et de la morphogenèse de division chez plusieurs Genres de trichomonadines primitives. Protistologica, 4, 457–468.

Dallai, R. and Afzelius, B.A. 1991. Sperm flagellum of *Dacus oleae* (Gmelin) (Tephritidae) and *Drosophila melanogaster* Meigen (Drosophilidae) (Diptera). Int. J. Insect Morphol. Embryol., 20, 215–222.

Delgado-Viscogliosi, P., Brugerolle, G. and Viscogliosi, E. 1996. Tubulin post-translational modifications in the primitive protist *Trichomonas vaginalis*. Cell Mot. Cytoskel., 33, 288–297.

Diamond, L.S. 1957. The establishment of various trichomonads of animals. J. Parasitol., 43, 488–490.

Drochmans, P. 1962. Morphologie du glycogène. Étude au microscope electronique de colorations négatives du glycogène particulaire. J. Biochem. Biophys. Cytol., 8, 553–558.

Eichenlaub-Ritter, U. and Tucker, J.B. 1984. Microtubules with more than 13 protofilaments in the dividing nuclei of ciliates. Nature (Lond.) 307, 60–62.

Grimstone, A.V. and Cleveland, L.R. 1965. The fine structure and function of the contractile axostyles of certain flagellates. J. Cell Biol., 24, 387–400.

Heggeness, M.H., Simon, M. and Singer, S.J. 1978. Association of mitochondria with microtubules in cultured cells. Proc. Natl. Acad. Sci. USA, 75, 3863–3867.

Hepler, P.K. 1980. Membranes in the mitotic apparatus of barley cells. J. Cell Biol., 86, 490–499.

Heuser, J.E. 1986. Different structural states of a microtubule cross-linking molecule, captured by quick-freezing motile axostyles in protozoa. J. Cell Biol., 103, 2209–2227.

Honigberg, M.B. and Brugerolle, G. 1990. Structure. In: Honigberg, M.B. (ed) Trichomonads Parasitic in human. Springer-Verlag, Munich, 5–35.

Honigberg, M.B., Mattern, C.F.T. and Daniel, W.A. 1971. Fine structure of the mastigont system in *Tritrichomonas foetus* (Riedmüller). J. Protozool., 18, 183–198.

Juliano, C., Monaco, G., Rubino, S. and Cappuccinelli, P. 1986. Inhibition of *Trichomonas vaginalis* replication by the microtubule stabilizer taxol. J. Protozool., 33, 255–260.

Kreis, T.E. 1990. Role of microtubules in the organization of the Golgi apparatus. Cell Mot. Cytoskel., 15, 67–70.

MacIntosh, J.R. 1974. Bridges between microtubules. J. Cell Biol., 61, 166–187.

Matteoni, R. and Kreis, T.E. 1987. Translocation and clustering of endosomes and lysosomes depends on microtubules. J. Cell Biol., 105, 1253–1265.

Mizuhira, V. and Futaesaku, Y. 1972. New fixation for biological membranes using tannic acid. Acta Histochem. Cytochem., 5, 233–235.

Mogensen, M.M., Tucker, J.B. and Stebbings, H. 1989. Microtubule polarities indicate that nucleation and capture of microtubules occurs at cell surfaces in *Drosophila.* J. Cell Biol., 108, 1445–1452.

Mooseker, M.S. and Tilney, L.G. 1973. Isolation and reactivation of the axostyle. Evidence for a dynein-like ATPase in the axostyle. J. Cell Biol., 56, 13–26.

Porter, K.R. and MacNiven, M.A. 1982. The cytoplast: a unit structure in chromatophores. Cell, 29, 23–32.

Queiroz, R.C.B., Silva Santos, L.M., Benchimol, M. and De Souza, W. 1991. Cytochemical localization of enzyme markers in *Tritrichomonas foetus*. Parasitol. Res., 77, 561–566.

Ribeiro, K.C., Monteiro-Leal, L.H. and Benchimol, M. Contributions of the axostyle and flagella to closed mitosis in the protists Trichomonas foetus and Trichomonas vaginalis, in press.

Robinson, J.M. and Karnovsky, M.J. 1983. Ultrastructural localization of several phosphatases with cerium. J. Histochem. Cytochem., 31, 1197–1208.

Robson, S.J. and Burgoyne, R.D. 1989. Differential localisation of tyrosinated, detyrosinated, and acetylated α-tubulins in neurites and growth cones of dorsal root ganglion neurons. Cell Motil., 12, 273–282.

Schrader, M.G., Burkhardt, J.K., Baumgart, E., Lüers, Spring, H., Völkl, A. and Fahimi, H.D. 1996. Interaction of microtubules with peroxisomes. Eur. J. Cell Biol., 69, 24–35.

Silva-Filho, F.C. and De Souza, W. 1986. Effect of colchicine, vinblastine, and cytochalasin B on cell surface anionic sites of *Tritrichomonas foetus*. J. Protozool., 33, 6–10.

Smith, D.S., Järlfors, U. and Cayer, M.L. 1977. Structural cross-bridges between microtubules and mitochondria in central axons of an insect (*Periplaneta americana*). J. Cell Sci., 27, 255–272.

Terasaki, M., Chen, L.B. and Fujiwara, K. 1986. Microtubules and the endoplasmic reticulum are highly interdependent structures. J. Cell Biol., 103, 1557–1568.

Viscogliosi, E. and Brugerolle, G. 1994. Cytoskeleton in trichomonads. III Study of the morphogenesis during division by using monoclonal antibodies against cytoskeletal structures. Eur. J. Protistol., 30, 129–138.

Viscogliosi, E. and Delgado-Viscogliosi, P. 1996. Cytosquelette, division et évolution dans un groupe de protistes primitifs, les Trichomonadines. Ann. Biol., 35, 146–161.

Walz, B. 1983. Association between cytoskeletal microtubules and Ca^{2+}-sequestering smooth ER in semper cells of fly ommatidia. Eur. J. Cell Biol., 32, 92–98.

Woodrum, D.T. and Linck, R.W. 1980. Structural basis of motility in the microtubular axostyles: implications for cytoplasmic microtubule structure. J. Cell. Biol., 87, 404–414.