Mass Analysis of Individual *Spiroplasma* Cells and their Cytoskeletons

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The mollicutes are wall-less bacteria that evolved by regressive evolution and genome reduction from the genus *Clostridium* [1]. They are the simplest free-living and self-replicating forms of life. *Spiroplasma* has helical symmetry [2] and an internal, contractile cytoskeleton that functions as a linear motor by differential contraction of its fibril components. Here we take advantage of this cell's helical geometry to quantify annular dark-field STEM images in terms of the mass and organization of whole cells and their cytoskeleton. A *Spiroplasma* cell can be viewed as a membranous tube to which a flat cytoskeletal ribbon of parallel fibrils is attached along the shortest helical line on the inner surface of the tube [3]. An average cell of ~4 helical turns has a tube and coil diameter of ~0.2 and 0.6 μm, respectively, a period ~0.9 μm and a contour length of ~6 μm [4]. The average linear-mass-density of a freeze-dried cell is ~3.74 MDa/nm (Fig 1a, 2a) and the total mass of an average cell is ~2.22×10^{10} Da/cell, equivalent to ~3.69×10^{-14} gram/cell. The average mass density of a double layered, collapsed, cytosol-free, membrane vesicle is ~11.08 kDa/nm^2 (Fig 1b, 2b), while the linear mass density of individual cytoskeletal fibrils (Fig 1c, 2c) is 13.5 kDa/nm [5]. The number of fibrils per ribbon, deduced from the mass per length of straight, uniform ribbons divided by the linear mass density of individual fibrils, is therefore 13.8 (Fig 1c, 2d). The total mass of the cytoskeletal ribbon per average cell is ~1.12×10^6 Da, accounting for only 5% of the total cell mass. Diffraction patterns of a monolayered, cytoskeletal ribbon (Fig 1e) reveal axial and lateral repeats of ~1/8.7 and ~1/10.2 nm^{-1}, respectively. Cytoskeletal fibrils are assembled from a 59 kDa protein with an average volume per mass of 1.2×10^{-3} nm^3/Da and an equivalent sphere diameter of ~5.1 nm. This information, combined with the present results, support the following functional model: A 59 kDa protein occupies an average distance, F_L, along a fibril with a measured linear mass density M_L=13.5±0.2 kDa/nm of F_L=MW/M_L=4.36±0.06 nm. The number of monomers, N, contained within an axial repeat, P=8.7 nm, along a fibril is N=P/F_L=1.99±0.03. Thus, we conclude that the basic structural repeat along the fibril is a dimer. The lateral repeat within a monolayered ribbon is ~10.2 nm corresponding to a pair of fibrils. The structural building block of the ribbon is a chain of dimers, while the functional unit is a pair of dimeric chains forming tetrameric rings. A total of ~14,000 molecules may be expected in the average cell. We suggest that generation of motive force involves a conformational change in the linked tetrameric subunit from a circular to elliptical arrangement, possibly with the participation of other proteins [6].

References

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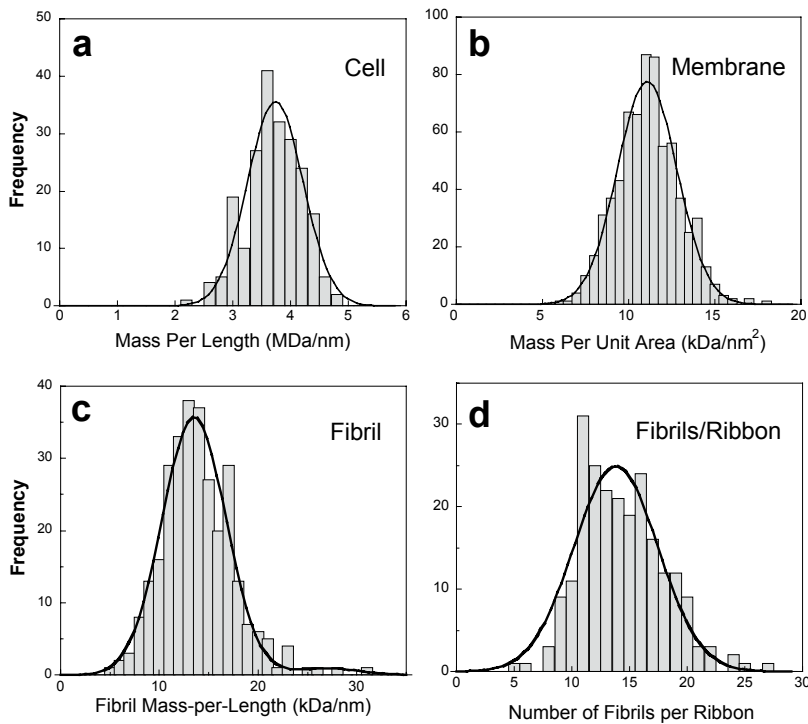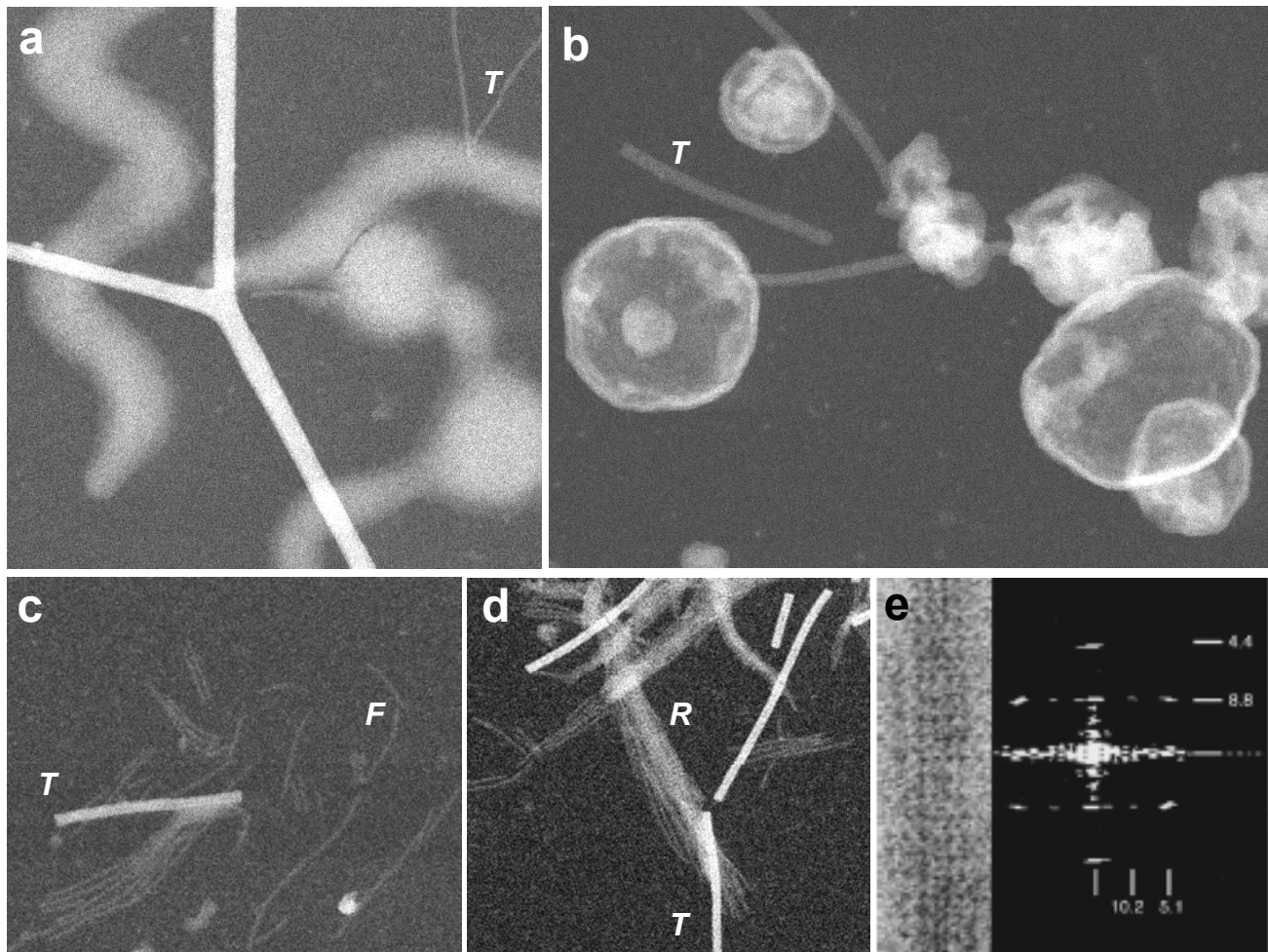[6] Supported by the Israel Science Foundation (ST) and the NIH intramural program.

Fig. 1 (above) -– STEM images of (a) whole cells; (b) membrane vesicles; (c, d) cytoskeletal ribbons (*R*) and fibrils (*F*). The diameter of the TMV particles (*T*) in (a-d) is 18 nm. (e) A negatively stained ribbon and its diffraction pattern.

Fig. 2 (left) – Frequency distribution histograms of mass densities (a-c) and fibril/ribbon measurements (d).