Chapter 2: Force-induced cell shaping of bacteria?

In this chapter we will provide background information on the mechanisms of growth and division in rod-shaped Gram-negative bacteria, in particular the bacterium *E. coli*. For these processes, a possible role of (cytoskeletal) forces is discussed.

The instantaneous shape of cell-walled bacteria is determined by its peptidoglycan (PG) cell wall. This is illustrated in Figure 2.1, where EM pictures of three isolated cell walls (also termed “sacculi”) are shown. In all three cases, the shape of the cell wall was found to be identical to the shape of the bacterial cells they were isolated from. That it is the only determinant of cell shape in bacteria can be easily demonstrated by enzymatic degradation of the cell wall (e.g. by the PG hydrolase lysozyme), after which cells become spherical (termed “spheroplasts”) (Zinder and Arndt 1956; Malamy and Horecker 1964; Neu and Heppel 1964).

The sacculus of the dividing cell (Figure 2.1B) also makes it clear that division in bacteria is somewhat different from mammalian cells, as during division not only the cytoplasmic membrane needs to be constricted, but also cell wall synthesis needs to take place to form the new cell poles. Thus, to understand bacterial cell division, understanding cell wall growth is essential.

A key experiment was performed in 1971 (Schwarz and Leutgeb 1971): An *E. coli* mutant strain was used that requires an externally added particular precursor for PG cell wall growth, in the absence of which PG hydrolases break down the cell wall. As mentioned above, this results in the formation of spheroplasts. After re-addition of the precursor, the cells started to build a new cell wall, which (after isolation) was found to be spherical! (Figure 2.1C) Thus, not only does the wall shape the cell, but apparently the cell can also shape the wall. This suggests that the shape-information is not encoded in the molecular structure of PG. Instead, cell wall deposition appears determined by the location of the PBPs (penicillin-binding proteins, named after their penicillin binding property), the enzymes that synthesize new cell wall, and are typically anchored in the cytoplasmic membrane (Note that although in this thesis, we refer to PBPs when we mean PG synthesizing enzymes, actually some PBPs are PG hydrolases).
As already mentioned in the introduction (Chapter 1), the discovery of cytoskeletal homologues of tubulin and actin in bacteria (FtsZ and MreB, respectively) has had a major impact on bacterial cell biology. In this chapter we discuss experimental evidence that suggest an active force-generating modus operandi of the Z-ring. Interpreted as a force-induced formation of the new cell poles, bacterial cell division can be seen as one aspect of bacterial morphogenesis, i.e. the processes that underlie the creation and maintenance of bacterial shape.

There are various factors that play a role in shaping of the cell wall. The main factors are the cellular turgor pressure, due to the osmotic difference inside and outside the cell, the cytoskeleton, built from protofilaments of MreB (actin) and FtsZ (tubulin), and the enzymes that make (PBPs) and break (hydrolases) covalent bonds in the cell wall. How

\[\text{Figure 2.1: Isolated } \textit{Escherichia coli} \text{ cell walls (sacculi):} \text{ PG Cell walls have the same shape as the cells from which they are isolated. (A) Isolated rod-shaped PG sacculus imaged by electron microscopy (Schwarz and Leutgeb 1971) (B) Isolated PG sacculus of a dividing bacterium (de Pedro et al. 1997). (C) Isolated PG sacculus of a spheroplast cell allowed to rebuild its cell wall (Schwarz and Leutgeb 1971).}\]
these factors (potentially) affect the (shape of the) cell wall is depicted schematically in Figure 2.2. In this chapter, we will review what is known about these factors, limiting ourselves to information relevant for a coarse mechanistic understanding of morphogenesis. We start with a brief description of what is known about the structure and properties of the cell wall itself.

The PG cell wall
The peptidoglycan (PG) cell wall (or “sacculus”) is a covalent macromolecule in the shape of the bacterium from which it originates (Figure 2.1). In *E. coli*, the PG cell wall has a thickness of 3-8 nm (Matias et al. 2003). It consists of approximately a monolayer (Wientjes et al. 1991) of stiff glycan chains cross-linked via flexible peptide cross-bridges. Its 3D structure has been elusive for many years, and various models have been proposed (Vollmer et al. 2008).

Light scattering experiments on isolated sacculi have shown that it is flexible, with a capacity to expand over 300% (Koch and Woeste 1992). This flexibility is mainly ascribed to flexibility in the peptide cross-bridges (Boulbitch 2000; Boulbitch et al. 2000). The elastic properties (the tendency to deform reversibly) of a material are defined through its elastic moduli \( \lambda_{ij} \). When stress (force per area; unit pressure) is applied, a certain amount of strain
(length change/length) will occur in the material. The Young’s modulus $\lambda_{22}$ (a material property) is the proportionality factor. For sufficiently small deformations, the response is linear.

$$\frac{F}{A} = \lambda_{22} \frac{\Delta l}{l}$$

For cell-walled bacteria and yeasts, the Young’s modulus has been determined using various techniques, and typically values around $10^7$-$10^8$ Pa are found (Thwaites and Mendelson 1985; Mendelson and Thwaites 1989; Thwaites and Mendelson 1989; Yao et al. 1999; Arnoldi et al. 2000; Mendelson et al. 2000; Smith et al. 2000). This allows one to calculate e.g. the force required to increase the length of a bacterium by 10% through pulling at its ends. Assuming a cylindrical bacterium with radius 500 nm and PG thickness of 5 nm, and assuming $\lambda_{22} = 10^7$ Pa, one finds that 15 nN of force needs to be applied. As such a force is way outside the range what is typically generated inside cells (~pN range), division by a direct elastic deformation of the PG cell wall to “pinch” a static non-growing cell into two daughter cells is unlikely to take place.

Since in Chapter 3, molecular constructs are created that need to go through the PG cell wall, information about the permeability of the cell wall is important. Fluorescently labeled dextrans were used to estimate the pore size in isolated sacculi. It was found that the mean radius of the (unstretched) pores was ~2 nm (Demchick and Koch 1996). After stretching of the wall due to the intra-cellular turgor pressure (see below), possibly globular proteins up to ~50 kDa can pass through the PG pores. A sub-set of cellular protein content <100 kDa is released when cells are osmotically "shocked". The authors suggested that the pores in the PG cell wall acts as a molecular sieve (Vazquez-Laslop et al. 2001). The cell envelope contains several huge protein complexes that bridge the periplasm, such as the flagellar motor. For several such complexes it appears that dedicated PG hydrolases enlarge the PG locally for the complex to fit in (for references see Vollmer et al. 2008). An older but more comprehensive review regarding issues with PG permeability pertaining to large protein complexes also exists (Dijkstra and Keck 1996).

**Turgor pressure**

Osmotic pressure is the hydrostatic pressure that is produced by the difference in concentrations of solutes on both sides of a semi-permeable membrane, such as a lipid bilayer, or the Gram-negative cell envelope (see also below). Since not all particles
contribute to osmosis, the osmole unit is used to count the number of particles that contribute. The osmolality difference between inside and outside the cell causes a turgor pressure (force/area) on the cell envelope:

$$\Delta \Pi = RT(Osm_{cyt} - Osm_{ext})$$

This exerts a force on the PG cell wall, which is thought to be important for wall growth. For gram-negative bacteria, the turgor pressure is reported to range from 1-5 atm (Koch and Pinette 1987) (1 atm = $10^5$ Pa = $10^5$ N/m$^2$ = 100 pN/μm$^2$). In addition, the turgor pressure depends on the osmolality of the growth medium (Cayley et al. 2000).

As briefly explained in the previous chapter, Gram-negative bacteria have an additional compartment surrounding the cytoplasm called the periplasm. The osmotic pressure in the periplasm has long been assumed to be lower than that of the cytoplasm, resulting in a force exerted outwards on the cytoplasmic membrane (Koch 1998). This was thought to compress the contents of the periplasm, explaining the concentrated periplasm (observed in EM pictures of freeze-substituted bacteria) that led to the periplasmic gel concept: The periplasm was proposed to consist of a compressed, gel-like, viscous, protein-dense matrix that reduced diffusion coefficients two orders of magnitude compared to the cytoplasm (Hobot et al. 1984; Brass et al. 1986; Koch 1998).

Recently, both the osmotic pressure difference over the cytoplasmic membrane, as well as the periplasmic gel concept, has been challenged by new experimental evidence. Cryo-transmission electron microscopy of frozen-hydrated sectioned cells shows that the periplasm is relatively empty compared to the cytoplasm (Matias et al. 2003). The authors suggest that the cytoplasmic membrane has some capacity to float freely in the periplasm. In addition, new, more direct measurements of diffusion coefficients (using GFP) in the periplasm gave values similar to that of the cytoplasm (Mullineaux et al. 2006). Finally, from cytoplasmic and total cell volume measurements it was recently concluded that the cytoplasm and periplasm are iso-osmotic (Cayley et al. 2000). It follows that the turgor pressure is exerted on the outer membrane (this would “explain” the high amount of covalent lipoprotein trimers tethering the OM to the PG cell wall).

The cytoskeleton: FtsZ

FtsZ is a prokaryotic tubulin-homologue (Erickson 1995) which polymerizes into protofilaments (Mukherjee and Lutkenhaus 1994) in a GTP-dependent manner
(RayChaudhuri and Park 1992). Depending on the conditions, FtsZ can polymerize in a wide variety of higher order shapes: straight protofilaments, tubules, double-stranded filaments and curved and/or circular shapes have been reported (see (Horger et al. 2008) for references). Experimental evidence indicates that GDP in between two monomers inside a protofilament can be exchanged with GTP (see (Mingorance et al. 2005) for references). The details of GTP hydrolysis, such as the effect of nucleotide phosphorylation state on the monomer-monomer bond strength and angle (and thus intrinsic curvature) are not fully understood yet. Phosphate release after GTP hydrolysis could cause a conformational change to a more curved shape (Erickson 1997; Lu et al. 2000). It is hypothesized that this can generate force (Lu et al. 2000). However, AFM images of FtsZ in the presence of GTP or in the presence of GDP with aluminum fluoride (an analog of the γ-phosphate of GTP) were observed to be structurally similar (Mingorance et al. 2005).

In vivo, FtsZ filaments localize to mid-cell in a ring, termed the Z-ring (Ma et al. 1996). In EM pictures, it can be seen that FtsZ localizes underneath the leading edge of a constricting cytoplasmic membrane (Bi and Lutkenhaus 1991). Recently, FtsZ from *E. coli* was expressed in fission yeast and found to form rings, suggesting that a cylindrical geometry is enough to cause the helical filaments to condense into a ring (Srinivasan et al. 2008). Z-spirals that condensed into constricting rings were also observed when YFP-tagged FtsZ fused to an amphipathic membrane binding α-helix (originating from the MinD protein), was present in tubular vesicles (Osawa et al. 2008).

Cryo EM images (Li et al. 2007) of *Caulobacter* indicate that the FtsZ ring consists of a few separate, arc-like protofilaments. This does not match with the continuous FtsZ spirals in *E. coli* observed with fluorescence microscopy *in vivo* (Thanedar and Margolin 2004). Furthermore, experiments also show that lateral interactions are important for Z-ring formation (Lan et al. 2008). The location of the Z-ring determines the site of constriction. Interestingly, in vivo, an FtsZ mutant that does not condense into a ring but remains spiral also forms spiral invaginations (Addinall and Lutkenhaus 1996). In spherical cells, FtsZ formed arcs that locally constricted the cells. This suggests that complete circumference of the Z-ring is not required for constriction (Addinall and Lutkenhaus 1996). FRAP experiments on GFP-tagged FtsZ have shown that the FtsZ ring is highly dynamic, with a recovery on a second time-scale (Stricker et al. 2002). This is consistent with continuous polymerization and depolymerization. An FtsZ mutant with a 100-fold reduction in
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GTPase activity forms stable polymers and surprisingly, supports in vivo constriction (Mukherjee et al. 2001). If this is true, then the GTPase activity is not important for its function.

The cytoskeleton: MreB

The actin-homologue MreB forms helical cables in the cytoplasm, just below the cytoplasmic membrane (Jones et al. 2001). MreB cables are dynamic structures as well: it was found that in vivo, MreB filaments are ~400 nm in length and exhibit treadmilling, in which monomers are assembled on one end, and depolymerize on the other end (Kim et al. 2006). In the absence of MreB, cell width increases and the cells become more round (Wachi and Matsuhashi 1989). Thus, it was concluded that MreB is responsible for the maintenance of constant cell width during growth. Consistent with this idea is the absence of MreB-like genes from coci species (round). However, MreB is also present in cell wall-less species such as Spiroplasma (Cabeen and Jacobs-Wagner 2005).

The prevalent view is that treadmilling MreB helical tracks in the cytoplasm guide the PG synthesizing complexes (PBPs) and cause disperse helical insertion of new cell wall material during cell elongation (den Blaauwen et al. 2008). However, in the absence of MreB or Mbl, localization patterns of nascent PG (fluorescently stained) or some PBPs (typically a single bacterial species has ~5-10 different PBPs) are unaltered (Scheffers et al. 2004). Localization by substrate recognition was subsequently proposed to explain the observed PBP localization patterns (Scheffers and Pinho 2005). For a more extensive discussion of this subject see a recent review (Cabeen and Jacobs-Wagner 2007).

PBPs and PG hydrolases: making and breaking bonds in the sacculus

After division of a rod-shaped bacterium such as E. coli, first an elongation phase takes place before the next round of division is initiated. This elongation phase was found to consist of two sequential sub-phases: (i) only “diffusive” elongation along the cylindrical sidewalls, followed by (ii) a phase in which, on top of the diffusive elongation, also FtsZ-dependent zonal elongation at mid-cell takes place (de Pedro et al. 1997).

“Diffusive” elongation occurs through insertion of new PG everywhere along the cylindrical part of the cell. As already mentioned, PBPs (penicillin-binding proteins) are the cell’s workers that perform the enzymatic reactions required to synthesize new PG cell
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wall, and to maintain the existing cell wall (den Blaauwen et al. 2008). In E. coli, as many as 12 different PBPs exists. When 8 of the 12 PBPs were deleted, cells were still viable (Denome et al. 1999). This indicates a very robust wall deposition mechanism.

The new cell wall material (“precursor”) is synthesized in the cytoplasm in a set of sequential enzymatic steps, is then attached to the membrane (“Lipid II”) and translocated to the periplasmic side (by a “flippase” membrane protein (Ruiz 2008)). In the periplasm, PBP’s incorporate the new material into the pre-existing cell wall.

Per cell cycle, 50% of the PG is turned over (Park 1993; Park 1995). As the PG is a covalent structure, and in E. coli only a monolayer thick, new PG precursor insertion is thought to require the breaking of bonds. Enzymes that break the covalent bonds in the PG cell wall are called PG hydrolases. However, multiple deletions of PG hydrolases, e.g. all (six) known lytic transglycosylases or all (three) known amidases are viable, and only cell separation appears affected, causing chain formation (Heidrich et al. 2002). Apparently also among the PG hydrolases a large redundancy exists, and the degradation of a cross-linked structure might be performed either by breaking peptide bonds or glycan bonds.

It is tempting to speculate that wall growth in E. coli occurs in a similar manner as in B. subtilis, i.e. in an inside-to-outside manner, with deposition on the IM side of the PG cell wall, and degradation by PG hydrolases (tethered to the OM) at the OM side of the wall. However, such a discussion is beyond the purpose of this chapter.

Cell division

Here, we discuss the details of the division process in E. coli. As mentioned in the introduction (Chapter 1), the envelope consists of a thin PG cell wall sandwiched in between two lipid membranes. The inner cytoplasmic membrane surrounds the cytoplasm; the outer membrane is overlaid on the PG cell wall and forms the bacterial cell surface. The outer membrane is an asymmetric bilayer, with the inner leaflet containing lipids and the outer leaflet containing lipopolysaccharide (LPS), a compound with extended sugar chains that are exposed on the cell surface.
Rod-shaped bacteria grow by elongation at approximately constant width, and then switch to a “division” mode at mid-cell. In Gram-positive bacteria such as *B. subtilis*, a thick septum (septal plate) is deposited while the Z-ring constricts inwards. Only after the septum is complete do the PG hydrolases cleave the septum to separate the daughter cells (Figure 2.3A).

In contrast, in Gram negative bacteria, all three layers of the cell envelope appear to invaginate simultaneously (Figure 2.3B, “simultaneous”), as judged from EM pictures. Bi
During division, cell wall addition occurs with a progressively decreasing diameter. This results in the formation of two new polar cell caps, which are approximately hemi-spherically shaped (Reshes et al. 2008). Nevertheless, already in the 1970s experiments indicated that Gram-negative bacteria also form a septum during division (Burdett and Murray 1974). Final proof came when strains lacking particular PG hydrolases were shown to form un-cleaved septa (Heidrich et al. 2001; Holtje and Heidrich 2001; Heidrich et al. 2002) (Figure 2.3B “delayed”). The currently held view is that wild-type *E. coli* does form a septum, but cleavage occurs “almost” simultaneously with cell division, resulting in a V-shaped constriction instead of the Gram-positive septal plate. Recent results suggest that the septum consists of 3 or 4 PG monolayers instead of two (assuming a monolayer thickness for the cylindrical cell wall), of which one or 2 layers are degraded as constriction proceeds (Uehara and Park 2008).

What is known about the steps that finally lead to the formation of two daughter cells can be summarized as follows: During septum formation, addition of new PG is thought to occur specifically at the leading edge (Wientjes and Nanninga 1989). On the OM side of the PG layer, PG hydrolases start to cleave the double-layered PG wall, and the layers will separate. Then proteins that tether the OM to the PG move in and make sure that the OM follows the invaginating PG. In highly constricted *Caulobacter* cells (Judd et al. 2005), cryo-EM images show that the distance between IM and OM increased during (late) constriction, from 30 nm to ~ 60 nm. Although the PG layer could not be imaged, it is possible that the septal splitting at the late stage starts to “lag” behind with constriction. At the final stages, the inner membranes fuse, the PG septum closes, and the OM connection is severed (Judd et al. 2005).

**Evidence for force-generation by the Z-ring**

In biology, non-motor protein elements exist that can generate force by polymerization. An example of a polymerizing structure that can generate forces is a microtubule, which can generate up to several pN of force (Dogterom and Yurke 1997; Janson and Dogterom 2004). Working together, molecular motors such as kinesins that individually can only generate 4-6 pN can pull tubes out of vesicles, which required forces >18 pN (Koster et al. 2003). Not only polymerization, but also depolymerization of microtubules can produce force (Grishchuk et al. 2005). A collection of small (de)polymerizing FtsZ filaments might locally generate similar amounts of force along a concentric circle.
Thus, as soon as was found that FtsZ polymerized into higher order structures (Bramhill and Thompson 1994), models for “active” constriction were proposed (Bramhill 1997). However, since in bacteria, constriction of the cytoplasmic membrane and PG septum formation occur simultaneously, it is difficult to separate cause and effect. For example, one could argue that the observed cell envelope constriction can also be caused by an inward “pushing” of the newly synthesized PG, followed by a dynamic remodeling (decrease in diameter) of the Z-ring. The Z-ring’s function would then be limited to the mid-cell localization of the PG synthesizing complexes (“scaffold function”).

More and more evidence accumulates that the Z-ring actively constricts the cytoplasmic membrane, and that it can do so without simultaneous PG septum formation. For example, FtsZ is highly conserved within the bacterial kingdom, and even bacteria that have no cell wall, such as the *Mycoplasma* species, contain FtsZ (Wang and Lutkenhaus 1996). Furthermore, it has been shown that in a mutant deficient in cell separation (ΔAmiABC), the PG inward growth was stalled, but that in some cells, the cytoplasmic membrane could still be constricted, presumably by the Z-ring (Heidrich et al. 2002) (see Figure 2.4). Thus, inward growth of PG is not a prerequisite of Z-ring constriction. Additional evidence that neighboring cells are compartmentalized in these mutant cells with stalled inward PG growth was reported recently using Fluorescence Loss In Photobleaching (FLIP) techniques on cytoplasmic GFP in the chained ΔAmiABC cells (Priyadarshini et al. 2007). Also in *B. subtilis* cytoplasmic membrane invagination could be separated from PG septum formation in a PBP2B (PBP3 in *E. coli*) mutant (Daniel et al. 2000). Recently, it was demonstrated that when FtsZ was mixed with lipids, Z-rings inside tubular vesicles formed, and dependent on GTP hydrolysis, constriction occurred (Osawa et al. 2008). Together, these data suggests that FtsZ by itself can generate a constrictive force.

**Modeling the Z-ring**

Models can focus on two different aspects of Z-ring mediated division: the actual force-generating mechanism of the Z-ring itself, or the ability of a force-generating Z-ring to drive wall synthesis inwards to form the new polar caps.

For models in the first category several experimental observations need to be taken into account. These are: the (perceived) absence of motor proteins such as myosin or kinesin in bacteria, the dynamic polymerization and depolymerization of the ring, and the
ability of Z-arcs to locally constrict. Recently, such a model was formulated (Ghosh and Sain 2008): it assumes that GTP-FtsZ protofilaments prefer to be straight, and GDP-FtsZ protofilaments to have an intrinsic curvature. The authors estimated that the ring could generate a radial contractile force of the order of 0.5 pN/nm. Another model was recently reported (Horger et al. 2008): The model emphasized lateral interactions, in which energetically favored fitting of parallel filaments produces an inward force.

In the second category, also two models have been reported. As discussed earlier, in Gram-negative cells such as *E. coli* and *Caulobacter*, Z-ring constriction and PG growth occur at similar timescales. Therefore, both models combine small constriction forces with PG growth, and show that division can be accomplished this way (Fero et al, Biophysical Society Meeting 2006) (Lan et al. 2007). Fero *et al.* assume a small constant contractile “pressure” (force/area) that induces a deflection on the PG, which combined with the sequential additional of rings of new PG material results in division shapes similar to those observed for *Caulobacter* using Cryo-electron Tomography (Judd et al. 2005). A more comprehensive and detailed model was presented by (Lan et al. 2007). In this work, turgor pressure, as well the elasticity of the cell wall is taken into account. The idea is that the Z-ring slightly changes the wall radius and in doing so establishes the direction in which the new wall is added. To explain how a Z-ring that generates only ~8 pN of force can constrict
the cell, the authors propose that over time, the wall is remodeled by the PBPs (“turnover”) to remove the elastic stresses. This is similar to the concept of “morpho-elastic” materials used to theoretically describe the alteration of equilibrium shapes of plant tendrils and bacterial filaments under externally imposed stresses (Goldstein and Goriely 2006).

**Force-induced cell wall shaping**

From the experimental results and models on FtsZ, a picture emerges in which the Z-ring generate constrictive force to control the cell shape, in this case the shape of the new cell poles. As no significant differences can be detected in the PG building blocks present in dividing and non-dividing cells using HPLC (de Jonge et al. 1989), this argues for a physical mechanism that controls new cell wall deposition and thus shape.

In a second key experiment, it was found that when cells were restricted in agarose chambers (created by a PDMS mold) and filamentation was induced, cells formed straight filaments until a barrier was hit, after which they slowly adopted the shape of the chamber. After release from the chambers, helical, spiral and zig-zag shaped bacteria were obtained (Takeuchi et al. 2005) (Figure 2.5). Astonishingly, the change in shape was not elastic but plastic! This provides indirect evidence that external forces can influence the shape of the cell wall, which is (re-) modeled such that stresses are minimized. Furthermore, the remodeling occurred dispersed throughout the filament, indicating forces are felt throughout the cell, and can affect growth locally.

Shape changes upon growth during confinement have also been observed for fission yeast, which is also rod-shaped with a rigid glycan-based cell wall (Terenna et al, ASCB Meeting 2007). However, whether the shape changes are elastic or plastic is not known yet. It might well be possible that in *E. coli* and fission yeast similar mechanisms work to accomplish cell division. Fission yeast contains an acto-myosin ring that is thought to generate force to “guide” the septation process, and is tightly coupled to septum formation that occurs simultaneously (Vjestica et al. 2008).

The experiment mentioned in the introduction in which cells had rebuild spherical cell walls, actually restored their rod-shape after continuation of growth. If a proper cylindrical cell shape is required as template to maintain this shape, this observation is difficult to explain. Instead, it points to a local mechanism that can restore severely distorted cell shapes. As the turgor pressure is equal in all directions, and PBPs themselves appear “dumb” and just synthesize cell wall where they happen to sit, it is expected that
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the default shape is a round cell wall. What brings back and maintains the rod-shape? Or: what generates the forces to maintain the rod-shape? Just as the Z-ring provides the force to direct growth inwards, we can hypothesize that MreB helices provides the inward force to maintain the rod-shape. Local force might be exerted on the growing cell wall that favors a gradual change of cell shape back to rod-shape. Perhaps mechanical forces of MreB exerted on the IM would be responsible for the gradual conversion back to rod-shape. Interestingly, a similar slow process back to rod-shape is observed after MreB is re-expressed in spherical cells depleted from MreB (T. den Blaauwen, unpublished observations).

A recent theoretical study has focused on the polymerization of polymers on curved membranes, with the observed behavior of FtsZ and MreB polymers in mind (Andrews and Arkin 2007). If e.g. MreB binds strongly with one face to the membrane, it can in polymerized form impose its preferred radius on to the membrane, and as such, generate inward force. It will be interesting to polymerize MreB inside model membranes and see

Figure 2.5: External forces can shape a growing bacterium. (A) After constraining E. coli cells to micron-sized chambers and inducing filamentation, the growing filaments would adopt the shape of their confining geometry. Releasing the cells from the chambers showed that the cells were not bent elastically, but that the new shape was encoded in their cell walls. (B) Time sequence of a single filament. Only when the filament is constrained end-to-end does the filament shape start to adopt the shape of the chamber. Images reproduced from (Takeuchi et al. 2005).
whether cylinders are formed, and which helical period and radius those helices have.

As indicated in Figure 2.2, there are at least two ways imaginable in which forces can influence cell wall growth. The forces can displace/relocate the PBPs in the cytoplasmic membrane, in this way controlling the location/direction where new cell wall is deposited (e.g. inwards during division). Alternatively, (small) forces are exerted directly on the PG cell wall, locally creating (small) displacements. Subsequent PG turnover and remodeling then could make these displacements permanent (“morpho-elasticity” (Goldstein and Goriely 2006)).

The experiments that are aimed at in this thesis might be used to distinguish between these alternatives. If the shape is regulated internally by modulating the distance of the (inner membrane) PBP’s to the PG cell wall, then we expect that higher forces are needed to influence wall growth when exerting them from the outside. This is because the external force needs to displace the cell wall, whereas the internal force (such as the Z-ring) needs only to displace the cytoplasmic membrane. The latter is expected to require much smaller forces than the former. However, if small displacements are made in the cell wall that are subsequently remodeled to become permanent, then it does not matter if force is exerted on the outside or on the inside.