Spiroplasma Fibrils

ROD TOWNSEND, Ph.D.

John Innes Institute, Norwich, England

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Spiroplasmas contain long flexuous fibrils composed of a protein, molecular weight 55,000, which is specific to Spiroplasma and is highly conserved among different species. The protein cannot be detected in other wall-less prokaryotes reported to contain actin-like proteins and is unrelated to eukaryotic cytoskeletal components. Fibrils occur in similar concentrations in helical and nonhelical strains of Spiroplasma citri. Proposals that fibrils are responsible for maintenance of helical cell shape and rotary motility are discussed in the light of these findings. Evidence is presented which suggests that fibrils may be arrayed as one or more bundles in intact cells and a consistent association of these structures with DNA filaments is noted. These observations are discussed in relation to possible models to account for the maintenance of helical morphology and to the segregation of chromosomes during cell division.

Spiroplasmas apparently lack structures such as rigid outer envelopes, flagella, or periplasmic filaments associated with maintenance of cell shape and motility in other prokaryotes. However, when spiroplasma cells are lysed, they release long flexuous fibrils 3.6 nm in diameter, which show a marked tendency to associate in pairs [1]. Paired fibrils have a prominent axial repeat every 8.5 nm along their length. These structures may represent a novel form of microbial motility.

PURIFICATION AND PROPERTIES

Large numbers of fibrils can be released from the honeybee spiroplasma BC3 by treatment with Triton X-100. They are associated with two proteins of molecular weight (MW) 39,000 and 45,000 and another of 26,000 MW, which is antigenically related to spiralin [2], the major membrane protein in Spiroplasma citri [3]. These proteins can be dissociated by treatment with high concentrations of potassium chloride in the presence of EDTA and the fibrils purified by isopycnic centrifugation [4]. Purified fibrils form ordered aggregates which may be tubular and maintain the 8.5 nm repeat, giving them a striated appearance. They are incompletely denatured by 8M urea but are soluble in sodium dodecyl sulphate (SDS). Fibrils can be dissociated into subunits by treatment with 2,3-dimethylmaleic anhydride at pH 8.2 and will re-associate into short bundles, showing the 8.5 nm repeat when the pH is lowered to 6.0.

Purified fibrils are composed of a single protein, MW 55,000, as determined by SDS polyacrylamide gel electrophoresis (PAGE). Its amino acid composition distinguishes it from prokaryotic proteins including flagellin and eukaryotic cytoskeletal components such as actin and tubulin.

DISTRIBUTION

Using protein (Western) “blotting” and enzyme-linked immunodetection techniques, Townsend and Archer [5] have shown that proteins antigenically related to
SDS denatured BC3 fibril protein occur in eleven different spiroplasmas. These include representatives of four of the five _Spiroplasma_ serogroups as well as the BREV1 spiroplasma which probably represents a sixth group [RE Davies, personal communication]. Organisms belonging to the remaining serogroup II are non-cultivable parasites of _Drosophila_ spp and were not available to the investigators but have been shown to contain fibrils [1]. Fibril antigen could not be detected in any _Mycoplasma_ spp or _Acholeplasma_ spp examined, including those reported to contain filamentous structures [6,7] or “actin-like” proteins [8,9,10].

**QUANTIFICATION**

On the basis of yield of purified protein, it has been estimated that fibrils account for between 1 and 2 percent of total BC3 cell protein [4]. Subsequent determinations based on the amount of 55,000 MW antigen resolved by two-dimensional immunoelectrophoresis have shown that as much as 3 percent of cellular protein is fibrils. Some spiroplasmas apparently have much less than this: _S. citri_ SP-A contains only 0.5 percent fibril protein [5]. A comparison of the properties of these two closely related organisms provides circumstantial evidence that fibrils are involved in maintenance of shape and motility. For instance, BC3 is more motile as judged by direct microscopy and its ability to spread through soft agar and, whereas the helical morphology of SP-A is only maintained in media with high osmolarities, BC3 retains its helical shape in hypotonic media.

**SEQUENCE CONSERVATION**

Fibril protein is highly conserved among serogroup I spiroplasmas. Limited proteolysis with _Staphylococcus aureus_ V8 protease [11] of fibril proteins from SP-A, BC3, corn stunt spiroplasma E275, and tick spiroplasma 277F generates identical sets of peptides, all of which react with antibodies to SDS denatured BC3 fibril protein. One extra peptide, which also binds antibodies, is produced by digestion of the tick spiroplasma SMCA (serogroup V) fibrils. Fibril proteins from flower spiroplasmas BNR1 (serogroup III) and SR3 (serogroup IV) digest to give slightly different sets of peptides, but at least three are common to all spiroplasmas and bind antibodies directed against BC3 fibrils.

**A MODEL FOR ARRANGEMENT AND FUNCTION**

A mechanism to account for spiroplasma morphology and motility based on a model of helical cell development in bacteria [12] proposes that fibrils are attached to and wound helically around the inner surface of the cell membrane. By varying the tension in the fibrils, a helix could be maintained and contraction and relaxation of the fibrils in the correct sequence could generate the changes in shape necessary to achieve motility [13]. This model is supported by the observation that spiralin, an intrinsic membrane protein, co-purifies with the fibrils and that antibodies directed against spiralin cause cell deformation [3]. Proteins antigenically related to spiralin are widely distributed among spiroplasmas, which suggests that they have functional similarity [5]. However, cytochemical localization of spiralin antibody binding sites on the cell surface has failed to reveal any ordered arrangement of the protein which would be concomitant with a functional association [2].

**SIGNIFICANCE OF NONHELICAL SPIROPLASMAS**

Several nonhelical and consequently non-motile variants of _S. citri_ have been isolated [14,15]. One of these, ASP-1, has been shown by electron microscopy to
contain fibrils [16] and fibril protein has been detected in two others by antibody binding. Helical and nonhelical strains contain the same amount of 55,000 MW antigen. Fibril protein has also been detected in cells of SP-A which have lost helicity as a consequence of extended incubation and in spiroplasmas induced to grow in micro-colonies which contain only ovoid mycoplasma-like cells. These findings support the conclusion that if fibrils are involved in maintenance of helicity, they do so by interaction with other cell components, possibly those with which they co-purify. One of these may be similar to a 39,000 MW protein which is absent from the membrane of ASP-1 and other nonhelical variants. It has been suggested [16] that this protein forms part of an “anchor” structure linked at one end to the fibril and embedded in the membrane at the other. Absence of this protein or perturbations in membrane organization such as may arise in “aged” cells or spiroplasmas grown in nutritionally unbalanced media [17] would impair fibril function and result in cell deformation.

**ALTERNATIVE MODELS**

An approach which may prove of some value in resolving the questions of fibril arrangement and function is the application of the Kleinschmidt nucleic acid spreading technique to spiroplasma cells. Osmotically sensitized spiroplasmas are lysed and spread on an air-water interface and collected on support films. Metal shadowed preparations (Fig. 1) show three distinct fibrilar structures: thin, highly flexuous strands; thicker, apparently more rigid filaments: and thick bundles about 10 nm in diameter. If such spreads are exposed to DNase prior to shadowing, both thin and intermediate fibrils disappear, showing them to contain DNA, and only the bundles remain. The uniform spreading of DNA fibers suggests that these bundles do not represent a collapsed contractile peripheral sheath. Negative staining causes dissociation of these structures, releasing paired flexuous fibrils which react with antibodies raised against native BC3 fibril protein subunits. About five times more fibrils are released from BC3 than SP-A cells.

Fibril bundles are invariably associated with material which often assumes an apparently helical configuration. Presumably it does not originate from the membrane because it fails to bind antibodies against spiralin. Fibril bundles are frequently sheathed in this material (Figs. 2 and 3). Cells which fail to lyse form membrane

![FIG. 1. Metal shadowed spread of spiroplasma (BC3) cell contents showing thin DNA fibrils (t), intermediate DNA fibrils (i), fibril bundle (f), ribosomes (r), and membrane fragment (m). Bar represents 500 nm.](image)
ghosts which contain a twisted but apparently flat ribbon which, in BC3 cells, contains about nine fibrils (Fig. 4); it has not been determined if these are single or paired. Occasionally this ribbon is partially sheathed (Fig. 5). Some thin sections of spiroplasma cells suggest the presence of a fibrilar structure which is more evident where it abuts the cytoplasmic membrane on the inside curvatures of a cross-sectioned cell. This may represent the same 6.7 nm wide inner layer with internal content, suggesting striations observed by Cole et al. [18] occurring irregularly in sections of S. citri. These observations are consistent with the presence of an endocellular sheathed ribbon of fibrils which is closely associated with the cytoplasmic membrane and twisted to produce a helix. The helix has the same wavelength as the cell but the wave amplitude is reduced by a factor equal to the diameter of the cell. Hence, it is always proximal to the longitudinal axis of the cell. If the filament were securely anchored at either end of the cell, it could provide the tension necessary to hold the cell cylinder in a helical shape. Such an arrangement implies that an independent mechanism is responsible for maintaining the cell in a cylindrical form. Although membrane ghosts are not cylindrical, the presence of actin-like proteins in the spiroplasma membrane [19] suggests there could be a contractile mechanism capable of fulfilling this function. If the helical shape of the intact cell is determined by an endoplasmic filament, it should be possible to demonstrate differences between filaments from helical and nonhelical spiroplasmas. Alternatively, the function of endoplasmic filaments could be associated with cell motility. Unless the filament moves around the cytoplasmic membrane, which seems unlikely, it is difficult to envisage how they might function to generate the rotary element of spiroplasma motility. However, contraction of the filament, brought about by shortening of individual fibrils or sliding of one fibril over another, could produce the flexing movements seen in both helical and nonhelical spiroplasmas [14].

A further possibility is that the function of spiroplasma fibrils is totally unrelated to morphology or motility but is associated with the segregation of chromosomes at
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FIG. 4. Negatively stained membrane ghost (BC3) containing a twisted flat ribbon of fibrils. Bar represents 250 nm.

FIG. 5. As in Fig. 4 but fibril ribbon is partially sheathed. Bar represents 250 nm.

cell division. It has been suggested that the association of the cell wall with the membrane of *Escherichia coli* is required to provide the necessary physical strength for chromosome segregation [20]. In the absence of a cell wall, it is possible that spiroplasma fibrils fulfill a similar function. A preliminary study of DNA binding proteins in spiroplasmas has shown that fibril protein is among those able to bind DNA.

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