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Intracellular cytoskeletal elements and cytoskeletons in bacteria

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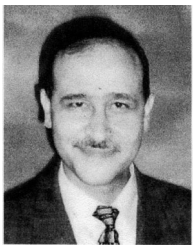
ABSTRACT

Within a short period of time after the discovery of bacterial cytoskletons, major progress had been made in areas such as general spatial layout of cytoskeletons, their involvement in a variety of cell functions (shape control, cell division, chromosome segregation, cell motility). This progress was achieved by application of advanced investigation techniques. Homologs of eukaryotic actin, tubulin, and intermediate filaments were found in bacteria; cytoskeletal proteins not closely or not at all related to any of these major cytoskeletal proteins were discovered in a number of bacteria such as Mycoplasmas, Spiroplasmas, Spirochetes, Treponema, Caulobacter. *A structural role for bacterial elongation factor Tu was indicated. On the basis of this new thinking, new approaches in biotechnology and new drugs are on the way.*

Keywords*: bacterial cytoskeletons, actin-homologs, tubulin homologs, intermediate filament homologs, EF-Tu , cell shape, motiliy, chromosome segregation, cell division, biotechnology*



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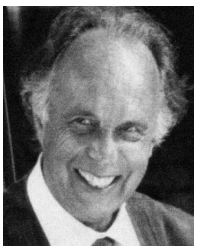

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New ideas, new discoveries, new thinking in bacterial cell biology: cytoskeletons!

The preservation of shape of typical multicellular organisms, with all its functional implications, is ensured by skeletons: bones in vertebrates; a cuticule in insects; a carapace in crustaceae; shells in mollusks. Certain elements also function as skeletons in plants as the cell walls exert shape preservation and provide stability for the entire organism. This is true also for unicellular plants such as algae, and for yeasts and fungi in general.

The optimization of preparative and imaging techniques for biological samples by light and electron microscopy revealed that the individual cells of eukaryotes, making up the organism, contain, by themselves, a highly differentiated and complex additional – intracellular – skeleton, the cytoskeleton. It is composed of a framework of at least three classes of fibers that consist of protein, *i.e.* actin, tubulin (organized as microtubules) and intermediate filaments[1] (Figure 1). One function of this cytoskeleton is

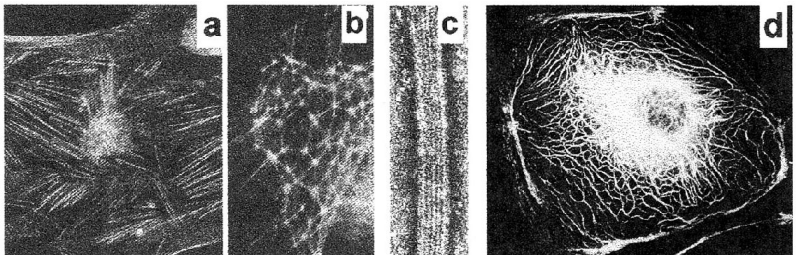

Fig. 1. *Cytoskeletal elements and cytoskeletons in eukaryotic cells, depicted by light microscopic immuno fluorescence (a, b, d) and by electron microscopy (c).* **a,b***, Actin filaments in fibroblast cells. a, stress fibers, three-dimensional network (Geodom). From ref. 1 with permission.* **c***,Tubuli.. Microtubulus. Magnification bar: 50 nm. From ref. 53 with permission.* **d***, Intermediate filaments. Network of keratin filaments. From ref. 54 with permission.*

to help maintain cell shape by providing mechanical support. In animal cells, the cytoskeleton also assists the function of the extracellular matrix that surrounds the cell and consists of collagen, proteoglycans, and further components that provide rigidity not only to the cell *per se* but also to tissues and organs. In addition, the cytoskeleton offers anchoring sites for other cellular components and serves as a track along which motor molecules are able to move. The cytoskeleton provides a structural framework against which muscles work to elicit movement. It is not a static structure, the most obvious dynamic functions being the actions of muscles, the formation and degradation of spindle fibers mediating mitosis and cytokinesis, the movement of amoeba and macrophages, and the streaming of protoplasma in plant cells. Recent progress in sequencing and annotation techniques did show that 850 different types of protein can be assumed to be structural or functional components of the cytoskeleton of eukaryotes (or at least proteins interacting with it), and that over 350 types of protein may belong to the groups of motor proteins involved in functions of the cytoskeleton.

Although the importance of a cytoskeleton for cells of eukaryotic organisms was evident, for decades research in the field of biology of prokaryotic microorganisms did not consider the search for similar cytoskeletons in bacteria to be a topic of major importance. After all, it is known that bacteria possess a strong cell wall held together by covalent bonds. It consists of peptidoglycan, the ''murein sacculus''. It is located outside the cytoplasmic membrane, engulfs the entire cell, and protects the bacterium from the stresses resulting from the higher osmotic pressure of its cytoplasm as compared to its environment. A huge body of data was collected over the years regarding many details of the construction of this sacculus, its biochemistry, its physical properties, its role in shape determination, growth, and division of the cells. In a review, Koch[2] outlines major aspects of the respective work. Though the bacterial wall fulfils various functions characteristic for a skeleton, it is not a cytoskeleton in the proper sense because it is not located within the interior of the cell, *i.e.* the body of the cell enclosed by the cytoplasmic membrane, and it does not consist of proteins. Rather, it can be called an exoskeleton. This review concentrates on elements of typical bacterial cytoskeletons. Therefore, the bacterial cell wall is not a topic of this review.

As mentioned above, a search for cytoskeletons in bacteria was not in the focus of interest. In 1972, a very uncommon bacterium was discovered[3]. It was called *Spiroplasma*; it shares various major

properties with some other bacteria detected a few years before. It is motile, helical, lacks a cell wall and was identified to belong to the Mollicutes, together with *Mycoplasma* and *Acholeplasma*. One year after the discovery of *Spiroplasma*, this bacterium was found to contain a unique and well-defined internal cytoskeletal element, a core organized as a flat, monolayered ribbon made up of filaments, composed of a 59-kDa protein. Electron micrographs revealed that this ribbon follows the shortest helical line of the polar cell from end to end[4]. Such a ribbon appeared to be a good candidate for a structural element involved in the observed motility and dynamic fluctuations of the *Spiroplasma* cell, though the 59-kDa protein was not actin or tubulin. In *Mycoplasma pneumoniae*, the presence of cytoskeletal elements somehow related to actin was proposed by Neimark[5]. Before this, in 1964, Domermuth *et al.*[6] had observed, by electron microscopy, an electron-dense straight or slightly bent rod within the *M. pneumoniae* terminal organelle, although an interpretation as a cytoskeletal element was not immediately made. In 1981, Göbel *et al.*[7] observed filamentous structures in adherent *Mycoplasma pneumoniae* cells treated with non-ionic detergents. Such a kind of protein complex within a cell was indicative for the presence of a cytoskeletal apparatus.

Mollicutes, in general, are unique bacteria, the phenomenology of which is reminiscent of wall-less eukaryotic cells in terms of swimming, gliding and dynamic fluctuations in shape. The lack of a cell wall and the presence of intracellular cytoskeletal elements as described above enhance this resemblance and did evoke a long search for eukaryotic-like contractile proteins not only in Mollicutes but also in typical eubacteria. This early search anticipated the recent findings of the bacterial cytoskeletal homologs of actin, tubulin and intermediate filaments in cell-walled bacteria[4,8].

In a study aimed at the optimization of the conditions for the production of a starch-degrading enzyme with a bacterium named *Thermoanaerobacterium* spec. (former name: *Clostridium* sp. strain EM1), an observation was made that gave rise to an extensive search for a bacterial cytoskeleton different from those supposed to exist in Mollicutes[9]. It was found that limitation of starch in the growth medium, combined with phosphate limitation, gave best results in a continuous culture of the bacteria. A careful electron microscopic investigation of the subsequent states of growth, including the state where highest enzyme production was measured, revealed that the bacteria did produce the highest amounts of active enzyme (α-amylase) in a state where they had completely lost their entire cell wall (Figure 2). In this situation, the enzyme complexes

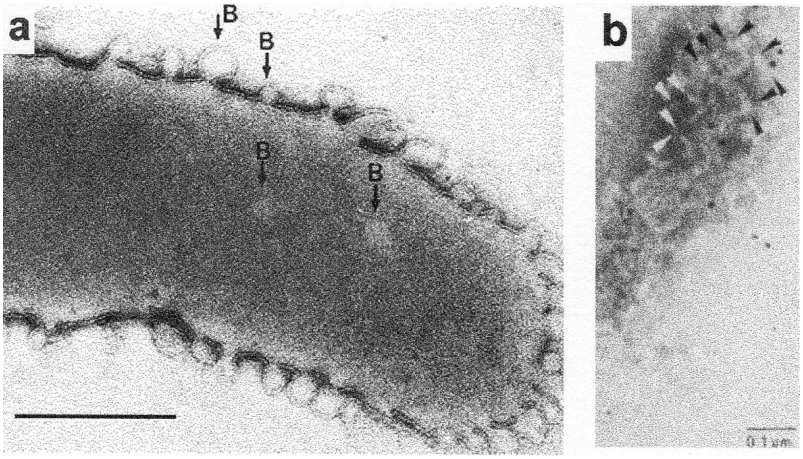

Fig. 2. *Cytological aspects of the eubacterium Thermoanaerobacterium sc. (former name: Clostridium sp. strain EM1).* **a**, *cell grown under starch and phosphate limitations. The cell, still viable, has lost its cell wall (surface layer, peptidoglycan sacculus), but still remained its elongated shape. B, bleb formation by the exposed cytoplasmic membrane. Magnification bar: 0.5 µm. From ref. 9 with permission.* **b**, *cytoskeletal framework (arrowheads), discovered by electron microscopy, after a cell in the state shown in Fig. 2a was gently lysed on the electron microscopic support film. The small round black dots are projections of colloidal gold markers with a diameter of 14 nm. They were used for labelling of anti-actin antibodies that were applied for a test on cross reactivity of these antibodies with the cytoskeletal framework. Weak cross reactivity was observed. From ref. 10 with permission.*

could be seen to be attached to the exposed outer face of the cytoplasmic membrane, covering the entire cell surface. Although the cells had lost their cell wall, they maintained their typical elongated shape. This was a surprising observation. After all, it was common knowledge that a bacterium with an elongated cell shape would be expected to be transformed into a sphere (a "spheroplast") without the shape-determining cell wall. Hence, it was assumed that there exists an additional intracellular structural system that is strong enough to warrant the preservation of the elongated cell shape without the cell wall. Hints for such a structure – in fact a cytoskeleton – were obtained by electron microscopy[10] (Figure 2b). The experimental findings described above represented the first case where, in a typical rod-shaped eubacterium not belonging to the Mollicutes, strong indications were obtained for the existence of a true shape-preserving cytoskeleton engulfing the entire cell body.

The data on cytochemical elements in Mollicutes, and on a cytoskeleton enclosing the entire cell body of *Thermoanaerobacterium* spec., did decisively influence further investigations into composition, structural organization, and function of bacterial cytoskeletons, enclosing the entire cell body, in Mollicutes and in walled eubacteria. However, there were additional reasons for the intensification of a search for bacterial cytoskeletal elements and cytoskeletons. Many of the structural and functional traits of the eukaryotic cell have evolved from earlier forms of life. Why should the various elements of the eukaryotic cytoskeleton not also have their evolutionary origin in prokaryotes (archaea, eubacteria)[11], and could we expect to identify these precursors in todays prokaryotes? One of the unsolved questions was the process of chromosome segregation in bacteria[12]: is there a bacterial mitotic apparatus comparable with that of the eukaryotic cell?[13] And could the structural elements that can be expected to exist have similarities with the proteins making up the eukaryotic mitotic system? In 1978, Beck *et al.*[14] described novel properties of bacterial elongation factor Tu (EF-Tu), known to have a function in translation, *i.e.* in cellular protein synthesis. To their surprise, they found that the isolated protein was able to form filaments *in vitro*. This observation was confirmed by extended studies[15]. Electron microscopy and image processing were used for the investigation of regular polymerization products of EF-Tu isolated from *E. coli*. A structural role of EF-Tu filaments in a bacterial cytoskeleton was discussed, but not further investigated in *in vivo* studies. The idea was abandoned after Schilstra *et al.*[16] had observed, by immuno electron microscopy on ultrathin sections, a nearly homogeneous distribution of EF-Tu in *E. coli*. Such a distribution would exclude the existence of EF-Tu filaments in the cell and, hence, an EF-Tu-based bacterial cytoskeleton. Observations of the Lutkenhaus group[17,18] revealed that ftsZ is an essential division gene in *Escherichia coli*, and that an FtsZ protein ring structure, a kind of cytoskeletal element, is located at the site where the septation membrane and wall are formed. In these early investigations, a functional or structural relationship with tubulin did not become evident. No detailed explanation was found, prior to the findings described by Jones *et al.*[19] on bacterial cell shape-determination by helical, actin-like filaments, for the observation made by Doi *et al.*[20] that disruption of the mreB gene of *Escherichia coli* caused a change in cell morphology from the normal rod shape to a spherical form, indicating a direct or indirect role of the MreB protein in cell shape determination.

Bearing in mind the experimental data, summarized above, on cytoskeletal elements and the indications for the existence of intracellular cytoskeletons engulfing the entire cell body, several research groups intensified their efforts aimed at more detailed investigations on the occurrence of these structures, their spatial organization, their functions, their composition, their relationships to cytoskeletal elements and cytoskeletons in eukaryotic cells, and their evolution. Besides established basic experimental approaches such as conventional light and electron microscopic preparation and imaging techniques and mutagenesis methods, a range of additional and refined, more recent, approaches are applied, often in combination. Examples are video light microscopy, confocal laser scanning microscopy, often combined with immunofluorescence or techniques involving green fluorescent protein (GFP), fluorescence recovery after photobleaching, flow cytometry, fluorescence resonance energy transfer measurements, scanning-transmission electron microscopy, immunoelectron microscopy, cryopreparation of samples, cryoelectron tomographic imaging and image reconstruction, refined X-ray crystallography, nuclear magnetic resonance measurements, improved mutagenesis methods, PCR (polymerase chain reaction), refined sequencing techniques, automated search for sequence comparisons, mass spectrometry, genome annotation procedures, improvements in characterization of protein properties and interactions, and in computer modelling of proteins in complexes.

A very uncommon complex cytoskeleton in *Mycoplasma pneumoniae*

One of the convincing examples for the progress in knowledge on cytoskeletal elements and cytoskeletons are the advances made in the group of the *Mycoplasmas*[21–26].

Based on early observations, made by conventional electron microscopy, on the structural organization of the (wall-less) *Mycoplasma* cells and the variations of cell shape (coccoidal or filamentous)[6,27], on the function of its attachment organelle, on the internal electron-dense rod structure and on the gliding phenomenon, in several research groups a detergent-insoluble fraction (the Triton X-100 fraction) of the cell components was prepared and investigated[7]. This fraction was found to be filamentous, but actin-like structures were not observed. The electron-dense core was a major component of the insoluble material, in addition to a network of finer filaments. An investigation by 2-D gel electro-

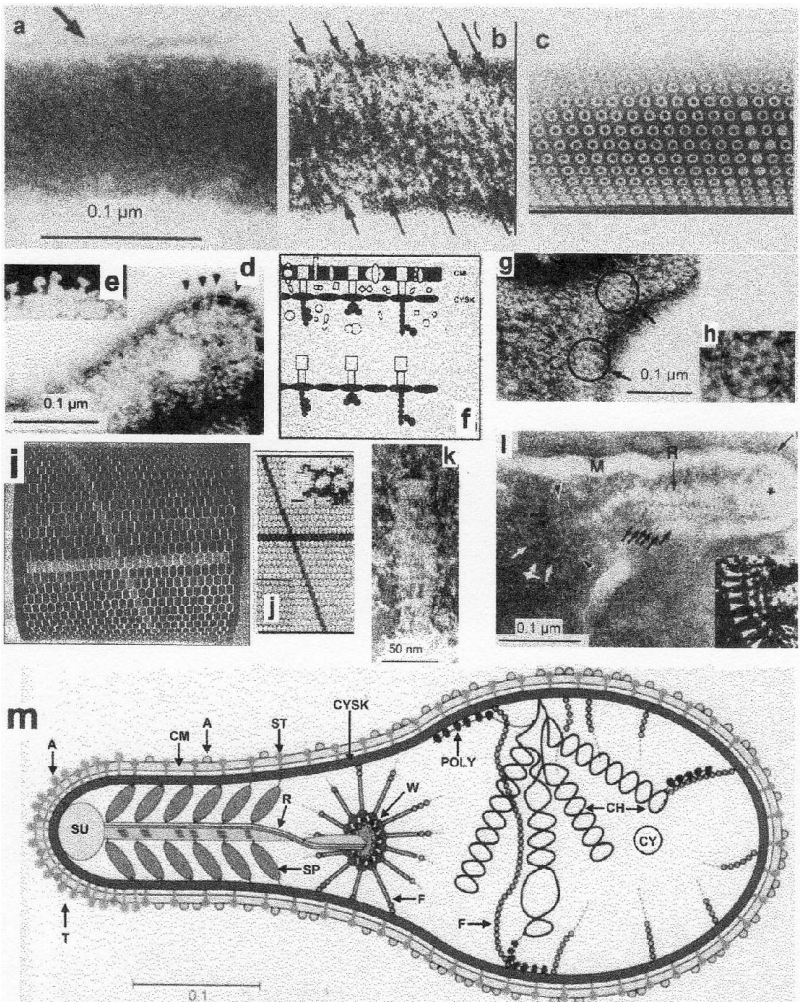

Fig. 3. *The cytoskeleton of Mycoplasma pneumoniae (from ref. 23. with permission).* **a**, *part of a cell with well preserved cytoplamic membrane (arrow) as the outermost cell layer.* **b**, *part of a cell body from which the cytoplasmic membrane had been removed by treatment with formaldehyde and a non-ionic detergent (Triton X-100). Note that the cytoplasmic membrane is no longer visible. Instead, the outermost cell layer is now formed by a layer that appears "rough"; it is composed of helically arranged rows of protein molecules (arrows).* **c**, *diagrammatic presentation of the helical organization of the outermost layer exposed after removel of the cytoplasmic membrane (see Fig. 3b).* **d,e**, *Short stalks (arrowheads in d, depicted at higher magnification In e), with knobs at their tip, extend from the "rough" layer shown in Fig. 3b.* **f**, *Interpretation of the observations depicted in Figs. 3b,d. and Top: diagrammatic view of the organization of the cell periphery prior to removal of the cytoplasmic membrane. The cytoplasmic membrane (CM) is connected to the layer below*

(a kind of lining, CYSK) by the stalks. The knobs at the ends of the stalks are depicted to be integrated into the cytoplasmic membrane. Bottom: situation after removal of the cytoplasmic membrane. The CM is no longer present, the majority of the soluble cell components are removed by treatment with non-ionic detergent and blotting-dry of the sample during the preparation procedure. The lining with the attached stalks is preserved. It is interpreted to be an intracellular cytoskeleton engulfing the entire cell body, situated immediately below the cytoplasmic membrane. The stalks, with their basis in the lining, are assumed to support the cytoplasmic membrane and keep it in place. **g,h**, *After partial removal of the stalks, the basic structural organization of the lining became visible. It could be seen that it is composed of a network of regularly arranged meshes (encircled in g, shown at higher magnification in h).* **i,j**, *Diagrammatic views of the structural organization of the lining. Inset in j: structure of a few meshes; the image was obtained by superposition of several original electron micrographs. Magnification bar: 10 nm.* **k**, *Electron micrograph of an isolated rod from the tip of a cell. The rod exhibits striations perpendicular to its long axis. The rod is composed of fine fibrils running parallel to the long rod axis. The striations are brought about by a regular arrangement of protein masses present along the parallel fine fibrils. The interpretation is that the fine fibrils are very long individual protein molecules with a number of mass concentrations exhibiting a conformation different from that in the linear stretches between the mass concentrations.* **l**, *Electron micrograph of a cryo ultrathin section through the tip region of a cell. M, cytoplasmic membrane; R, rod, connected to the cell periphery by a group of linkers (group of black arrows); two black arrowheads point to a wheel-like inclusion attached to the end of the rod; white arrows, fine fibrils connecting the wheel with the cell periphery. Inset: electron micrograph of a wheel-like complex depicted at high magnification and in reversed contrast. Note its composition of prorein molecules (white arrowheads) with circular arrangement, indicating that the wheel has a round shape. Unpublished results, obtained by J.Hegermann, prove that ioslated wheels, in flat projection,exhibit round projections.* **m**, *diagrammatic view of a cross section through a cell of Mycoplasma pneumoniae.*

Abbreviations: a, proteins exposed at the surface of the cytoplasmic membrane, probably involved in attachment of the cell; CH, chromosome; CM, cytoplasmic membrane; Cy, cytoplasm; CYSK, lining of the cytoskeleton; F, fine fibrils, belonging to the cytoskeleton, crossing the cytoplasm and connecting the wheel to the lining; P, polysomes attached to fine fibrils or to the lining; R, rod; SP, linkers connecting the rod to the lining; ST, stalks with terminal knobs, with their basis, each linker is attached to the lining, the knob is integrated into the cytoplasmic membrane; SU, unidentified protein mass located in the tip, providing the site of attachment of the rod; T, cell tip.

phoresis and mass spectrometry of the Triton X-100 fraction, assumed to contain all elements of the *Mycoplasma* cytoskeleton[26], did initiate a more detailed electron microscopic study[23] that gave surprising results (Figure 3). The interior of the *Mycoplasma* cell exhibited a highly organized cytoskeleton composed of the rod, spokes connecting the rod with the cell periphery, fine fibrils crossing the cytoplasm, and a complex, at the proximal end of the rod, that appeared to be wheel-like and connected, by fibrils, to the cell periphery. It could be seen that the rod is, in fact, composed of two matching flat halves. During initiation of cell division, these two flat halves separate by splitting open. Each of them is, in a subsequent phase of cell division, the stabilizing internal structure for the tip of one daughter cell. A further detail could be observed in chemically fixed cell bodies that were obtained by treating intact cells in such a way that the cytoplasmic membrane was totally removed. Even without any support of cell shape by the cytoplasmic membrane, the elongated form of the cell was preserved. A regular network of fine fibrils could be detected, located, in untreated cells, in immediate neighbourhood to the inner face of the cytoplasmic membrane, forming a lining engulfing the entire cell body. It appeared to be the site of attachment of the fibrils crossing the cell cytoplasma, and of the stalks attached to the rod. Numerous regularly arranged short stalks, extending outward from the plane of the lining, had carried the cytoplasmic membrane prior to its removal. Immuno electron microscopic labelling studies on this kind of membrane-less cell bodies with gold-conjugated anti-*Mycoplasma pneumoniae* EF-Tu-antibody resulted in dense labelling of the exposed surface of the lining (Figure 4e). Occasionally, a label was observed to be attached to short fibrillar extensions of the partially destroyed network, originating from the mechanical stress during sample preparation. Control labelling experiments with antibodies raised against MreB protein or tubulin were negative. Respective cross-reactivity control experiments with anti-actin-antibodies gave weak positive cross reactivity. This was explained with the fact that EF-Tu and actin both belong to the actin superfamily. Control experiments with gold-conjugated anti-EF-Tu antibodies applied to untreated cells were negative. Figure 3m depicts a diagrammatic view of a longitudinal section through a cell of *M. pneumoniae* in an elongated state, exhibiting the components of the cytoskeleton as described above. Major features of the model are that a lining is postulated, *i.e.* that the lining is not just a cytoskeletal element but a cytoskeleton (called "cytoskeletal web") that encloses the entire cell body, that it is located right below the

cytoplasmic membrane, that it is not composed of actin or a precursor of actin, or of FtsZ, or some other cytoskeletal building material known from eukaryotic organisms or bacteria. Presuming that this cytoskeletal web contains, as the main protein, EF-Tu as indicated by the immuno electron microscopic results, this would mean that a very primordial protein in living systems might have at least a double function. After all, EF-Tu plays an important role in cellular protein synthesis. Further experiments were undertaken with the aim to demonstrate the presence of a cytoskeletal web also in typical walled bacteria, and the involvement of EF-Tu in this web. To this end, typical bacteria (Gram-positive and Gram-negative) were used for a detailed analysis (see below).

Again a unique bacterial cytoskeleton in Mollicutes: the linear motor in *Spiroplasma*

Spiroplasma are, like *M. pneumoniae*, wall-less bacteria also belonging to the class Mollicutes. Their cell body exhibits a helical shape. They are motile; however, motility is not brought about by gliding as in *M. pneumoniae*, but by active swimming, and it is the helical cell shape that mediates this property[4]. The basis for helicity and for swimming is an intracellular cytoskeletal ribbon, consisting of seven pairs of parallel fibrils. The fibrils are composed of rows of protein molecules – not related to actin or tubulin or proteins in intermediate filaments – that were shown to dynamically alter their axial ratios. Nearly circular to nearly flattened ellipses were observed, by electron microscopy of isolated ribbons, as projections of the proteins making up the rows. This dynamic variation of diameter appears to be the underlying mechanism of fibril length changes (contraction and expansion), and the length changes have as a consequence an alteration of the swimming behaviour. It is reasonable to assume that the length changes are a response to signals from the outside (chemotaxis). Some of the structural properties of this linear motor are depicted in Figures 7e,f,g, together with several cytoskeletal elements of other bacteria. It should be noted that *Spiroplasma citri* is the only known Mollicute that contains MreB. (5 MreB homologs). Their presence in a species without a wall suggests that MreB is involved in determining the helical shape of the cell by a cell-wall-independent mechanism, perhaps as constituents of the linear motor characteristic for this organism[28].

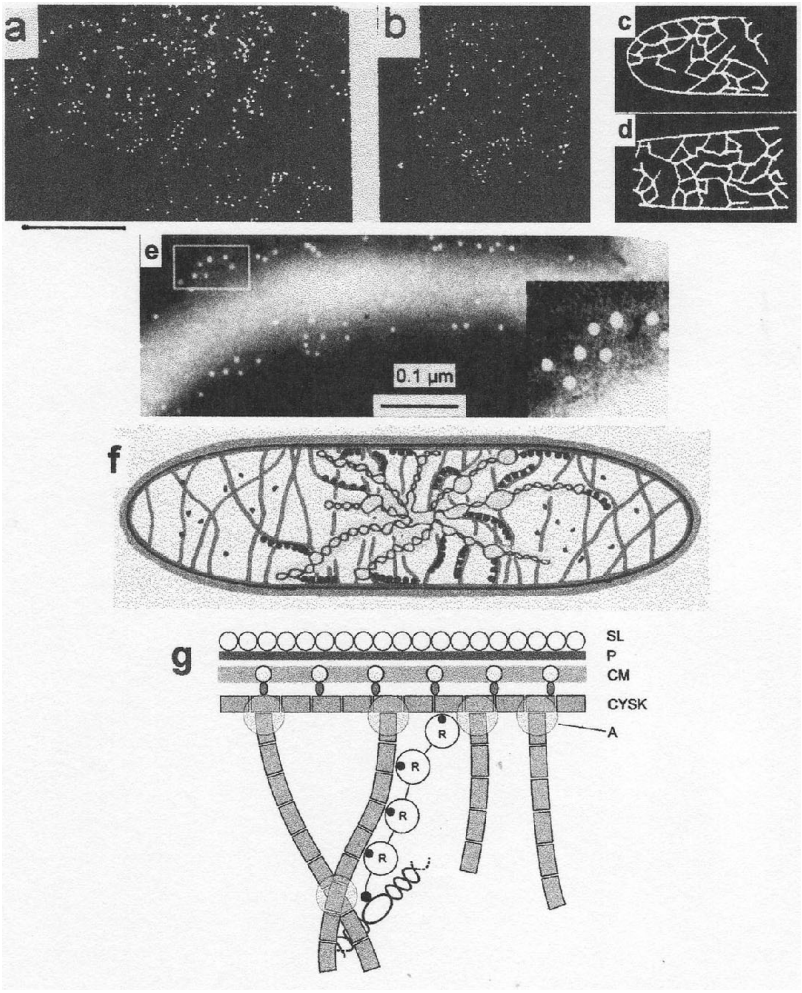

Fig. 4. *Electron microscopic immuno labelling experiments and deduced cell models.* **a,b**, *cellular location of bacterial elongation factor EF-Tu in Thermoanaerobacterium sp. Note non-homogeneous distribution of EF-Tu. The colloid gold marker particles did form rows and networks thereof. Magnification bar: 0.5 µm. Printed in reversed contrast. From ref. 40 with permission.* **c,d**, *sketch illustrating the distribution of the EF-Tu marker. The rows of particles were connected to form rows in the drawing, indicating the existence of a lining and a network containing EF-Tu. From*[43] *with permission.* **e**, *Localization of EF-Tu markers at the outside of Mycoplasma pneumoniae cell bodies lacking the cytoplasmic membrane (compare with Fig. 3b). Insets: colloidal gold markers attached to fibrillar constituents extending from the lining due to partial rupture of the lining, caused by the preparation procedure for electron microscopy. Printed in reversed contrast.* **f**, *Simplified model of a eubacterial cell (longitudinal section). The cell periphery is composed of the wall and the cytoplasmic membrane. In close vicinity to the inner face of the cytoplasmic membrane,*

Cytoplasmic filament ribbon of *Treponema*: A member of an intermediate-like filament protein family

Treponema bacteria belong to the class of spirochete genera. The cell is composed of an outer membrane, a periplasmic space where the motility-associated flagellar filaments are located, an inner or cytoplasmic membrane, and the cytoplasmic cylinder. Just underneath the cytoplasmic membrane, a cytoplasmic filament ribbon can be seen[29]. The helical periodicity of the ribbon is equivalent to that of the cell. The ribbon spans the length of the cell. Electron tomography of ribbons isolated from *Treponema phagedenis* indicated that the ribbon is formed of individual filaments that are continuous within the cell volume; the filament do not interface or touch each other; bridging proteins connect filaments two by two on the cytoplasmic side of the ribbon, and anchor proteins are present on the inner-membrane side of the ribbon. In Figure 7h, purified isolated filaments are depicted. The constitutive protein of *Treponema pallidum* (CfpA, cytoplasmic filament protein A) was isolated and partially sequenced. BLAST and FASTA searches did not discover significant sequence similarity of the protein to other open reading frames in GenBank database available at the time of investigation. A comparison with the sequences obtained from several other *Treponema* strains revealed a remarkable property: there is a conserved coiled-coil segment organization (see also below) among the CfpA proteins, with four segments distributed along the overall protein sequence. This fact allowed the conclusion that the *Treponema* cytoplasmic filament ribbon can be classified together with intermediate filaments (IF) in eukaryotic cells, exhibiting a similar sequence organization. In general, the main proteins of intermediate and intermediate-like filaments have in common coiled-coil domains in the section of their peptidic sequence.

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a ling (part of a cytoskeleton engulfing the entire cell cytoplasm body) is drawn. Fine fibrils, also parts of the cytoskeleton, are shown to cross the cytoplasm; polysomes, containing active ribosomes, are attached to the lining and to the fine fibrils. In active ribosomes are not attached. The central part of the nucleoid (see Fig. 3m) is not crossed by cytoskeletal fibrils. **g**, *Diagrammatic few of the structural organization of the cell periphery and the cell cytoplasm. A, not yet identified proteins postulated to make connections between cytoskeletal fibrils; CM, cytoplasmic membrane; P, peptidoglycan sacculus; R, ribosomes in polysomes; SL, surface layer of the cell wall. From ref. 40 with permission.*

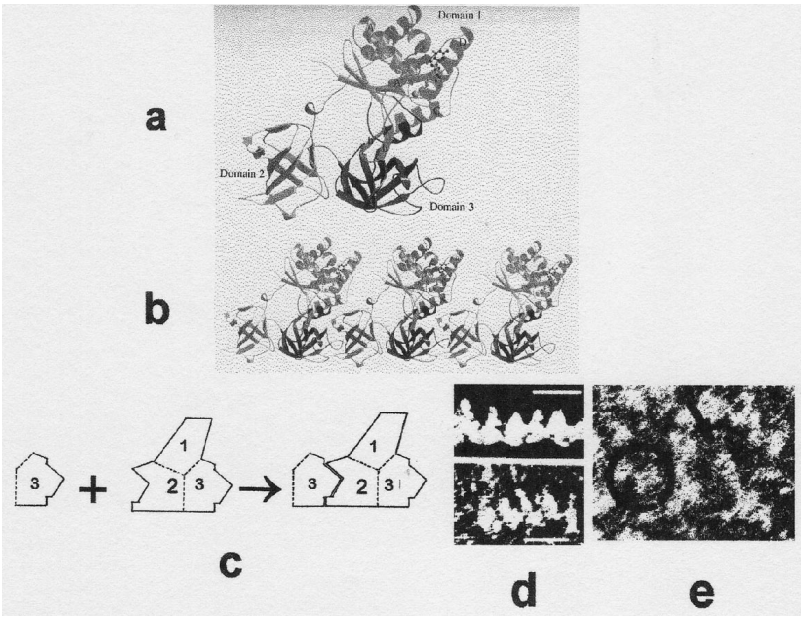

Fig. 5. *EF-Tu and complexes thereof.* **a**, *Ribbon blot of full-size EF-Tu in the GDP state. Note: the protein exhibits three domains. Domain 1 is, together with parts of domain 2, involved in translation. Domain 3 has an exposed loop extending from its surface; domain 2 has a cleft. From ref. 42 with permission.* **b**, *Model for the formation of a row (by polymerization) of EF-Tu protein molecules, taking advantage of the fact (confirmed by computer simulation) that the loop of domain 3 fits into the cleft of domain 2 of a neighbouring full-size EF-Tu protein. From ref. 40 with permission.* **c**, *Prevention of row formation by insertion of a kind of EF-Tu that is truncated; this molecule was designed and synthesized in Escherichia coli by Schwienhorst and Hempel using recombinant techniques. It consists solely of domain 3 of the full-size EF-Tu. As soon as such a molecule is bound to a full-size EF-Tu, row elongation in one direction is prevented because there is no possibility for the binding of the domain of a neighboring full-size EF-Tu. At a numerical ratio of one to one in a mixture of full-size and truncated EF-Tu the formation of rows longer than two or three building units can be neglected; this was shown in respective experiments (see Fig. 5e). Further explanations, main text. Original drawing by F.M.* **d**, *Electron micrographs, printed at high magnification and at reversed contrast. Top: projection of one of the fibrils depicted in Fig. 2b. Note the similarity with the overall shape of a row of EF-Tu proteins in Fig. 5b. Bottom: row of full-size EF-Tu proteins, formed in vitro. Note the similarity with the rows depicted in Fig. 5b and Fig 5d top. Magnification bars: 10 nm. From ref. 24 with permission.* **e**, *Electron micrograph depicting the result of an experiment on row formation in a mixture of full-size and truncated EF-Tu as described in Fig. 5c. Note the absence of typical rows as seen in Fig. 5d. Printed in reversed contrats. Original micrograph by F. Mayer and A. Schwienhorst.*

Mutant strains of *T. denticola*, obtained by disruption of the open reading frame *cfpA*, did not express CfpA. No filamentous ribbon was present. The phenotypic consequences affected multiple cell functions. Cell shape was not altered. The most noticeable phenotype was a segregation defect with condensation of the chromosomal DNA. However, the actual role of these cytoplasmic filaments in the cell division process and the relation to the chromosomal DNA is unclear. It is speculated that the mechanism that spatially and temporally controls and directs the oriC region (*i.e.* the site on the chromosome from where the two replication forks initiate) might involve the CfpA ribbon. Further work is required to demonstrate that the filamentous ribbon of treponemes is part of a permanent spindle or mitotic-like apparatus. Such an apparatus has not yet been found in bacteria[13].

Another intermediate filament-like cytoskeletal element : Crescentin, a coiled coil-rich protein in the bacterium *Caulobacter crescentus*[30]

Caulobacter crescentus is a dimorphic aquatic bacterium. Its name reflects its characteristic cell shape. Both cell types, the sessile stalked cell and the motile swarmer, have a curved cell shape resembling a moon crescent. Cells in the stationary growth phase of the culture may adopt a helical morphology. This change is assumed to be part of a specific developmental programme acting against cell death by starvation and aging. The inactivation of a specific gene, designated creS, encoding crescentin, resulted in the loss of cell curvature in different mutants. Crescentin is a 430 amino acids long α-helical protein. Most of its sequence consists of heptad repeats (*abcdef*)n in which hydrophobic residues occupy positions *a* and *d*. The regular repetitive pattern of alternating hydrophobic and polar residues results in the formation of a hydrophobic stripe on one face of the helix. Proteins with this feature adopt a coiled-coil conformation, where individual α-helices align the complementary hydrophobic surfaces and form a double or triple superhelix. In general, short coiled-coil stretches usually function as protein dimerization domains. Long coiled-coil domains are often found as building blocks of large mechanically rigid structures, such as cytoskeletal filaments and levers of motor proteins in eukaryotic cells[30]. It should be noted that no domains with similarity to motifs conferring enzymatic or nucleotide-binding activities could be detected in the crescentin sequence.

Detailed cytological studies of curved and helical *Caulobacter crescentus* cells both by electron microscopy and by application of a light optical evaluation of the sites where crescentin-GFP protein is found revealed that the crescentin filament is found asymmetrically located at the inner curvature of curved and helix-shaped cells. This observation gave rise to the assumption that crescentin is involved, together with other factors, in the synthesis of the murein sacculus. The recently discovered intracellular actin-like cytoskeleton, encoded by mreB-related genes (see below) and the membrane-anchored MreCD proteins together might provide a helical framework for the extracellular wall synthetic machinery. The result of such a mechanism is a cylindrical murein sacculus and a rod-shaped cell. The inhibition of peptidglycan synthesis along only one lateral side – that one where the crescentin filament is located – could cause the formation of a bent rod-shaped sacculus. Hence, crescentin could be a key factor to modulate cell wall building by interacting with other proteins or membrane components. Not yet answered is the question how the asymmetric localization of the crescentin filament is brought about. It was proposed that, instead of sequence similarity-based searches for cytoskeletal elements in bacteria, identification and analysis of all coiled-coil-rich proteins could be a more useful approach for providing candidate proteins for novel cytoskeletal roles.

Bacterial FtsZ protein: The ancestral homolog of eukaryotic tubulin

Bi and Lutkenhaus[18] were the first to report on a discovery that was decisive for major advances in the understanding of the molecular details of bacterial cell division. They described a ring structure (the Z ring) at the leading edge of the septum of a dividing bacterium. This ring was the first molecular structure associated with bacterial cell division, and FtsZ (filamenting temperature sensitive Z) was the first protein to be localized to this discrete cellular address. In a later report[31], the authors report and discuss the major properties of the system with a specific view to the earliest stages of the formation of the bacterial septum involving formation of the Z ring and its topological regulation. The reader is also referred to an overview of FtsZ in cell division[13]. Since the discovery of the Z ring, fusion of FtsZ to GFP and immunofluorecence microscopy have been used for visualization of FtsZ and other cell division proteins. Most of them associate with the Z ring to form a complete septal apparatus; others, which spatially

regulate cell division, like the Min proteins[32], are dynamically distributed in the cell (*Escherichia coli*) or associated with the cell poles (*Bacillus subtilis*).

FtsZ and tubulin share only 10% sequence identity; however, they exhibit surprising similarities in the crystal structures[33] and in the GTPase mechanisms; they polymerize into structurally similar protofilaments (Figure 7d). Tubulin subunits have strong lateral interactions leading to the assembly of adjacent protofilaments; the result is the formation of a microtubule (Figure 1b). FtsZ does not form microtubules. However, lateral association of protofilaments was observed. Evidence for the physiological relevance of lateral associations comes from analysis of FtsZ mutants with alterations in lateral amino acids. These alterations do not prevent the GTPase activity and polymerization into protofilaments but do prevent FtsZ from functioning in division. FtsZ polymers, like tubulin polymers present in microtubules, are dynamic in nature owing to GTP hydrolysis; subunits of FtsZ are constantly cycling in and out of the polymers. This dynamism was revealed by studies utilizing FRAP (fluorescence recovery after photobleaching) in live *Escherichia coli* and *Bacillus subtilis* cells. This technique demonstrated that the subunits of the Z ring are constantly exchanging with the pool of cytoplasmic FtsZ. This flux occurs the entire time the Z ring is at the septum, both before and during constriction. The half-life of individual subunits of FtsZ in the ring is around 8–9 s. This is on the same order as the rate of GTP hydrolysis, suggesting that the *in vivo* turnover rate is dictated by the GTPase activity. In case of the FtsZ ring (and also of the mitotic spindle in eukaryotic cells) it is not clear how the ring (or the microtubules in the spindle apparatus) can maintain coherence and intergrity while at the same time undergoing such rapid turnover. Calculations based upon the high GTPase activity associated with FtsZ assembly indicated that an average protofilament length of only 30 subunits (about 120 nm) can be sustained. This suggests that the FtsZ ring consists of multiple short filaments of FtsZ, presumably held together by FtsA or ZipA[34] (ZipA is a membrane protein that is involved in attachment of the Z ring to the membrane, an essential requirement for the formation of the Z ring), and/or by lateral contacts that polymers make with each other. One of the problems that remains to be solved is a more detailed analysis of the processes leading to the regulation of the Z ring assembly, especially with respect to the contribution of interacting proteins. A recent proposal (Figure 7c) suggests that polymers of FtsZ associate with the membrane through FtsA, a protein distantly

related to actin), and this occurs throughout the cell. However, only at midcell is there a sufficient concentration of these membrane-bound polymers for a Z ring to form. This is where the inhibitory effects of Min and Noc (nucleoid occlusion), both negative effectors, are at their lowest and, hence, structures growing perpendicular to the long cell axis have a chance to persist. The model implies that regulation is exerted over self-organization of the Z ring which involves the cooperative association of protofilaments assembling on the membrane, mediated by lateral interactions or interacting proteins.

Bacterial actin homologs

Already in 1988, Doi *et al.*[20] performed experiments that resulted in the notion that MreB is an important cell shape determinant in rod bacteria; its disruption caused a change in cell morphology from the normal rod shape to a spherical form. This indicated that MreB may be involved in cell wall synthesis (see below). The finding was also a first clue for the speculation that MreB could have a function as a cytoskeletal element; after all, a change in cell morphology would mean that a possibly existing intracellular scaffold and its weakening could be involved. Hence, MreB was a candidate for a possible building entity for such a scaffold, and a similarity of MreB with eukaryotic actin, one of the major components of the eukaryotic cytoskeleton, could support this notion. A breakthrough regarding the relationships between MreB and eukaryotic actin was achieved in 2001 by Errington and coworkers[36], and by Van Den Ent *et al.*[35] Though sequence alignments of various MreB proteins revealed that their similarities with eukaryotic actin were not striking, the overall shape of MreB, as indicated by X ray analysis, was surprisingly similar. The projections of both proteins could indeed be superimposed. Both proteins polymerize into protofilaments that pair lengthwise. Basically the same interaction that has been proposed for the longitudinal interaction in the two strands of F-actin was found for MreB protofilaments, but MreB double filaments are not nearly as helical as actin double filaments. Numbers of copies of MreB and Mbl (MreB-like protein, absent in *Escherichia coli*, present in *B. subtilis*, together with MreB) were determined; an average *Bacillus subtilis* cell contains about 8000 molecules of MreB and 13000 molecules of Mbl. MreB and Mbl form distinct helical structures (Figure 7a) that often extend the entire length of the cell. It was postulated that the protofilaments (Figure 7b), the building elements of the helical bands supporting

cell shape, might provide sufficient mechanical stability when organized as bands. With a few exceptions, MreB orthologs are present only in bacterial species with rod or helically shaped cells; they are usually absent in species with round-shaped cells (cocci). So, the question was to be answered of how the MreB/Mbl-based cytoskeletal framework could define the shape of the bacterial cell. In a review , Margolin[28] did present and discuss the state of research. Daniel and Errington (2003)[37] could give first answers to this question. They did link the MreB/Mbl localization patterns with the pattern of wall biosynthesis. They used a fluorescently labelled derivative of vancomycin, an antibiotic that binds to a precursor of peptidoglycan, the main constituent of the bacterial wall. Because the drug cannot penetrate the outer membrane of Gram-negative bacteria, *Bacillus subtilis*, a Gram-positive bacterium was used for staining as it lacks the outer membrane. The staining exhibited two patterns: a helix that extended the length of the cell, and bands at cell division septa. Elimination of Mbl abolished the helical staining by vancomycin, while increasing the septal staining. Inactivation of MreB had little effect on the staining. This result indicated that Mbl, not MreB, directs the helical topology of cylindrical wall extension.

What defines the helical path taken by Mbl and the peptidoglycan synthesis machinery? Other proteins, such as the Min proteins of *Escherichia coli*,[32] also form intracellular extended helices. This implies that there may be multiple helical tracks in the cell. Just as Min proteins move forth and back along their track, MreB helices are also dynamic, both in terms of subunit turnover and movement of the helices across the cytoplasmic membrane. Such a sweeping motion may be essential to be able to synthesize new wall material all over the cell as it grows.

Emerging evidence has linked this group of actin-related cytoskeletal proteins not only to shape determination and shape stabilization and to cell wall synthesis, but also to chromosome partitioning. This connection was first observed in ParM[38], an MreB homologue. It is a plasmid-encoded partitioning protein, which forms dynamic filaments that actively move the plasmid copies apart to opposite cell poles during cell division. Subsequent cytological and flow cytometry experiments revealed that *Escherichia coli* cells depleted of MreB, fail to segregate their chromosomes and therefore produce paired chromosomes. Recently, it was suggested that these defects might not be mediated directly by MreB, but through a polar effect on genes downstream of mreB. In *Caulobacter crescentus* MreB was shown to be

important for the segregation of the chromosomal origin region, but not for partitioning of origin-distal loci. It was concluded that the origin-proximal region is segregated *via* an MreB-dependent mechanism, whereas segregation of the rest of the chromosome occurs through an MreB-independent mechanism. An alternative interpretation for MreB-dependent origin segregation is that MreB is required for the assembly of a yet-to-be-discovered mitotic apparatus[39], but once assembled, the presence of MreB is no longer needed for chromosome separation. In contrast to a concept that supports the existence of a mitotic apparatus, speculative alternative models suggest that energy released during essential processes, such as replication and transcription, provides the driving force that moves the daughter chromosomes apart. However, for *Caulobacter crescentus* it was suggested, on the basis of experimental data, that it is unlikely that the force that is generated by the replication is the driving force for segregation.

A kind of intracytoplasmic bacterial cytoskeleton that might be very primordial

As outlined above (see the section on the complex cytoskeleton in *Mycoplasma pneumoniae*), wall-less bacteria exhibit a kind of cytoskeleton that engulfs the entire cytoplasmic cell body like a lining[1,23,24,40]. Electron microscopic studies showed that this lining is organized as a net-like structure; it was called cytoskeletal web. Immuno electron microscopic labelling experiments indicated that a protein, elongation factor Tu (EF-Tu), may be the main component of the web. This protein is known to have a function not related to a cytoskeleton, but to the process of translation during cellular protein synthesis. Such a double function might be surprising, but, as mentioned above, already in 1978 it was shown that the protein is able to form, *in vitro*, filamentous polymers. Hence, it was speculated that this protein could be a candidate protein for a bacterial cytoskeleton. Later experiments, using low-temperature preparation techniques for electron microscopic samples, could not establish that EF-Tu-based filaments are present inside of bacterial cells until the results of the experiments with *Mycoplasma pneumoniae* described above were available. Though it is generally accepted that the cytoskeletal elements and cytoskeletons in Mollicutes are very specific for these wall-less bacteria, it was interesting to see whether also for typical walled bacteria indications for such a kind of cytoskeletons could be obtained.

To this end, studies were done with *Thermoanaerobacterium* spec., the bacterium described above to keep its rod shape, exist and stay alive even without a surrounding cell wall. Immuno electron microscopic investigations revealed that application of gold-labeled EF-Tu-specific antibody resulted in strong labelling (Figure 4a,b) and that the pattern of labelling allowed to suggest that the labelled antigen, EF-Tu, has a cellular location along lines (Figures 4c,d). Such a labelling result cannot be explained when it is assumed, as communicated by Schilstra *et al*., that EF-Tu is monomeric and nearly homogeneously distributed throughout the cytoplasm. In fact, this labelling pattern demonstrates that nearly no "free" EF-Tu is present in the cell, but that the majority of EF-Tu is "bound" to linear structures located along the inside of the cytoplasmic membrane and crossing the cytoplasm. This observation is in agreement with the results obtained by respective immunolabelling experiments performed with membrane-less *Mycoplasma pneumoniae* cells (see above). EF-Tu is known to be transiently complexed with ribosomes in a 1 : 1 ratio during the elongation step in translation. On the basis of the classic view, one would expect that many of the EF-Tu copies (those that are not engaged in translation) should be free in the cytoplasm. The fact that the number of EF-Tu copies per cell is about four times higher than needed by the cell in context with translation[41], would even more give rise to the expectation that at least around 75% of the EF-Tu copies would not be complexed with ribosomes at a given moment, and that they should be found in immuno electron microscopic investigations homogeneously distributed in the cytoplasm. As this is obviously not the case, it allows the conclusion that nearly all the EF-Tu of a cell is not present in a monomeric state, but that it is located within fibrillar structures or complexed with ribosomes that are, themselves, attached to fibrillar structures. This postulate was the start signal for an additional set of experiments that made use of recombinant techniques. In 1999, Song *et al*.[42] had made the X ray data available that were used for the calculation, at 2.05 Å resolution, of a ribbon plot of full-size EF-Tu. The protein exhibits a three-domain structure (Figure 6a). By visual inspection of the plot and subsequent computer modelling it turned out that the exposed loop of domain 3 fits well into the cavity of domain 2 of a neighboring full-size EF-Tu[40]. Such a fit, extended to many full-size EF-Tu molecules, could lead to a filament (Figure 6b). This notion had never before been described, and it could be tested. Specifically designed truncated EF-Tu, consisting solely of domain 3 of the protein (see Figure 6c), was

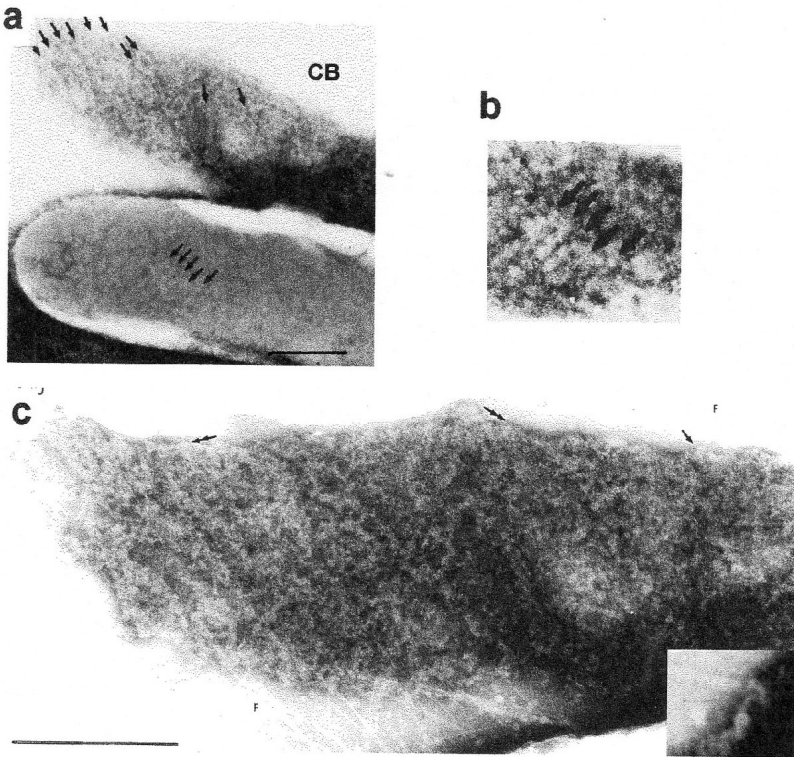

Fig. 6. *Induced lysis of Escherichia coli cells.* **a**, *At an early state of lysis induced by induction of truncated EF-Tu as shown in Fig. 5c (see also text for further explanations), E. coli cells could be observed in various states of lysis. The cell at the bottom of the figure was still surrounded by an electron translucent cell envelope. Rows of particles with sizes comparable to ribosomes (polysomes; group of arrows) were seen at various sites of the cell; the orientation of the row shown here, and of other rows not depicted, was at an angle relative to the long axis of the cell. The cell at the top of the figure was devoid of the wall and the membrane (see also Fig. 6c). It was interpreted to represent a later state of induced lysis. The groups of arrows point to band-like structures also running at an angle relative to the cell axis. It was deduced that such an orientation indicates that these structures are, in fact, parts of bands helically wound around the cell body. This helical organization was visible over the entire surface of membrane-less cell bodies (CB). Magnification bar: 0.5 µm. From ref. 43 with permission.* **b**, *Group of polysomes (group of arrows) depicted in Fig. 6a, cell at the bottom, shown at higher magnification. The composition of the polysomes out of individual ribosomes can be seen.* **c**, *The cell body shown in Fig. 6a (bottom), depicted at higher magnification. The double-arrows point to sites along the periphery of the cell body where the absence of wall and cytoplasmic membrane is evident, the outermost layer of the cell body is a lining visible as the projection of fine fibrils. At this magnification, more details on the ultrastructure of the fibrils making up the helically organized fibrillar network can be detected.*

expressed and harvested from respective recombinant *Escherichia coli* cells. Full-size EF-Tu was used to repeat the *in vitro* process of filament formation of monomeric EF-Tu as described earlier (Figure 6d, lower part). When full-size EF-Tu and truncated EF-Tu were mixed, filament formation was inhibited (Figure 6e) by a simple competition mechanism as illustrated in Figure 6c.

Elongation of the filament was no longer possible because the truncated EF-Tu bound to a full-size EF-Tu as depicted was devoid of the necessary binding site located on domain 2. A crucial experiment was to obtain this situation *in vivo*[40]. For this series of experiments, two *Escherichia coli* strains were used: one was the wildtype strain, harbouring the gene set that codes for full-size EF-Tu. The other strain was the one which had been used for the production of the truncated EF-Tu. This second strain was designed in such a way that initiation of synthesis of the truncated EF-Tu could be achieved by induction at a state of growth of the bacterial culture when the cells were in the logarithmic phase. Up to this moment, only full-size EF-Tu was present in the cell. However, upon induction of expression of the truncated EF-Tu, the resulting truncated EF-Tu molecules now present in the cell in addition to full-size EF-Tu were expected to compete, similar to the *in vitro* situation, for binding sites in the filaments. It could be observed that cells treated this way, after a short delay, started to exhibit signs of lysis (Figure 6). Within one generation time, most of the cells in the culture had lost their wall and their cytoplasmic membrane (Figure 6a and c), whereas many of them had still maintained their elongated form or were in an early state of decomposition. In a systematic electron microscopic analysis of bacterial samples withdrawn from the cell culture at short intervals it could be shown that, after a short delay, the ratio of walled cells was more and more diminished after induction. A close inspection of Figures 6a and 6c revealed that, after loss of the cytoplasmic membrane, the exposed surface of the remaining cell body did exhibit a lining (marked by double-arrows), and the entire cell body did show a helical organization (Figure 6a, group of arrows). Several hours later, these cell bodies had also lost their rod

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Numerous fimbriae are attached to the lower side of the cell body. F marks a site where such a fimbria remained inserted though the cytoplasmic membrane was absent. The inset depicts this site at higher magnification. The fimbria is, in fact, inserted into the lining. An insertion complex is seen in the plane of the lining. Magnification bar: 0.5 μm. From ref. 43 with permission.

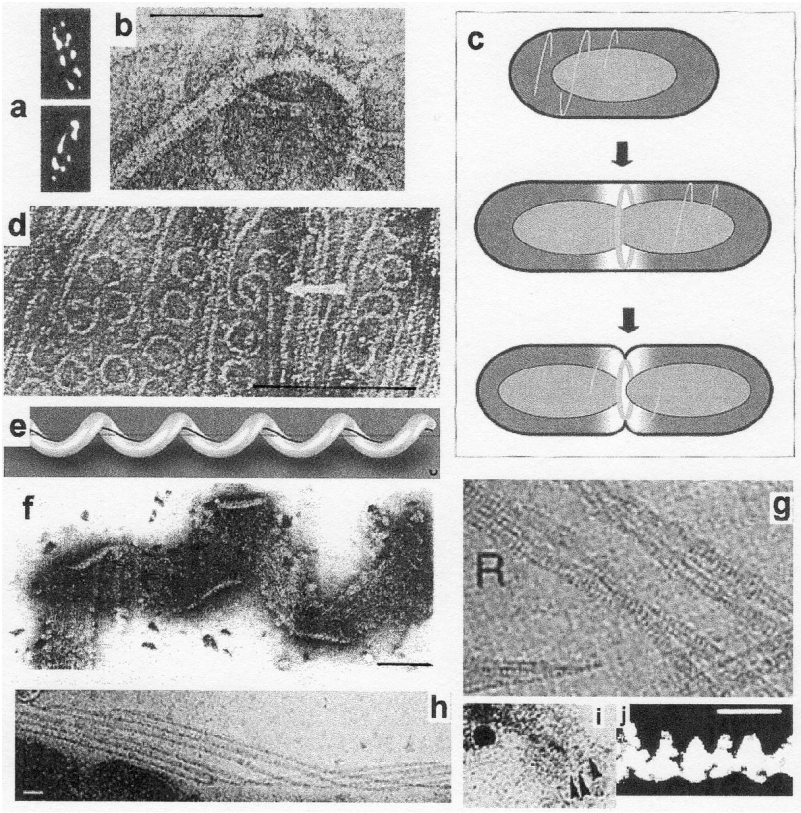

Fig. 7. *Cytoskeletal elements and cytoskeletons in bacteria.* **a**, *Subcellular localization of protein Mbl in Bacillus cells, depicted by immuno fluorescence. The helical organisation is evident. From ref. 19 with permission.* **b**, *Electron micrograph of isolated MreB filaments. Single and double filaments can be seen. Magnification bar: 100 nm. From ref. 35 with permission.* **c**, *Spatial and temporal control of Z-ring assembly. Recent observations of FtsZ-GTP indicate that dynamic helices form at the membrane throughout the cell. Some of these helices coalesce at midcell where the negative effects of the Min and Noc system are lowest. Further explanations, main text. From ref. 31 with permission.* **d**, *Protofilaments, small sheets, and minirings formed by isolated FtsZ. The arrow marks a protofilament that has separated from a sheet, remaining straight for some length, and then changing abruptly to the curved confirmation. Magnification bar: 0.2 µm. From ref. 55 with permission.* **e**, *A scaled, three-dimensional model of an average Spiroplasma cell. The cytoskeletal ribbon is depicted by the black line following the shortest helical line on the tube's surface. The axis about which the tube is coiled is depicted in grey. From ref. 4 with permission.* **f**, *Ribbon-like cytoskeleton obtained by ongrid lysis and detergent extraction of whole Spiroplasma cells. Magnification bar: 0.1 µm. From ref. 56 with permission.* **g**, *Isolated cytoskeletal elements from Spiroplasma embedded in vitrified buffer. Electron micrograph. Note the longitudinal and axial periodic repeats*

shape, and they were completely lysed and disintegrated. These observations, taken together with the results of the *in vitro* studies described above, are strong support for the notion that EF-Tu filaments are parts of an intracellular cytoskeleton supporting cell shape preservation, and that induction of truncated EF-Tu weakens this cytoskeleton. Structural similarities of cell bodies as that depicted in Figure 6c, with membrane-less *Mycoplasma* cells (see Figure 3b) are evident. Two additional remarkable features could be observed: fimbriae (marked F in Figure 6c), said to be inserted into the cytoplasmic membrane, were still at their place, obviously inserted in the lining. More interesting was that rows of ribosomes (polysomes), identified by their size and shape (Figures 6a and 6b), could be seen to be aligned along the helical substructures[43]. This was surprising; after all, for bacteria it was not known that ribosomes are attached to fibrillar cell components. Provided these helical fibrillar structures have the cellÇs EF-Tu as structural components, this would mean that filament-bound active ribosomes and EF-Tu (responsible for translation) are immediately complexed.

EF-Tu carries a GTPase activity; in this respect, it is similar to FtsZ and tubulin. For FtsZ it was measured that a rapid exchange of subunits of a given protofilament occurs, even when no growth takes place[31] (see above, chapter on Bacterial FtsZ protein). A similar exchange of units in an EF-Tu-based protofilament would have as a consequence – in the presence of free truncated EF-Tu – that the initially intact EF-Tu chains are more and more disturbed, loosing their stability within a relatively short period of time. Provided this cytoskeleton is of major importance for cell stability, one would expect a final cell collapse and loss of viability. In the experiments described above, this was the case. It may be mentioned that the experimental findings supporting the notion of a possible additional role of bacterial EF-Tu led to applications

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and the straight state of the relaxed ribbons (R). From ref. 4 with permission. **h**, *Isolated and purified cytoplasmic filaments of Treponema. A band of six filaments shows a twist similar to that seen in vivo (not shown). Magnification bar: 30 nm. From ref. 29 with permission.* **i**, *Group of cytoskeletal filaments (group of arrows) obtained by ongrid lysis of Thermoanaerobacterium sp. cells (see Fig. 2b). The black dot is the projection of a 14-nm colloidal gold particle. From[10] with permission.* **j**, *Higher magnification of one of the filaments depicted in Fig. 7i. Note the triangular shape of the polymerized individual proteins. Magnification bar: 10 nm. From ref. 24 with permission.*

in biotechnologs[40]. A structural role of EF-Tu as described may also be an explanation for bleb formation in *Thermoanaerobacterium* sp. depicted in Figure 2a. The blebs were found to be created from a surplus of cytoplasmic membrane carrying a high amount of starch-degrading enzyme. The blebs were continually transformed into vesicles that were set free. Both blebs and vesicles did not contain cytoskeletal elements. Hence, the cell proper did remain stable[44] during this phase of intense enzyme production.

A comparison of the bacterial and archaeal sequences and those in chloroplasts, coding for EF-Tu or functionally related proteins, reveals that the EF-Tu sequence is very much conserved[40].

With these data in mind, one could include the possible existence of an EF-Tu-based cytoskeleton in discussions on the evolution of the cytoskeleton[35,45–48]. One could speculate that an EF-Tu-based cytoskeletal web, enclosing the entire cell body and exhibiting a helical organization, might have been created by nature in a very early state of the evolution. A function of EF-Tu in cellular protein synthesis might also have been a very basic property. In principle, EF-Tu might first have been just a cell stabilizing protein due to its ability, aquired early in evolution, to form protofilaments; the additional function, *i.e.* that as an elongation factor, might have evolved later. However, the sequence of evolutionary steps could very well have been the other way around. An additional speculation could imply that the helices formed by other cytoskeletal proteins (see above, chapter on bacterial actin homologues) take the basic helical organization of the postulated EF-Tu-based cytoskeletal web as a template and scaffold.

Conclusions and future prospects

Within the last few years basic discoveries in the field of bacterial cytoskeletal elements and cytoskeletons have been made[49,50]. Present and future work will have to concentrate on a search for even more cytoskeletal elements in bacteria, and their properties. After all, cytoskeletons have already turned out to have decisive functions in aspects such as cell shape control, cell division, chromosome segregations, motility. However, one of the most important problems to be solved, step by step, is the identification and detailed characterization of cellular components interacting with the cytoskeletons. None of the functionalities of the cytoskeletal structures is isolated and all of them require partnership of other cell components. The availability of a multitude of advanced investigation techniques will warrant major successes. Not only

basic research will be concerned. Also searches for new biotechnological applications will result. An example is the work on the proposed structural role of elongation factor Tu; the data obtained so far indicate that a new class of antibacterial drugs will be created, and that a new and very gentle mode of cell lysis can be designed for bacteria used for the synthesis of recombinant products[40]. In general, the new understanding from protein molecule to system organization will facilitate the design of small molecules that inhibit protein complex function for cell biology study and drug discovery[51,52].

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