The Mechanical Properties of Bacteria and Why they Matter

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"It behooves us always to remember that in physics it has taken great scientists to discover simple things. They are very great names indeed which we couple with the explanation of the path of a stone, the droop of a chain, the tints of a bubble, the shadows in a cup. It is but the slightest adumbration of a dynamical morphology [of biological systems] that we can hope to have until the physicist and the mathematician shall have made these problems of ours their own."

– D'arcy Thompson, On Growth and Form.

Abstract

I review recent techniques to measure the mechanical properties of bacterial cells and their subcellular components, and then discuss what these techniques have revealed about the constitutive mechanical properties of whole bacterial cells and subcellular material, as well as the molecular basis for these properties.

Keywords

Mechanobiology · Cell growth · Cellular morphogenesis

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1.1   Introduction

Bacteria are the smallest, simplest, and most successful (that is, most numerous) class of living organisms on Earth. It is reasonable to assume that these three traits are intimately connected with each other, and with bacteria's foundational role in our understanding of molecular biology. However, these traits have historically been "selected against" by those interested in biomechanics: their small size renders bacterial cells intractable to many biophysical assays used on eukaryotic cells or tissues, and their relative simplicity (for example, their lack of a true cytoskeleton) along with a historical focus on their genetics and molecular biology have perhaps caused scientists to underestimate the richness of their mechanics and the value of studying them.

Over the last 10 years there has been a realization of the importance of mechanical sensing and signaling in bacteria (reviewed elsewhere in this

volume; also see Persat et al. 2015). In parallel, several fundamental measurements have begun to elucidate the intriguing mechanical properties of bacterial cells. Due to the size of bacteria, these measurements have typically required Herculean efforts in assay development to make what are relatively crude mechanical measurements compared to what can be measured for eukaryotic cells or non-living materials. Yet these seminal measurements have already demonstrated that bacteria possess many novel materials from a mechanics perspective, and underscore the importance of endeavoring to characterize these materials.

While the field of bacterial cell mechanics is still in its infancy, in certain cases it is clear how the mechanical properties of subcellular material are adaptive with respect to subcellular physiological processes or survival of the cell in complex environments. Here, I will review what we have learned about the mechanical properties of bacteria, beginning with measurements of whole-cell mechanical properties and proceeding to those of each subcellular material. Instead of simply listing the absolute quantitative values of mechanical properties (stiffness, viscosity, etc.), I will focus on discussing the **constitutive properties** (Box 1.1) of the cell and its subcellular materials, that is, the functional form of the quantitative dependence of the deformation of a material on the forces applied to it. Along the way, I will highlight the current methods available for assaying bacterial mechanics. Finally, in each case, I will discuss the relevance of the mechanical properties to cellular physiology.

Box 1.1: A Brief Glossary of Mechanics

Mechanical Stress (σ): A force distributed over an area. Stress has dimensions of pressure.

Mechanical Strain (ε): The degree to which a material is stretched. Strain has no dimensions – it is a fractional change in length, area or volume.

Constitutive property: The specific, quantitative relationship between the magnitude of deformation of a material and the

magnitude of mechanical stresses applied to it.

Elastic: Possesses the simplest constitutive relationship for a solid material in which strain is proportional to stress, $\sigma = E\varepsilon$, and deformation is reversible when the force is removed. E is the "Young's Modulus" and has dimensions of pressure.

Nonlinear elastic: Possesses a constitutive relationship in which deformation increases with applied stress but not proportionally.

Strain-stiffening: A specific type of nonlinear elasticity in which the amount of additional force required to stretch a material a given amount increases as the material is stretched.

Viscoelastic: Has properties of both a solid and a liquid. A viscoelastic material behaves as a solid immediately after a stress is applied, but flows like a liquid after longer periods.

Plastic: Deforms irreversibly if the applied stress exceeds a certain threshold or is applied for long enough. A plastic material is a solid.

Glass: A material that behaves as a viscous liquid or rubbery material above a certain critical temperature and a brittle solid below it.

Anisotropic: Possesses different structural and mechanical properties in different directions.

Flexural Rigidity (κ): The degree to which a material (like a cell) resists bending when a deflection force is applied to it. Flexural rigidity has dimensions of force times area.

1.2 Mechanical Elements of the Bacterial Cell

Most readers of this chapter will be familiar with the structural components of the bacterial cell. I will briefly outline the bacterial cell features relevant to the topic of mechanics.
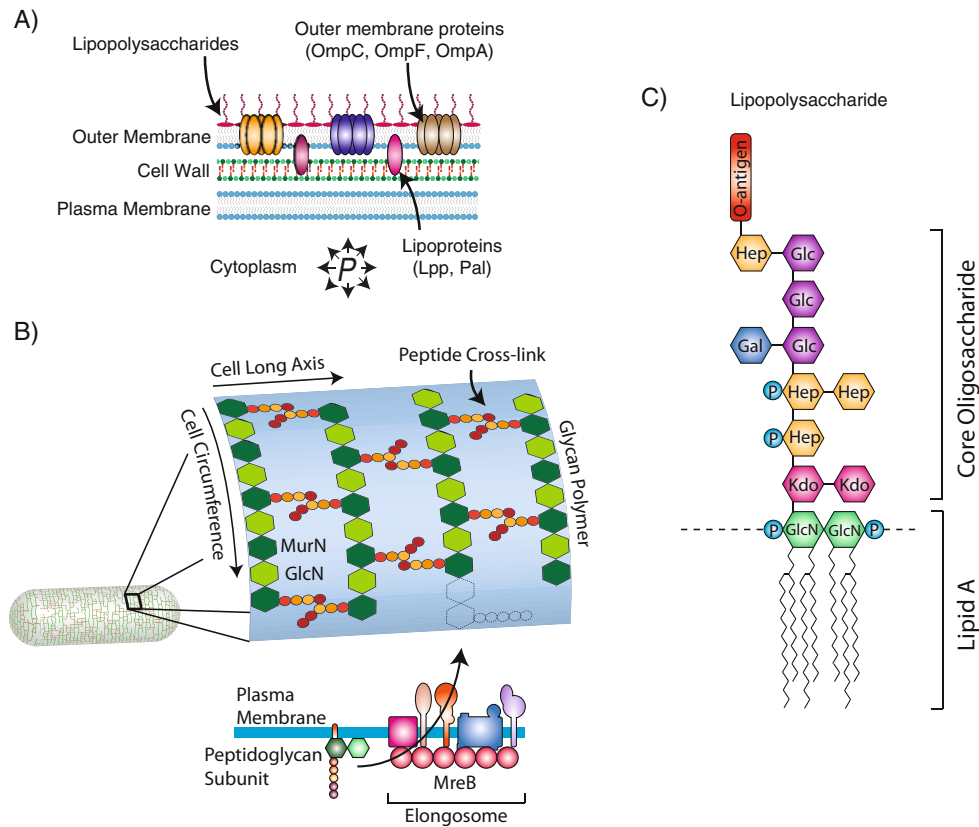
Fig. 1.1 The bacterial cell envelope. (**a**) Layers of the Gram-negative bacterial cell envelope, including proteins that bind the outer membrane. *P* turgor pressure. (**b**) Peptidoglycan and the peptidoglycan biosynthetic machinery, i.e., the "elongosome." *GlcN* N-acetyl glucosamine, *MurN* N-acetyl muramic acid. Adapted from Cho et al. 2014. (**c**) *E. coli* K12 lipopolysaccharide. *P* phosphate, *Kdo* 3-deoxy-D-*manno*-oct-2- ulosonic acid, *Hep* L-*glycero*-D-*manno*-heptose, *Glc* glucose, *Gal* galactose

Bacteria are small and, as mentioned above, it is likely that their small size is selected for by nature. As a result, bacterial cells must concentrate all the biomolecules required for life into a very small volume, resulting in a large concentration differential between the inside and the outside of the cell. This differential results in a hydrostatic, osmotic pressure, called the turgor pressure (Fig. 1.1), which is between about 1 atm (for Gram-negative bacteria; Deng et al. 2011) and 10 atm (for Gram-positive bacteria; Whatmore and Reed 1990) above atmospheric pressure. The highly concentrated nature of the cytoplasm, and the resulting turgor pressure, has important consequences for both the mechanical properties of the cytoplasm and those of the cell envelope.

For the sake of this review, the cell envelope (Fig. 1.1) will be defined as all the layers of the cell surface *outside* the plasma membrane. The cell wall is superficial to the plasma membrane, and is primarily composed of peptidoglycan, a covalently cross-linked network of polysaccharides and short peptides (Fig. 1.1b). Depending on the species, other components of the cell envelope may also be present. For example, Gram-positive bacteria covalently attach teichoic acids – anionic polymers of alternating phosphate and sugar-alcohol residues – to their cell walls. Besides the cell wall, Gram-negative bacteria additionally possess an outer membrane (Fig. 1.1a, c); the inner leaflet of the outer membrane is composed of phospholipids but the outer leaflet is composed of lipopolysaccharides, which are themselves complex molecules that possess acyl chains, phosphate, and several sugar moieties (Fig. 1.1c). Largely due to the phosphate groups, the outer membrane is highly anionic and thus binds cations such as magnesium, which stabilize the membrane by preventing repulsive in-

teractions between the lipopolysaccharides. Like the plasma membrane, the outer membrane is rich in proteins, especially β-barrel porins, and several abundant proteins such as Lpp, OmpA, and Pal also link the cell wall and the outer membrane (Fig. 1.1; Sonntag et al. 1978; Mizuno 1979). Other Gram-indeterminate bacteria, such as the *Mycobacteria* also have outer membrane-like structures, but with different chemical composition.

Many bacteria have still other envelope layers, such as S-layer and capsule, but very little is known about the mechanical contributions of these structures and so I will not treat them in this review.

1.3 Whole-Cell Measurements

1.3.1 Cell Bending

Galileo Galilei became the first quantitative biophysicist when he astutely applied his new theory of bending beams to animal bones (Galilei 1914). It then seems appropriate to begin our discussion of the new bacterial biomechanics with similar bending experiments at the microscale performed almost 400 years later. These experiments were enabled by inventive applications of optical tweezers (Wang et al. 2010) and microfluidics (Amir et al. 2014). In the optical tweezers assay, a positively charged polysterene microsphere was suspended with a laser, and was then used to apply and measure bending forces to filamentous *E. coli* cells that were adhered to a cover glass at one end (Fig. 1.2a). This was performed on an inverted microscope, which allowed simultaneous measurement of the deflection of the cell. In the microfluidic assay, filamentous *E. coli* cells (created by genetically inhibiting cell division) were grown in long dead-end channels (Fig. 1.2b). When one end emerged from the open end of the channel, they were subjected to an orthogonal fluid flow; displacement was measured and force was calculated using the theory of viscous drag. Both studies observed **elastic** (Box 1.1) deformation in response to short periods of deformation. By treating the cell as an elastic rod, the **flexural rigidity** (Box 1.1) of the cell could then be calculated, and the two measurements agreed well with each other (3×10^{-20} and 5×10^{-20} Nm^2, respectively). The molecular basis for this bending rigidity will be discussed later (see *Cytoskeletal proteins* and *Outer membrane*).

The microfluidics-based assay was also used to question whether the cell as a whole had **plastic** properties (Box 1.1): if you bent a cell for long enough, or applied large enough forces, would the cell stay bent? It was found that *E. coli* cells were indeed plastic, but that this plasticity required deformation on the time scale of the cell cycle and that cell growth had to be taking place during this period for plasticity to occur. That is, growth seemed to "fix" deformation in place. Conversely, growth also was able to straighten cells on similar time scales once the force was released. These data agreed with qualitative and quantitative studies observing the growth of *E. coli* cells trapped and released in micron-scale wells (Takeuchi et al. 2005).

Because the shape of the bacterial cell is conferred by the peptidoglycan cell wall, the connection between plastic deformation and active cell growth points to an intimate connection between the mechanical properties of the bacterial cell (its plasticity) and the physiology of cell-shape maintenance via peptidoglycan synthesis. To sum, cell shape, an adaptive feature of bacteria (Young 2006), is robust to mechanical forces that act over time scales that are less than the time scale of cell growth (approximately the doubling time), yet if forces are applied for longer cell shape will conform to the mechanical environment. This is likely adaptive as well, allowing bacteria to grow in highly constrained environments (Takeuchi et al. 2005; Männik et al. 2009).

1.3.2 Cell "Squeezing"

Another microfluidic device was developed to assay whole-cell mechanics by measuring how far cells could be driven into wedge-shaped traps as a function of applied pressure (Fig. 1.2c; Sun 2014a). This assay could distinguish between the

Fig. 1.2 Methods for measuring the mechanical properties of bacterial cells
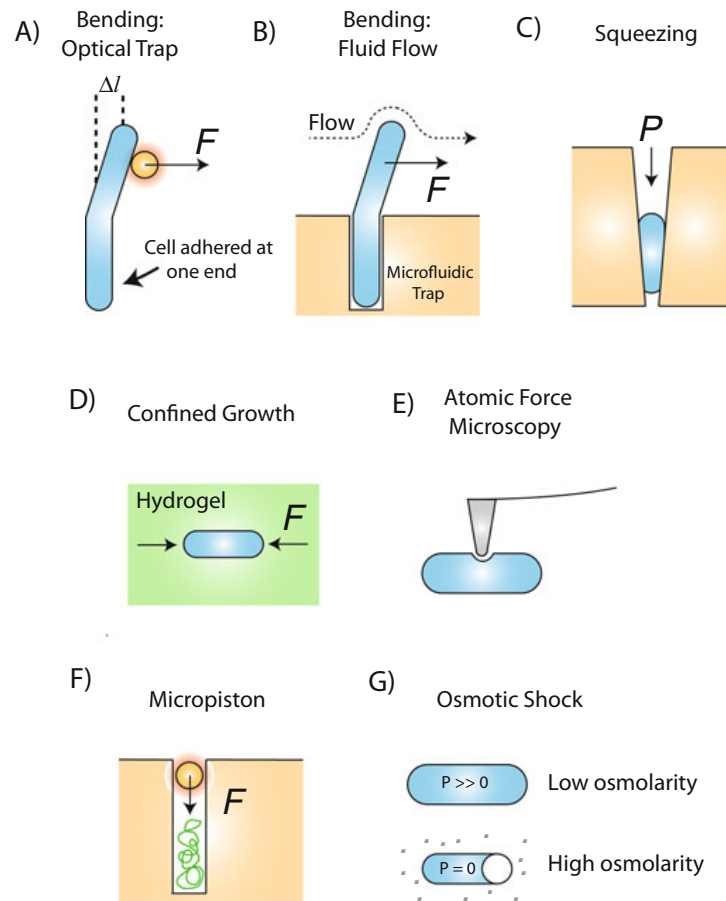
model Gram-negative and Gram-positive bacteria, *E. coli* and *B. subtilis*, with *E. coli* cells moving ≈20–50% further into the wedges, depending on pressure. That is, *E. coli* cells are "softer" than *B. subtilis* cells. While many factors could be mediating cell softness viz-à-viz this assay, this result is consistent with *E. coli* cells having less stiff cell envelopes and lower turgor pressures than *B. subtilis* cells. Thus, while more sophisticated modeling would be required to assess the material properties of the cell quantitatively from this assay and compare them with those obtained by other assays, the "squeezing" assay does provide a relative measurement of whole-cell mechanical compliance. Physiologically, this compliance is surely related to the ability of *E. coli* cells to grow through constrictions well below their typical cell diameter in search of nutrients (Männik et al. 2009), which could provide an adaptive value in natural niches with similar spatial constraints, such as microvilli in the gut.

1.3.3 Growing Cells in Agarose Hydrogels

Whole-cell mechanics were also assayed indirectly by embedding growing cells in an agarose gel and measuring the effect of the gel on single-cell growth (Fig. 1.2d; Tuson et al. 2012). The gel reduces growth rate to a degree that increases with increasing gel stiffness; analytical and finite-element based models were used to quantitatively calculate the stiffness of the whole cell. As for the "squeezing" assay, *B. subtilis* had a modestly higher stiffness (about twofold) than *E. coli*; interestingly, though, the stiffness of *P. aeruginosa*, a Gram-negative bacterium, was similar to that of *B. subtilis*.

While the meaning of whole-cell stiffness is unclear physiologically, the key advantage of the hydrogel assay is that it was easily adaptable to a high-throughput format, allowing cell mechanics to be measured across a non-essential gene

deletion library, thereby connecting genetics and mechanics in bacteria for the first time (Auer et al. 2016). What genes contributed significantly to cell stiffness? As expected, deletion of genes involved in cell envelope synthesis often resulted in less stiff cells. These included the top hit, *mrcB*, a gene that encodes PBP1b, a protein that plays a key role in the incorporation of peptidoglycan subunits into the cell wall (Fig. 1.1b). However, cell-envelope related genes accounted for only a fifth (9/46) of the hits in the screen. Other highly represented categories of genes included genes involved in energy production and DNA replication. How genes from these latter categories affect stiffness is an open question. It is possible that they do so through an effect on turgor pressure; lower turgor would lower the force that the cell could exert on the hydrogel, potentially resulting in a slower growth and therefore a lower effective value of cell stiffness. However, a surprising finding in this study was that de-energizing cells using the uncoupler CCCP made cells stiffer. The mechanistic basis for this was not explored.

1.3.4   Atomic Force Microscopy

Atomic force microscopy (Fig. 1.2e) has been used extensively to measure the mechanical properties of whole bacterial cells. In this method, a microscopic cantilever is used to locally indent the cell, and force-displacement relationships are calculated by measuring the degree to which the cantilever bends as a function of indentation distance. The effective "stiffness" of the cell assayed using AFM is dependent on many factors, including the turgor pressure and the mechanical properties of all of the envelope components. Thus, while it is difficult to dissect the molecular basis for whole-cell AFM measurements, this method has been useful for making phenomenological measurements of whole-cell mechanics. Both **viscoelastic** and **plastic** (Box 1.1) properties have been reported (Vadillo-Rodriguez and Dutcher 2009; Gaboriaud et al. 2008). Furthermore, AFM has been used to measure the effects of various antimicrobial agents on global cell mechanics. For example, it has been observed that bacteriophage,

EDTA, chitosans, and antibiotics each have a softening effect on bacteria (Chen et al. 2009; Eaton et al. 2008; Perry et al. 2009; Francius et al. 2008).

1.4   The Cytoplasm

I will now discuss what is understood about the mechanical properties specific to each subcellular component of the bacterial cell, beginning with the cytoplasm and ending with the outer membrane.

The cytoplasm of bacteria is composed of about ≈70% water, 15% proteins, and 7% nucleic acids by mass (Todar 2006), with the remaining content composed of sugars, ions and other small molecules. Thus, it stands to reason that any deviation of the mechanical properties of the cytoplasm from the incompressibility of water is due largely to proteins, with potential lesser contributions from DNA and RNA. Few studies have addressed the mechanics of the bacterial cytoplasm directly, and the wealth of data concerning the mechanics of the eukaryotic cytoplasm is irrelevant since it is clearly dominated by the properties of the cytoskeleton (Janmey 1991), which is absent in bacteria despite the presence of homologues of the eukaryotic cytoskeleton proteins.

1.4.1   Gross Mechanical Properties of the Cytoplasm

The bacterial cytoplasm is largely composed of water and, as such, has fluid properties: the shape of the cell is determined by the geometry of the cell wall, which is adopted by the cytoplasm. However, more complex mechanical properties have been inferred from detailed experiments in which the motion of fluorescent molecules in the cytoplasm was tracked. Several such experiments found that many molecules move "sub-diffusively" through the cytoplasm: whereas a molecule exhibiting a Brownian random walk in a fluid would diffuse away from its initial position with the mean squared displacement proportional

to time, MSD $\sim t$, molecules undergoing sub-diffusive motion move randomly with MSD $\sim t^{\alpha}$, where $\alpha < 1$ (Metzler et al. 2014).

Sub-diffusive motion can result from a number of causes, including obstruction by the cytoskeleton (in eukaryotic cells; Saxton 1994) and "molecular crowding," that is, when macromolecular concentration is approximately equal to that of free water. However, in one case sub-diffusion pointed directly to the mechanical properties of the bacterial cytoplasm. In this study, chromosomal loci were observed to move sub-diffusively, with MSD $\sim t^{0.4}$ (Weber et al. 2010). This could not be explained by obstruction by the cytoskeleton, nor could the constraint imposed on the locus by the chromosome itself account for all of the effect. The key result that elucidated the basis of the sub-diffusive motion was that the direction of locus motion was anti-correlated with the direction of motion less than one second in the past. This suggested that the loci were "rebounding" from the cytoplasm, that is, the cytoplasm is **elastic** (Box 1.1) at time-scales less than a second and therefore the cytoplasm has **viscoelastic** properties (Box 1.1) as a whole. Similar results were obtained with RNA-protein complexes. While this study clearly demonstrated viscoelasticity, technical limitations prevented the calculation of the elastic or loss moduli from these data, preventing quantitative comparison to that of other cellular components or to the eukaryotic cytoplasm.

It is unlikely that there is a specific adaptive value (or cost) of sub-diffusive motion in bacteria. Rather, given their small size, sub-diffusive motion is almost certainly not selected against strongly since diffusing species explore the entire cell within milliseconds (Milo and Phillips 2015). However, if bacteria are selected to be small, and viscoelasticity results from crowding effects, then viscoelasticity is an inherent byproduct of selection. While viscoelasticity inhibits molecular transport via diffusion, this inhibition is acceptable evolutionarily so long as there are not great distances over which transport needs to occur.

Observation of sub-diffusive motion revealed that whether or not the cytoplasm behaved as a solid or a fluid depended on the time-scale at which its mechanics are assayed (Weber et al. 2010). A separate study demonstrated that cytoplasmic mechanical properties also depend on the length-scale at which they are assayed by tracking the cytoplasmic motion of particles of a range of sizes. "Anomalous diffusion," in which the distribution of random step sizes was not Gaussian, as would be expected from Brownian diffusive motion, was observed for particles larger than 30 nm (Parry et al. 2014). The distribution of random motion was also heterogeneous within the same cell, that is, different particles obeyed different distributions of random motion. These properties suggested that the cytoplasm has **glass-like** properties (Box 1.1), whereby even though it is a fluid, that it is "close to" a fluid-to-solid phase transition. What does "close to" mean? Remarkably, de-energizing cells with metabolic uncouplers resulted in highly constrained diffusion of large particles, characteristic of a solid. This is consistent with earlier results demonstrating that metabolism increased the mobility of chromosomal loci (Weber et al. 2012). That is, metabolism "fluidizes" the cytoplasm, allowing large particles to explore the entire volume of the cell.

Because the random motion of particles underlies biochemical reactions, it is very likely that the specific characteristics of the glassy cytoplasm, such as the degree to which metabolism can fluidize it, is highly adaptive. Biomolecules that undergo biochemistry must be able to move randomly in the cytoplasm and thus the cytoplasm must stay in the fluid phase with respect to the size scale of these molecules. It was suggested that this may set an upper limit to the size of biomolecules in the cell. Since the glassy nature of the cytoplasm is a direct result of molecular crowding, there may also be an evolutionary trade-off between cell size and how glassy (how close to the solid phase) the cytoplasm is.

1.4.2 Cytoskeletal Proteins

While bacteria do not have cytoskeletons, that is, cytoplasmic polymeric networks that can bear load and actuate forces, they do have homologs of

the three main eukaryotic cytoskeleton proteins: actin, microtubules, and intermediate filaments, that perform other functions (Shih and Rothfield 2006). It was natural, then, to question whether these proteins contribute to the mechanical properties of the cell. This was done in the case of MreB, the actin homolog found in most rod-shaped bacteria (Margolin 2009). MreB forms short (<200 nm) polymers (Billaudeau et al. 2019) that bind to the plasma membrane and orchestrate peptidoglycan synthesis, likely by acting as a scaffold for various biosynthetic enzymes (Fig. 1.1b; Shi et al. 2018). Depolymerization of MreB using a chemical inhibitor, A22, causes aberrant peptidoglycan synthesis and loss of the cell's rod shape (Gitai et al. 2005).

The contribution of MreB to cell mechanics has been probed in several ways. First, optical tweezers were used to bend *E. coli* cells and measure force-displacement curves (Fig. 1.2a; Wang et al. 2010). By treating the cell as an elastic rod, the **flexural rigidity** (Box 1.1) of the cell was then calculated. Remarkably, when MreB was depolymerized with A22, the flexural rigidity was reduced by ≈50%, and this effect was rapidly reversible by washing out the inhibitor. At the time of this study, it was thought that MreB formed a helical "cytoskeleton" that ran the length of the cell, and it was shown theoretically that this would be sufficient to confer the observed contribution to the flexural rigidity of the cell. We now know that MreB forms many short independent filaments rather than a single helix (Garner et al. 2011; Domínguez-Escobar et al. 2011; van Teeffelen et al. 2011). As such, it remains unclear how MreB is mechanically coupled to the cell envelope at the molecular scale such that it can contribute so strongly to flexural rigidity. It is possible that flexural rigidity depends not only on the structural state of the cell but also on active cell wall synthesis such that rigidity decreases when this process is perturbed with A22; this idea would be straightforward to test.

While MreB makes a strong contribution to flexural rigidity, its contributions to other modes of deformation are more modest. In the cell squeezing experiment (Fig. 1.2c), A22 caused the cells to move marginally more (≤10%)

into the traps than untreated cells, depending on pressure (Sun 2014a). The effect of A22 on cell growth in an agarose hydrogel (Fig. 1.2d) is negligible (Tuson et al. 2012). Since the squeezing assay, but not the hydrogel assay, causes slight bending of the cell envelope, together these data suggest that MreB's sole contribution to cell mechanics could be to confer flexural rigidity to the envelope. Combined with the fact that applying bending forces to cells over many minutes caused a persistent, plastic deformation, MreB then maintains the cell's straight rod shape in two independent ways: biochemically by coordinating organized peptidoglycan synthesis and biomechanically by restricting bending.

The contributions of the other cytoskeleton homologues, FtsZ (a tubulin homolog) and crescentin (an intermediate filament homolog) to global cell mechanics have not been measured but they are less likely to be important since they form highly localized polymers that govern specific physiological functions: division and morphogenesis, respectively. Furthermore, while it is clear that polymers of both proteins can apply forces (Osawa et al. 2008; Cabeen et al. 2009), it is unclear whether these forces, per se, are important for their function.

1.4.3 The Chromosome

The mechanics of DNA in various contexts has been studied extensively (Benham and Mielke 2005). However, it is important to study the chromosome *in situ* to understand its physiology. This was done for *E. coli* by developing a hybrid microfluidics/optical tweezers assay (Fig. 1.2f; Pelletier et al. 2012). In this experiment, single bacterial cells whose chromosomes had been fluorescently labeled were trapped in long dead-end microfluidic channels, similar to those used in the microfluidic bending experiment described above. Once trapped, they were subjected to a lysis buffer that released their cytoplasm and allowed their chromosomes to expand to their rest lengths, which was found to be approximately tenfold longer than their confined lengths in the cell. The rate of expansion and

chromosome morphology depended heavily on the physiological state of the cell, with exponentially growing cells possessing globular chromosomes that expanded slowly while those from stationary phase cells possessed featureless chromosomes that expanded rapidly. Although not explicitly shown, it was suggested that these differences were due to the effect of the physiology of the chromosome (i.e., transcription and replication) on its mechanical properties.

The chromosome can be thought of as an "entropic spring": when it is confined to a small volume it gives rise to a pressure similar to that created when a gas is trapped in a balloon. By using optical tweezers to create a "micropiston" in the microfluidic channel, this magnitude of this pressure could be measured directly (Fig. 1.2f). It was found that even when the chromosome was confined to its *in vivo* size (i.e., tenfold compression), this created a pressure that was still only about one thousandth as large as the turgor pressure within the cell. This implies that the chromosome is extremely soft compared to the cell envelope, which bears the turgor pressure. In fact, it was demonstrated that forces arising from molecular crowding in the cytoplasm are alone enough to cause compaction of the chromosome to the *in vivo* size. These forces result from the fact that the chromosome excludes many proteins from within its volume, causing them to exert an entropic, osmotic pressure on the chromosome and compact it. That is, the chromosome may not even require constraint by the cell envelope!

While the mechanical properties of the chromosome are clearly correlated with the physiological state of the cell, it needs to be determined whether these properties are just a consequence of that physiology or also dictate certain physiological processes.

1.5 The Plasma Membrane

The plasma membrane is perhaps the best-studied biological structure from a biomechanics perspective, and yet there has been very little investigation into the specific mechanics of the bacterial plasma membrane. The clear exception

to this statement is that the properties of stretch-activated ion channels, which are mechanically gated channels that are thought to mediate turgor pressure relief in bacteria, have been extensively studied (Martinac 2004). While mechanics at the protein level is not the focus of this review, it is likely that these ion channels actually do contribute to the gross mechanical properties of the membrane by providing "slack" (Rojas et al. 2017). When the genes encoding stretch-activated ion channels in *B. subtilis* are deleted, moderate (0.5 M) hypoosmotic shocks cause cells to swell and lyse (Hoffmann et al. 2008). However, when channels are present, cells can survive enormous (1.5 M) hypoosmotic shocks, and swell to sizes well beyond those that would cause them to lyse in the absence of channels (Rojas et al. 2017). Notably, the presence of channels does not cause cells to shrink after hypoosmotic-shock induced swelling, that is, there is no evidence that the channels actually relieve turgor pressure after shocks *in vivo*. This suggests that the role of the ion channels to "soften" the membrane by decreasing the force experienced by the phospholipid bilayer for a given extension, thereby preventing rupture. While consistent with the observed data, this mechanism needs to be tested more thoroughly.

A micropipette aspiration experiment explicitly measured the mechanical properties of the plasma membrane in *E. coli* spheroplasts (wall-less and outer membrane-less cells; Sun et al. 2014b). This measurement revealed that, as opposed to pure phospholipid bilayers, the *E. coli* membrane had viscoelastic properties, with stress relaxation occurring in about a minute. The *E. coli* membranes were also about twice as soft as pure phospholipid bilayers. While a "membrane reservoir" was invoked to rationalize these results, the protein content of the membrane, including stretch-activated ion channels, could also explain them. In any case, in concert with the ion channels, its soft viscoelastic nature likely underlies the plasma membrane's ability to accommodate large deformations and therefore confers a fitness advantage in fluctuating osmotic environments, which many bacteria regularly endure in the gut, the soil, or in natural bodies of water.

1.6   The Cell Wall

If the bacterial plasma membrane is the least well studied bacterial structure from a mechanics perspective, the cell wall is certainly the best studied. This is because the cell wall serves two primary, critical roles that are both mechanical in nature: (i) it protects the cell from osmotic lysis and (ii) it confers shape to the cell. To accomplish these functions it has to be relatively strong (resistant to rupture) and it has to be a solid material. As such, across species, the cell wall is a covalently cross-linked macromolecule. However, while peptidoglycan is the major component of the cell wall in most bacterial species, the specific chemical composition of the wall and its microscopic structure is dependent on taxum, and this has important consequences for its mechanical properties.

A key difference between the mechanics of the cell wall and other materials in the cell is that the wall is **anisotropic** (Box 1.1), and this feature is directly dependent on its microscale architecture and directly related to its function. The glycan polymers, which are thought to be stiffer than the peptide species, are oriented circumferentially around the cell axis (Fig. 1.1b; Verwer et al. 1978; Gan et al. 2008). Two studies confirm that cell-wall stiffness is anisotropic, with a larger stiffness in the longitudinal direction (parallel to the cell axis) than in the circumferential direction. First, cell walls of *E. coli* were isolated and placed on a substrate with microscopic grooves (Yao et al. 1999). Then, atomic force microscopy was used to measure the force required to indent the cell walls into the grooves (similar to the way that standing on a trampoline causes an indentation in the trampoline surface). Importantly, this force depended on which way the cell walls were laying across the groove: the force was higher if the long axis of the wall was parallel to the groove. By using simple mechanical equations, the stiffness in the circumferential direction was found to be ≈80% higher than that in the longitudinal direction. This agreed qualitatively with a second study that used large hyperosmotic shocks (Fig. 1.2g) to plasmolyze the cells, thereby relieving their turgor pressure, and then measured the resulting contraction in the circumferential and longitudinal directions (Rojas et al. 2014). Although the contractions were roughly equal in both directions, the mechanical stress in the circumferential direction is twice as large as that in the longitudinal direction for a pressurized cylindrical surface, such as a rod-shaped cell (Love 2013). That is, the stiffness of the envelope in the circumferential direction was about twice as stiff as that in the longitudinal direction.

The circumferential orientation of glycan polymers in the cell wall is due to their oriented synthesis by the "elongosome" complex (Fig. 1.2b), which moves circumferentially along the plasma membrane as it catalyzes glycan synthesis (Garner et al. 2011; Domínguez-Escobar et al. 2011; van Teeffelen et al. 2011). When this oriented motion is chemically perturbed (but biosynthesis is allowed to continue), the cell loses its rod shape and grows amorphously (without specific shape; Gitai et al. 2005). This demonstrates that the microscopic anisotropy of the wall, and probably the resulting mechanical properties, are critical for rod-shaped maintenance.

In addition to anisotropy with respect to the circumferential and axial directions, the cell wall also likely has *helical* anisotropy. In one creative experiment, charged microspheres were adhered to opposite ends of a filamentous *E. coli* cell and it was found by measuring the relative motion of these microspheres that cells "twist" as they grow (Wang et al. 2012). Furthermore, when turgor pressure was relieved in these cells by hyperosmotic shock, the cell envelope twisted in the opposite direction as the cell envelope contracted. A mechanical model of cell wall expansion demonstrated that both the helical anisotropy and the twisting growth could result from the fact that the elongosome complexes (Fig. 1.1b) actually move with a slight helical pitch with respect to the axis of the cell (Garner et al. 2011; Domínguez-Escobar et al. 2011; van Teeffelen et al. 2011).

In addition to being highly anisotropic, the cell wall also has highly **nonlinear elastic** properties (Box 1.1). This was demonstrated most clearly by a delicate atomic force microscopy (Fig. 1.1e) experiment in which force-distance responses were measured for single *E. coli* cells (Deng et al.

2011). This measurement was performed on intact cells and on cell wall-less blebs that were induced pharmacologically, allowing for the direct measurement of turgor pressure. Additionally, applying a complex mechanical model allowed the specific mechanical properties of the cell wall to be calculated *in vivo* for the first time (rather than measuring the properties of the whole cell or the isolated cell wall). It was found that the cell wall exhibited a high degree of **strain-stiffening**, in which the additional force required to make incremental deformations increased continuously as the deformation increased (Box 1.1). This result was consistent with osmotic shock-based experiments whereby hyperosmotic shocks (relieving turgor pressure, Fig. 1.1g) caused much more contraction than hypoosmotic shocks (increasing turgor pressure) of the same magnitude caused extension of the cell wall (Rojas et al. 2014, 2017). In fact, large hypoosmotic shocks caused no extension of the *E. coli* cell envelope, although rapid down-regulation of turgor pressure by stretch-activated ion channels could also account for this result.

While the specific advantage of the cell wall's nonlinear properties has not been explicitly tested, it is reasonable to speculate that these properties are important for the wall's function in protecting the cell from osmotic lysis. If the wall is present, then any extension of the plasma membrane will be limited by the extension of the cell wall that surrounds it. That the *E. coli* cell envelope is essentially inextensible beyond the length prescribed by steady-state growth guarantees that the plasma membrane is never stretched *in vivo* by osmotic fluctuations, making it virtually impossible to lyse the cell except by chemically undermining the envelope or the plasma membrane itself.

1.7 The Outer Membrane

Historically, it was textbook dogma that the cell wall was the dominant mechanical element in the cell (Madigan et al. 1997), but this view came into question after several anomalous observations suggested that the outer membrane could

also bear significant loads. First, the outer membrane is not a fluid (in the plane of the membrane) like the plasma membrane; this was concluded from the observation that outer membrane proteins do not freely diffuse in the membrane (Rassam et al. 2015). Rather, it is likely that the outer membrane is an "ionic hydrogel," a solid phase wherein neighboring divalent anionic lipopolysaccharide molecules are bound to one another via divalent magnesium cations (Herrmann et al. 2015). Second, it was discovered that a protein complex that disrupts the outer membrane was required for cell lysis by bacteriophage λ (Berry et al. 2012). Without these proteins, the phage could digest the cell wall, causing spheroplasts to form, thereby transferring the entire load imposed by turgor pressure to the two membranes, but the cells did not lyse, suggesting that the outer membrane was bearing the load. Finally, it was demonstrated that treatment of *E. coli* cells with vancomycin, a cell wall-targeting antibtiotic caused blebbing of the protoplast (the plasma membrane and cytoplasm); that is, the protoplast escaped from the cell wall. However, this did not immediately cause cell lysis, which took minutes to hours after protoplast escape (Yao et al. 2012). Similar phenomena have now been observed many times in response to drug treatment of bacteria.

The mechanical properties of the *E. coli* outer membrane were explicitly assayed by using several methods to apply forces to the entire cell envelope (i.e., the composite cell wall-outer membrane complex), measuring the deformation in response to these forces, and then repeating these experiments while perturbing the outer membrane via a variety of chemical and genetic techniques to determine if the deformations were greater than they were in the absence of perturbation (Rojas et al. 2018). For example, when turgor pressure was depleted by subjecting cells to a large hyperosmotic shock, the cell envelope contracted, as expected. However, when the outer membrane was subsequently dissolved by subjecting the shocked cells to a detergent, the naked cell wall contracted again, and the two contractions were roughly equal. This suggested that the outer membrane, which is connected to

the cell wall by numerous proteins (Fig. 1.1a), was stabilizing the cell wall above its rest length after hyperosmotic shock; in order to do this it had to be bearing compressive stress commensurate with that borne by the composite cell envelope during the turgid state. A simple mathematical analysis revealed that, remarkably, the outer membrane was *at least* as stiff as the cell wall. Atomic force microscopy, a cell bending assay, and another osmotic shock assay yielded similar results. It was expected that the anionic lipid-A moiety of lipopolysaccharide (Fig. 1.1c) would be the primary load bearing species within the outer membrane because it can form intermolecular bonds in the presence of divalent cations. While this hypothesis was correct, the protein and sugar species within the outer membrane also contributed greatly to outer membrane stiffness. Finally, the Tol-Pal complex, which binds both the outer membrane and cell wall, was found to be an essential mechanical link between the two structures.

The stiffness of the outer membrane raises the obvious possibility that, like the cell wall, this structure is critical for prevention of osmotic lysis of the cell. Interestingly, it was found that during steady-state growth in chemostatic conditions, the outer membrane was under no load, even though the cell wall is highly stretched (Rojas et al. 2018). This was found by digesting the cell wall, measuring the surface area of the remaining outer membrane, and comparing this area to the surface area of cell before cell wall digestion; the areas were precisely equal. However, during osmotic fluctuations, the outer membrane was mechanically engaged and strongly protected the cells from lysis. Furthermore, outer membrane stiffness was important for L-form proliferation. L-forms are wall-less bacteria that proliferate in the presence of β-lactam antibiotics as long as the medium osmolarity is high (Lederberg and Clair 1958), and they are thought to be important to the pathology of several bacteria such as those that cause urinary tract infections (Errington et al. 2016). As hypothesized, L-form proliferation was drastically inhibited upon chemical or genetic perturbation of outer membrane stiffness.

1.8   Conclusion and Outlook

Like the mechanics of non-living material hundreds of years ago, within the last 10 years the field of bacterial mechanics began with simple yet important questions: how do bacterial cells deform when you bend, poke, squeeze and deflate them? The measurements that have addressed these questions clearly point to the fact that "bacterial materials" are mechanically rich: they include at least elastic, nonlinear elastic, viscoelastic, and plastic materials. We will surely look back on these seminal measurements just as D'arcy Thompson fondly remembered the scientists who made fundamental mechanical measurements of non-living material (Thompson 1992).

In one sense, bacteria are ideal systems with which to study the mechanics of living material because the many molecular tools available allow us to make very fine-scale perturbations to the chemical composition and architecture of the materials that constitute the cells. Additionally, bacteria are incredibly diverse in terms of their subcellular material. While *E. coli* has been used as a model system for most of the mechanical studies described above, we stand to find ever more novel materials by expanding our scope to other bacteria; just as each bacterial species has a metabolic ecological niche, so does it have a mechanical niche for which its mechanical properties are highly adapted. A good example is *Myxococcus xanthus*, which uses a unique gliding mechanism of motility to assemble into multicellular communities (Zhang et al. 2012). It is apparent from single-cell time lapse micrographs that *M. xanthus* cells are easily deformable and a theoretical analysis suggests that the flexibility of the cells is critical for their multicellular organization (Harvey et al. 2011). There are surely myriad other examples than this and the ones reviewed above where mechanical properties are adapted specifically for specialized physiological processes. Finally, even within the best-studied systems, many materials are waiting to be probed mechanically: teichoic acids, capsule, and the S-layer, for example.

From a different perspective, bacteria are the most challenging systems with which to study mechanics of living material because their size often inhibits our ability to make precise measurements of their mechanical properties, especially *in vivo*. But this is then a call for highly innovative developments in experimental technology to enable the measurement of mechanical properties as precisely as we can tune them. This will require continual collaboration between microbiologists and experimental soft condensed matter physicists.

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