

# Ring, helix, sphere and cylinder: the basic geometry of prokaryotic cell division

## Workshop on Manufacturing Bacteria: Design, Production and Assembly of Cell Division Components

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This workshop took place at the Juan March Institute, Madrid, Spain, between 16 and 18 December 2002 and was organized by Miguel Vicente, Piet de Boer and Jeff Errington. It was the 146th in the series of International Biology meetings of the Juan March Institute for Study and Research; it marked the retirement of Andrés González, who has, until now, been responsible for their technical organization. In the name of all the scientific organizers of these meetings, we express our gratitude to him for facilitating our task to the point of making it a pleasure, and we dedicate this report to him.

modified for eukaryotic roles less related to those that they performed in their prokaryotic ancestors. Molecular microbiology has been transformed during the past ten years by the availability of high-powered microscopes, green fluorescent protein (GFP)-fusion proteins, and immunostaining procedures that allow proteins to be localized within bacteria. The result of this revolution is the finding that many proteins localize to defined regions or oscillate between two locations. Fluorescence labelling has also shown that bacterial DNA is segregated in a rapid and ordered fashion, very much as it is during mitosis in eukaryotes. We are now faced with the task of determining how the rapid localization of these cell constituents is orchestrated—a problem that traditionally has been associated with eukaryotic cell biology. This workshop gathered together 18 speakers and 32 participants to discuss recent progress in the description of the elements and processes that effect and coordinate cytokinesis and DNA segregation (karyokinesis) in prokaryotic cells and in some eukaryotic organelles.

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### Introduction

Bacteria have sophisticated molecular machineries dedicated to regulating their growth and division with superb accuracy. Although many components of the prokaryotic division and DNA segregation apparatus are conserved, some of them have distinct functions in different bacteria. Unsurprisingly, some elements of bacterial division have been incorporated into the division machinery of eukaryotic organelles, whereas others have been

### Cell growth: how a cylinder emerges from a helix

Before septation, the walls of rod-shaped bacteria grow by elongation. J.M. Ghuysen (Liège, Belgium) described some of the bacterial enzymes that synthesize peptidoglycan, the macromolecule that confers rigidity to this structure. These enzymes are penicillin-binding proteins (PBPs) and belong to the SxxK superfamily of serine proteases. Class A SxxK peptidases act independently, whereas class B associate with either glycosyl transferases or acyl transferases. This latter class also associates with other morphogenetic proteins (for example, RodA) to form part of an enzymatic complex. Depending on the specific composition of the complex, different cell-wall structures are produced through various cross linkages of peptidoglycan; for example, the 3-3 peptidoglycan synthesis mode of *Mycobacteria* allows them to grow in the presence of penicillin.

The growth of the *Escherichia coli* cell wall takes place at spatially defined areas along its length. M. de Pedro (Madrid, Spain) described how, once they are formed during division, the cell poles become fixed structures with no peptidoglycan turnover. Moreover, even the outer-membrane proteins at the poles remain fixed to the peptidoglycan cap. He raised the provoking proposition that the

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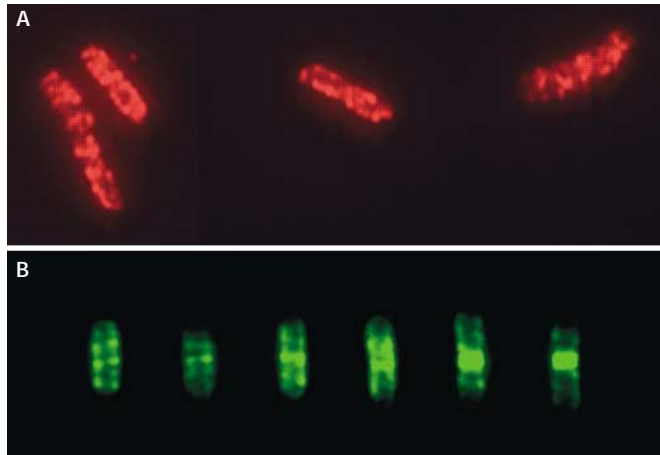
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**Fig. 1 | The helix. (A)** Helix of MreB that has been revealed by immunostaining with a specific antibody in *Escherichia coli* cells. (Figure provided by M.V.) **(B)** Time course of the helical and ring structures in the peptidoglycan of *Bacillus subtilis* as revealed by staining the newly synthesized peptidoglycan with fluorescently labelled vancomycin (presented at the workshop by R. Daniel).

polar areas of the outer membrane are differentiated domains that might have a morphogenetic role by acting as a template for the synthesis of the cylindrical wall.

Recent evidence indicates that some of the proteins involved in cell elongation and rod-shape maintenance are found as helical structures along the length of the cell. T. den Blaauwen (Amsterdam, The Netherlands) has studied the intracellular localization of MreB, a protein closely related to eukaryotic actin that has been associated with the maintenance of rod shape in both *E. coli* and *Bacillus subtilis* (Jones *et al.*, 2001; van den Ent *et al.*, 2001, 2002). In *E. coli*, MreB usually forms a longitudinal helix throughout the cytosol (Fig. 1A), but it also localizes at the mid-cell at the time of division. PBP2, the penicillin-binding protein that is responsible for most of the peptidoglycan synthesis during elongation and septation, localizes in the lateral wall and at the septum, but not at the old cell poles (den Blaauwen *et al.*, 2003). Although PBP2 does not form helical structures itself, den Blaauwen speculated that this protein associates with the MreB helix.

Direct evidence for a helical pattern of peptidoglycan synthesis has been provided by the study of *B. subtilis*, a Gram-positive bacteria that is larger and has a thicker wall than the Gram-negative *E. coli*. R. Daniel (Oxford, UK) took advantage of the fact that only newly synthesized peptidoglycan contains D-alanyl-D-alanine to visualize the areas in which the new peptidoglycan is located. Labelled vancomycin, which binds to D-alanyl-D-alanine, stains both a helical region along the cell length and a central ring in the cell centre (Fig. 1B). The helix is present even in MreB-defective strains but not if Mbl—another MreB-like protein related to actin and associated with the maintenance of the rod shape (Jones *et al.*, 2001)—is absent.

### Segregation of nucleoids defines a DNA-free space

The replicon theory proposed by François Jacob stated that replicated nucleoids segregate to the two halves of the cell by anchoring to the cell wall, whereby they are passively moved apart as the cell elongates (Jacob *et al.*, 1963). However, proteins

such as MukB have been found to have a role in nucleoid segregation (Hiraga *et al.*, 1998). Moreover, plasmids, and even nucleoids, have been found to segregate rapidly and independently of cell growth. Examples of these contrasting, but not necessarily mutually exclusive, views of nucleoid segregation were discussed at the workshop. C. Woldringh (Amsterdam, The Netherlands) challenged the idea that segregation is an active mechanism. His thesis is that Brownian movement of supercoiled chromosome segments is constrained by transection—the co-transcription, translation and insertion of the membrane proteins into the membrane. A spatial bias would be generated as the replication of the chromosome proceeds, because of the transient nature of the constraints, the competition for membrane space and the cooperativity between neighbouring genes on the same strand. This bias would provide bidirectionality to the movement of replicated daughter strands, whereas Brownian diffusion would provide the driving force for the gradual movement of the bulk of non-transcribed DNA. By contrast, J. Møller-Jensen (Odense, Denmark) described a mitosis-like mode of segregation in the partitioning of the *E. coli* R1 plasmid. This involves ParM, a protein that forms filaments along the length of the cell. It is structurally related to actin and forms double helical filaments that are similar to F-actin *in vitro*.

The packaging of a full chromosome within *B. subtilis* spores also requires an active segregation mechanism. J. Errington (Oxford, UK) described this complex machinery, which contains translocators as well as anchoring elements. During sporulation, a region of the *B. subtilis* chromosome that spans the replication origin becomes attached to the cell pole by an anchoring protein complex containing Spo0J, RacA (equivalent to YwkC; Ben Yehuda *et al.*, 2003) and DivIVA. DivIVA is a protein that is targeted to the cell centre and to both poles. Besides its role in determining the cell centre (Table 1), it is needed to specifically anchor the *oriC* region during sporulation. Another element of the anchoring complex, Soj, is a protein that jumps from one nucleoid to the other and counteracts the binding of Spo0J to DNA outside the replication origin. RacA and Soj seem to be redundant, and a strain defective in RacA, Soj and Spo0J shows a massive failure in sporulation. The anchoring complex is thought to localize a 1-Mb region of the chromosome containing *oriC* to the sporulating pole, and SpoIIIE, a DNA translocase, is required at a later stage to transfer the rest of the chromosome to the prespore.

### Exploring the middle

The placement and completion of a septum in the middle of the cell are the final events of bacterial cell division and in fast-growing cells, these processes occupy most of the life of a bacterium (Donachie *et al.*, 1995). Septation does not proceed normally in cells in which segregation is altered, which indicates that a DNA-free space is required to position a septum (Yu & Margolin, 1999). In addition, a positive placement mechanism is required to correctly localize the septum. *E. coli* contains a system that is able to find the cell centre in the absence of any other regulator. Hence, it is self-contained and there is no need for an endless regulatory chain. The system involves an oscillating mechanism, similar to the pattern formation mechanisms that operate to produce snail shell decorations (Meinhardt, 2003). In *E. coli*, this mechanism involves four proteins: MinC is a division inhibitor activated by MinD, and together these proteins prevent the assembly of the

**Table 1** | Biology of the homologue proteins MinE and DivIVA

Organism	Protein	Localization	Activity	Function
<i>Escherichia coli</i>	MinE	Surrounding the cell centre	Follows the track of and dislodges the membrane-bound MinD	Blocks division at the poles. Maintains the cell centre free from MinCD
<i>Bacillus subtilis</i>	DivIVA	Poles and cell centre	Recruits MinD into the septum as a late event. Traps <i>oriC</i> during sporulation	Blocks division at the poles. Maintains the cell centre free from MinCD
<i>Streptomyces coelicolor</i>	DivIVA	Hyphal tip	Unknown	Involved in polar growth

FtsZ complex, which is required for septation; MinC and MinD are relentlessly pushed away from one cell pole to the other by MinE, and in doing so, a central zone that coincides with the nucleoid-free region is liberated from the inhibitor; FtsZ assembles in this zone and forms a Z ring that nucleates the remaining components of the septum (Meinhardt & de Boer, 2001).

MinD causes MinC to associate with the cell membrane, whereas MinE mediates its subsequent release. These interactions have been reproduced *in vitro* using phospholipid vesicles by P. de Boer (Cleveland, OH, USA). Membrane-bound MinC is cleared from the membrane by MinE before the hydrolysis of the nucleotide in the MinC–MinD–ATP–membrane complex. J. Lutkenhaus (Kansas City, KA, USA) described how the *in vitro* association of MinD with phospholipid vesicles converts them into tubules. Results similar to those discussed by de Boer and Lutkenhaus were also reported by D. RayChaudhuri (Boston, MA, USA).

### Geometry in non-cylindrical bacteria

Septum positioning and its subsequent constriction are a paradigm for the conservation or the reassignment of tasks of phylogenetically related proteins in different organisms. In rod-shaped bacteria such as *E. coli* and *B. subtilis*, elongation (involving MreB and specific PBPs) and septation (mainly orchestrated by the MinCDE system and FtsZ) are the two most important morphogenetic events. Variations of this theme are seen in bacteria that deviate from the bacillar (rod) shape (Table 1).

Cells of round *E. coli* mutants (*rodA*) have been used by J.A. Dillon (Ottawa, Canada) to show that the MinD and MinE proteins from the normally round cells of *Neisseria gonorrhoeae* also oscillate. Due to the nucleoid positioning in *N. gonorrhoeae*, the oscillation of the MinCD inhibitor occurs in alternating perpendicular planes so that cell division in *Neisseria* yields tetrads (Ramírez-Arcos *et al.*, 2002).

*Streptomyces coelicolor* is an actinomycete bacterium that grows vegetatively as a branched mycelium in which septa are sparingly positioned. Mycelial expansion occurs by the duplication of tips, which are the areas of active growth. K. Flårdh (Uppsala, Sweden) has found that DivIVA (the *Bacillus* equivalent of MinE) localizes to the *S. coelicolor* hyphal tips and that its activity is essential. DivIVA is likely to be involved in the apical growth of the hyphae because their tips become swollen when it is overproduced, or fail to extend properly and branch excessively when it is depleted.

Although the formation of the vegetative septa requires the presence of FtsZ, the mycelium of *S. coelicolor* can grow sparsely in its absence. Eventually, mycelial crowding is accompanied by the production of aerial hyphae, in which nucleoid segregation is followed by synchronous septation to generate spores. K. Chater (Norwich, UK) has found that, in addition to FtsZ being required for septation during *S. coelicolor* sporulation, its accumulation is

also a developmental checkpoint to upregulate earlier sporulation genes (*parAB*) that are needed for nucleoid partitioning.

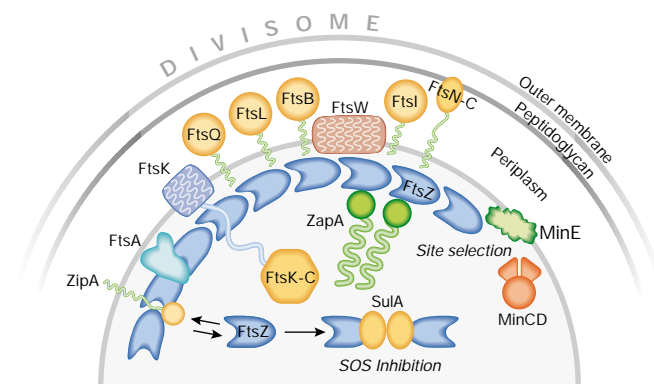
Y. Brun (Bloomington, IN, USA) described an unexpected effect of cell division: it regulates differentiation in *Caulobacter crescentus*, a bacterium in which non-dividing swarmer cells differentiate into stalked cells that become fixed to a support. The stalked cell then grows, divides and differentiates to produce a flagellated swarmer at the pole opposite to the stalk. Differentiation of the flagellar pole is dependent on the processing of PodJ, a protein that regulates polar organelle development and that is required initially for the pole to develop pili and subsequently as an adhesive holdfast. Therefore, PodJ is required for the differentiation of the daughter swarmer cell into a stalked cell. If cell division is prevented, the stalked cell grows but PodJ processing is inhibited and the differentiation programme is arrested (Hinz *et al.*, 2003).

### Congregating at the ring

The first visible evidence of septation is the formation of a cytokinetic ring at the mid-cell containing FtsZ. This protein is relatively abundant; estimates vary from a minimum of 4,000 molecules per cell, up to a maximum of 15,000 (Rueda *et al.*, 2003; Lu *et al.*, 1998). A host of other proteins then follow suit and assemble into the ring (Fig. 2). Although the division ring has been revealed by immunostaining, no ultrastructural information is available at present. Consequently, a great deal of effort has been devoted to the elucidation of the molecular intricacies of the *in vitro* assembly of the FtsZ complex.

FtsZ is a structural homologue of eukaryotic tubulin, and both of these proteins are GTPases that assemble into filaments. However, differences between the two are also apparent, as they are each adapted to functioning in the different environments of bacteria and eukaryotic cells, respectively. J.M. Andreu (Madrid, Spain) has found that FtsZ obtained either from *E. coli* or *Methanococcus jannaschii*, in stark contrast to tubulin, is able to refold spontaneously, independently of chaperones (Andreu *et al.*, 2002). In addition, FtsZ from *M. jannaschii* cooperatively assembles to form double filaments. A possible mechanism to effect the transition from a straight to a curved protofilament arrangement has been suggested by a molecular dynamics simulation, which showed that the presence of GDP in the *M. jannaschii* FtsZ binding site causes the T3 loop to push on helix H8 of the adjacent monomer (Díaz *et al.*, 2001).

Microcalorimetric measurements presented by H. Erickson (Durham, NC, USA) suggest that there is a cooperative mechanism for *E. coli* FtsZ assembly in the presence of GTP. However, previous results from this group had indicated that the assembly of *E. coli* FtsZ yields filaments that are only one molecule thick (Romberg *et al.*, 2001), which is more compatible with an isodesmic, rather than a cooperative, process. Results obtained



**Fig. 2** | Schematic representation of a transverse section of a dividing *Escherichia coli* cell. The proteins that assemble at the division site, which are believed to form a structure called the divisome, are shown. Some events that may modulate the assembly of the FtsZ ring, namely septum-site selection and SOS-induced division inhibition, have been labelled. (Figure presented at the workshop by J.L.)

using ‘cap’ mutants (in which the top or bottom surfaces of the molecule are modified) indicate that the assembly of FtsZ into the monofilaments might be directional. Assembly of FtsZ into the division ring is a rapid process, GFP–FtsZ fusion protein turnover rates as short as 7 s have been measured using fluorescence recovery after photobleaching (FRAP).

Division does not take place under conditions in which the chromosome of the *E. coli* cell is damaged. Induction of the subsequent SOS response triggers the synthesis of SulA, a division inhibitor (Bi & Lutkenhaus, 1993; Huang *et al.*, 1996; Higashitani *et al.*, 1997). J. Löwe (Cambridge, UK) proposed a model in which a SulA dimer poisons the Z ring, causing it to disassemble due to the high turnover rate of the FtsZ polymer *in vivo*. His crystal structure shows that SulA dimers block the T7 loop side of the protofilament interface of FtsZ.

The division ring of *E. coli* is nucleated initially by the assembly of FtsZ, and then the rest of the ring proteins are incorporated sequentially: FtsA and ZipA, FtsK, FtsQ, FtsB and FtsL, FtsW, FtsI, and finally FtsN. N. Buddelmeijer (Boston, MA, USA) presented evidence showing that FtsL and FtsB (equivalent to YgbQ) form a complex in the division ring and that their localization occurs simultaneously. FtsL is able to dimerize through its coiled-coil leucine zipper, which spans a region from the cytoplasmic membrane to the peptidoglycan. Löwe has found a similar spatial localization for FtsN, and the nuclear-magnetic-resonance-derived structure of this protein shows a large unstructured linker region that is compatible with its proposed role in connecting the inner membrane and the peptidoglycan.

The temporal sequence in which the FtsZ, FtsA and ZipA proteins assemble into the central ring has been analysed by J. Mingorance (Madrid, Spain) using age-selected synchronous cultures. He found that the concentration of the three proteins is constant at all ages. Although localization studies show that the recruitment of FtsA and ZipA depends on the presence of FtsZ, the results could not distinguish the ages at which the three proteins assemble into a ring. A recent publication reports that a point mutation in the S13  $\beta$ -strand of FtsA can compensate for the loss of ZipA

function, which suggests that the roles of both of these proteins in *E. coli* may be partially redundant (Geissler *et al.*, 2003). The study of a complete deletion of the S12–S13  $\beta$ -strands, presented at the meeting by A.I. Rico (Madrid, Spain), indicates that the mutated protein provokes the abnormal placement of the Z ring.

AmiC is a periplasmic amidase that participates in the separation of the daughter cells by cleaving some of the crosslinks in the septal peptidoglycan. T. Bernhardt (Cleveland, OH, USA) has found that AmiC forms a periplasmic division ring only after FtsN, the last element that assembles into the division ring in the cytoplasmic and inner membrane, has been recruited.

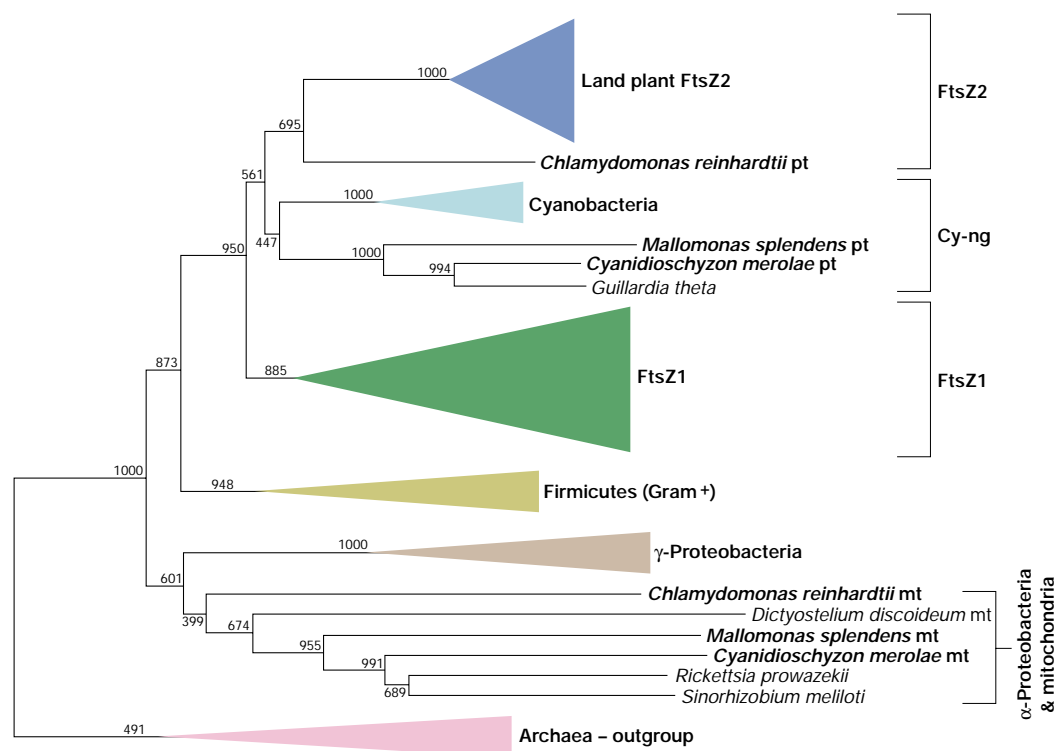
### The phylogeny of the ring

In *E. coli* and other bacilli, many of the genes that encode proteins that are responsible for septation, as well as most of those that code for the enzymes that synthesize the peptidoglycan precursors in the cytoplasm and translocate them to the periplasm, are grouped in the *dcw* cluster (Ayala *et al.*, 1994). The phylogeny of the *dcw* cluster as described by M. Vicente (Madrid, Spain) is a feature that unifies the rod-shaped bacteria independently of their Gram-staining properties (Tamames *et al.*, 2001). The cylindrical rod shape, in which cell growth occurs by elongation of the wall, is advantageous as it maintains a constant surface-to-volume ratio. The hypothetical ancestral bacterium would have maintained its rod shape by means of morphogenes such as *rodA*, which map outside the cluster and encode proteins specifically dedicated to cell elongation. In this situation, the *dcw* clustering would facilitate the supply of adequate quantities of scarce precursors to the septation machinery. *N. gonorrhoeae*, morphologically classified as a coccus, is a notable exception to the proposed phylogeny, as it has a prominent *dcw* cluster. However, in contrast to *E. coli*, in which there is no transcriptional terminator along the cluster, the *dcw* cluster of *Neisseria* is functionally disintegrated and transcribed as five separate units (Francis *et al.*, 2000).

*Chlamydiae* are obligate intracellular parasites that do not contain FtsZ. Do *Chlamydiae* contain peptidoglycan? This was the challenging question raised by D. Rockey (Corvallis, OR, USA). Peptidoglycan has never been isolated from these microorganisms, even though their genome contains most of the genes required for peptidoglycan synthesis, and their development inside the host is sensitive to  $\beta$ -lactams, which inhibit peptidoglycan synthesis. A central ring containing an antigen called SEP (in reference to the apparent septum) can be visualized in the middle of the dividing cell. When division is inhibited, SEP disassembles and is distributed around the periphery of the cell. As Western blotting or immunoprecipitation cannot reveal SEP, there is a possibility that it consists of peptidoglycan. *Chlamydiae* contain other proteins that have roles in cell division in other bacteria: MinD and FtsK; proteins involved in the synthesis of septal peptidoglycan such as FtsI (equivalent to PBP3) and FtsW; and even proteins with peptidoglycan recycling activities such as PapQ and AmiA. What is even more puzzling is that PBP3, AmiA and PapQ colocalize in the cytosol of *Chlamydiae*.

### The ring in the eukaryotic world

The success of bacterial cells in the living world resulted in their incorporation into more complex eukaryotic cells. For example, plant chloroplasts are derived from cyanobacterial endosymbionts. Indeed, one of the components of the bacterial division



**Fig. 3** | Phylogenetic tree of the *ftsZ* genes present in the genomes of different organisms. Plants have two *ftsZ* genes that have been designated Z1 and Z2. A small multigene family encodes FtsZ in plants. The proteins clearly group into two families. One family (FtsZ2) groups with its cyanobacterial ancestors, whereas the origin of the other family (FtsZ1) remains to be elucidated. However, the abundance of different FtsZ proteins in a given plant species already suggests that the different proteins may have (slightly) different functions in plants. (Figure adapted from Reski, 2002.). Mt, mitochondrial; pt, plastid.

machinery, FtsZ, can be identified in the organelles of present-day eukaryotic cells (Fig. 3). Some components of this machinery might have been converted to other proteins in eukaryotic cells, such as MreB and FtsZ to actin and tubulin, respectively, which are important in cytokinesis and karyokinesis. A surprising recent discovery is that verrucomicrobia contain genes that are unmistakably related to eukaryotic  $\alpha$ - and  $\beta$ -tubulin (Jenkins *et al.*, 2002).

R. Reski (Freiburg, Germany) described the formation of a 'plastoskeleton' in the plastids of *Physcomitrella*. Remarkably, FtsZ in the plastids of this moss shows similar behaviour to FtsZ in bacteria. A large excess of the protein blocks plastid division, whereas moderate overproduction results in plastids that divide at smaller sizes. If FtsZ is artificially targeted to the cytosol by deleting the transit peptide, it is no longer able to form plasto-skeletal structures, which indicates that compartment-specific cofactors are required for this process.

K. Osteryoung (East Lansing, MI, USA) tried to identify proteins in the *Arabidopsis* chloroplast similar to FtsA and ZipA, which colocalize in *E. coli* at the division ring, immediately after FtsZ. Instead, she found that chloroplasts need other unrelated proteins for division to occur. Arc5 is one such protein, and although it is unable to enter the chloroplast, it associates with the membrane and localizes to the middle of the organelle at the time of division. The gene for Arc5 belongs to the dynamin family (Gao *et al.*, 2003).

## Homework

Despite this recent progress, many questions still need to be answered to gain a full understanding of how bacteria grow and divide. Do the *E. coli* Min proteins oscillate in reconstituted membrane systems? How do the cytosolic components of the division machinery interact with those in the membrane and in the periplasm? Which molecules or conditions prevent the initiation of a septum when the nucleoids are not segregated? Is there a specific structural arrangement of proteins at the septation site? Can such a structure, if present, be isolated and purified? Will it show any cell-division-related activity *in vitro*?

Little is known about cell division in cocci. Their shape is different from the commonly studied models, *E. coli* and *B. subtilis*, and they may have selected mechanisms that deviate from our present-day paradigms. However *Streptococcus pneumoniae*, one possible cocci model, is even smaller than *E. coli* and this might prove problematic as much of the progress reported at this meeting relied heavily on the visualization of the molecules that take part in division.

The field of molecular microbiology is still relatively small, but exciting progress is being made towards understanding division and DNA segregation at the molecular level. As some elements of the bacterial division apparatus are precursors of the eukaryotic cytoskeleton—some are involved in mechanisms that are similar to those present in eukaryotes, whereas others are surprisingly different—participants of the meeting departed feeling part of a very rewarding international endeavour.

REFERENCES

- Andreu, J.M., Oliva, M.A. & Monasterio, O. (2002) Reversible unfolding of FtsZ cell division protein from archaea and bacteria. Comparison with eukaryotic tubulin folding and assembly. *J. Biol. Chem.*, **277**, 43262–43270.
- Ayala, J.A., Garrido, T., de Pedro, M.A. & Vicente, M. (1994) In *New Comprehensive Biochemistry. 27. Bacterial Cell Wall* (eds Hakenbeck, R. & Ghuysen, J.-M.), 73–101. Elsevier Science, Amsterdam, The Netherlands.
- Ben-Yehuda, S., Rudner, D.Z. & Losick, R. (2003) RacA, a bacterial protein that anchors chromosomes to the cell poles. *Science*, **299**, 532–536.
- Bi, E. & Lutkenhaus, J. (1993) Cell division inhibitors SulA and MinCD prevent formation of the FtsZ ring. *J. Bacteriol.*, **175**, 1118–1125.
- Den Blaauwen, T., Aarsman, M.E.G., Vischer, N.O.E. & Nanninga, N. (2003) Penicillin binding protein PBP2 of *Escherichia coli*, localizes preferentially in the lateral wall and at mid cell in comparison with the old cell pole. *Mol. Microbiol.*, **47**, 539–547.
- Díaz, J.F., Kralicek, A., Mingorance, J., Palacios, J.M., Vicente, M. & Andreu, J.M. (2001) Activation of cell division protein FtsZ. Control of switch loop T3 conformation by the nucleotide  $\gamma$ -phosphate. *J. Biol. Chem.*, **276**, 17307–17315.
- Donachie, W.D., Addinall, S. & Begg, K. (1995) Cell shape and chromosome partition in prokaryotes or, why *E. coli* is rod-shaped and haploid. *Bioessays*, **17**, 569–576.
- Francis, F., Ramírez-Arcos, S., Salimnia, H., Victor, C. & Dillon, J.R. (2000) Organization and transcription of the division cell wall (*dcw*) cluster in *Neisseria gonorrhoeae*. *Gene*, **251**, 141–151.
- Gao, H.D., Kadirjan-Kalbach, D., Froehlich, J.E. & Osteryoung, K.W. (2003) ARC5, a cytosolic dynamin-like protein from plants, is part of the chloroplast division machinery. *Proc. Natl Acad. Sci. USA*, **100**, 4328–4333.
- Geissler, B., Elraheb, D. & Margolin, W. (2003) A gain-of-function mutation in *ftsA* bypasses the requirement for the essential cell division gene *zipA* in *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **100**, 4197–4202.
- Higashitani, A., Ishii, Y., Kato, Y. & Koriuchi, K. (1997) Functional dissection of a cell-division inhibitor, SulA, of *Escherichia coli* and its negative regulation by Lon. *Mol. Gen. Genet.*, **254**, 351–357.
- Hinz, A.J., Larson, D.E., Smith, C.S. & Brun, Y.V. (2003) The *Caulobacter crescentus* polar organelle development protein PodJ is differentially localized and is required for polar targeting of the PleC development regulator. *Mol. Microbiol.*, **47**, 929–941.
- Hiraga, S., Ichinose, C., Niki, H. & Yamazoe, M. (1998) Cell cycle-dependent duplication and bidirectional migration of SeqA-associated DNA-protein complexes in *E. coli*. *Mol. Cell*, **1**, 381–387.
- Huang, J., Cao, C. & Lutkenhaus, J. (1996) Interaction between FtsZ and inhibitors of cell division. *J. Bacteriol.*, **178**, 5080–5085.
- Jacob, F., Brenner, S. & Cuzin, F. (1963) On the regulation of DNA replication in bacteria. *Cold Spring Harb. Symp. Quant. Biol.*, **28**, 329–348.
- Jenkins, C., Samudrala, R., Anderson, I., Hedlund, B.P., Petroni, G., Michailova, N., Pinel, N., Overbeek, R., Rosati, G. & Staley, J.T. (2002) Genes for the cytoskeletal protein tubulin in the bacterial genus *Prostheco bacter*. *Proc. Natl Acad. Sci. USA*, **99**, 17049–17054.
- Jones, L.J., Carballido-López, R. & Errington, J. (2001) Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell*, **104**, 913–922.
- Lu, C., Stricker, J. & Erickson, H.P. (1998) FtsZ from *Escherichia coli*, *Azotobacter vinelandii*, and *Thermotoga maritima*—quantitation, GTP hydrolysis and assembly. *Cell Motil. Cytoskeleton*, **40**, 71–86.
- Meinhardt, H. (2003) *The Algorithmic Beauty of Sea Shells*. Springer, Heidelberg, Germany.
- Meinhardt, H. & de Boer, P. A. (2001) Pattern formation in *Escherichia coli*: a model for the pole-to-pole oscillations of Min proteins and the localization of the division site. *Proc. Natl Acad. Sci. USA*, **98**, 14202–14207.
- Ramírez-Arcos, S., Szeto, J., Dillon, J.R. & Margolin, W. (2002) Conservation of dynamic localization among MinD and MinE orthologs: oscillation of *Neisseria gonorrhoeae* proteins in *Escherichia coli*. *Mol. Microbiol.*, **46**, 493–504.
- Reski, R. (2002) Rings and networks: the amazing complexity of FtsZ in chloroplasts. *Trends Plant Sci.*, **7**, 103–105.
- Romberg, L., Simon, M. & Erickson, H.P. (2001) Polymerization of FtsZ, a bacterial homolog of tubulin. Is assembly cooperative? *J. Biol. Chem.*, **276**, 11743–11753.
- Rueda, S., Vicente, M. & Mingorance, J. (2003) Concentration and assembly of the division ring proteins FtsZ, FtsA and ZipA during the *Escherichia coli* cell cycle. *J. Bacteriol.*, **185**, 3344–3351.
- Tamames, J., González-Moreno, M., Mingorance, J., Valencia, A. & Vicente, M. (2001) Bringing gene order into bacterial shape. *Trends Genet.*, **17**, 124–126.
- van den Ent, F., Amos, L.A. & Löwe, J. (2001) Prokaryotic origin of the actin cytoskeleton. *Nature*, **413**, 39–44.
- van den Ent, F., Møller-Jensen, J., Amos, L.A., Gerdes, K. & Löwe, J. (2002) F-actin-like filaments formed by plasmid segregation protein ParM. *EMBO J.*, **21**, 6935–6943.
- Yu, X.-C. & Margolin, W. (1999) FtsZ ring clusters in *min* and partition mutants: role of both the Min system and the nucleoid in regulating FtsZ ring localization. *Mol. Microbiol.*, **32**, 315–326.



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