Dynamic proteins and a cytoskeleton in bacteria

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The application of modern fluorescence microscopic methods to bacteria has revolutionized our view of their subcellular organization. Many proteins are now known to be targeted with exquisite precision to specific locations in the cell, or to undergo rapid directed changes in localization. Structural and functional homologues of tubulin (FtsZ) and actin (MreB) are now indisputably present in bacteria, overturning the textbook view that the cytoskeleton is unique to eukaryotes. These advances are stimulating a radical rethink about how various fundamental processes are organised in bacteria.

In the last ten years or so, our understanding of the subcellular organization of bacterial cells has undergone a revolution through the increasing application of cell biological methods, principally fluorescence microscopy[1]. Previous studies, based mainly on transmission electron microscopy (TEM), had given rise to the belief that bacterial cells have a simple architecture, distinct in various fundamental ways from that of eukaryotes. In particular, the general absence of intracellular membrane-bound organelles (including the nucleus), the absence of a cytoskeleton and the presence of a prominent external peptidoglycan cell wall. Recently, our view of several fundamental aspects of bacterial organization has needed to be revised. This commentary summarises current thinking in relation to three aspects of bacterial cytosol organization that I think have undergone particularly marked changes. First, the specialized localization of major macromolecular synthetic machineries. Second, the existence and function of various cell cycle proteins that undergo rapid and directed spatial relocation. Finally, the discovery that bacterial cells contain not only a tubulin homologue (FtsZ), but also actin (MreB), another key cytoskeletal protein previously thought to be restricted to eukaryotes.

Localized transcription, translation and DNA replication

Central to the expression of genetic material in all cells are the processes of transcription and translation. The clear absence of a nuclear membrane, together with the evidence for close coupling of transcription and translation (for example, transcriptional attenuation mechanisms, in which translating ribosomes regulate the progression of RNA polymerase[2]), suggested that transcription and translation were intimately interconnected in bacteria, in contrast to their striking separation into distinct compartments in eukaryotes. This view was overturned by simple experiments with green fluorescent protein (GFP) fusions to RNA polymerase or ribosomal subunits, which showed a clear separation of these activities in live bacteria[3] (Fig. 1a, b). As in eukaryotes, the central part of the cell turned out to be highly enriched in RNA polymerase and therefore the most active part of the cell in terms of transcription. In contrast, the peripheral zone of the cell turned out to be highly enriched in ribosomal proteins, presumably engaged in protein synthesis (Fig. 1b). Recent labelling studies have confirmed the existence of separate zones of activity, with cold-shock proteins and other ribosomal proteins also being localized in the peripheral zone[4,5].

Although the existence of regulation by transcriptional attenuation highlights the need for a close interaction between the transcriptional and translational machineries, the current view of bacterial cell organization does not preclude such mechanisms, because coupling between transcription and translation could still occur at the interface between the inner and peripheral zones. Intriguingly, very recent results with eukaryotic cells have shown that a low level of translation can be detected within the nucleus[6], reinforcing the possibility of fundamental similarities between the two groups of organisms, despite their different membrane architectures.

In the last decade or so, the notion of fixed sites for DNA replication, so-called 'replication factories', has emerged in studies of eukaryotic cells[7]. This notion received a considerable boost when it was discovered that several different DNA replication proteins were localized in a single focus located at about the mid-cell in actively growing *Bacillus subtilis* cells[8] (Fig. 1c). These observations suggest not only that replication proteins are targeted to specific sites in the cell, but also that a single site is responsible for replication of both clockwise and anticlockwise forks on the circular chromosome. This model could have important consequences, because it suggests that during replication, chromosomal DNA is fed through the fixed replication complex and that the daughter replicated duplexes are extruded[8,9]. If the daughter duplexes were channelled towards opposite cell poles, this could form the basis for a simple mechanism of chromosome segregation[9,10]. However, as discussed below, there are probably additional mechanisms involving dynamic proteins.

Dynamic movement of cell cycle proteins

In eukaryotic cells, chromosome segregation is affected by motor proteins that actively move chromosomes via attachment sites (centromeres) on the microtubules of the mitotic spindle. The clear absence of the spindle in bacteria had long supported the idea that these cells use a fundamentally different segregation mechanism from that of eukaryotes. A widely prevailing model held that the *oriC* regions of sister chromosomes are gradually separated by attachment to sites on either side of the cell envelope growth zone[11]. The first application of time-lapse imaging of protein localization in bacterial cells was with Spo0J, a protein that turned out to be associated with replication origin (*oriC*) regions of the chromosome[12] (Fig. 1d). These experiments revealed that newly replicated sister *oriC* regions move apart abruptly. Many other experiments visualizing *oriC* regions in various ways (originally by the elegant use of LacI–GFP fusions bound to chromosomal *lacO* arrays[13]), all support the notion that origin regions are actively positioned in the cell and that they are separated actively and early in the round of DNA replication[9,14]. Similar experiments have been performed for various plasmids of *Escherichia coli* and a number of distinct active positioning and segregation mechanisms have been identified[14]. To date, however, the mechanisms responsible for origin and plasmid movement and positioning remain largely
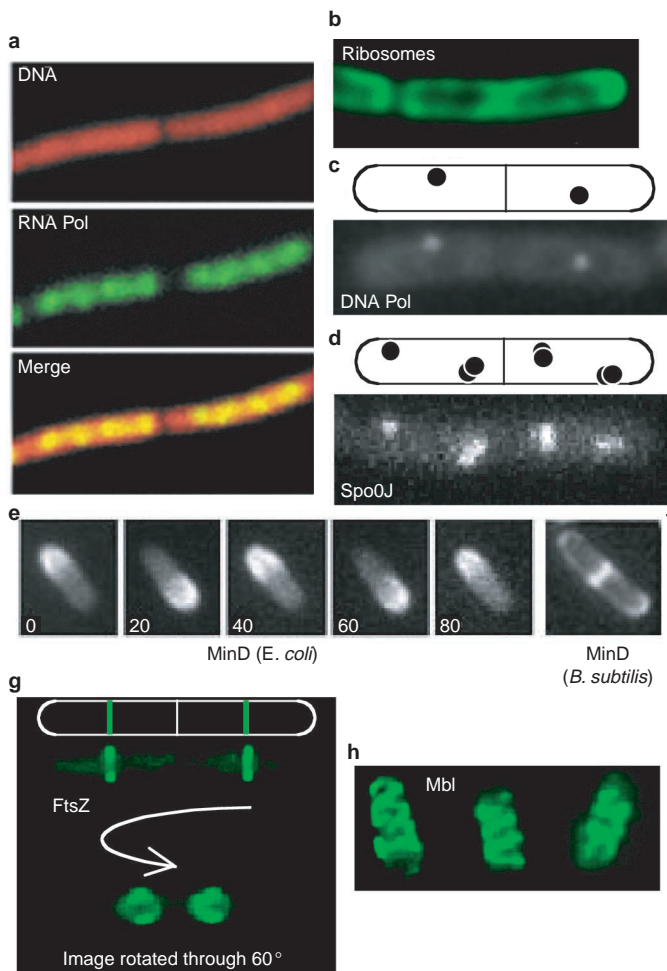
Figure 1 **Distinct cellular addresses of cell cycle and morphogenic proteins in bacteria.**
a, **Immunofluorescence microscopy images of *B. subtilis*, showing RNA polymerase labelled with GFP (green) and the nucleoid stained with DAPI (red). A merged image of the two signals is also shown. b, Ribosomes labelled with GFP. c, Localization of the DNA replication machinery (DnaE–GFP). d, Localization of the *oriC* regions of the chromosome (SpoOJ–GFP). Some of the foci have a bilobed appearance, probably caused by the onset of new rounds of DNA replication. e, Oscillation of GFP–MinD in *E. coli* (images are reproduced from ref. 15 © (1999) with permission from the National Academy of Sciences, USA). The elapsed time in seconds is shown. f, Static MinD localization at mid cell (band) and at both cell poles in *B. subtilis*[25]. g, A pair of cells with mid-cell FtsZ bands (GFP-labelled). A 3D reconstruction was obtained and the image was rotated through 60° to reveal the ring-like nature of the bands. h, Three short cells, showing helical filaments of the cell shape protein, Mbl (Mbl–GFP).**
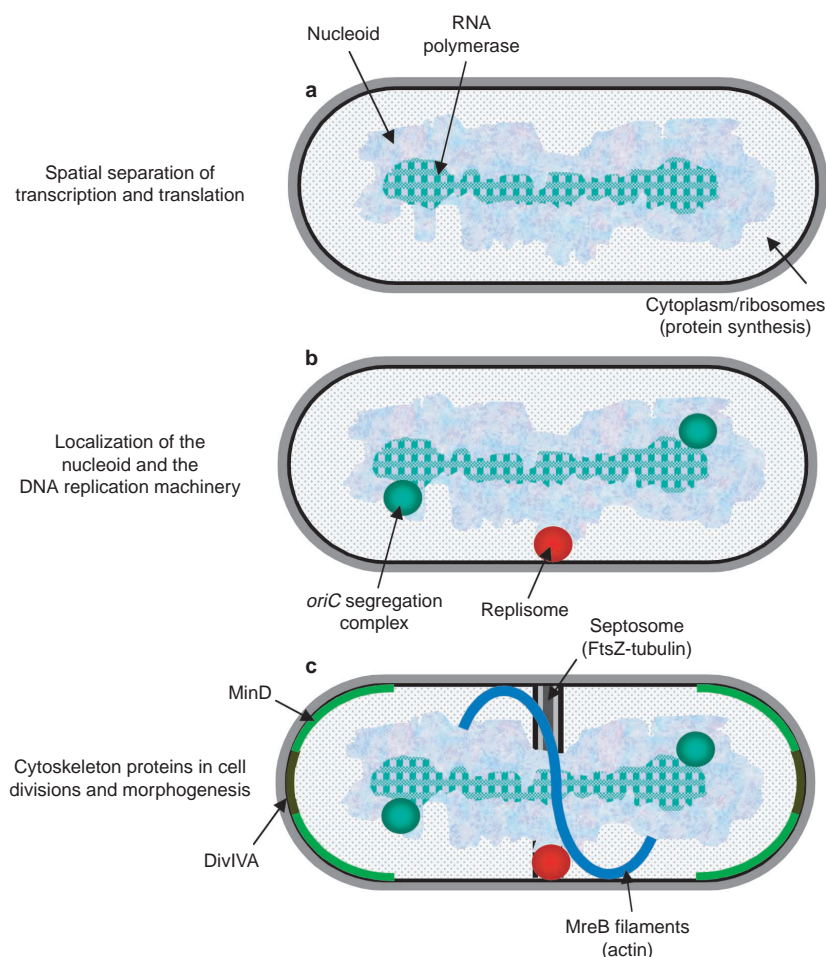
Several groups have developed mathematical models that can faithfully reproduce the oscillation and predict how effects such as mutations in key components will alter the behaviour of the system[22–24]. Bacterial cells may offer an exciting playground for mathematical modellers in the next few years, offering advantages of simplicity of components together with a detailed level of biological understanding.

Although the oscillating system clearly serves the purpose of specifying the division site in *E. coli*, it is unclear why such an apparently extravagant oscillating mechanism has evolved in this organism. Another bacterium, *B. subtilis*, seems to achieve the same result by the apparently simpler strategy of fixing MinCD to both cell poles in a static manner, through association with a polar anchor protein DivIVA[25] (Fig. 1f).

MinD is certainly not the only bacterial protein that is capable of rapid relocation in the cell. Again in *B. subtilis*, the Soj protein, which is distantly related to MinD but is involved in chromosome segregation and transcriptional regulation, also undergoes dynamic, co-operative movement, though on a longer time scale of many minutes[26,27]. In *Caulobacter crescentus*, several protein kinases and their substrates involved in regulation of the cell cycle undergo marked changes in localization at specific points in cell cycle progression. In this case, various proteins alternate between a dispersed cytoplasmic distribution and a targeted localization at either both poles or at a specific single cell pole[28].

Cytoskeletal proteins in bacteria

The discovery that the key player in bacterial cell division, FtsZ, forms a ring structure at the sites of incipient cell division (Fig. 1g) was one of the most influential events in the history of bacterial cell biology[29]. The field of bacterial cell division, in particular, was completely invigorated by evidence that a complex protein machine assembled at a particular sub-cellular location as part of this process. Systematic studies of the other eight or so proteins involved in bacterial cell division have subsequently revealed that they do indeed all assemble at the site of impending division, marked by the Z ring, and the hierarchy for this assembly has been elucidated[30,31]. The complex of division proteins then drives constriction of the various envelope layers of the cell, resulting in division and cell separation[31,32]. It had been known for several years that there was weak sequence similarity between FtsZ and eukaryotic tubulins. Furthermore, biochemical studies of FtsZ demonstrated tubulin-like, GTP-dependent polymerization *in vitro*[33,34]. The crystal structure of FtsZ was then solved, revealing that it had the same fold as tubulin and confirming that it is a bacterial homologue of tubulin[35].

obscure, and this is an important area for future research.

Other bacterial cell cycle proteins undergo even more marked movements: the *E. coli* MinD protein actually oscillates from cell pole to cell pole on a time scale of seconds[15] (Fig. 1e). The division machinery seems to have an intrinsic ability to assemble and act not just at the correct mid-cell sites, but also incorrectly near the cell poles[16]. The function of the Min system is to prevent incorrect assembly at the poles, which would produce aberrant anucleate 'minicells' (ref. 17). MinD

carries with it an inhibitor of division (MinC), which prevents assembly of the FtsZ ring at each pole in an alternating manner. Recent work has begun to reveal the biochemical basis for MinD oscillation. The protein seems to assemble into ordered structures, possibly polymers, at the cell membrane, in a reaction mediated by the binding and hydrolysis of ATP[18]. During oscillation, another mobile protein, MinE, forms a ring that sweeps across the cell, removing MinD from one pole and thereby driving its assembly at the opposite pole[19–21].

Figure 2 Some patterns of protein targeting in a typical bacterial cell (*B. subtilis*).
a, **Spatial separation of the nucleoid (chromosomal DNA) and RNA polymerase in the central core of the cell from ribosomes in the peripheral region.** b, **Organization of the chromosome, with replicated *oriC* regions oriented towards the cell poles, and the terminus (not shown) and replication machinery (replisome) localized near the mid cell.** c, **Locations of cytoskeletal elements involved in cell shape (the actin-like MreB protein) and the cell division proteins FtsZ, MinD and DivIVA.**

helical filaments that run around the circumference of the rod-shaped cells *in vivo*[39] (Fig. 1h). Again, the crystal structure of an MreB protein (from *Thermotoga maritma*) then provided final confirmation that MreB is homologous to actin[40]. Thus, bacteria possess homologues of both major players of the eukaryotic cytoskeleton — actin and tubulin — and these molecules must now be viewed as having arisen early during the course of evolution, rather than being recent innovations of the eukaryotic lineage.

Very recently, another bacterial actin homologue has been shown to be involved in segregation of plasmid R1. The ParM protein forms filamentous structures *in vivo* that are required for plasmid segregation. *In vitro*, the protein was shown to have very similar properties to that of actin[41] and again the protein has been shown to have structural congruence with actin[42]. This raises the interesting possibility that bacterial actins may have a range of functions in addition to determination of cell shape.

Perspective

To date, the discovery of dynamic protein movement in bacteria has been technically difficult because of limitations of resolution and sensitivity. It seems likely that many more examples of dynamic localization will emerge in the near future. The rather featureless electron microscopy images of bacterial cytoplasm that forged opinion about their functional organization for 40 years have been replaced by a rich new tapestry of diverse targeting patterns and dynamic movements. Figure 2 integrates knowledge of some of the better-characterized proteins in one organism (*B. subtilis*). However, many more proteins are being similarly studied in an increasingly wide range of organisms. Indeed, systematic studies of protein localization, taking advantage of complete genome sequences and powerful genetic systems, are now in progress. So far, the main impact of this new dimension of research has been focused on problems in the areas of cell cycle, cell morphogenesis and cell differentiation. There are hints that protein targeting, or at least the existence of preferred sub-cellular domains (for example, core versus peripheral domains), could be the norm for many, or even most, bacterial proteins. Furthermore, there are increasing examples of bacterial proteins that undergo dynamic changes in localization in accordance with their normal activity. The next few years are likely to see dramatic advances in our understanding of the biochemical basis for protein localization and dynamic behaviour, and the emergence of a more global and integrated view of bacterial sub-cellular organization. □

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Recently, the application of fluorescence recovery after photobleaching (FRAP) has led to a paradigm shift in our view of division machinery organization[36]. These experiments showed that the FtsZ ring undergoes continuous turnover with a half time of approximately 30 s. This has important implications, because it means that our view of Z ring 'assembly' and its associated factors is a much more dynamic process than previously envisaged.

In the last 18 months, compelling evidence has emerged for the existence of the other major player in the eukaryotic cytoskeleton, actin, in prokaryotes. Bacteria come in a wide range of shapes, of which the rods of *E. coli* and *B. subtilis* are well known. In most bacteria, the external cell wall of peptidoglycan is responsible for the shape of the cell[37]. Treatments that remove the wall result in a loss of cell shape, with the 'default' round shape being assumed if osmotic lysis of the protoplast is prevented. Furthermore, isolated cell wall sacculi retain the shape of the cells from which they are obtained. Most mutations affecting cell shape also turned out to be in genes associated with cell wall synthesis. However, one cell shape gene, *mreB*, independently identified several decades ago in both *E. coli* and *B. subtilis*, seemed to encode a soluble protein with no obvious role in wall synthesis. It was pointed out that MreB proteins possess key residues that are diagnostic of proteins belonging to an actin superfamily[38]. A functional equivalence to actin was established last year by the finding that two different *mreB* homologues in *B. subtilis* were not only involved in cell shape determination, but, more importantly, also formed

commentary

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