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Mycoplasma and Bacterial Proteins Resembling Contractile Proteins: A Review

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The basis of gliding motility in prokaryotes including certain mycoplasmas and the ability of mycoplasmas to retain their characteristic cell shapes in the absence of a supporting cell wall is unexplained. This review examines the available studies describing proteins resembling contractile proteins and cytoskeletal proteins in prokaryotes. Proteins with a significant degree of amino acid sequence homology to the myofibrillar proteins actin and myosin Al light chain and to tropomyosin have been described in prokaryotes. In addition, protein preparations from *Mycoplasma pneumoniae* have been shown to bind heavy meromyosin fragments, anti-actin antibody, and phalloidin; however, it remains to be proved that proteins in these preparations sharing properties with actin are synthesized by the mycoplasma.

Among prokaryotes, various bacteria are capable of movement although they lack flagella. These bacteria glide on solid surfaces by a mechanism(s) that remains unexplained. No locomotor organelle or structure has been identified as being responsible for this movement [1], and the molecular basis for movement in these bacteria is not understood. The ability to glide is found in Myxobacteria, Cytophaga, phototrophic green bacteria, and Cyanobacteria [2]. Certain mycoplasmas are also capable of gliding motility, and spiroplasmas are capable of flexing and rotational movement [3].

In eukaryotes, proteins similar to muscle contractile proteins are found in a wide range of non-muscle cells where they are believed to function in the fundamental cell processes of motility, chromosomal movement, amoeboid movement and cytoplasmic streaming, maintenance of a cytoskeleton, and organization of membrane proteins. The widespread occurrence of actomyosin-like proteins across a wide range of eukaryotic phyla including fungi and algae indicates that these proteins provide a general mechanism for motility and contractility in all higher cells [4]. We were interested in how gliding mycoplasmas moved and how they maintain their cell shape in the absence of a cell wall. Particularly curious was the fact that *M. pneumoniae* moves apparently with a definite anterior-posterior orientation with its electron-dense terminal rod structure always forward at the anterior end [3]. We wondered whether motility in mycoplasmas and perhaps other gliding bacteria might be explained by a contractile protein system possibly resembling actomyosin. Among contractile proteins, actin is known to have changed very little during evolution and seemed a suitable choice for a search. We did find a protein in extracts of *M. pneumoniae* that resembled actin and shared several characteristics with actin. These included *(1)* similar solubility properties, *(2)* an electrophoretic mobility very

close to that of vertebrate muscle actin, *(3)* treatment with ATP-Mg^{2+} allowed separation of long curvilinear filaments 5–6 nm wide that closely resembled eucaryotic actin, and *(4)* the filments could be "decorated" with vertebrate muscle heavy meromyosin (HMM) to form hybrid complexes with the characteristic shape of periodic repeating arrowheads, and no HMM was bound in the presence of ATP [5].

About the time our work was presented [6] Minkoff and Damadian [7] independently reported the extraction of a protein fraction from *E. coli* "with characteristics reminiscent of muscle actin." The notion that a contractile system similar to actomyosin might be present in motile bacteria is not new [8,9], but no evidence had been presented. More recently, Henrichsen [10] sought evidence for contractile activity in gliding and twitching bacteria by treating these bacteria with cytochalasin B, but no inhibition of movement was detected. A search for an effect on motility by cytochalasin B in *M. pneumoniae* similarly was unsuccessful [11]. The discovery of the terminal electron-dense rod in *M. pneumoniae* [12] led workers to suggest that this structure might function in reproduction or locomotion [3,12,13]. Our discovery of long filaments in *M. pneumoniae* raised the possibility that these filaments might function in maintenance of cell shape [5]. The fact that *M. pneumoniae* changes its direction of movement when the anterior end bearing the rod-shaped structure bends in a new direction suggests some contractile process that would allow rapid shortening and lengthening of filaments (or possibly sheets) longitudinally anchored in the envelope.

Subsequently, additional related reports appeared. Ghosh et al. [14] found that cytochalasin B inhibited the growth of *M. gallisepticum* and Searcy and co-workers [15] found cytochalasin B inhibited growth of *Thermoplasma acidophilum.* Maniloff and Chaudhuri [16] extended the study to several mycoplasmas, acholeplasmas, and a spiroplasma species, and found the growth of only the three gliding species was inhibited. (However, cytochalasin B is known to have more than one inhibitor activity in contrast to cytochalasin D which only inhibits actin-like proteins.) Searcy et al. [15] described an actin-like protein extract from *Thermoplasma* that underwent repeated reversible clearing and precipitation under the influence of ATP. Maniloff and Chaudhuri [16] also sought a filamentous structure, and found a protein fraction in *M. gallisepticum* that reversibly polymerized in 0.6 M KCl, depolymerized in low salt, and contained a 46,000 dalton protein. The interesting work of Kahane on contractile-like proteins from *A. laidlawii* is discussed in detail elsewhere in this symposium. Other reports have appeared [17].

Early on, we nad considered the possibility that the *M. pneumoniae* actin-like protein might be a known bacterial protein. Possibilities with molecular weights near that of actin included protein elongation factor Tu (EF-Tu), flagellar basal body hook protein [18], and a protein in the membrane-DNA complex of *Bacillus subtilis* [19]. Rosenbusch and co-workers [20] had raised the possibility that *Escherichia coli* EF-Tu and actin might share a common evolutionary ancestor. Their thought was based on the fact that both proteins are precipitated in a characteristic manner by vinblastin and also share several physical and chemical properties. However, they were unable to show similarity in tryptic peptide maps, and although Wurtz et al. [21] were able to produce filamentous paracrystalline aggregates of EF-Tu, the surface lattices bore little resemblance to filamentous or paracrystalline actin; also, immunochemical tests detected no cross-reactions. Finally, no amino acid sequence homology has been detected [22].

Beck et al. [23] utilized the procedure of Minkoff and Damadian [7] and identified the actin-like protein as EF-Tu. They also demonstrated some new properties of EF-Tu including polymerization to form filaments or, more often, arrays of filaments (the filament diameter was not stated and is difficult to estimate from the published photographs). EF-Tu was also the predominant component of a complex of proteins that underwent reversible polymerization in the presence of KCl and $MgCl_2$. They also showed that purified EF-Tu binds to DNase I in the presence of 10 mM $MgCl_2$. In the presence of EF-Ts, some of the EF-Tu formed large bundles of filaments that differed from bundles formed in the absence of EF-Ts by their paracrystalline appearance; these showed a 6 nm periodic repeat along the filament axis. They suggested the EF-Tu may have certain actin-like properties and that it may have cellular function in addition to its role in protein synthesis. A related study of interest is that of H. Sugino and N. Sakabe (Nagoya University, Japan) in which they found three-dimensional models constructed from X-ray diffraction patterns obtained from elongation factor Tu-DNAase crystals and actin-DNAse crystals are very similar in structure [personal communication]. Mallot and McCurdy [24] also applied the procedure of Minkoff and Damadian [7] to *E. coli* and reported decorating filaments with HMM, but the pictures reproduced were unclear.

In contrast to the uncertain relationship between EF-Tu and actin, another bacterial protein, the M protein of group A streptococci, has unequivocally been related to the muscle contractile protein tropomyosin [25,26]. In addition, computer analysis of the partial sequence of staphylococcal protein A, another biologically active surface protein, reveals significant homology with two other contractile proteins, actin and myosin Al light chain [25]. Also, homology may exist between a *Streptococcus faecalis* K^+-dependent ATPase and actin [27].

We prepared two-dimensional peptide maps of the *M. pneumoniae* actin-like protein (eluted from acrylamide gels, labeled with ^{125}I, combined with purified actin, and digested with trypsin) and found few coincidental peptides in common with muscle actin. Possibly, internal labeling and digestion with other proteases might have revealed additional common peptides, but extensive amino acid homology appears unlikely [Neimark, unpublished]. Also, Rodwell et al. [28] attempted to demonstrate α-actin in several mycoplasmas, but they were unable to show its presence by two-dimensional gel electrophoresis or by affinity for DNase I.

Filaments have also been detected in spiroplasmas lysed with the detergent sodium deoxycholate [29], and these have been characterized [30]. Subsequently, Williamson and co-workers [31] reported staining whole spiroplasma cells with an invertebrate actin antiserum coupled to horseradish peroxidase.

When our results were first presented [6], I suggested that it might prove profitable to lyse *M. pneumoniae* cells adhering to glass with the neutral detergent Triton X-100, and search for cytoskeleton-like filaments similar to those found in higher cells [32]. Recently, Meng and Pfister [33] demonstrated the presence of a network of filaments (ca. 6 nm in diameter) apparently attached to the rodlike tip in detergent-lysed *M. pneumoniae* cells. They also showed by indirect immunofluorescence that anti-actin antibody reacted with whole cells in colonies; fluorescence was most pronounced at the tips of filamentous cells. At about the same time, Göbel, Speth, and Bredt [34] obtained results similar to those of Meng and Pfister [33]. They also found a rodlike tip structure and a filamentous network in the cytoplasm, and the tip structure appeared to consist of filaments about 5 nm wide arrayed in parallel. In addition, they showed that the filaments were protein,

that they were stable in 1.6 M KCl but were destroyed by 0.6 M KI, that fixed cells extracted with Triton X-100 reacted specifically with highly purified anti-actin antibody, and that detergent-treated cells bound rhodamine-labled phalloidin. All these properties are consistent with an actin-like protein but the authors were unable to demonstrate binding of the S_1 fragment of myosin.

It must be pointed out that some of the studies employing highly specific anti-actin antibody reviewed here did not describe controls in which the antiserum was absorbed with actin before application to the mycoplasmas, and, conversely, where the serum was absorbed with mycoplasmas before being used to stain actin cables in cultured animal cell controls. However, the study of Göbel et al. [34], in particular, utilized antibody purified by actin affinity chromatography and it would be very surprising if these controls alter their results.

From this review it can be seen that mycoplasma cells contain filaments with some striking similarities to actin filaments. But it also appears that no protein sharing extensive homology with muscle actin has been identified in mycoplasmas. Yet the actin antibody studies and the phalloidin study indicate that mycoplasma cells contain antigenic determinants and steric groups in common with actin. This review shows that the concept of a cytoskeletal system involving filaments anchored in the envelope is still attractive. At this point, a very helpful study would be either an immunoelectron microscope examination of mycoplasma skeletal filaments with purified anti-actin antibody or the demonstration that an internally radiolabeled mycoplasma protein can bind anti-actin antibody or phalloidin.

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