SUMMARY

A ring consisting of microfilaments was found in the apical region of Tetrahymena thermophila wild-type strain B and janus mutant. This ring, about 0.4 μm wide and 0.2 μm thick, is located at the bases of the anterior, non-ciliated basal bodies of the apical ciliary couplets. The apical ring is made of fine filaments showing a banded pattern, the distance between bands depending on the fixation procedure and ranging from 50-200 nm. The bands are made of small beads fastened to the filaments. The microfilaments of the apical ring are attached to the bases of the basal bodies. No connection with the cell membrane was found.

In dividing cells in the incipient furrow region a filamentous band originates from the epiplasmic fibrogranular meshwork. This contractile ring is about 0.4 μm wide and 0.8 μm thick. It is formed by circumferentially aligned microfibrils. During constriction the contractile ring remains associated with the epiplasmic layer, which in turn adheres to the inner alveolar membrane. The microfilaments of both the apical and the division-furrow rings have diameters ranging from about 3.8-7.1 nm.

INTRODUCTION

In recent years there has been increasing interest in the structure and function of the sub-surface and cytoplasmic cytoskeletons in eukaryotic cells (Goldman & Knipe, 1973; Fujiwara, Porter &Pollard, 1978; Ishikawa, 1979; Lazarides, 1980; Sanger & Sanger, 1980; Wolosewick & Porter, 1979). In Tetrahymena, a widely studied representative of the ciliated protozoans, several morphologically different filamentous structures have been identified (Allen, 1967; Sattler & Staehelin, 1979; Jerka-Dziadosz, 1982) and characterized biochemically (Gavin, 1977; Numata, Yasuda, Hirabayashi & Watanabe, 1980; Williams, Vaudaux & Skriver, 1979).

In an ultrastructural investigation undertaken in order to describe the fine structure of surface organelles in the janus mutant of Tetrahymena thermophila, 2 filamentous structures, not described previously in this ciliate, were detected (Jerka-Dziadosz, 1979). These were the apical filamentous band underlying the anterior termini of the ciliary rows (the so called 'apical crown', McCoy, 1974) and a microfilamentous contractile ring appearing transiently in the division-furrow region during cytokinesis.

Recently, Yasuda, Numata, Ohnishi & Watanabe (1980) described a contractile ring of microfilaments present in the division furrow of Tetrahymena pyriformis.
strain W in cells dividing synchronously after heat-shock synchronization. Biochemical studies showed that the contractile filaments are probably made from a fibre-forming protein different from actin, myosin and tropomyosin (Numata et al. 1980).

The present paper describes the ultrastructure of the apical ring and division-furrow ring in 4 strains of *T. thermophila* from exponentially growing cultures, including the *janus* mutant, which shows partial duplication of cortical patterns (Frankel & Jenkins, 1979; Jerka-Dziadosz, 1981; Jerka-Dziadosz & Frankel, 1979). Detailed knowledge concerning the spatial arrangement of filamentous structures in morphostatic cells and their rearrangement during cytokinesis may be helpful in understanding the function of these different fibrillar structures.

**MATERIAL AND METHODS**

*T. thermophila* strain B and 3 lines of the *janus* mutant were used in the present experiments. These were: the original CU-127 (jan/jan) line isolated after *N*-methyl-*N'*-nitrosoquandine mutagenesis by Dr P. Bruns; lines no. 30 and no. 46 of *jan/jan* mutants, resulting from crosses between different lines; line no. 30 is a homozygous *jan/jan* clone derived from a cross between a *jan/jan* heterozygote descendant from CU-127, and line A* (jan*/jan*). A* is a defective line described by Weinruch & Doerder (1975). Line no. 46 (also *jan/jan*), resulted from a cross between another *jan/jan* and clone A* (jan*/jan*) (Frankel & Jenkins, 1979).

Culture media were standard 1 or 2 % proteose-peptone with yeast extract (PPY), and Fe**+**-EDTA + glucose enriched medium originally developed by Thompson (1967). The origins of the stocks and media, and standard culture procedures have been published (Jerka-Dziadosz, 1981; Frankel & Jenkins, 1979; Jerka-Dziadosz & Frankel, 1979).

For electron microscopic observations, cells in the early exponential phase of growth were pelleted in a laboratory centrifuge and fixed in a 1:1(v/v) mixture of 2 % osmium tetroxide and 3 % glutaraldehyde dissolved in cacodylate buffer, pH 7 ('cocktail'). After fixation for 1 h in a refrigerator (4°C), cells were rinsed in buffer, collected into agar blocks and dehydrated through a graded series of ethanol followed by 2 changes in propylene oxide. They were then embedded in Epon 812. Some samples were additionally stained with tannic acid (0.1 %) before collection into agar blocks, as this is known to give better contrast to microtubular (Burton, Hinkley & Pierson, 1975) and microfilamentous structures (Begg, Rodewald &
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Rebhun, 1978; Goldman, Chojnacki & Yerna, 1979). Fixation in cocktail preserved the microtubular, microfilamentous and membraneous structures rather well but causes quite a substantial extraction of the cytoplasm. Therefore, some samples were fixed in glutaraldehyde first, and then postfixed in osmium tetroxide following standard methods (Aufderheide, 1979). Sections were stained with lead citrate and uranyl acetate. The material was sectioned on an LKB ultramicrotome with a diamond knife and examined under a JEM100B transmission electron microscope.

RESULTS

The fine structure of the apical ring in Tetrahymena

Tetrahymena is a small ciliate protozoan about 50 µm long. The cell typically possesses 17–21 longitudinal rows of ciliary basal bodies, all but 2 (postorals) of which extend from the anterior to the posterior pole. All ciliary rows except the 2 postorals and 3 adjacent to the oral apparatus possess at their anterior termini a ciliary couplet (McCoy, 1974), composed of 2 basal bodies linked together and having common microtubular derivatives. The posterior basal body is ciliated, the anterior one is not (Jerka-Dziadosz, 1981).

A microfilamentous ring underlying the anterior kinetosomes from the apical couplets was detected in cross-sections and tangential sections of the anterior part of Tetrahymena in all the lines used in this study (Figs. 1–4). This ring is formed by a ribbon of parallel microfilaments. The width of this ribbon in tangential sections appears to be slightly greater than the diameter of the basal body (0.4 µm at its maximum). The depth of this band is about half of its width. The anterior margin of this band is bordered by cisternae of endoplasmic reticulum with their smooth surfaces adjacent to the microfilaments. The opposite sides of these membranes are decorated densely with granules resembling ribosomal structures (Fig. 6). The apical microfilamentous ring is located about 0.8 µm below the cell surface, adjacent to the proximal level of the basal bodies forming the anterior components of the apical couplets (McCoy, 1974; Jerka-Dziadosz, 1981). Microfilaments radiate from the bases of the kinetosomes towards the apical microfilamentous band (Figs. 6, 7). The diameters of the filaments were measured on the negative glass plates at a magnification of ×13, giving a final magnification ranging from ×150 000–×312 000. The

Fig. 6. Higher magnification of an apical ring (ar) from the cell shown in Fig. 2. The ciliary couplet is seen on the left. Note that the microfilaments of the apical ring are connected with the proximal regions of the basal bodies (bb). The anterior margin of the filamentous band is bordered by endoplasmic reticulum (er). kd, kinetodesmal fibre. × 60 000.

Fig. 7. A. Cross-section through the apical filamentous ring of a cell from line CU-127 janus mutant of T. thermophila, grown in 1 % PPY, fixed in cocktail. A longitudinally sectioned basal body (bb) is seen. Three arrowheads point to the bands. × 21 500. B. Higher magnification of a part of the apical ring shown in Fig. 7; arrowheads point to the bands. × 60 000.

Fig. 8. Section through an oral apparatus of T. thermophila janus line CU-127, grown in 1 % PPY, fixed in cocktail. Banded microfilaments underlying the oral ribs are visible. × 90 000.
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Diameter of the most frequently occurring filaments was in the range between 4.8 and 7.1 nm. Filaments with larger diameters, although present, were not measured, due to a high error in the estimation of their sharp edges. This measurement is only an approximation since the sections were mounted on Formvar-coated grids.

On sectioned material, especially in cells where the cytoplasmic content was substantially extracted, the microfibrillar ribbon shows a banding pattern with different distances between stripes ranging from 100 to 200 nm (Fig. 7A, B). The bands in the ribbon are made of knots or small balls situated one on top of the other. These knots are seen most frequently on the inner part of the ribbon; the peripheral filaments are more uniform (Fig. 6). No connection between the apical ribbon and the epiplastic fibroreticular sheet was found. As can be judged from the micrographs of cells grown in different media and fixed in different fixatives, the localization and gross morphology of the apical ring appears similar in all *Tetrahymena* strains, but the fine detail of the ultrastructural image depends on the method of fixation of the material. In cells fixed in glutaraldehyde and postfixed in osmium tetroxide the microfilaments appear more 'compact', i.e. the banding seems to be more condensed (Figs. 4, 5). On the other hand, in some cells fixed in a cocktail of glutaraldehyde and osmium tetroxide and washed extensively in cacodylate buffer a clear banding of 'stretched' apical ring can be seen (Fig. 7A, B).

It has not been established whether the microfilamentous apical ring is contractile; the anterior region of *Tetrahymena* shows great variability in shape, from rostrum-like tip to a rounded oval shape. A fibroreticular sheet of banded filaments is known to exist in the oral apparatus of *Tetrahymena* (Sattler & Staehelin, 1979; Jerka-Dziadosz, 1981). The banding pattern of these filaments (Fig. 8) is more regular than that found in the apical ring in this study and in the division-furrow microfilaments described by Yasuda et al. (1980).

The fine structure of the contractile ring present in the division furrow in Tetrahymena

In several dividing *janus* mutant cells of *Tetrahymena* with advanced division furrows, a filamentous ring closely resembling the so-called 'contractile ring' (Schröeder, 1970) was found. The contractile ring appears as a structure composed of...
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Circumferentially aligned microfilaments encircling the zone of cell constriction. The filaments seem to originate in the fibrogranular material underlying the inner alveolar membrane, i.e. the epiplasm (Figs. 9–11). Membranes of rough endoplasmic reticulum were not found in the vicinity of the contractile ring. The filaments in the contractile ring showed no banding pattern. The ring in our material was much more delicate and it was not as thick as that described by Yasuda *et al.* (1980) in dividing *Tetrahymena* synchronized by heat shock. The lateral stripes and beads were not found. Whether the differences result from the heat-shock division synchronization procedure, or from different fixation procedures remains to be resolved. The diameters of measurable filaments in the division-furrow ring ranged between 3.8 and 7.1 nm, which is very close to the average diameter of microfilaments of the apical ring.

Examination of serial sections of dividing *Tetrahymena* made it possible to compare the apical filamentous ring present in the anterior product of division and the contractile ring present in the division furrow. In such cells differences between both microfilamentous structures were confirmed. These were: the lack of a banding pattern and the lack of the accompanying endoreticular membranes in the division-furrow ring.

The earliest stages of the formation of the contractile ring are not precisely known. Based on serial section of doublet *janus* cells, such as those shown in Figs. 9 and 10, it can be stated that the division-furrow filamentous ring is formed in close spatial association with the anterior kinetosomes of the ciliary couples, which appear in the incipient furrow region. A clear microfibrillar band is seen immediately anterior to the anterior, non-ciliated component of the ciliary couplet. Serial sections revealed that the microfilamentous band reaches from the surface to at least the middle of the anterior basal body. During cleavage the contractile ring in the division furrow remains associated with the epiplasmic layer of fibrogranular material, underlying the inner alveolar membrane (Figs. 15–17). Subsidence of the ring towards the inside of the cell (Yasuda *et al.* 1980) was not observed.

During constriction, both products of division grow and elongate. In *Tetrahymena*, in the late stages of cleavage the new anterior region of the posterior product of

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Figs. 13–17. Sagittal section through a dividing cell of *T. thermophila janus* line CU-127, grown in 1% PPY, fixed in cocktail and stained with tannic acid.

Fig. 13. A. Section through the division furrow and the ciliary couplet (ac). Note the delicate filamentous structures located at the bases of the anterior kinetosomes of the ciliary couplets (ar). × 16,000. B. Higher magnification of the apical ring from the cell shown in A. × 53,000.

Figs. 14, 15. Higher magnification of the division-furrow region from cell shown in Fig. 13. Arrowheads point to the microfilaments of the contractile ring. × 60,000.

Fig. 16. Serial section of the same dividing cell as that shown in Fig. 13. Note the positions of the apical couplets (ac) with derivatives. kd, kinetodesmal fibre; pc, postciliary microtubules; im, longitudinal microtubular band; al, cortical alveolae in the furrow. × 16,000.

Fig. 17. Higher magnification of the division-furrow region from the cell shown in Fig. 16. Remnants of the contractile ring (cr) in the anterior product of division are visible. × 40,500.
division is formed between the constriction region and the termini of the ciliary rows (Figs. 13, 16). The contractile ring of fine filaments is seen in the constriction region (Figs. 13-15). At the same time a microfilamentous band can be seen at the bases of the most-anterior of the basal bodies of the posterior product of division (Fig. 13A, B). This means that during constriction the filamentous material formed in the incipient furrow region is separated from the anterior kinetosomes of the ciliary couplets differentiated in the posterior product of division. Whether the apical microfilamentous ring is formed from the same material as the contractile ring remains to be resolved.

Suitable sections through dividing cells of Tetrahymena wild-type strain B cells were not found. Therefore, it is not known whether the contractile ring differs from that found in janus cells. Based on the fact that there is no difference between the structures of the microfilamentous bands in the apical regions of janus and wild-type cells (Figs. 1-4), it seems reasonable to assume that the formation and structure of the contractile ring in Tetrahymena strain B does not differ from that described in janus cells.

**DISCUSSION**

In the present paper 2 microfilamentous structures, one permanent (the apical ring) and the other transient (division furrow ring), are described. The filamentous structures were discovered in a broader study of ultrastructural specializations in cells bearing the mutated single recessive gene (jan/jan), the phenotype and genetic background of which have been described (Frankel & Jenkins, 1979; Jerka-Dziadosz & Frankel, 1979; Jerka-Dziadosz, 1981). It is important that these structures were found in cells growing in 2 different media after 3 different fixation procedures.

The janus gene brings about the formation of atypical doublets that differ from the typical ones in 2 fundamental ways: (1) the oral structures produced along one oral longitude are completely normal, whereas those produced along the opposite longitude are always abnormal and frequently display a mirror-image reversal of the normal arrangement of membranelles. (2) janus cells frequently manifest 2 sets of contractile-vacuole pores (an excretion organelle), one to the right (as usual) of the normal oral axis and the other to the left of the abnormal oral axis of the cell (Jerka-Dziadosz & Frankel, 1979). The ciliary meridians of janus cells are all of normal polarity and asymmetry, and the basal bodies of all the oral and ciliary structures show the same microtubular pattern (Jerka-Dziadosz, 1981). Similarly, the microfilamentous structures present in the apical ring do not differ morphologically in janus and wild-type cells.

The existence of the contractile ring in T. pyriformis strain W, synchronously dividing after the heat-shock procedure, was recently documented by Yasuda et al. (1980). It is evident, therefore, that the contractile ring of the division furrow does exist in Tetrahymena as in Nassula (Tucker, 1971), in spite of the fact that it was not detected in many earlier ultrastructural studies of this ciliate.

The microfilamentous ring of the division furrow found in this study is optically not as dense as that described by Yasuda et al. (1980). This may be related to the fact
that these authors studied cells dividing synchronously after heat shocks, and some sort of accumulation of fibrillar material may occur during the synchronization procedure.

Examination of sections through dividing cells of *T. thermophila* in different stages of constriction revealed that the contractile ring is initially formed in connection with the epiplasmic layer in the incipient division-furrow region. When the ciliary meridians 'break' in the division region (Nelsen, Frankel & Martel, 1981), microfilaments oriented circumferentially appear in the vicinity of the anterior kinetosomes of the ciliary couplets of the posterior product of division. These fibrils form a ring that encircles the cleavage region.

The diameters of the thinnest microfilaments found in this study in cells fixed in cocktail (3.1–7.1 nm) are close to those found in other dividing cells, in spite of the rather crude measurements performed in this study. At this point a qualification should be made. Measurements of diameters of filaments *in situ* without glycerination and on sections mounted on Formvar-coated grids give only a rough approximation of the sizes of filaments. In this study only the thinnest of the filaments were measured; whether the thicker ones represent another kind of filament or are artifacts due to structures superimposed on the micrographs remains unresolved.

It is generally accepted that the filaments seen in the electron microscope, about 5–7 nm in diameter, usually contain actin. From biochemical studies performed on isolated pellicles of *Tetrahymena*, it follows that the fibrogranular layer of epiplasm contains protein (Williams *et al.* 1979) similar to the spectrin–actin meshwork found in the inner surface of the erythrocyte membrane. Numata *et al.* (1980) isolated a fibre-forming protein, FFP-38, from whole cells of *Tetrahymena*. This protein could be assembled into 14-nm filaments *in vitro*; by immunohistological methods *in vivo* it was found to be present in the pellicle, the oral apparatus and the division-furrow region. This fibre-forming protein in *Tetrahymena* does not seem to be sensitive to cytochalasin B, since Gavin (1976a, b) found that cells can divide in the presence of this drug. In this respect *Tetrahymena* differs from other dividing cells (Schroeder, 1970).

The microfibrillar ring underlying the apical crown of ciliary couplets in *Tetrahymena* is described here for the first time. Similar structures are known to exist in a close relative of *Tetrahymena*: a scuticociliate ciliate *Dexiotricha* described by Peck (1977). The ultrastructure of this ring in *Tetrahymena* appears morphologically very similar to the ultrastructure of the 'contractile ring' found in the furrow region in late dividing cells of *T. pyriformis* by Yasuda *et al.* (1980). The general appearance and the banding pattern of the apical ring of our study resemble the morphology of the contractile ring described by Yasuda *et al.* It is interesting to note, therefore, that anti-FFP-38 antiserum combined with fluorescent dye does not stain the apical microfilament band (Numata *et al.* 1980). Instead, it binds to the filamentous meshwork present in the oral apparatus. It should be noted, however, that the stained band in the division-furrow region is much wider than the actual microfibrillar ring, suggesting that it probably also comprises the microfibrillar apical ring, that is being formed in the posterior product of division (compare fig. 4 of Numata *et al.* 1980). The apical
ring in non-dividing cells might have gone undetected. The other possibility is that the 2 structures may be built from different filament-forming proteins, or else the combination of components may be different. This may be related to the fact that the division-furrow ring is attached to the epiplasm whereas the apical ring is attached to the proximal regions of the basal bodies.

The role of the microfilamentous ring present in the anterior region of *Tetrahymena* is not known. It may be contractile and is probably involved in changes of cell shape especially during 'rapid swimmer' transformation (Nelsen & Debault, 1978) and during mating (Wolfe & Grimes, 1979).

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