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Regulation of microbial growth by turgor pressure

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Rapid changes in environmental osmolarity are a natural aspect of microbial lifestyles. The change in turgor pressure resulting from an osmotic shock alters the mechanical forces within the cell envelope, and can impact cell growth across a range of timescales, through a variety of mechanical mechanisms. Here, we first summarize measurements of turgor pressure in various organisms. We then review how the combination of microfluidic flow cells and quantitative image analysis has driven discovery of the diverse ways in which turgor pressure mechanically regulates bacterial growth, independent of the effect of cytoplasmic crowding. In Gram-positive, rod-shaped bacteria, reductions in turgor pressure cause decreased growth rate. Moreover, a hypoosmotic shock, which increases turgor pressure and membrane tension, leads to transient inhibition of cell-wall growth via electrical depolarization. By contrast, Gram-negative *Escherichia coli* is remarkably insensitive to changes in turgor. We discuss the extent to which turgor pressure impacts processes such as cell division that alter cell shape, in particular that turgor facilitates millisecond-scale daughter-cell separation in many Actinobacteria and eukaryotic fission yeast. This diverse set of responses showcases the potential for using osmotic shocks to interrogate how mechanical perturbations affect cellular processes.

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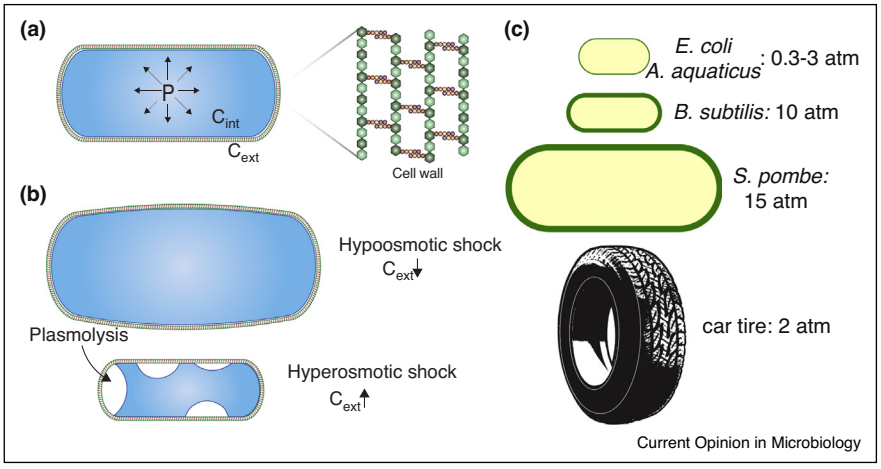
Introduction

In walled organisms such as bacteria, cell volume and surface area are defined by the size and shape of the cell envelope, including the membrane(s) and the cell wall [1]. Therefore, expansion of the cell envelope is the ultimate process that determines the rate of cell growth. The envelope is inflated by turgor pressure, the intracellular hydrostatic pressure that results from the osmotic potential (concentration differential) across the membrane, which is balanced by mechanical stress in the cell envelope (Figure 1a). Since water is the primary cytosolic component, and bacterial cells do not have active water transporters, cells rely on osmosis for water import during cell growth. Indeed, the idea that swelling due to osmosis is fundamental to cell growth is centuries old [2]. However, recent progress has aimed to understand deeper functional relationships between water activity and cell growth. These studies demonstrated that, in many cases, osmotic potential is not simply required for water influx, but is required to generate turgor pressure that is used as a mechanical driver of cell deformation during growth or as a feedback signal regulating cell growth.

In principle, turgor pressure could regulate growth directly via a variety of mechanisms; evidence from plants provides important starting points for microbial research. Classic experiments by Green and others demonstrated that turgor pressure drives controlled mechanical expansion of the plant cell wall during cell growth in a process equivalent to plastic deformation [3]. In plants, hydrolysis of the cell wall via the expansin enzymes weakens the cell wall and thereby leads to turgor-dependent expansion [4]; similar processes have been proposed to be at play in microbes [5]. The ability to insert cell-wall precursors could be dependent on the physical stretching of the wall, which has been hypothesized to affect the ability of the *Escherichia coli* outer membrane lipoproteins LpoA/B to activate their wall synthase partners PBP1A/B [6,7]. Mechanical stresses in the cell envelope could also affect transport of nutrients, and the opening of channels could lead to loss of proteins or small molecules important for growth. When hyperosmotic shock causes plasmolysis (separation of the cytoplasmic membrane from the cell wall; Figure 1b), any coupling between the insertion of new material into the cell wall and membrane could be disrupted since stretching would be differentially affected in the two layers (as their spring constants are likely to be different due to their material properties). In sum, turgor pressure has myriad opportunities to affect the rate of growth through biomass, cell-wall, and/or membrane synthesis and through mechanical stretching, and osmotic shock represents a unique tool to probe coupling among these processes. Nonetheless, it is also possible that the biochemistry of growth is insulated from changes in turgor. Here, we review and analyze the effects of turgor pressure on the growth and division rates of several bacterial species in order to elucidate

Figure 1



Large turgor pressures inflate the cytoplasm of walled organisms. **(a)** Outward expansion of the cytoplasm due to turgor pressure P is balanced by mechanical resistance from the cell envelope, including the cell wall. C_{int} and C_{ext} are the internal and external concentrations, respectively, that determine P through the Morse equation $P = RT(C_{int} - C_{ext})$. **(b)** Bacterial cells often transition between environments with large differences in osmolarity, such as the exit of enteric bacteria from the gut into fresh water. Hypoosmotic shock due to this sudden decrease in external osmolarity causes water influx and cell swelling. By contrast, hyperosmotic shock due to a sudden increase in external osmolarity causes water outflux and plasmolysis of the cytoplasm (separation of the membrane from the cell wall). **(c)** Estimates of turgor pressure in various species [17,18,20,22••] (thickness of green contours qualitatively represents cell-wall thickness), as compared with a car tire.

fundamental principles of the mechanics of bacterial growth.

The significance, magnitude, and measurement of turgor pressure

Bacterial species often inhabit and transition between environments with dramatically different osmolarities: obvious examples are the soil before and after a rainstorm, and the exit from the gut to fresh water regularly experienced by enteric bacteria. In both cases, cells experience a hypoosmotic shock in which the external environment becomes more dilute, causing water to flow into and swell the cell to equilibrate internal and external concentrations (Figure 1b). Bacterial cells express an array of osmoregulatory proteins that regulate turgor, including osmosensors that produce or import osmolytes used specifically for turgor homeostasis [8••] and mechanosensitive channels that act as release valves during hypoosmotic shock [9]. During the response to an osmotic shock from a change in the concentration of a compound to which the membrane is not completely permeable, water flux occurs within milliseconds [10,11], while osmolyte transport requires minutes [12]. The adaptation period can last several hours depending on the osmolyte and growth conditions [13], and recovery from hypoosmotic shock can involve shrinking of the cell to below the pre-shock volume in a mechanosensitive channel-dependent manner [14•]. Thus, the connections between turgor pressure and growth are a major component of osmoadaptation.

Turgor pressure (P) results in cytoplasmic swelling, and energy is required to overcome turgor and to compress the cytoplasm by a volume ΔV. The higher the concentration difference between the outside and inside of the cell, the more work is necessary. Turgor pressure is defined by the ideal gas law-like Morse equation, $P = RT(C_{int} - C_{ext})$, where R is the gas constant, T is the temperature, and $C_{int/ext}$ are the internal/external osmolarity, respectively (Figure 1a). One atmosphere of pressure is equivalent to ~100,000 N/m^2, or 14.7 pounds per square inch (psi); this value can be compared to the pressure in a car tire, which is generally inflated to ~35 psi (Figure 1c). Pressure has the same units (force per unit area) as Young's modulus, the parameter used to measure the stiffness of a three-dimensional material (analogous to the spring constant k for a Hookean spring where $F = kx$). One way to measure whether turgor pressure is 'large' is to compare the work required to reduce the volume by an amount ΔV against turgor pressure, $W = P\Delta V$, to thermal and biochemical reaction energy scales. For P = 1 atm = 0.1 pN/nm^2, the work required to displace 1 nm^3 of volume is W = 0.1 pN nm, which is 2.4% of the thermal energy k_BT = 4.2 pN nm. Thus, thermal fluctuations can induce a change in volume of 42 nm^3, and hydrolysis of a single ATP (which is equivalent to ~20 k_BT) can induce a volume reduction of ~800 nm^3. These volumes are miniscule fractions of the cellular volume of a bacterium, which is on the scale of 10^9 nm^3, demonstrating that sustaining turgor pressure requires a large energy investment. As we will discuss, many species make the most of this investment by

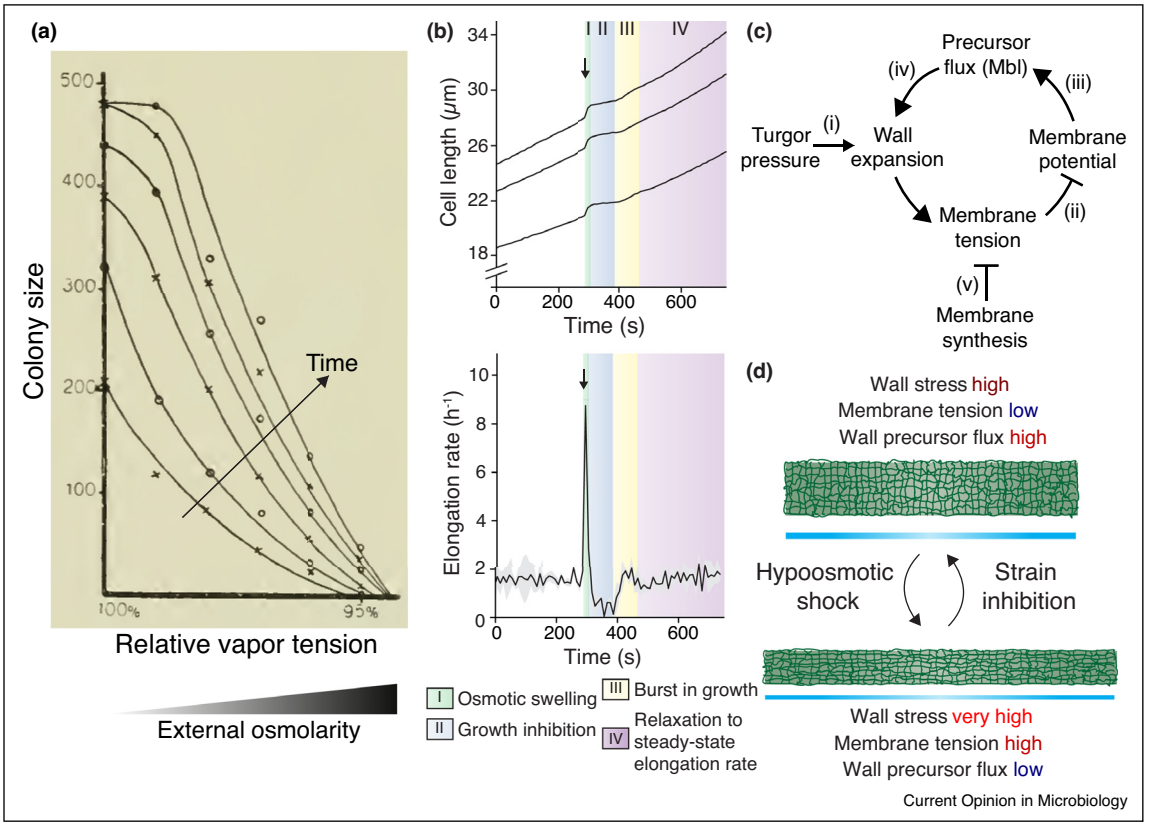
exploiting turgor pressure to regulate cell growth and division.

Plant cells are sufficiently large for turgor to be measured directly from the ability of the cell to compress gas trapped in the closed end of a capillary, the open end of which is in the cell vacuole [3], yielding measurements of a few atmospheres [3,15]. Such measurements are not currently possible in bacteria due to their small size; nevertheless, several clever methods have been devised to indirectly estimate turgor using the collapse of gas vesicles [16], water content measurements [17], and atomic force microscopy [18]. For the Gram-negative (thin-walled, ~2–4 nm [19]) *E. coli*, turgor pressure has been estimated at ~0.3–3 atm [17,18] depending on measurement technique and on medium [17], in approximate agreement with another Gram-negative species, *Ancylobacter aquaticus* [16]. By contrast, turgor in the Gram-positive (thick-walled, ~30 nm [5]) *Bacillus subtilis* was estimated at 10 atm [20], while the fission yeast *Schizosaccharomyces pombe* has a thick cell wall (hundreds of nm [21]) and turgor pressure ~15 atm [22••]. While it is tempting to speculate about the connections among turgor, wall thickness, and phylogenetic relatedness, we currently only have measurements in these few organisms from which to extrapolate (Figure 1c).

The role of turgor pressure in regulating growth rate

Almost a century ago, quantitative studies by Heinrich Walter showed that the size of a *Bacillus mycoides* colony was inversely proportional to the osmolarity of the surface on which it was grown (Figure 2a) [23]. One possible interpretation of this finding is that, based on the Morse

Figure 2



Hypoosmotic shock induces transient growth inhibition in rod-shaped Gram-positive bacteria by coupling electrical depolarization to cell-wall synthesis. **(a)** *B. mycoides* colony diameter is proportional to relative vapor tension, and hence inversely proportional to external osmolarity. Modified from [23]. **(b)** In *B. subtilis*, *L. monocytogenes*, and *C. perfringens*, hypoosmotic shock (arrow) induces cell swelling (green rectangles) and then transiently inhibits growth (blue rectangles, top is cell length traces of *B. subtilis*). This response is followed by an overshoot in elongation rate (yellow rectangle in bottom, computed from length traces of *B. subtilis*), which finally settles back to the original growth rate (purple rectangle in bottom). Modified from [27••]. **(c)** Hyperosmotic shock of *B. subtilis* cells slows wall expansion (i), demonstrating that growth rate is turgor dependent. Hypoosmotic shock results in electrical depolarization (ii), and depolarization alone slows the motion of the MreB homolog Mbl (iii) and arrests growth (iv). Synthesis of excess membrane reduces growth inhibition (v), demonstrating that increased membrane tension is responsible for growth inhibition. Modified from [27••]. **(d)** Feedback in which membrane tension and cell-wall stress compete to regulate cell-wall growth rate ensures balanced synthesis of the layers of the cell envelope (green mesh represents cell wall, blue represents membrane).

equation, increasing C_{ext} leads to a decrease in turgor pressure, and hence growth rate would be directly proportional to P. However, such an argument ignores the fact that after hours or days, any number of transcriptional, translational, and structural changes could occur in response to osmotic shifts. To distinguish between turgor-mediated effects and indirect, pressure-independent effects of osmolarity changes, a microfluidic flow cell can be used to rapidly change osmolarity while quantifying instantaneous elongation rate via single-cell imaging [24••].

B. subtilis is a rod-shaped, Gram-positive bacterium with a thick (~30 nm) cell wall [25] that ceases growth upon a large increase in extracellular osmolarity [26]. A single hyperosmotic shock reduced *B. subtilis* growth rate for tens of minutes [27••], and this reduced growth rate was well below the steady-state growth rate in the higher-osmolarity medium [27••]. This observation suggested that the reduction in turgor pressure, and not the increase in external osmolarity per se, was the critical factor determining growth rate in this bacterium: turgor pressure may be driving plastic deformation of the cell wall during cell growth, as for plant cells. Interestingly, after a short period of cell swelling, a hypoosmotic shock also reduced *B. subtilis* growth rate, albeit for a shorter amount of time (~1–2 min; Figure 2b) [27••]. The same behavior occurred in *Listeria monocytogenes* and *Clostridium perfringens* [27••], suggesting that this behavior may be conserved in Gram-positive rods. During the period of inhibition, the motion of the MreB homolog Mbl, a reporter of cell-wall synthesis [28,29], also halted [27••]. The behavior of *B. subtilis* cells under hypoosmotic shocks of different magnitudes agreed quantitatively with a model in which the increase in membrane tension induces growth arrest [27••]. In support of this model, applying a hyperosmotic shock to reduce membrane tension before hypoosmotic shock relieved growth arrest in *B. subtilis* [27••].

How is hypoosmotic shock, which mechanically induces an increase in membrane tension and cell-wall stress, transduced into the biochemical response of growth arrest? Dissipation of the membrane potential with the proton ionophore carbonyl cyanide m-chlorophenyl hydrazine (CCCP) resulted in rapid delocalization of MreB in *B. subtilis* [30], and also affected membrane organization [31••]. Intriguingly, hypoosmotic shock also electrically depolarized *B. subtilis* cells, and depolarization using the proton ionophore 2,4-dinitrophenol slowed the motion of Mbl and arrested growth, independent of any osmotic shock (Figure 2c) [27••]. Thus, turgor pressure is integrated with cell-wall expansion in an elegant manner by which membrane tension regulates wall synthesis via the membrane electrical potential. This homeostatic mechanism dictates that growth can occur only when membrane tension and cell-wall stress are in optimal ranges, ensuring balanced syntheses of the membrane and cell wall (Figure 2d).

E. coli maintains cell-wall insertion for several minutes after hyperosmotic shock

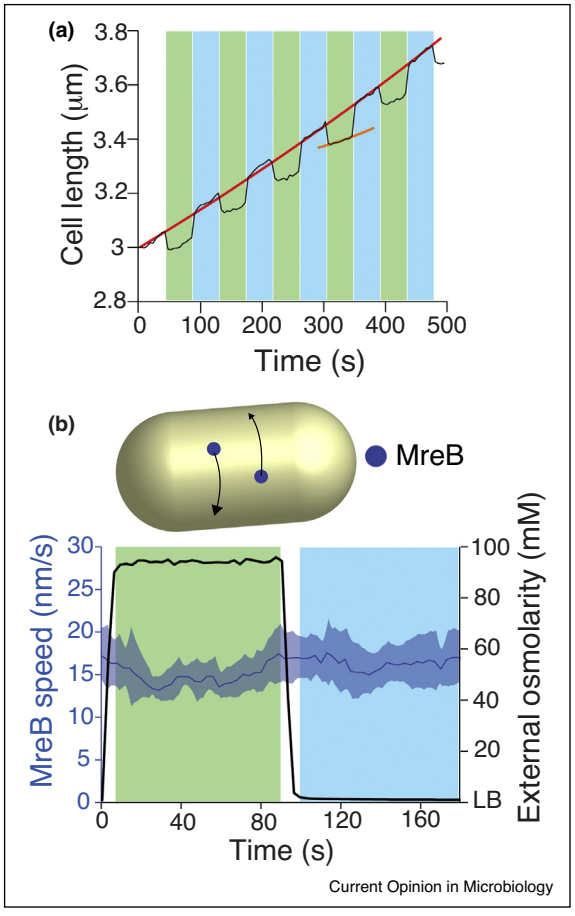
In contrast to *B. subtilis* [27••], the growth rate of *E. coli* cells was initially unaffected by a single hyperosmotic shock, remaining higher than the steady-state growth rate in the higher-osmolarity medium for tens of minutes [24••]. To determine the extent to which turgor pressure affects growth rate in this organism, the osmolarity of the medium was varied periodically on the minute time scale using a microfluidic flow cell. During these oscillatory shocks, the widths of cells (which would normally be constant [32]), oscillated along with the osmolarity [24••], reflecting switches in turgor pressure that did not adapt on the ~5 min time scale. Although hyperosmotic shock-induced plasmolysis caused apparently slower cell elongation, cells nevertheless exhibited a 'stored growth' behavior: upon reestablishment of turgor, they expanded to the length that they would have attained without the osmotic shocks (Figure 3a) [24••]. During periods of low turgor pressure, motion of the bacterial actin homolog MreB, a signature of the rate and location of cell-wall synthesis [33,34], continued unabated (Figure 3b) [24••]. Thus, cell-wall synthesis in *E. coli* is surprisingly robust to turgor fluctuations, despite the decrease in steady-state growth that occurs on longer time scales in response to increased osmolarity [24••,35]. Measurements of cell volume as a function of the osmolarity of the growth medium also indicated that turgor does not directly control growth rate [36•]. On the other hand, a recent study showed that mechanical strain sensing could, in principle, account for cell-shape recovery in cells forced into a bent shape [37], and sufficiently large compressive forces slowed growth rate [38•]. How the intrinsic couplings among turgor, mechanical strain and stress, and cell geometry ultimately affect cell shape and growth remains to be fully understood.

The role of turgor pressure in cell separation

For a bacterial cell with size w, the stress σ (force per unit area) in the wall can be approximated as Pw/d, where d is the envelope thickness. For a rod-shaped cell with $P = 1$ atm = 0.1 MPa, $w = 1$ μm, and $d = 10$ nm, σ is approximately 10 MPa. Estimates of the stiffness (Young's modulus) for bacterial cell walls mostly lie in the range of 10–100 MPa [39,40], indicating that stretching of the envelope is 10–100% if the envelope behaves as a linear elastic material (as has been observed for *B. subtilis* [27••]). In addition to stretching, turgor stresses can drive fracture (material breakage) or plastic deformation (permanent change without fracture) in the envelope during growth.

In *E. coli*, cell constriction and separation occur concurrently [41], while in many Gram-positive bacteria,

Figure 3



E. coli cell-wall growth rate is not dependent on turgor. **(a)** Trace of mean length changes after scaling to an initial 3-μm length of a population of *E. coli* cells during oscillatory osmotic shocks. Cells exhibit 'stored growth': despite the apparent slower growth rate at lower turgor (compare orange to red curve), upon turgor reestablishment with hypoosmotic shock, cells expand to the length that they would have attained without the osmotic shocks (modified from [24••]). Green and blue rectangles represent intervals of growth in LB + 100 mM and LB + 0 mM sorbitol, respectively. **(b)** The speed of the bacterial actin homolog MreB (blue curve; shading is ±1 standard deviation), a signature of the rate and location of cell-wall synthesis [33,34], averaged over several osmotic shock cycles with a period of 180 s, is the same during low (green rectangle) and high (blue rectangle) turgor (modified from [24••]).

construction of a septal wall by the division machinery to separate the two daughter cells precedes cell separation [25]. In the round, Gram-positive bacterium *Staphylococcus aureus*, daughter-cell separation occurs incredibly quickly, within a millisecond (Figure 4a) [42••,43••], in a process that relies on mechanical fracture of the cell wall. The dependence on turgor-generated stresses was demonstrated by showing that cells undergoing oscillatory osmotic-shock cycles synchronized separation events with the hypoosmotic and hyperosmotic shocks (Figure 4b), as these were moments when turgor pressure (and hence stress in the cell wall) suddenly increased and decreased, respectively [42••]. This ultrafast cell separation has since been shown to occur in several Actinobacteria (Figure 4c) [44•], as well as in the fission yeast *S. pombe* (Figure 4c) [22••]. It remains to be seen whether turgor pressure plays a role in other large morphological changes, for instance by creating envelope defects that lead to the formation of branches in species that form hyphae [45,46].

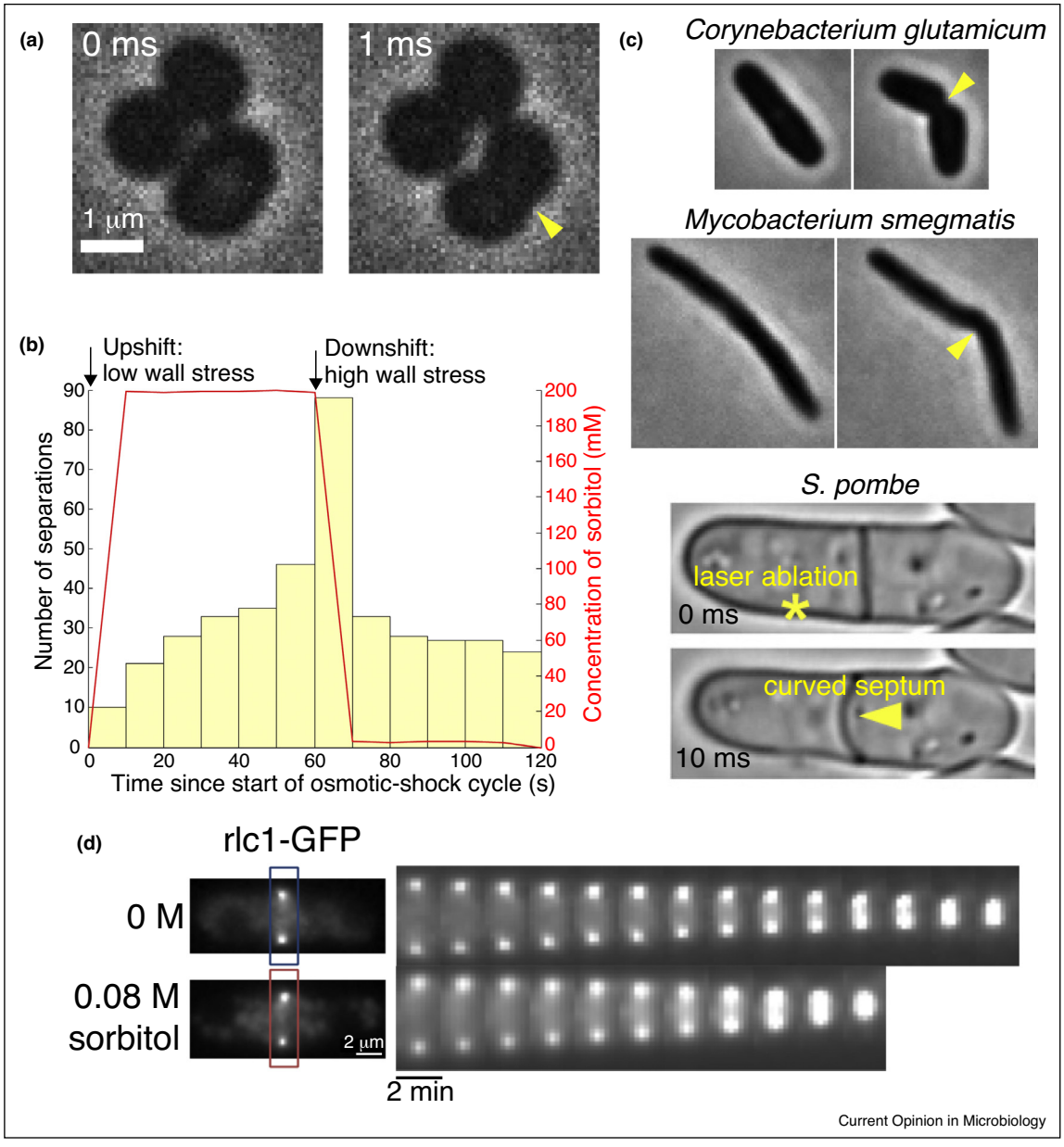
The role of turgor pressure in cell constriction

While turgor pressure can drive growth (as in *B. subtilis*) and cell separation, it may inhibit processes, such as cell division, that involve inward deformations of the cell envelope. In fission yeast, decreasing turgor pressure in adaptation-deficient cells by adding osmolytes to the growth medium increased the cleavage rate during cell division (Figure 4d) [47], suggesting that the inward force generated by the cytokinetic ring is resisted by outward forces due to turgor pressure. It is unknown whether this scenario occurs in bacteria as well, although in general, the inward construction of the cell wall during constriction faces resistance from turgor if the volume of the cell is otherwise unchanging. Is turgor a major roadblock to division progression? A back-of-the-envelope estimate reveals that a single ATP ($\approx 20\, k_B T$) can induce a volume change of $\approx 800\ \mathrm{nm}^3$, equivalent to the size of a polymer of the key division protein FtsZ that is 5 nm × 5 nm × 32 nm (approximately 6–7 subunits long); this estimate ignores the energetic contributions of membrane bending, which will depend heavily on the local composition of the membrane. An FtsZ dimer has been shown to undergo GTP hydrolysis-induced bending [48] that can generate 10–20 $k_B T$ of energy [49], suggesting that FtsZ polymers can bend membranes even against turgor pressure, although it remains unclear whether FtsZ-related constrictive forces are important for cell division. Regardless, constriction must be reinforced by cell-wall synthesis [50,51], which is the rate-limiting step in division [52•]. This requirement suggests the potential for interplay between septal cell-wall synthesis and turgor, although such a connection has yet to be explored.

Discussion

Clearly the role of turgor pressure in microbial growth varies across species, and we have only scratched the surface of phenomenology. As a start, it would be informative to pin down whether the response to changes in turgor is conserved phylogenetically, similar to the analysis of ultrafast separation that revealed conservation across Actinobacteria [44•]. While growth inhibition induced by hypoosmotic shock may be widespread among rod-shaped Gram-positive bacteria [27••], it remains to be seen whether the slowdown in growth is generally mediated by membrane depolarization. Moreover, it will be intriguing to probe the extent to which non-turgor-related mechanical perturbations also regulate

Figure 4



Turgor-dependent ultrafast separation of daughter cells. **(a)** Daughter-cell separation (yellow arrowhead) in the round, Gram-positive bacterium *S. aureus* occurs within a millisecond (modified from [42••]). **(b)** During oscillatory osmotic shocks with sorbitol, separation events occur more often during hypoosmotic shocks, corresponding to increases in turgor, than during hyperosmotic shocks (modified from [42••]). **(c)** Ultrafast daughter-cell separation also occurs in several Actinobacteria (modified from [44•]) and in the fission yeast *S. pombe* (modified from [22••]). The images of bacteria show daughter cells snapping into a kink (arrowheads) within a single 5-min frame. *S. pombe* images display the rapid curving of the septum (arrowhead) 10 ms after the left daughter cell was laser ablated (asterisk). **(d)** In osmoadaptation-deficient *S. pombe gpd1*Δ cells, the actomyosin contractile ring (marked by rlc1-GFP) progresses more rapidly when sorbitol is added to the medium, demonstrating that ring contraction is inhibited by turgor pressure.

growth through membrane electrical potential. Finally, the molecular sensors that transduce the mechanical effects of turgor fluctuations are as yet undiscovered.

A major open question is the response of other enteric bacteria; most of these species naturally face rapid transitions from highly concentrated environments like the gut to fresh water. Because most gut commensals prefer anaerobic environments, probing their response requires imaging in conditions without oxygen. Differential responses to osmotic changes may lead to reconfiguration of the microbiota, both spatially and compositionally,

which could have important impacts on the response of host and microbiota to osmotic diarrhea.

The turgor insensitivity of *E. coli* growth presents a stark contrast to the use of turgor for regulating growth and cell separation in *B. subtilis* and *S. aureus*, respectively. Do Gram-negative bacteria closely related to *E. coli*, such as *Salmonella*, similarly store growth during turgor oscillations? For that matter, how general is the response of *E. coli*? It is unknown whether stored growth occurs in different media, and whether stored growth is a general response of all *E. coli* strains, particularly pathogenic strains that may have different osmotic requirements for growth than commensals due to the lifestyles for which they have evolved. Given that *E. coli* MG1655 cells can continue to insert cell-wall material at the same rate after hyperosmotic shock in LB [24••], one would expect to generally detect stored growth unless rapid negative feedback stops precursor synthesis, or unless the structure of the cell wall in certain strains or environments precludes insertion of the precursors.

Changes in water activity coupled to fluctuations in turgor pressure can also affect growth rate indirectly. Given the change in water content, it is possible (perhaps likely) that intracellular density generally changes during osmotic shocks, as has been shown for *E. coli* [53]. Since hyperosmotic shocks cause changes to both the diffusion of cytoplasmic proteins [54] and cell shape, it stands to reason that proteins involved in a reaction-diffusion mechanism would have altered patterning. The Min system in *E. coli*, which utilizes a Turing pattern [55,56] to generate pole-to-pole oscillations that result in placement of the division site at midcell [57,58], may be altered by osmotic shock in such a manner as to relocalize or even completely inhibit the division machinery. Perhaps turgor fluctuations caused by repeated osmotic shocks can alter the morphology of certain microbes by perturbing the localization of the wall-synthesis machinery. Beyond cell shape and growth, myriad other cellular processes, such as DNA organization, metabolism, membrane transport, and the state of the cytoplasm itself [59] could be dramatically affected by osmotic shocks; these are fertile grounds for discovery in both basic and applied research.

Extrapolating our knowledge about turgor-dependent regulation of bacterial growth to walled eukaryotes, and vice versa, may yield exciting new insights. Many hypotheses for how bacteria respond to turgor shifts have been based on existing theories in plants, for which it is well accepted that turgor drives growth [60]. However, it is now clear that the role of turgor pressure in regulating bacterial growth can be simple or complex, depending on the organism. Many more species must be studied to build a comprehensive picture of how turgor factors into growth. Future 'shocking' discoveries promise to shed light on the fascinating evolutionary possibility that wall thickness, turgor pressure, and the mechanism of cell-wall expansion (pressure-driven vs. non-pressure-driven) co-evolved across the tree of life.

Conflicts of interest

The authors confirm that there are no known conflicts of interest associated with this publication.

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- • of special interest
- •• of outstanding interest

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