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Wanderley de Souza
Editor

Structures and Organelles in Pathogenic Protists

Springer

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Preface

Parasitic protozoa comprise a large number of species, including some which are agents of human and veterinary diseases such as malaria, leishmaniasis, Chagas disease, African trypanosomiasis, amebiasis, trichomoniasis, giardiasis, toxoplasmosis, coccidiosis, theileriosis, and babesiosis, to mention only the more important ones. Some of these protozoa, as is the case with *Trichomonas*, present a simple life cycle. For others, however, as occurs with Apicomplexa (which includes *Plasmodium, Toxoplasma, Eimeria*, etc.) and some trypanosomatids, the life cycle is relatively complex, displaying several developmental stages in the vertebrate host and, in some cases, in invertebrate hosts. These protozoa are also of interest from a cell biology point of view, as they present special cytoplasmic structures and organelles that have been studied in some detail during the last several years, providing new information of general biological interest. These studies have discovered new metabolic pathways that take place in these organelles and open up alternate possibilities for the identification of different drug targets and innovative drugs to be used for the treatment of patients and animals with diseases caused by protozoa.

There are currently only a few publications that review the available data on the cell biology of pathogenic protozoa. This Microbiology Monographs volume covers the current and most recent advances made on relevant cytoskeletal structures and organelles found in parasitic protists. Renowned scientists, some of whom were directly involved in the discovery and characterization of these organelles, have contributed reviews that incorporate recent results obtained using modern cell biology and molecular approaches, including genomics and proteomics. Some important organelles such as the hydrogenosome and mitosomes were not reviewed here as they were examined in detail in a previous volume of this series (Tachezy 2008).

The first group of reviews deals with cytoskeletal structures such as the mastigont system found in trichomonads (written by Marlene Benchimol), the subpellicular microtubules, better characterized in trypanosomatids and in some Apicomplexa (written by Wanderley de Souza and Marcia Attias), and the paraflagellar rod, a characteristic feature of the flagellum of some protists (written by Johana Buisson and Philippe Bastin).

The second group deals with structures and organelles involved in the synthesis and secretion of macromolecules, as well as in the uptake of molecules through an endocytic process. These include the flagellar pocket of trypanosomatids (written by Paul McKean and Keith Gull), the reservosome of *Trypanosoma cruzi* (written by Narcisa Leal Cunha-e-Silva, Celso Sant'Anna, Miria Pereira, and Wanderley de Souza), the megasome found in *Leishmania* (written by Diane McMahon-Pratt, Tania Ueda-Nakmura, and Yara Traub-Cseko), the various organelles and the traffic of vesicles in *Entamoeba histolytica* (written by Sherri Smith and Nancy Guillen), the secretory organelles found in members of Apicomplexa (written by Jean François Dubremetz), and the secretory events that take place during the process of encystation of *Giardia lamblia* (written by Fernando Rivero, Dana Muller, and Hugo Lujan).

The final group of reviews deals with various organelles, which are characteristic features of protozoa. These include the kinetoplast–mitochondrion complex of trypanosomes and related flagellates (written by Julius Lukes, Hassan Hashimi, Zdenek Verner, and Zdenka Cicov), the apicoplast, an ancient organelle found in Apicomplexa (written by Swaiti Agrawal, Sethu Nair, Lilach Sheiner, and Boris Striepen), the glycosomes found in Kinetoplastida (written by Fred Opperdoes), and the acidocalcisome found in several protozoa (written by Paul Ulrich, Rozana Cintrón-Moret, and Roberto Docampo).

Rio de Janeiro, Brazil Wanderley de Souza

Reference

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The Mastigont System in Trichomonads

Marlene Benchimol

Contents

Abstract Trichomonads are protists that are found in several environments. Some of them are parasites, whereas others live in the gut of different animals without provoking any apparent infection. The trichomonad mastigont system is formed by the basal bodies and several structures composed of rootlets and proteinaceous fibrils; the function of many of these structures is not yet determined. The main structures in the mastigont system consist of the following: the pelta–axostyle system, made of microtubules; the costa, a periodic rootlet; the parabasal and sigmoid filaments; and several other filaments. The axostyle supports the axis of the cell and participates in karyokinesis, the costa supports the flagellar movements of the recurrent flagellum, and several fibrils provide an anchoring system for the Golgi and the nucleus. The pelta, a microtubular structure, supports the flagellar

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canal. The trichomonad mastigont system has been studied with different techniques such as plasma membrane extraction, high-voltage electron microscopy, field emission scanning electron microscopy, the cell-sandwich technique, freeze-etching, and immunocytochemistry.

1 Introduction

Trichomonads are protists found in several environments. Some are parasites, whereas others live in the gut of different animals, such as mammals, birds, snakes, and insects, without provoking any apparent infection. Among the most important trichomonads are *Tritrichomonas foetus* (Figs. 1 and 2) and *Trichomonas vaginalis* (Fig. 3), which are flagellated parasites of the urogenital tracts of cattle and humans, respectively. Trichomoniasis, caused by *T. vaginalis*, is the most common non-viral sexually transmitted infection associated with adverse consequences for women's health (World Health Organization 2001). Molecular phylogenetic studies using large and small subunit ribosomal RNAs indicate that these organisms are among the most early diverging eukaryotes (Cavalier-Smith and Chao 1996), belonging to the Phylum Parabasalia, although it is possible that phylogenetic repositioning took place (Embley and Hirt 1998). It was demonstrated that *T. foetus* and *Tritrichomonas suis* belong to the same species (Mattos et al. 1997; Tachezy et al. 2002).

T. foetus (Figs. 1a, b and 2) and *T. vaginalis* (Figs. 3a, b and 4) are extracellular parasites, and they must overcome the mucus barrier and parasitise the vaginal epithelium for infection to occur. When grown in axenic medium (Figs. 1a, b, 8a, and 9a–d), *T. foetus* is characterised by a pear-shaped body that is 16.5-μm long and 5.0–6.7-μm wide (Mattos et al. 1997), with one anterior nucleus (Figs. 1, 2, and 9c, d).

2 The Mastigont System

Trichomonads present unusual organelles, such as the hydrogenosomes, mitochondria-like organelles that produce ATP and molecular hydrogen, the pelta, the axostyle, the costa, and several rootlet fibres concentrated in the mastigont system, as shown in the illustration in Fig. 2. This system is localised to the anterior region of the protist's body, as we will present in detail below.

The complex mastigont system (Figs. 1b, 2a, b, 5a, b, 6a, b, 9a–d, and 10–14) is comprised of several skeletal structures, including the following (1) the flagellar axonemes and their respective basal bodies (kinetosomes), located at the most anterior region of the cell body (Figs. 1b, 5a, b, 6a, b, and 12–14); (2) several appendages and rootlet filaments around the basal bodies (Figs. 1b, 2, 5a, b, 6a, b,

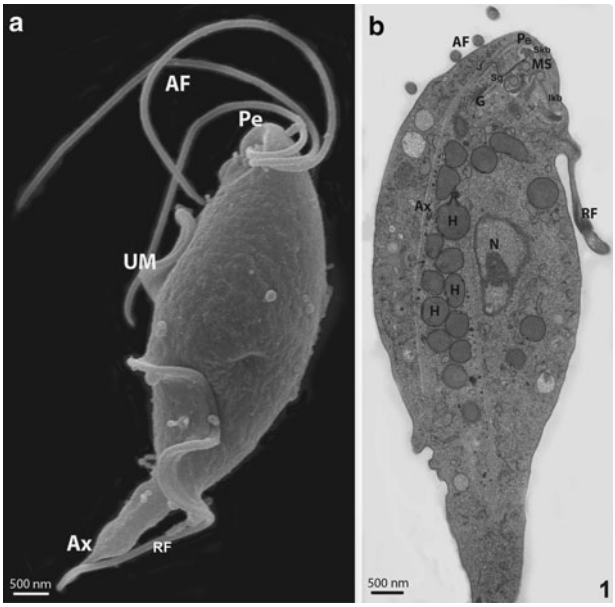

Fig. 1 Routine preparation for scanning (**a**) and transmission electron microscopy (**b**) of *Tritrichomonas foetus*. Figure (**a**) shows a general view with the anterior flagella (AF), the recurrent flagellum (RF) forming the undulating membrane (UM), the flagellar canal supported by the pelta (Pe), and the region of origin of the anterior flagella. The axostyle (Ax) forms the posterior tip of the cell. Figure (**b**) shows the cell interior and it is possible to note the following structures: an anterior single nucleus (N), the axostyle (Ax), the pelta (Pe), and the mastigont system (MS), which includes kinetosomes (basal bodies) and associated structures such as the supra-kinetosomal body (Skb) and the infra-kinetosomal body (Ikb). Note that the sigmoid filaments end in the pelta–axostylar junction (J). The anterior flagella (AF), the recurrent flagellum (RF), the Golgi complex (G), and the hydrogenosomes (H) aligned near the axostyle (Ax) can be seen clearly. *Bars*, 500 nm (Pereira-Neves and Benchimol unpublished)

9d, 13, and 14); (3) the costa (Figs. 2a, b, 7a–c, 9a–d, and 14), a periodic proteinaceous structure that underlies the recurrent flagellum along the undulating membrane (UM); (4) the parabasal filaments (PBF) (Figs. 2a, b, 3b, 5a, b, and 9b–d), which support a single Golgi complex, forming a Parabasal apparatus (Figs. 9b, c, and 14); (5) a pelta (Figs. 1a, b, 2a, b, 5a, b, 6a, b, 7a, 9b–d, 13, and 14) supporting the flagellar canal (Figs. 1a and 9b); and (6) a single ribbon of microtubules forming the axostyle (Figs. 1–5, 9a–d, 10, and 14), which runs from the basal bodies to the cell tip, forming, together with the pelta, the pelta–axostyle complex. Trichomonads also present other structures not directly related to the mastigont system, such as a nucleus, hydrogenosomes, glycogen granules, a Golgi complex, lysosomes, an endoplasmic reticulum, and a cell matrix that contains an extensive and elaborate three-dimensional skeletal network (Figs. 1b, 2a, b, 3, and 5a, b).

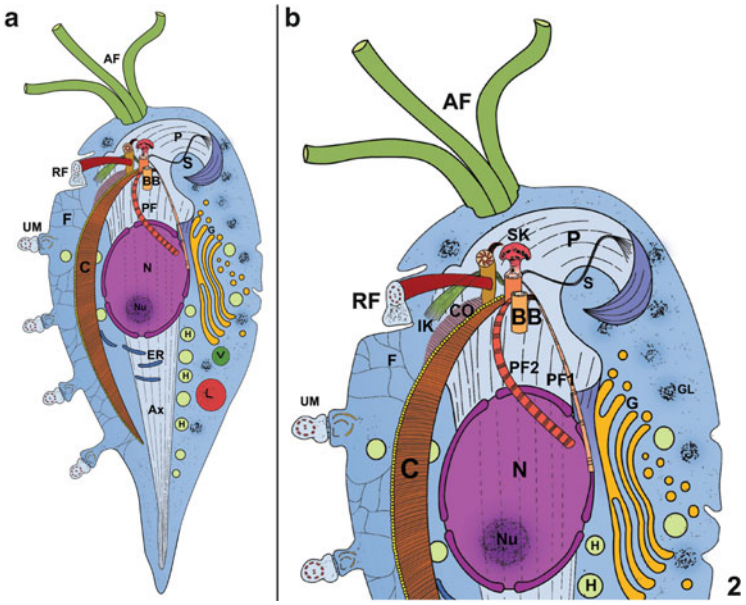

Fig. 2 Schematic diagrams of a whole view of *Tritrichomonas foetus* (**a**) and detail of the anterior region (**b**) showing the main cell structures and a close view of the mastigont system. *AF* anterior flagella; *Ax* axostyle; *BB* basal body; *C* costa; *Co* comb; *ER* endoplasmic reticulum; *F* filaments connecting the costa to the plasma membrane, next to the recurrent flagellum; *G* Golgi; *GL* glycogen granules; *H* hydrogenosomes; *IK* infra-kinetosomal filament; *L* lysosome; *N* nucleus; *Nu* nucleolus; *P* pelta; *PF* parabasal filament; PF_1 parabasal filament number 1; PF_2 parabasal filament number 2; *RF* recurrent flagellum; *S* sigmoid filaments; *SK* supra-kinetosomal body; *UM* undulating membrane; *V* vacuole (Benchimol unpublished)

Several procedures that provide three-dimensional images have been employed to better analyse the mastigont system in trichomonads; these include routine preparation for either conventional (Figs. 1b, 3, 5, and 6) or high-voltage transmission electron microscopy (HVTEM) (Fig. 9a), detergent-extraction procedures and observation with scanning electron microscopy (SEM) (Fig. 9b–d), physical fixation by freezing methods (Figs. 7c, 10, and 11b, c), and techniques such as immunocytochemistry (Fig. 6a) and tomography (Benchimol 2004, 2005, in press; Benchimol et al. 1992, 1993, 2000; Lee et al. 2009).

2.1 Basal Bodies and Associated Filaments

2.1.1 Basal Body (kinetosomes)

The kinetosomes or basal bodies are microtubular structures located in the most anterior region of the cell, where the flagella, the spindle, and several other rootlet

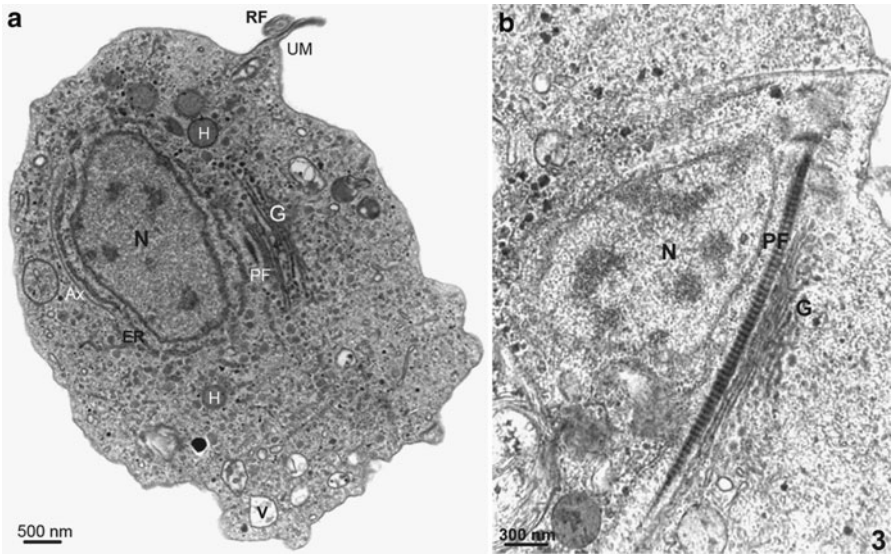

Fig. 3 Routine preparation for transmission electron microscopy of *Trichomonas vaginalis* showing a general view of the cell (**a**) and detail of the Golgi complex (G) and its parabasal filament (PF), a periodic structure that follows the Golgi in trichomonads in (**b**). *N* nucleus, recurrent flagellum (RF), forming the undulating membrane (UM), the endoplasmic reticulum (ER), Golgi and its parabasal filament (PF), vacuoles (V), hydrogenosomes (H), and the axostyle (Ax). *Bar*, 500 nm (**a**), 300 nm (**b**) (Benchimol unpublished)

structures originate (Figs. 1b, 2a, b, 5a, b, 6a, b, 9c, d, 13a, b, and 14). The number of kinetosomes varies according to the *Trichomonas* species; for instance, there are five kinetosomes in *T. vaginalis* (Fig. 4) and four in *T. foetus* (Figs. 1b and 2a, b). All these kinetosomes are embedded in a peripheral material of greater density compared to the surrounding cytoplasm.

Several proteinaceous accessory structures, such as striated roots and filaments in the form of hook-shaped lamellae, are associated with the basal bodies (Figs. 1b, 2a, b, 5a, b, and 6b); they include the sigmoid filaments (Figs. 1b, 2a, b, 5, 6a, 9d, 13a, b, and 14), the supra-kinetosomal (Figs. 1b, 2a, b, 5a, b, and 6b) and infra-kinetosomal bodies (Figs. 1b, 5a, b, and 6b), the PBF (Figs. 3a, b, 5a, and 14), the costa (Figs. 2a, b, 7a–c, 9a–d, and 14), the comb (Figs. 2a, b and 5b), and the pelta–axostyle complex (Figs. 1b, 2a, b, 5a, b, 9a–d, 14, and Table 1). None of these fibres are considered contractile (Honigberg and Brugerolle 1990; Bricheux et al. 2000; Viscogliosi and Brugerolle 1994), with the few exceptions outlined below.

The basal bodies present a structure common to the basal bodies of many organisms, including higher eukaryotic cells, with nine triplets of microtubules (Figs. 1b, 5a, and 6b). In the transitional zone, the basal bodies present a wheel-like aspect comprising a central hub and spokes (Benchimol 2004). Each flagellar axoneme emerges, with its typical structure consisting of 9+2 microtubules.

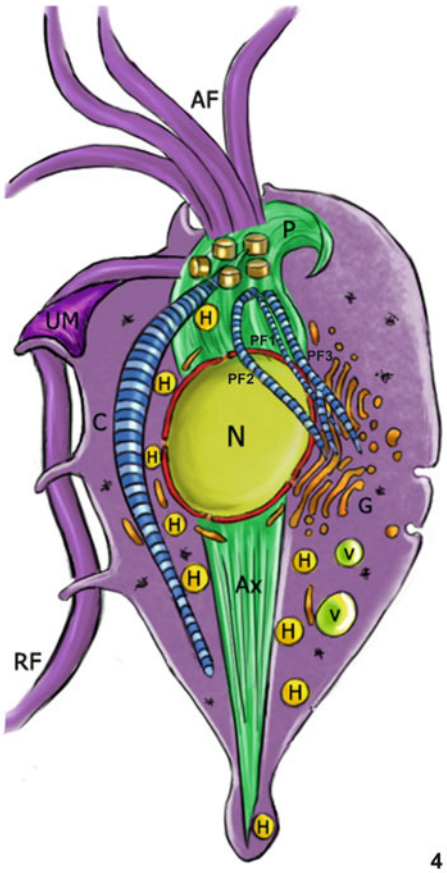

Fig. 4 Schematic diagram of *T. vaginalis* showing the undulating membrane (UM) which is formed by a fold of the plasma membrane in contact with the recurrent flagellum (RF). Note the difference with the undulating membrane of the *T. foetus* in Figs. 1 and 2. *AF* anterior flagella; *Ax* axostyle; *BB* basal body; *C* costa; *H* hydrogenosome; *N* nucleus; *PF1* parabasal filament 1; *PF2* parabasal filament 2; *PF3* parabasal filament 3

In trichomonads, the basal bodies/kinetosomes are assembled during the S phase (Ribeiro et al. 2000).

2.1.2 Kinetosome R

The recurrent flagellum (Figs. 1a, 2a, b, 3a, 4, 9a–c, and 11a) originates from the R kinetosome (Figs. 4, 6a, and 7a), which is situated at a right angle to the kinetosomes of the anterior flagella.

2.1.3 Kinetosomes #1, #2, #3, #4

Depending on the species (Figs. 1b, 2a, b, 5a, b, 6a, b, and 13a, b), there are several other kinetosomes that are located in the anterior region and connect to

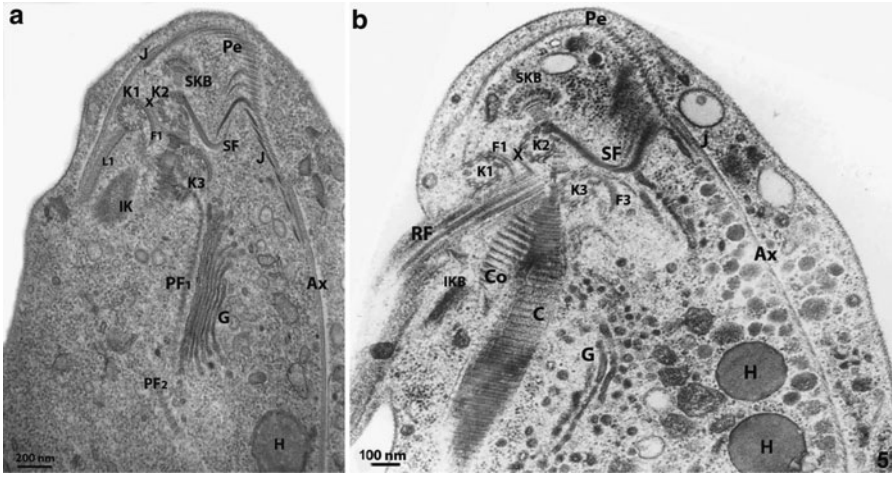

Fig. 5 (**a**, **b**) Routine thin sections of *T. foetus* through the anterior region. These two sequential sections display the complex structures that form the mastigont system. The basal bodies or kinetosomes, K_1, K_2, and K_3, are seen. Originating from kinetosome 1 (K_1), lamella 1 (L_1) is located towards the posterior region. The sigmoidal filaments (SF) emerge from kinetosome 2 towards the pelta (Pe). The costa (C) originates in the region of kinetosomes R (not seen here) and K_2–K_3, close to the comb (Co). The pelta (Pe) and the axostyle (Ax) overlap in the pelta–axostylar junction (J). The basal body of the recurrent flagellum (RF) is in an orthogonal position in relation to the other three basal bodies of the anterior flagella (K_1–K_3). Note the presence of rootlet filaments (F_1–F_3) emerging from basal bodies 1 and 3. Another fibrillar structure (**X**) courses between and connects kinetosome #2 and F_1. The sigmoid filaments (SF) emerge from the region of basal body 2 and the supra-basal body (SB). The supra-basal (or supra-kinetosomal) body (SKB) appears as a stalk connected to basal body 2, whereas the infra-basal (or infra-kinetosomal) body (IKB) lies below the whole basal body complex. The parabasal filaments (PF_1–PF_2) follow the Golgi complex (G). *H* hydrogenosome. (**a**, **b**) Benchimol (unpublished). *Bars* (**a**) 200 nm, (**b**) 100 nm

several other mastigont structures All kinetosomes are formed by microtubule triplets (Fig. 6b) in a configuration similar to that seen in the centrioles of higher eukaryotes.

2.1.4 Clockwise Filaments

There are several types of filaments and lamellae originating from the kinetosome region (Figs. 1b, 2a, b, 5a, b, 6a, b, and 9d). These filaments have a clockwise orientation and emerge from each kinetosome. Kinetosome #1 is the origin of the clockwise F_1 (filament 1) as well as other filaments known as the marginal lamellae (ML) of the UM (Figs. 1a, 2, and 5a, b), which emerge from the left side of kinetosome #1. There is also IK1, a filament that emerges from kinetosome #1 to the right of the ML (Figs. 2a, b and 5a).

Kinetosome #2 is the origin of the F_2 sigmoid filaments and the X filament (Figs. 1b, 2a, b, 5a, b, 6a, b, 9d, and 14). The sigmoid filaments course ventrally and

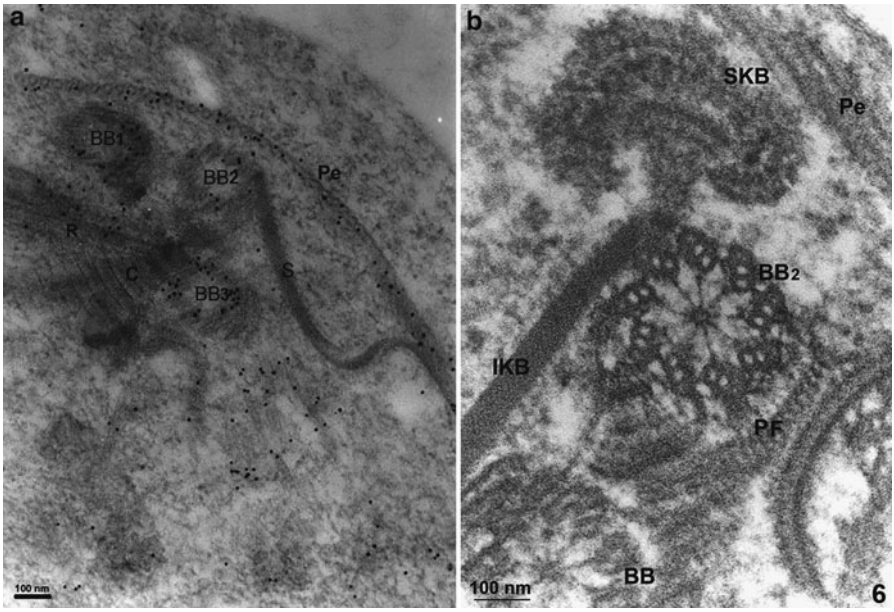

Fig. 6 Immunolabelling (**a**) and routine thin section (**b**) through the anterior region of *T. foetus,* showing some structures that form the mastigont system. The basal bodies, BB_1, BB_2, and BB_3, are seen labelled by gold-conjugated anti-acetylated tubulin monoclonal antibody. The sigmoidal filaments (SF) emerge from the basal bodies towards the pelta (Pe). The costa (C) emerges in the region of the R kinetosome (*not seen here*) and BB_2–BB_3. The pelta (Pe) is formed by microtubules and is also gold-labelled. The recurrent flagellum (R) is situated in an orthogonal position in relation to the other three basal bodies of the anterior flagella. The supra-basal body (SB) and the sigmoid filaments (SF) emerge from the region of basal body 2. Neither the costa nor the sigmoid filaments present labelling for anti-tubulin antibody; therefore, they are not made of microtubules. The supra-basal (or supra-kinetosomal) body (SKB) appears as a stalk connected to basal body 2, whereas the infra-basal (or infra-kinetosomal) body (IKB) lies below the whole basal body complex. The parabasal filaments (PF) are also seen. *Bars*, 100 nm (Benchimol unpublished)

towards the pelta–axostylar complex until they reach the pelta–axostylar junction (Figs. 1b, 5a, b, and 14). The X filament courses between and connects kinetosome #2 and F_1 (Fig. 5a, b). Kinetosome #3 is the origin of the clockwise periodic filament F_3 (Fig. 5a, b).

The costa originates between kinetosomes R and #2 (Figs. 2a, b, 5b, 6a, and 7a), and its broad base (Fig. 5a) is better seen in that region. In cells that present a comb (Fig. 5b), such as those of *T. foetus*, this structure originates from the R kinetosome and the costal base.

2.1.5 Sigmoidal Filaments

Several filamentous lamellae anchor the basal bodies and connect them to other cell structures such as the pelta and the cytoplasm. The largest of the lamellae is a

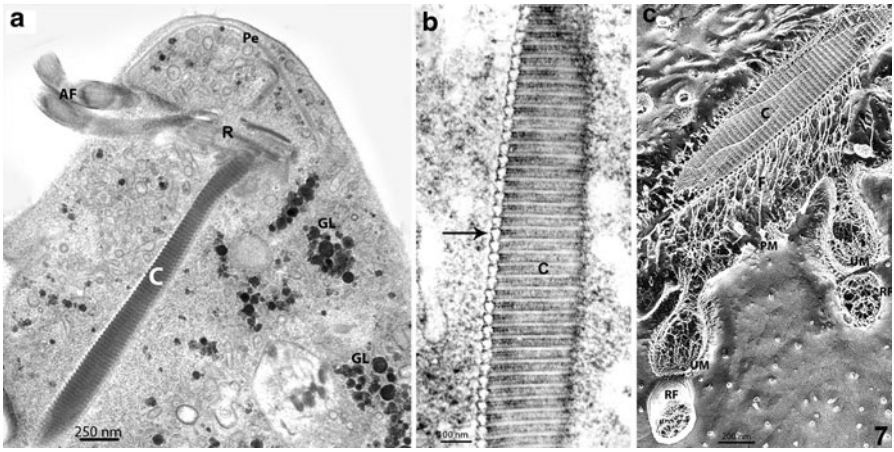

Fig. 7 Three different views (**a–c**) of the costa, a proteinaceous periodic structure present in trichomonads. This structure originates from the basal body region, between kinetosomes #2, R, and #3; it courses near the cell surface, in the region of the undulating membrane. Its periodic structure can be seen clearly (**a–c**). (**b**) Longitudinal thin section of the *T. foetus* costa (C) under high magnification, showing its periodic structure with dense bands alternating with lucent bands. Pits (*arrow*) are seen on the peripheral border of the costa. **(c)** Costa (C) of *T. foetus* cell treated by fast-freezing and freeze-fracture followed by freeze-etching. This preparation allowed the visualisation of 15-nm-thick filaments (F) emanating from the costa (C) and connecting it with the plasma membrane (PM). *GL* glycogen granules; *Pe* pelta; *AF* anterior flagella. *Bars*, (**a**) 250 nm; (**b**) 100 nm, (**c**) 200 nm [(**a**, **b**) Benchimol (unpublished); (**c**) from Benchimol et al. (1993)]

sigmoid sheet (Figs. 1b, 2a, b, 5a, b, 6a, 9d, 13a, b, and 14) composed of parallel filaments in a curved distribution that connect kinetosome #2 to the pelta and fan out at the first bend. These filaments end just in the region of the pelta–axostylar junction (Figs. 1b, 5a, b, 6a, and 14). A part of the sigmoid sheet of filaments originates from kinetosome #2, extends towards the pelta, and continues along the pelta–axostylar junction (Figs. 1b, 5a, b, and 14), which presented a positive reaction for centrin, and an unknown function. Tomographic images obtained by Lee et al. (2009) showed that the sigmoid filaments are composed of several individual filaments. The curve of each sigmoid filament gradually becomes smooth (Lee et al. 2009).

2.1.6 Supra-Kinetosomal Body or Supra-Basal Body

The Supra-kinetosomal bodies (SKB) (Figs. 1b, 2a, b, 5a, b, and 6b) is a stalked structure restricted to *Tritrichomonadinae*. It is connected to basal body #2 in the region of attachment of the sigmoid filaments (Figs. 1b, 2a, b, and 5a, b). In many views, it is observed to be crescent-shaped. Its function and composition are unknown but anti-tubulin antibodies do not stain this structure.

Table 1 Fibrillar structures in *Trichomonas*

Name	Characteristics	Function
Axostyle	Microtubular ribbon	Participates in karyokinesis
Pelta	Microtubular sheet; overlaps with the axostyle; anterior region of the cell	Reinforces the wall of the periflagellar canal
Costa	Periodic proteinaceous structure; courses near the cell surface; connects basal bodies #2 and #3	Supports the movement of the recurrent flagellum; serves as an axis for trichomonad cells
Comb	Periodic structure located between the costal base and basal body R; present only in *Tritrichomonadinae*	Unknown
Basal bodies (kinetosomes)	Nine microtubule triplets	Origin of the flagellar axonemes
MTOC (atractophore)	Electron-dense material found in the costal base (*T. foetus*) or at the origin of the parabasal filaments (*T. vaginalis)*	Origin of the spindle microtubules
Axonemes	Motile microtubule structures of flagella in typical 9+2 arrangement; four in *T. foetus* and five in *T. vaginalis*	Participates in cell movement and division
Parabasal filaments PF_1 –PF_3	Periodic structures; follow the Golgi	Probable function in supporting the Golgi
Parabasal filament PF_1	Connects to the kinetosome #2; courses to the right of lamella F_3; extends towards the end of the cel; PF_1 is a long filament that splits into two strands	Probable function in supporting the Golgi
Parabasal filament PF_2	Presents a common base with the costa; origin in kinetosomes #2 and #R	Unknown
Parabasal filament PF_3	Presents a common base with the costa; origin in kinetosomes #2 and #R ; runs closely to PF_1	Probable function in supporting the Golgi
Sigmoidal filaments	Filaments in curved distribution; connect basal body #2 to the pelta; course ventrally and towards the pelta–axostylar complex	Unknown; positive for centrin
Infra-basal body (Infra-kinetosomal body)	Large and dense structure, below the basal body complex	Contributes to the proximal marginal lamella; restricted to *Tritrichomonadinae*
Supra-kinetosomal body (Supra-basal body)	Stalked structure; connects to the basal body #2 in the region of the attachment of the sigmoid filaments; crescent-shaped in many views	Unknown; restricted to *Tritrichomonadinae*
J	Junction between the pelta and the axostyle	Unknown
Clockwise F_1 (filament 1)	Origin from the kinetosome #1; comma-shaped	Unknown
Marginal lamellae (ML)	Origin from the kinetosome #1	Filaments following the undulating membrane
IK1	Filament that emerges from kinetosome #1 on the right of the ML	Unknown

(*continued*)

Table 1 (continued)

Name	Characteristics	Function
X filament	Origin from the kinetosome #2; courses between and connects the kinetosome #2 and F_1	Unknown
Filament F_3	Origin from the kinetosome #3; a clockwise turning periodic filament; comma-shaped	Unknown
Undulating membrane	Contains the proximal and distal marginal lamella	Supports the beating of the recurrent flagellum
Marginal lamella	Origin from the kinetosome #1; raises the plasma membrane; forms a thin, finlike dorsal fold	Adheres to the recurrent flagellum

2.1.7 Infra-Kinetosomal Body or Infra-Basal Body

The infra-kinetosomal body (IKB) (Figs. 1b, 2b, 5a, b, and 6b) is a large and dense structure located below the basal body complex and restricted to the *Tritrichomonadinae*. The IKB appears to contribute to the proximal ML (Fig. 5a) of the UM.

2.1.8 Marginal Lamella

Kinetosome #1 presents a left rootlet filament or lamella that runs ventrally to the recurrent flagellum (Fig. 5a). This structure is hardly seen in conventional preparation and exhibits a stack of fine filaments when observed properly in cross-sections. Its function and composition are unknown, but anti-tubulin antibodies do not stain this structure.

2.2 Costa

The costa (Figs. 2a, b, 5b, 6a, 7a–c, 9a–d, and 14) is a rod-shaped skeletal birefringent structure found only in trichomonads that possess an UM. It is assumed that its function is to provide mechanical support to the UM. This large striated root fibril emerges from the basal body region, between the kinetosome of the recurrent flagellum (R) (Figs. 5b, 6a, and 7a) and kinetosomes #2 and #3 (Honigberg et al. 1971). The costa is broader at the point at which it emerges. This proteinaceous structure courses under the UM and extends towards the posterior region of the cell under the dorsal cell surface. There are connections between the costa and the plasma membrane in the region of the UM (Fig. 7c).

2.2.1 Structure

The costa is formed by a complex array of filaments and globular structures (Fig. 7a–c). Through the use of fast-freezing methods, it was observed that the

costa is connected to the recurrent flagellum through a complex network formed by 15- and 10-nm wide filaments that emerge from the peripheral region of the costa and penetrate into the surface projections of the protozoan body to which the recurrent flagellum is attached (Fig. 7c) (Benchimol et al. 1993). As demonstrated by several methods, such as thin-sections, freeze-fracture, deep-etching, and negative staining, the costa is a complex structure in which 60-nm-wide bands alternate with thinner, 26-nm bands (Figs. 7a–c and 14) (Benchimol et al. 1993). The complex array of filaments that form the costa is more clearly seen in the thinner bands. The costa also contains incomplete secondary transverse bands. This proteinaceous structure presents basic proteins, as demonstrated by cytochemical methods such as the use of ethanolic phosphotunstic acid (Benchimol et al. 1982a).

Freeze-etching experiments (Fig. 7c) demonstrated that, in those regions facing the areas where the recurrent flagellum establishes a specialised junction with a surface projection of the cell body, a large number of filaments, about 15 nm thick, emerge from pits in the costa and make contact with a network of other filaments present in the surface projections. These latter filaments are shorter and thinner and about 10 nm wide (Benchimol et al. 1993; Monteiro Leal et al. 1993). In addition, in some species such as *T. foetus*, a filamentous branch can be seen originating from the costa; it is known as C_1F_1. Pits (Fig. 7b) are seen in the peripheral border of the costa. Moreover, the costa was observed to be connected to the cytoskeleton via filaments (Fig. 7c).

2.2.2 Types of Costa

Costae are classified into two types depending on the striation pattern, size, and shape at the structure's origin: type A (also known as type C) and type B (or C_1) (Honigberg 1978). *T. foetus* has an A-type costa (Figs. 7a–c, 9a–d, and 14) whereas *T. vaginalis* as well as all *Trichomonas* and *Pentatrichomonas* have B-type costae.

The periodicity of B-type costae is estimated at about 42–60 nm (Honigberg and Brugerolle 1990), depending on the preservation method used. In *Trichomonas* (B-type costa), the longitudinal filaments are arranged in a "herringbone" pattern, a finding based on observed alteration in horizontal rows (Honigberg and Brugerolle 1990).

2.2.3 Association with Other Structures

The close association of the costa with the UM and the recurrent flagellum (Figs. 7c and 9a–c) led to the hypothesis that its function is to support the UM. Such an association is especially seen in electron micrographs of mildly detergent-extracted cells (Benchimol et al. 1993).

The hydrogenosomes (Figs. 1b, 2a, b, 3a, 5a, b, 13a, b, and 14), which are important ATP-producing organelles in trichomonads, are observed to be associated

with the costa and the axostyle (Fig. 9c), which explains the old nomenclature for these organelles (paracostal and paraxostylar granules).

2.2.4 Function and Motility

It is assumed that the costa is a non-motile structure but provides mechanical support to the membrane of the recurrent flagellum. However, in some large species such as *Trichomonas gigantea* and *T. termopsidis*, the costa is a motile structure (Amos et al. 1979). In parasitic protists such as *T. vaginalis* and *T. foetus*, the costa seems to be an immotile structure, although in pseudocysts the costa is curved (Fig. 14) (Benchimol unpublished).

2.2.5 Chemical Composition

Studies by Viscogliosi and Brugerolle as well as by our group, in which monoclonal antibodies were produced (Viscogliosi and Brugerolle 1994; Monteiro Leal et al. 1993; Benchimol unpublished) and biochemical techniques such as cell fractionation were used to obtain pure costa fractions, revealed that B-type costa proteins in trichomonads are composed of several major polypeptides with molecular weights between 100 and 135 kDa, similar to those found in A-type costae. SDS-PAGE analysis showed that the costa contains several proteins with major bands corresponding to apparent molecular masses of 122, 115, 112, 93, 87, 82, 59, 44, 41, 32, and 26 kDa (Monteiro Leal et al. 1993; Viscogliosi and Brugerolle 1994). In addition, these techniques allowed the separation of several major protein components with molecular weights between 100 and 150 kDa (Viscogliosi and Brugerolle 1994). Monoclonal antibodies recognised five polypeptides with molecular weights of 135, 125, 114, 88, and 47 kDa by immunoblotting and the costa was labelled by immunofluorescence using these antibodies (Viscogliosi nd Brugerolle 1994). The authors did not find immunological cross-reactivity with other trichomonad genera; this indicates that the costae are not identical in their biochemical composition, which is in agreement with the differences in their respective fine structures. However, some proteins were similar, indicating the existence of shared epitopes (Viscogliosi and Brugerolle 1994).

2.3 Comb

In *T. foetus* and other related members of its subfamily, the simpler basal costa is replaced by a comb-like structure (Fig. 5b). This structure is located between the region of origin of the costa on the right and the IKB, close to the R kinetosome, on the left (Fig. 5b). The comb is also a cross-striated structure formed by periodic fibrils or lamellae that are thicker when seen towards the posterior area. The comb also

interacts with the IKB, on its posterior and ventral side. The function of the comb is unknown, and until now there have been no studies focused on this structure.

2.4 *Axostyle and Pelta*

2.4.1 Structure

The axostyle is an axial ribbon of longitudinally oriented microtubules, and in trichomonads (Benchimol et al. 2000) it runs from the anterior region to the other end of the cell (Figs. 1b, 2, 5a, b, 8, 9a–d, 10, and 14), The anterior part of the axostyle is wider and forms the capitulum. Posterior to the nucleus, the axostyle turns upon itself, forming a tube known as the axostylar trunk (Fig. 9a–d). The axostyle appears to narrow progressively until its terminal segment, which protrudes from the posterior cell as a thin tip covered by the cell membrane (Figs. 1 and 9a, b) (Honigberg et al. 1971; Ribeiro et al. 2000). The *T. foetus* axostyle contains a ribbon of about 150 microtubules with a diameter of 24 nm and is spaced by 40 nm (Fig. 10) (Benchimol 2004). These microtubules are connected by thin filamentous bridges that are 30–40-nm long and 10-nm thick, maintaining a uniform distance of 25 nm between them (Benchimol et al. 1993; Benchimol 2004). The chemical composition of these proteinaceous bridges has not been elucidated yet. Just posterior to the nucleus, the microtubular sheet of microtubules of the axostyle turns upon itself to form the axostylar trunk, which runs near the antero-posterior axis of the flagellum and extends into its caudal tip projection (Fig. 9a–d).

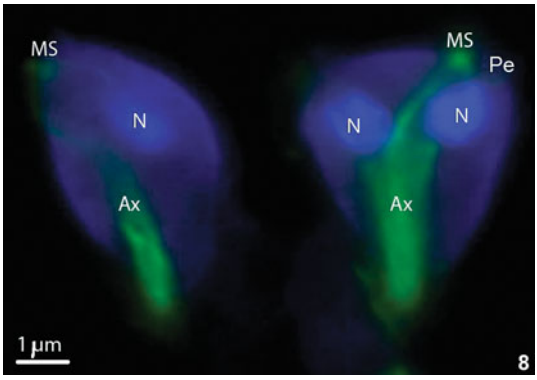

Fig. 8 Immunofluorescence image of an interphasic (*left*) and a dividing *T. foetus* cell using an anti-glutamylated tubulin monoclonal antibody. The nucleus (N) and microtubular structures such as the axostyle (Ax) are seen duplicated. The nuclei (N) were stained with DAPI. The axostyle (Ax) is seen running along the main axis of the cell, and is seen duplicated when the cell is dividing. The mastigont system (MS) is located on the upper anterior side of the cell. *Bar*, 1 µm (Benchimol unpublished)

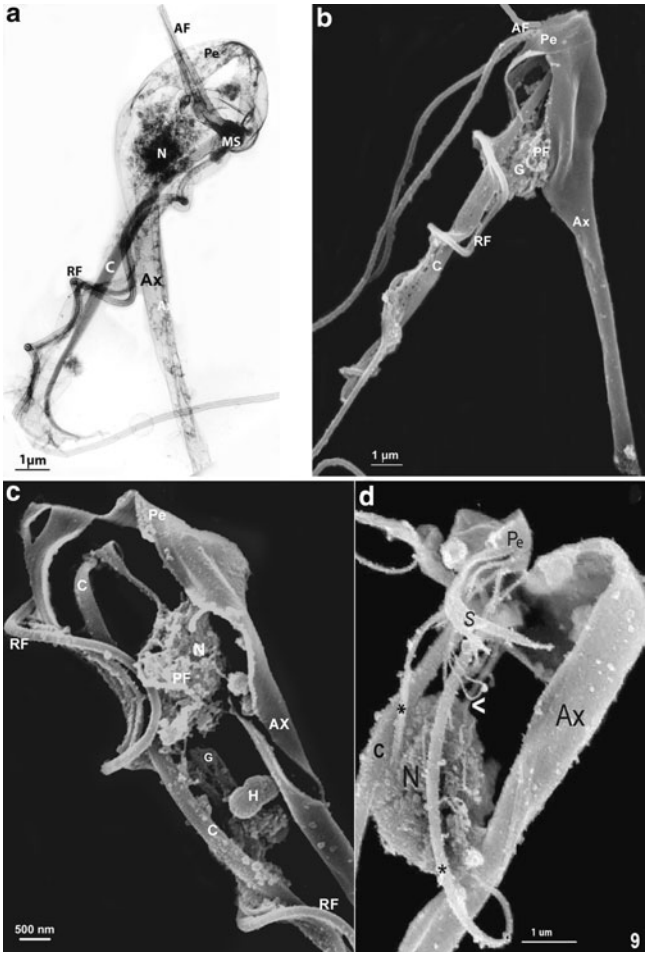

Fig. 9 Internal view of *T. foetus* using high-voltage transmission electron microscopy (HVTEM) (**a**) or detergent treatment to remove the plasma membrane and exhibit the cytoskeleton (**b–d**). (**a**) General view of Triton X-100-extracted trichomonads as observed with HVTEM. The microtubule ribbons that form the pelta (Pe) and the axostyle (Ax) are seen; the anterior part of the axostyle is wider and forms the capitulum. Posteriorly, the axostyle turns upon itself forming a tube, the axostylar trunk. Note that the costa (C) runs alongside the recurrent flagellum (RF). The anterior flagella (AF) emerge from the same point on the costa as the mastigont system (MS) does. *N* nucleus. Bar, 1 µm. (Benchimol and DeSouza unpublished). (**b**, **c**) Mastigont system of *T. foetus* after plasma membrane extraction, which allows better visualisation of the pelta (Pe), the anterior flagella (AF), and the axostyle (Ax) trunk. Note that the costa (C) follows the recurrent flagellum (RF), forming the undulating membrane. The costa bands can be seen in *panel* (**c**). Note the Golgi complex (G) associated with the parabasal filaments (PF) and the still-preserved nucleus (N). The hydrogenosomes (H) are depicted in panel (**c**); one of them (*asterisk*) is seen in the process of division. *Bars*, (**b**) 1 µm; (**c**) 500 nm (Benchimol unpublished). (**d**) High-resolution field emission scanning electron microscopy (FESEM) of *T. foetus* after detergent extraction. When the plasma membrane is removed and the cell is observed by FESEM, several fibrillar structures are seen forming the mastigont system. The axostyle (Ax) can be observed as a ribbon of microtubules

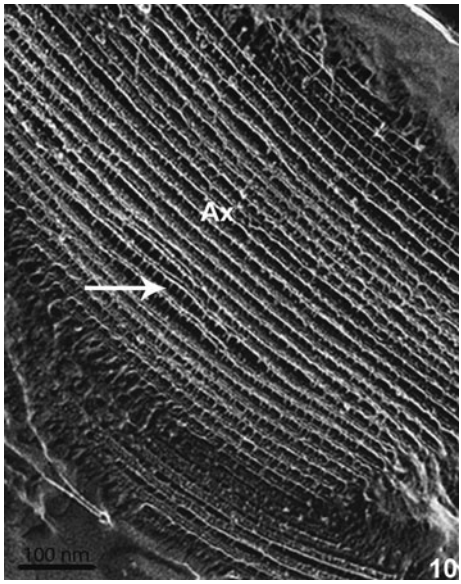

Fig. 10 *T. foetus* axostyle (Ax) observed by freeze-etching after quick-freezing by slam-freezing. The axostyle microtubules are seen connected by thin filaments (*arrow*). *Bar*, 100 nm (Benchimol unpublished)

A previous report (Benchimol et al. 2000) demonstrated that the trichomonad axostyle microtubules have a lateral projection formed by two protofilaments in addition to the 13 protofilaments normally found in microtubules. In addition, when different types of anti-tubulin antibodies were used to label microtubules in trichomonads (Fig. 6a), it was observed that the axostyle–pelta junction is a structure with high affinity for antibodies that recognise glutamylated tubulins in *T. foetus* (Lopes et al. 2001).

2.4.2 Pelta

The pelta (Figs. 1a, b, 2, 5a, b, 6a, b, 8, 9a–d, and 14) is a crescent-shaped sheet also formed by microtubules, and it overlaps with the axostyle in the anterior region of the cell. The pelta supports the wall of the anterior region of the cell and the flagellar canal from which the flagella emerges (Honigberg et al. 1971; Benchimol 2004) (Figs. 1a and 9b). At its broadest part, the pelta is composed of 35–40 microtubules (Figs. 5a, b and 9c), each one 25 nm in diameter. The pelta microtubules are internal to those of the axostyle (Figs. 5a, b and 9b–d), and when they encounter the axostyle microtubules they form the pelta–axostylar junction

Fig. 9 (continued) running from the anterior to the posterior region of the cell. The nucleus (N) is seen connected to the basal body region through an anchoring fibrillar structure (*arrowhead*), and it is also associated with the costa (C), an axial proteinaceous structure. Two parabasal filaments (*asterisks*), the pelta (Pe), the rootlet structures, and sigmoidal filaments (S) are also seen. *F* flagella. *Bar*, 1 μm (Benchimol unpublished)

(Fig. 14), which may also include sigmoidal filaments (Figs. 5a, b, 6a, and 14). As the pelta courses to the posterior region of the cell, the number of microtubules progressively decreases.

2.4.3 Functions

The main function of the axostyle in trichomonads is to support the axis of the cells. In addition, the axostyle also participates in karyokinesis. It was demonstrated in a previous study of cell division in *T. foetus* (Fig. 8b) (Ribeiro et al. 2000) that the axostyle in *T. foetus* presses the dividing nucleus, aiding in its division (karyokinesis). These processes were not observed by other authors in *T. vaginalis* (Viscogliosi and Brugerolle 1994; Delgado-Viscogliosi et al. 1996).

2.4.4 Stability

Some authors indicated that the axostyle is formed by labile microtubules since they depolymerised when the protozoa were incubated in the presence of colchicine early in the cell cycle (Brugerolle 1975; Juliano et al. 1986; Viscogliosi and Brugerolle 1994; Delgado-Viscogliosi et al. 1996). However, our group demonstrated that the microtubules of the axostyle are stable since they exhibited acetylated tubulins, did not depolymerise when treated with drugs affecting the cytoskeleton (e.g. vinblastine, colchicine, nocodazole, and taxol) (Silva-Filho and De Souza 1986; Batista et al. 1988; Ribeiro et al. 2000, 2002; Madeiro da Costa and Benchimol 2004), and were stable during mitosis (Ribeiro et al. 2000).

2.4.5 Movement

Unlike axostyles found elsewhere, the axostyle of trichomonads does not contract (Monteiro-Leal et al. 1996).

2.4.6 Composition

The axostyle is labelled after incubation with several antibodies recognising α- and β-tubulin (Figs. 6a and 8a, b) as well as glutamylated and acetylated α-tubulin (Delgado-Viscogliosi et al. 1996; Lopes et al. 2001; Boggild et al. 2002). It has been demonstrated that the most anterior region of the *T. foetus* pelta–axostyle exhibits glutamylated tubulin, whereas acetylated tubulin predominates in the posterior region (Lopes et al. 2001). Tyrosinated and polyglycylated microtubules were not found in trichomonads (Delgado-Viscogliosi et al. 1996; Schneider et al. 1999; Lopes et al. 2001; Boggild et al. 2002), and Brugerolle et al. (2000) have suggested that the sigmoid filaments could constitute an MTOC (microtubule-organising centre) of the pelta–axostylar system.

2.4.7 Association with Other Cell Structures

The axostyle is also associated with other cell structures such as the hydrogenosomes, the endoplasmic reticulum, the sigmoid filaments, and glycogen particles (Figs. 1b, 5a, b, and 9b, c) (Benchimol et al. 2000). The association of the hydrogenosomes with the axostyle explains the old nomenclature for these organelles (paraxostylar granules). These associations are aided by thin filaments that were only observed after the use of special techniques such as fast-freezing, followed by freeze-etching (Benchimol et al. 2000), and high-voltage electron microscopy (Benchimol and De Souza 1987; Benchimol 2004).

2.5 *The Parabasal Filaments*

Trichomonas vaginalis present three PBF: PF_1, PF_2, and PF_3 (Figs. 2a, b, 3b, 5a, 9b–d, and 14) (Honigberg and Brugerolle 1990; Lee et al. 2009). All PFs originate in the kinetosome region, near the costa's base (Fig. 5a). PF_1 arises between kinetosomes #2 and #3, whereas PF_2 has a common origin with the costa's base and originates from kinetosomes #2 and R (from the recurrent flagellum) (Honigberg and Brugerolle 1990). Lee et al. (2009) demonstrated by tomography that PF_1 is a long filament that extends towards the posterior region of the cell, where it splits into two strands, one of which curves towards the interior of the cell from the split point. They have shown that PF_1 and PF_3 appeared to be very close to each other. However, in 1971, Honigberg stated that PF_1 could be seen as two or three separate structures (Fig. 2a, b).

These two filaments present, at first glance, identical periodicity to that of the costa; however, their band pattern is quite different (Honigberg and Brugerolle 1990). The PFs have a periodic structure (Figs. 3b and 5a) that involves alternating transverse electron-dense and electron-lucent regions, and the dense area consists of four thin, dense lines. The PFs are located above the nucleus and below the well-developed Golgi complex in trichomonads (Figs. 3b, 9b–d, and 14). In the early literature, the term parabasal apparatus was applied to both PF_1 and PF_2, both of which are associated with the Golgi complex. It is believed that PFs accompany the Golgi, and thus that their function is to support the Golgi complex. However, until now, there is no clear evidence for this assumption.

2.6 *Flagella*

The flagella of trichomonads vary in number and size in each individual species. *T. foetus* has three anterior flagella (Figs. 1a and 2a, b), whereas *T. vaginalis* has four (Fig. 4); both have a recurrent flagellum (Figs. 1a, b, 3a, 4, 7a, 9a–c, and 11a–c) that runs towards the posterior region of the cell and adheres to the cell body, forming an UM (Figs. 1a, 4, 9a–c, and 11a) (Honigberg and Brugerolle 1990).