Tubular structures of *Mycoplasma gallisepticum* and their possible participation in cell motility

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In five strains of *Mycoplasma gallisepticum*, a protein with a molecular mass of about 40 kDa was detected by immunoblotting with anti-pig brain tubulin polyclonal and monoclonal antibodies. In eight other mycoplasma species similarly tested no reaction was observed. Thin serial sections of *M. gallisepticum* and *Acholeplasma laidlawii* cells examined by transmission electron microscopy revealed a submembrane system of tubules in *M. gallisepticum* but not in *A. laidlawii*. The intracellular spatial distribution of the tubular structures was reconstructed. Thin sections of *M. gallisepticum* treated with anti-tubulin antibodies and colloidal gold particles (immunogold labelling) revealed distinct labelling of the tubular system. Analysis of the tubular structures by high resolution electron microscopy and optical diffraction showed their helical organization to be: diameter 40 nm, helix pitch approximately 20 nm and electron-transparent core 10 nm in diameter. A possible involvement of the tubular system in mycoplasma motility is suggested.

Keywords: *Mycoplasma gallisepticum*, cytoskeleton, tubular structures, motility

INTRODUCTION

Mycoplasmas lack a cell wall and are amongst the smallest prokaryotes. However, despite the apparent simplicity of their organization, some mycoplasmas (e.g. *Mycoplasma gallisepticum*, *M. genitalium*, *M. mobile*, *M. pneumoniae*) possess so-called gliding motility, the molecular mechanisms of which are unknown. These motile mycoplasma species have flask- or club-shaped cells with specialized terminal structures ('tips' or 'blebs') which are apparently responsible for movement and adhesion of the mycoplasmas onto host-cell surfaces (Bredt, 1979; Kirchhoff & Rosengarten, 1984; Kirchhoff, 1992).

Probably, cytoskeletal elements participate in the motility of the non-spheroidal cells, as in eukaryotic cells. In *M. pneumoniae* cells, microfilaments resistant to Triton X-100 were found (Meng & Pfister, 1980). The possible existence of actin-like proteins as cytoskeletal elements in *M. gallisepticum* and *M. pneumoniae* has been discussed from biochemical (Ghosh *et al.*, 1978; Maniloff & Chauduri,

1979), ultrastructural (Collier, 1972; Neimark, 1977), immunological (Göbel, *et al.*, 1981) and DNA-hybridization (Chernova *et al.*, 1985; Göbel *et al.*, 1990) viewpoints. But the question of the presence of actin-like proteins in mycoplasmas is not yet clarified.

Tubulin is also a component of the eukaryotic cytoskeleton and is the major protein of microtubules, which participate in chromosome segregation (Simmonds *et al.*, 1983), compartmentalization and intracellular transport of various macromolecules and organelles (Vale *et al.*, 1985; Langford *et al.*, 1987), and in the maintenance of cell form (Kirschner & Mitchison, 1986). Tubular structures resembling eukaryotic microtubules have been described for several bacteria (for review see Pate, 1988), although the functions of these structures have not been specified. Helical or tetrahedral particulate arrays have also been described in the cytoplasm of *M. gallisepticum*. These were referred to as 'corn-cob' or 'helical polyribosomes' (Domermuth *et al.*, 1964; Maniloff *et al.*, 1965) and were later analysed by optical diffraction and rotational symmetry techniques (Maniloff, 1971).

In our study, screening of tubulin-like proteins in *M.*

Abbreviations: mAb, monoclonal antibody; pAb, polyclonal antibody.

gallisepticum and eight other mycoplasma species was undertaken using polyclonal and monoclonal antibodies against bovine brain and pig brain tubulins. A system of tubules was revealed and their intracellular spatial distribution reconstructed. The ultrastructural organization of the tubules is discussed.

METHODS

Organisms and cultivation conditions. *A. laidlawii* A, *M. hyorhinis* BT57, *M. hominis* H34 and five strains of *M. gallisepticum* (S6, A5969, MR3, U5 and R) were obtained from Dr G. Laber, Sandoz Research Institute, Vienna, Austria; *M. salivarium* PG20 and *M. orale* CH19299 were from Dr K. Jurmanova, Veterinary Institute, Brno, Czechoslovakia; *M. mycoides* subsp. *mycoides*, *M. pneumoniae* FH and *M. fermentans* were from Dr I. Rakovskaya, Institute of Epidemiology and Microbiology, Russian Academy of Medical Science, Moscow; and *M. arginini* R16 was from Dr V. Berdnik, Zonal Veterinary Station, Poltava, Ukraine. Mycoplasma cultures were grown on Oxoid Mycoplasma Broth Base (Oxoid) supplemented with 20% (v/v) preheated horse serum (Flow Laboratories), 10% (w/v) fresh yeast extract and 0.1% arginine (for growth of *M. salivarium*, *M. hominis*, *M. arginini* and *M. orale*) or 0.1% glucose (for growth of other mycoplasmas) at 37 °C. Organisms were harvested in the exponential phase of growth by centrifugation at 10000 g for 20 min and washed three times with phosphate-buffered saline (PBS), pH 7.2.

Gel electrophoresis. The washed mycoplasma cells were diluted with PBS to a final concentration of 4 µg mycoplasma protein ml⁻¹. Protein content was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard. Equal volumes of sample buffer (0.2% SDS; 15%, v/v, glycerol; 10 mM Tris/HCl, pH 7.5; 5%, v/v, β-mercaptoethanol) were added to mycoplasma suspensions. Samples were heated for 5 min at 100 °C. Gel electrophoresis of proteins was carried out on 12% (w/v) polyacrylamide gel, containing 0.1% SDS, by the method of Laemmli (1970). The molecular masses of the mycoplasma proteins were estimated relative to the electrophoretic mobility of low molecular mass markers (Pharmacia).

Immunoblotting. Electrotransfer of the proteins from the gel to nitrocellulose sheets was carried out by a standard method (Anderson & Thorpe, 1980). After transfer, the blots were soaked for 30 min at 20 °C in 0.1% Tween-20 in PBS (PBS-Tw). For immunological detection, the blots were incubated for 1 h at 20 °C with monoclonal (1:2000) or polyclonal (1:300) antibodies (mAbs, pAbs) against tubulin, diluted in PBS-Tw. The blots were then washed three times for 10 min in PBS-Tw and incubated for 1 h at 20 °C with peroxidase-conjugated goat anti-rabbit IgG (GAR-peroxidase) or peroxidase-conjugated rabbit anti-mouse IgG (RAM-peroxidase) used at a 1:2000 dilution in PBS-Tw. The blots were subsequently washed as described above and soaked at room temperature in the buffer containing 50 mM Tris/HCl (pH 7.5), 0.05% Tween-20, 1 mg 3,3'-diaminobenzidine.4HCl (DAB) ml⁻¹ and 0.0001% H₂O₂.

The mouse mAb TU-01 raised against pig brain tubulin was obtained from Dr V. Visklicky, Institute of Molecular Genetics, Prague, Czechoslovakia. Purified anti-bovine brain tubulin pAbs were from Dr V. Gelfand, Institute of Protein, Russian Academy of Science, Moscow, Russia. Anti-chicken α-tubulin mAbs (Amersham) were also used.

Preparation of mycoplasma cells for electron microscopy. (i) **Standard protocol.** Mycoplasma cells were grown, harvested,

washed as described above and fixed for 20 min at 20 °C in 2.5% (v/v) glutaraldehyde in PBS. Post-fixation was carried out in 1% (w/v) OsO₄ in PBS. Fixed cells were dehydrated in ethanol of ascending concentration (from 50% to absolute) and embedded in a mixture of Araldite-Epon (Fluka) in the volume ratio Araldite M:Epon 812:DDSA:DMP, 1:1.6:3.6:0.06. Embedding and polymerization was carried out by incubating specimens for 6 h at 37 °C followed by incubation for 12 h at 45 °C and 24 h at 60 °C. Thin sections were cut with a glass knife on an Ultramicrotome (LKB-III) and placed on formvar-coated cuprum grids. The sections were counterstained with uranyl acetate and lead citrate, and examined under JEM 100 and Hitachi H500 electron microscopes.

(ii) **Freeze-substitution.** Following prefixation with glutaraldehyde a pellet of mycoplasma cells was placed in an envelope made of aluminium foil, 10 µm thick. The envelope was dipped into liquid Freon-13 at -175 °C, transferred into absolute ethanol then cooled to -100 °C. Substitution of water for ethanol was carried out over 5 d at -95 ± 2 °C in a homemade cryostat. Then the temperature was slowly raised to 25 °C and the pellet was embedded in Araldite-Epon. The polymerization, cutting and staining were performed as described above.

(iii) **Immunogold labelling of thin sections.** Mycoplasma cells were grown, harvested and washed as described above and fixed in PBS containing 4% (w/v) paraformaldehyde and 0.2% glutaraldehyde, pH 7.2. The fixed cells were washed in PBS and incubated overnight in 100 mM glycine in PBS at 4 °C. The cells were gradually dehydrated in 30% (v/v) ethanol for 30 min at 4 °C, 50% and 70% ethanol for 30 min at -10 °C, 96% and absolute ethanol for 30 min at -30 °C. The dehydrated cells were embedded and incubated in the mixtures of Lowicryl K4M and absolute ethanol with an ascending concentration of Lowicryl (from 30 to 100%) at -30 °C. The cells were incubated overnight in 100% Lowicryl at -30 °C. Polymerization of Lowicryl was carried out by UV irradiation for 48 h at -34 °C with additional UV irradiation for 24 h at 20 °C. Thin sections were cut with a glass knife and mounted on formvar-covered nickel grids. The non-specific reactions in the sections were blocked with 0.5% BSA in PBS (PBS-BSA) for 30 min at 20 °C and incubations with the primary antibody diluted 1:10 for pAbs or 1:50 for mAbs in PBS-BSA were for 1 h at 20 °C. Grids were washed with PBS-BSA and incubated in the appropriate secondary antibodies (conjugate of 5 nm colloidal gold and goat anti-rabbit IgG (Sigma) or rabbit anti-mouse IgG) diluted 1:50 in PBS-BSA for 1 h at 20 °C. The sections were counterstained with aqueous uranyl acetate and lead citrate, or with 1.8% aqueous uranyl acetate and 0.2% methylcellulose (Roth *et al.*, 1990), and examined under the transmission electron microscope. Specificity of immunolabelling was controlled by excluding primary antibodies or by substituting them with non-immune sera.

Optical diffraction. Optical transforms were made from negatives of electron micrographs (×4200). To improve the signal-background ratio, only part of the picture (a circle 0.4 mm diam.) restricted by a diaphragm was analysed.

Analysis was conducted using an optical diffractometer (ИФ-124, Russia). A high-power argon laser (ЛГН-402, Russia), operating in a one-mode regime with a wavelength of 0.488 µm with a beam delivering 1.5 W was used as a source of light.

The diffraction pattern was analysed using the theoretical method developed for a general case of X-ray diffraction from discontinuous α-helices based on the Bessel function (Cochran *et al.*, 1952). In our application of this method to the interpretation of diffraction patterns of plane electron micrographs, the

Fig. 1. Identification of 40 kDa tubulin-like protein in *M. gallisepticum*. (a) PAGE of proteins of mycoplasmas *A. laidlawii* A (lane 1), *M. fermentans* (2), *M. hyorhinis* BTS7 (3), *M. mycoides* subsp. *mycoides* (4), *M. arginini* R16 (5), *M. hominis* H34 (6), *M. orale* CH19299 (7), *M. salivarium* PG20 (8), calf brain tubulin (9) and *M. gallisepticum* S6, A5969, R, MR3 and U5 (10–14, respectively). The gel is stained with Coomassie blue R-250. (b) Immunoblot of the same material with anti-pig brain α -tubulin mAb TU-01 stained with RAM-peroxidase.

principal equations, given in the paper cited were transformed to:

$$T\left(R, \frac{n}{p}\right) = J_j(2\pi Rr), \quad (1)$$

where T is the Fourier transform for the elementary unit of the helix, J_j is the j th-order Bessel function, n is an integer, P is the helix pitch, R is the helix radius and r is the helix radius in a reciprocal space. When function (1) is expanded in the Fourier series, we obtain:

$$F\left(R, \frac{n}{p} + \frac{m}{p}\right) = \sum T\left(R, \frac{n}{p} + \frac{m}{p}\right), \quad (2)$$

where m is an integer, p is the helix period and F is a structural factor, which corresponds to the intensity of diffraction maxima as $I = |F^2|$.

RESULTS

Immunoblotting of mycoplasma proteins with antibodies to eukaryotic tubulin

The results of immunoblotting all extracts of five *M. gallisepticum* strains (S6, A5969, MR3, U5, R) and eight other mycoplasma species (*M. hominis*, *M. orale*, *M. salivarium*, *A. laidlawii*, *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. mycoides* subsp. *mycoides*) with monoclonal TU-01 anti-pig brain α -tubulin antibody are given in Fig. 1. All *M. gallisepticum* strains reacted positively with mAbs, whereas the other eight mycoplasma species gave negative results.

The 40 kDa *M. gallisepticum* protein possessing epitopes

Fig. 2. Immunoblotting of proteins of *A. laidlawii* A (lane 1), *M. gallisepticum* A5969 (2), *M. arginini* R16 (3), *M. fermentans* (4), *M. pneumoniae* FH (5) and calf brain tubulin (6) with anti-bovine brain α -tubulin pAbs, stained with RAM-peroxidase.

similar to eukaryotic α -tubulin, is referred to hereafter as tubulin-like protein. These results were confirmed by similar experiments with purified anti-bovine brain tubulin pAbs (Fig. 2) and anti-chicken α -tubulin mAbs

Fig. 3. Thin section of *M. gallisepticum* A5969 cells cut at different angles to the long axis of the cell. M, Cell membrane; T, submembrane tubular structure; B, specialized terminal structure (bleb). Bar, 0.5 μ m.

(data not shown). *M. pneumoniae* FH, a motile strain, was also examined.

Electron-microscopic investigations of thin sections

These were carried out on two *M. gallisepticum* strains (S6 and A5969) and one *A. laidlawii* strain (A). Fig. 3 shows the profiles of *M. gallisepticum* cells differently oriented from the section plane; most of the profiles appear circular or ovoid. In Fig. 3, electron-dense areas are visible in the region of the specialized terminal structure (bleb) and in the central part of the cytoplasm. In

submembrane regions of the cytoplasm, sectioned tubules are revealed. Their fine structure can be seen in cell sections made at different angles and on serial sections (Figs 4, 5). Fig. 4 shows various profiles of tubular structures apposed to the cell membrane. The major parameters of these structures can be determined from the electron micrographs presented. Some profiles show 4–5 electron-dense units encircling an electron-transparent core 10 nm in diameter. These electron dense units seem to be helicoidally arranged. The helix possibly consists of a continuous line of units, which in most longitudinal profiles of the tubular structures seem to be elongated

Fig. 4. Elements of the tubular system of *M. gallisepticum* A5969. Tubules (T) are distributed along the membrane of the cell. Up to 4–5 units are revealed in cross-section (short arrowheads in a and b); longitudinal profiles of tubule units appear elongated (long arrowheads in a and b). The tubules diverging from the infrapleural area can be seen in (c). Tubules are located in the 'back' region of the cell in (d). Bars, 0.5 μ m.

(Fig. 4). The pitch of the helix is 23 nm, measured using 5 nm colloidal gold particles as marker.

Parameters of the tubular structure, measured by optical diffraction

The optical diffraction pattern made from an electron micrograph (Fig. 6a) of a cluster of tubules is shown in Fig. 6b. The optical transform is undoubtedly of a helix. Four meridional layer-lines (reflexes) of the transform correspond to a period of 82 nm. On a vertical axis of the transform another reflex (M) has been detected, which corresponds to the helix pitch of the structure examined (Fig. 6b). The assignments of the layer-lines and their

spacing, both observed and calculated, are listed in Table 1.

The distance from the vertical axis to position (E) of meridional layer-lines is determined by the distance between the axis of neighbouring helices (helix-to-helix distance). This distance can be measured directly on the electron micrograph (Fig. 6a). All these parameters are given in Table 2.

As follows from the theory (see Methods), it is possible on the transform to detect the reflexes corresponding to helix pitch (P) and period (p). According to our measurements, helix pitch (P) is 21 (or 23) nm, and helix period (p) is 82 (or 83) nm (see Tables 1, 2). The P/p ratio is 3.9 or 3.6,

Fig. 5. Thin sections of *M. gallisepticum* A5969 cells prepared by the freeze-substitution method. Some cross-sectioned profiles of the tubules with 4–5 globules are pressed back from the submembrane position by ice crystals (W). Bar, 0.5 μ m.

Fig. 6. Optical diffraction. (a) Electron micrograph (thin section) of a *M. gallisepticum* cell. Encircled is a cluster of tubular structures. Bar, 0.5 μ m. (b) Optical transform (diffraction pattern) of the region limited by the circle shown in (a). The figures on the righthand side of the picture show the layer-line (reflexes) assignment. M, basic meridional reflex; E, location of equatorial reflexes.

Table 1. Layer-line spacing (meridional reflexes) of the optical transform in Fig. 6(b)

Layer-line assignment	Spacing (nm)	
	Observed	Calculated (for 83 nm repeat)
1	82	83
2	38	41
3	28	28
4	22	21

respectively. The last figure is more correct, in our opinion, if one takes the distortion seen in the diffractogram (Fig. 6b) into account.

Table 2. Helix measurements

Parameters	Determined by optical diffraction (nm)	Measured on electron micrographs (nm)
Helix pitch	21	23
Helix-to-helix distance	39	42
Helix diameter	—	41

If P/p is an integer, on a discontinuous helix, there will be periods different from the helix pitch. According to our data, the P/p ratio is a non-integer, indicating the absence of such periods. These data possibly reflect the superposition of Bessel functions from the cluster of helices located in close proximity to one another.

Spatial distribution of the tubular system

The examination of sections of cells made at different angles (Figs 3–5) makes it possible to propose a model of spatial distribution of the tubular system inside the *M. gallisepticum* cell. To confirm this idea of the spatial organization of the tubules, serial sections were made. The thickness of the sections was 40–70 nm. The average diameter of *M. gallisepticum* cells is approximately 500 nm. Three of six serial sections across cells A and B, are shown in Fig. 7. These three sections make reconstruction of the spatial distribution of the tubular system possible (Figs 8, 9).

From analysis of the electron micrographs, the tubules appear loop-shaped. They are not visible in the bleb and infrable areas. Nevertheless, both ends of the tubules are located close to the infrable. Cell size varies, as well as the number, shape and position of tubular loops. The outline of the infrable area also probably undergoes changes during the cell division cycle. The scheme depicted in Fig. 8 is based on examination of numerous electron micrographs.

The tubular structures were also detected in their cross profiles (rings of 4–5 electron dense units) or longitudinal profiles on sections prepared by freeze-substitution of *M. gallisepticum* cells (Fig. 5a, b). Even at the high speed of freezing, the possibility of water crystallization inside some of the cells cannot be excluded. Crystals of ice are visualized on sections as electron-lucent regions, differing in shape and size. It looks as if the natural spatial distribution of tubules was perturbed during ice formation. As a result, tubules were pressed back from the submembrane position to the central part of the cell (Fig. 5a), thus suggesting that the tubular structures had been present in the native cell prior to freezing.

The tubular system in each strain of *M. gallisepticum* (S6 and A5969) was morphologically identical. However, in *A. laidlawii* cells treated simultaneously by the same methods of fixation and embedding, such a system was not found. The tubular structures detected in all the

Section 5

Section 2

Section 1

Fig. 7. Three sections (1, 2 and 5) from serial sections of *M. gallisepticum* A5969 cells. Spatial distribution of the tubular system can be followed on two cells (A, B). In cell A the transfer from cross-cut to longitudinal profiles of loop-shaped tubules is seen. In cell B different regions of cross-sectioned tubules are visible. Bar, 0.5 μ m.

Fig. 8. Schematic interpretation of sections 1, 2 and 5 shown in Fig. 7.

Fig. 9. Partial reconstruction of the tubular system of *M. gallisepticum* (cell B) based on a series of sections (1, 2 and 5) shown in Figs 7 and 8.

samples of *M. gallisepticum* cells fixed and embedded in Araldite or Lowicryl were similar.

Immunogold labelling of thin sections

To compare the immunoblotting data with ultrastructural observations, electron microscopy was carried out using immunogold labelling of anti-tubulin antibodies. Colloidal gold particles found over the tubular structures, using pAbs against bovine brain tubulin (Fig. 10a–c) and mAbs against chicken α -tubulin, usually gave similar results (Fig. 10d). Gold particles are aligned on the longitudinal sections of the tubules (Fig. 10a). In Fig. 10(b, c), gold particles are also visible in the profiles of cross-sectioned tubules. The labelling is distinctly visible

in the infrapleural area as well. It should be pointed out that the density of colloidal gold labelling is much lower when mAbs are used. These results, together with negative results of the control experiments without primary antibodies or using non-immune sera as a substitute, enable us to conclude that the binding of colloidal gold particles to the tubular structures is specific.

DISCUSSION

A protein detected in *M. gallisepticum* has common epitopes with bovine and pig brain tubulins. The molecular mass of this protein, determined by electrophoretic mobility, was about 40 kDa and differed from that of bovine brain tubulin which is known to be 50 kDa (for review see Burns, 1991).

Specificity of the anti-pig brain tubulin mAb Tu-01 has been demonstrated in many laboratories, in studies of eukaryotic microtubules (Viklicky *et al.*, 1982; Grimm *et al.*, 1987). Epitopes of α -tubulins of different organisms (bull, drosophila, yeasts and many others) recognized by mAb Tu-01, belong to a more conservative region of the polypeptide chain of tubulins (Little & Seehaus, 1988).

PAGE of total protein of *M. gallisepticum* (Fig. 1) shows that the band referred to as tubulin-like protein is one of the major bands in the protein spectrum. Tubulin-like protein was not detected in the other mycoplasma species we studied, including *M. mycoides* subsp. *mycoides*, which is non-motile but capable of reversible changes of cell shape (Bredt, 1979), and *M. pneumoniae* which is motile.

The mycoplasmas showing so-called 'gliding motility' are devoid of flagella or cilia. Thus, the existence of cytoskeletal structures seems to be crucial for mobility, and this possibility has been discussed for more than two decades (for review see Kirchhoff, 1992).

In this study, a system of tubular structures in *M. gallisepticum* has been revealed. It cannot be excluded that the tubular system is unique to *M. gallisepticum*. The intracellular 'rho' structure of *M. mycoides* (Rodwell *et al.*, 1975) also appears to be specific.

One may ask why *M. gallisepticum*, the slowest of the gliding mycoplasmas (gliding velocity $0.03\text{--}0.05\ \mu\text{m s}^{-1}$), possesses tubulin-like protein and a system of tubules, whereas these structures are absent in the faster-moving *M. pneumoniae* ($0.3\text{--}0.4\ \mu\text{m s}^{-1}$). Similarly, the fastest gliding mycoplasma, *M. mobile* ($2.0\text{--}4.5\ \mu\text{m s}^{-1}$), which has been extensively investigated (Kirchhoff & Rosengarten, 1984) also lacks tubular structures resembling those of *M. gallisepticum*. Possibly, motility is a comparatively new development in mycoplasmas which are considerably divergent in an evolutionary sense. The phylogeny of mycoplasmas and peculiarities of their evolution were reviewed recently (Maniloff, 1992).

Tubular structures have been observed previously in *M. gallisepticum*; however, they have been considered as 'ribosomal helices' and their spatial distribution inside the cell has never been studied (Domermuth *et al.*, 1964; Allen *et al.*, 1970; Maniloff, 1971, 1972; Maniloff & Chaudhuri, 1979).

Fig. 10. Immunoelectron microscopy of thin sections of *M. gallisepticum* A5969 cells. *M. gallisepticum* cells labelled with pAbs against calf brain tubulin (a–c) and mAbs against chicken α -tubulin (d) as the primary antibodies, and with 5 nm goat anti-rabbit IgG-colloidal gold or rabbit anti-mouse IgG-colloidal gold, respectively, as the secondary antibodies. Arrowheads indicate gold particles seen on profiles of the tubular structure. The infrapleural area or organizing centre of tubules is heavily labelled (a). Bar, 0.5 μ m.

In our control experiments, the tubular structures may be revealed prior to centrifugation of *M. gallisepticum* cells, following fixation directly in growth medium. These data suggest that the tubular system of *M. gallisepticum* is unlikely to be a result of non-physiological conditions of cell treatment, as was shown for spiral structures of *E. coli* cells treated with vinblastine (Kingsbury & Voelz, 1969) and spiral structures of *A. laidlawii* enzymes found in cell extracts (Kessel *et al.*, 1981). Tubular structures of *M. gallisepticum* were also detected in freeze-substitution experiments and the results enable us to conclude that they are native subcellular elements.

Our experimental approach to the measurement of the parameters of the tubular structures was similar to that of Maniloff (1971) and the major result obtained was the same: the optical transform of the electron micrograph of the structure was the diffraction pattern of a helix. But according to Maniloff, the P/p ratio was 3.0 (integer value), whereas our analysis of the transform shows that this value is essentially higher (3.6 or 3.9) and non-integer. Therefore, the ribosomal model suggested by Maniloff 23 years ago can hardly be correct. The elongated shape of units on some longitudinal profiles of the tubular structures and the existence of an electron transparent core in cross-sections also seem to disagree with the ribosomal model. In our opinion the structure is definitely helical but the helix is not ribosomal.

It is natural to speculate that the helical tubules are built up from subunits. It remains to be clarified how the electron-dense units seen in cross-sections of the tubular structure correspond to molecules of the tubulin-like protein (40 kDa) revealed on protein blots (Figs 1 and 2).

Further studies are necessary to determine the parameters of the subunits of the helical structure.

We are inclined to attribute the helical structures to the elements of the mycoplasma cytoskeleton for the following reasons.

1. Spatial reconstruction of the system of tubular structures (Fig. 11), shows that they are located mainly on the cell periphery and may be represented by smoothly curved loops. Such a system of tubules might perform the function of a cytoskeleton.

2. We assume that, analogous to eukaryotic microtubules (Farrell *et al.*, 1987), the structural polarity of *M. gallisepticum* tubules requires that the two ends of the tubule are different (minus end and plus end). The ends of the tubules are located in the infrapleural area forming a tubular 'organizing centre' (Fig. 11). Structural polarity makes it possible for the monomers of tubulin-like protein to associate predominantly at the plus end and to dissociate predominantly at the minus end. By analogy with the 'dynamic instability' of eukaryotic microtubules, monomeric protein molecules could be continually transferred from one end of the tubules to the other (tread-milling process). In this case, the concentration of the monomers of tubulin-like protein should be maximal in the organizing centre (infrapleural area). This presumption is in agreement with our data on immunogold labelling, which, in some cases, is most abundant in the infrapleural area (Fig. 10a).

3. It is known that some mycoplasma antigens are connected with cytoskeleton elements (Stevens & Krause, 1991) and it has also been suggested that some antigens

Fig. 11. A hypothetical scheme based on serial sections and on numerous random sections made at different angles to the long axis of the cell. The specialized terminal structure (bleb) (1), the electron-dense area (infrableb) (2), loop-shaped tubules (3) and plasma membrane (4) are shown.

Fig. 12. A scheme of the assumed 'caterpillar' type of mycoplasma cell motility. It can be supposed that due to the attachment (adhesion) of a mycoplasma cell to the solid surface (substrate), conformational changes of surface antigens (5) may take place. These changes may lead to an anchoring of surface antigens (6) in the tubule (3). Treadmilling of the tubule (see the text) ensures the directional movement of antigens along the membrane (4), making the cell move. The bleb (1) and infrableb or organizing centre of tubules (2) form the leading end of the cell. + and - designate the ends of tubules, at which association and dissociation, respectively, occur.

can move along the cell surface (Kahane, 1984). We further speculate that the tubular system found in *M. gallisepticum* cells may exercise a cytoskeletal function by means of reversible anchoring of surface proteins on tubules and directional movement along the mycoplasma surface membrane. We designated this type of mycoplasma cell motility 'caterpillar' movement. This idea is illustrated by Fig. 12. The existence of the connection between tubulin-like protein and surface antigens (ad-

hesins) of *M. gallisepticum* has yet to be experimentally determined.

4. An alternative biological role of the tubular system in chromosome segregation cannot be excluded. Such a role, as a primitive mitotic-like apparatus, has already been proposed for the specialized terminal structures (bleb and infrableb) of *M. gallisepticum* (Morowitz & Maniloff, 1966; Maniloff & Quinlan, 1974; Ghosh *et al.*, 1978; Raikov, 1985).

The protein revealed in this work has common epitopes with the tubulin of mammals; however, it differs in molecular mass and the electron microscopic parameters of the tubular structures do not correspond to those of classical microtubules. Nevertheless, antibodies to eukaryotic tubulin react not only with a corresponding band on the protein blot of *M. gallisepticum*, but also directly with the tubular structures in immunogold assay. We consider the protein as tubulin-like and suggest a cytoskeletal function of the tubular structures analogous to eukaryotic microtubules.

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