Microbiology Comment provides a platform for readers of *Microbiology* to communicate their personal observations and opinions in a more informal way than through the submission of papers.

Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others.

In other instances we may have a useful bit of methodology that we would like to share.

The Editors hope that readers will take full advantage of this new section and use it to raise matters that hitherto have been confined to a limited audience.

John Freer
Editor-in-Chief

Mycoplasma helical structures

Korolev *et al.* (1) have described and analysed helical cytoplasmic structures in *Mycoplasma gallisepticum* and suggested the helices are composed of tubulin-like proteins that may be associated with cell mobility. The authors note that we reported identical structures previously (5), but argue against our conclusions that these are preparation artifacts (5) and represent helical ribosomal structures (2), and disagree with our published ribosome helix model (2). Therefore, below I compare the two sets of published helix parameters and review the data demonstrating that the helical structures are preparation artifacts and are composed of ribosomes.

The helix parameters I reported previously (2) and those of Korolev *et al.* (1) are in general agreement. Both studies, using electron microscopy and image analysis by optical diffraction, report helical structures with a 40–41 nm diameter and a 7–10 nm transparent core, and 3.3–3.6 particles per turn. However, assuming the particles are roughly spherical, the pitch (an approximation of particle size) given by Korolev *et al.* (1) seems too small. The particle size should be about one-half the helix diameter minus the core diameter; i.e.

15–17 nm. The 17 nm pitch originally reported (2) is in better agreement with this figure than the 21–23 nm pitch calculated by Korolev *et al.* (1). In addition, the 17 nm particles making up the helices are orders of magnitude too large to be tubulin-like proteins, as suggested by Korolev *et al.* (1), and the *M. gallisepticum* helical structures are unlike any reported tubulin helices. Finally, the possibility of a meridional reflection on the 5th layer line suggested by Korolev *et al.* (1) is inconsistent with the model they construct from their optical diffraction data.

The absence of a cell wall structure in mycoplasmas makes these cells susceptible to morphological changes during preparation for electron microscopy [reviewed in (4)]. Almost 30 years ago, we noted that the morphology of wall-less *M. gallisepticum* cells is a function of when and how cells are fixed relative to harvesting by centrifugation (5). Rapid fixation followed by harvesting shows cells having an elongated shape, with a bleb structure at one end and cytoplasm containing fibrillar nuclear material and particles of about 17 nm, which were identified as ribosomes. This morphology was observed in negative-stain, thin-section and freeze-etch electron microscopic studies of growing *M. gallisepticum* cells (3,5). However, harvesting before fixation produces cells with an ellipsoidal or spherical shape, condensed nuclear material and helical cytoplasmic structures. Since the helical structures only appear in such poorly preserved cells, they are considered to be preparation artifacts. The observed alignment of helical structures in aggregates near the cell membrane is probably a result of Brownian motion.

Identification of the *M. gallisepticum* 17 nm cytoplasmic particles as ribosomes was based on data showing that they are composed of RNA and protein, have a sedimentation coefficient of 70S and dissociate into 50S and 30S particles (5). Assembly of ribosomes to form helical structures was studied by incubating growing cells in antibiotic-containing medium prior to harvesting by centrifugation to induce helix formation (2). Inhibition of transcription (by actinomycin D) and translation (by puromycin) so as to allow ribosome run-off did not affect helix formation, indicating the helices are not polyribosomes. Inhibition of translation by antibiotics that bind 30S ribosomes (streptomycin and tetracycline) did not affect helix formation, but antibiotics

that bind 50S ribosomes (chloramphenicol and lincomycin) prevented helix formation, suggesting interactions between 50S ribosomes are involved in helix assembly. Therefore, it was concluded that the helices are composed of 70S ribosomes and probably form by self-assembly during certain preparative procedures (2,5).

In summary, the conclusions of Korolev *et al.* (1) are wrong, *M. gallisepticum* ribosomes can self-assemble to form helical structures that are preparation artifacts. There are currently no data relating these, or any other structures, to the mechanism by which wall-less mycoplasmas maintain their shape and segregate subcellular components during cell division.

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1. Korolev, E.V., Nikonov, A.V., Brudnaya, M.S., Snigirevskaya, E.S., Sabinin, G.V., Komissarchik, Y.Yu., Ivanov, P.I. & Borchsenius, S.N. (1994). Tubular structures of *Mycoplasma gallisepticum* and their possible participation in cell mobility. *Microbiology* **140**, 671–681.

2. Maniloff, J. (1971). Analysis of the helical ribosome structures of *Mycoplasma gallisepticum*. *Proc Natl Acad Sci USA* **68**, 43–47.

3. Maniloff, J. (1972). Cytology of the mycoplasmas. In *Giba Foundation Symposium on Pathogenic Mycoplasmas*, pp. 67–87. Amsterdam: North-Holland.

► GUIDELINES

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of **Microbiology** do not necessarily agree with the views expressed in **Microbiology Comment**.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.

4. Maniloff, J. & Morowitz, H.J. (1972). Cell biology of the mycoplasmas. *Bacteriol Rev* **36**, 263–290.

5. Maniloff, J., Morowitz, H.J. & Barnett, R.J. (1965). Ultrastructure and ribosomes of *Mycoplasma gallisepticum*. *J Bacteriol* **90**, 193–204.

Authors' reply

It was interesting to read the evaluation of our paper (1) by Professor J. Maniloff, the pioneer of ultrastructural studies of *M. gallisepticum* (2,3).

In his letter above, J. Maniloff points out the general agreement in the parameters of the tubular structure measured by EM and optical diffraction, reported by him previously (2) and by us in the paper discussed (1). Although the optical transforms published in the two papers (2,1) are undoubtedly those of a helix, we cannot agree with his assertion, particularly regarding the quantity of particles (subunits) per turn. Values of 3.6 and 3.9 in our paper (1) do not correspond to the quantity of subunits per turn of the spiral. Meridional reflections (M) shown in Fig. 6(b) of our paper (1), considered by J. Maniloff as the 5th layer line, are actually the single meridional layer line reflecting the spiral pitch. Four layer lines on the right-hand side of the same Fig. 6(b) belong to another set of reflections, which is demonstrated by a non-integer (3.6 or 3.9) P/p (pitch/period) ratio, calculated from the two different sets of reflections. This is the reason why the results of our diffraction pattern

analysis do not allow us to predict the subunit structure of the helix and, more importantly do not permit the construction of models, based on ribosomal subunits or subunits of any other nature.

With regard to the electron-dense particles revealed by EM in our work (1) and shown previously by Maniloff (3), interpretation of the results is also different. We see 4–5 electron-dense units in cross-sections of the tubules. Using 5 nm colloidal gold particles (Sigma) as marker, we have determined the diameter of these particles to be 7 nm, certainly no more than 10 nm, which is different from Maniloff's estimation (17 nm) and does not correspond to the diameter of the 70S ribosomal particle.

Protein with a molecular mass of 40 kDa, reacting with antibodies to eukaryotic tubulin was found both on immunoblots and directly in the content of the tubular structures (1). Initially our working hypothesis was that some cytoskeletal spiral thread might be 'decorated' by ribosomes. But results of our image analysis by optical diffraction does not correspond to any ribosomal model.

The intracellular spatial distribution of the tubular structures of *M. gallisepticum* is highly organized. Loop-shaped tubules are arranged regularly along the cell membrane (1). In our EM experiments with *M. gallisepticum* cells, using standard chemical fixation or freeze-

substitution techniques before or after harvesting by centrifugation, tubular structures were visualized in all cases over several years. It is difficult to accept that the complex systems of tubular structures revealed is a result of a preparative artifact.

Our ultrastructural and immunological data indicate that tubulin-like proteins form part of the tubular structures of *M. gallisepticum*. However, we do not have enough data at the moment to propose any new subunit model for the tubular structures. The system of tubules revealed in *M. gallisepticum* is possibly involved in motility (1).

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1. Korolev, E.V., Nikonov, A.V., Brudnaya, M.S., Snigirevskaya, E.S., Sabinin, G.V., Komissarchik, Y.Yu., Ivanov, P.I. & Borchsenius, S.N. (1994). Tubular structures of *Mycoplasma gallisepticum* and their possible participation in cell motility. *Microbiology* **140**, 671–681.

2. Maniloff, J. (1971). Analysis of the helical ribosome structures of *Mycoplasma gallisepticum*. *Proc Natl Acad Sci USA* **68**, 43–47.

3. Maniloff, J., Morowitz, H.J. & Barnett, R.J. (1965). Ultrastructure and ribosomes of *Mycoplasma gallisepticum*. *J Bacteriol* **90**, 193–204.