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Mechanics and morphogenesis of fission yeast cells

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The integration of biochemical and biomechanical elements is at the heart of morphogenesis. While animal cells are relatively soft objects which shape and mechanics is mostly regulated by cytoskeletal networks, walled cells including those of plants, fungi and bacteria are encased in a rigid cell wall which resist high internal turgor pressure. How these particular mechanical properties may influence basic cellular processes, such as growth, shape and division remains poorly understood. Recent work using the model fungal cell fission yeast, *Schizosaccharomyces pombe*, highlights important contribution of cell mechanics to various morphogenesis processes. We envision this genetically tractable system to serve as a novel standard for the mechanobiology of walled cell.

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Introduction

Growth and form of biological matter ultimately relies on similar mechanical principles as for non-living matter. Cells and tissues grow and adopt defined shapes from the dynamic biochemical regulation of mechanical elements at multiple scales: from nanometric molecular motors, to millimetric tissue stress [1,2]. The shape of animal cells, for instance, is set by a balance between cortical tension and adhesion [3]. Cortical tension is mostly regulated by the actin cortex, a thin layer of branched actin filaments beneath the plasma membrane [4]. Opposite to animal cells, plants, bacteria and fungi, possess an extracellular case outside their plasma membrane called the cell wall, which has conceptual equivalence with the actin cortex and/or elements of the extracellular matrix. The cell wall is thin, typically hundreds of nm, and is made of heterogeneous polysaccharides and glycoproteins interwoven by hydrogen and covalent bonds. Walled cells are also characterized by high internal osmotic pressure, called turgor, typically 3–4 orders of magnitude higher than in animal cells [5–7,8•,9]. The balance between turgor and wall mechanics has long been recognized to influence the shape and growth properties of walled cells [10–12]. Recent advances in microscopy and biophysical approaches, have allowed to measure and manipulate relevant mechanical parameters of cell walls and turgor [6,8•,9,13••]. These technical progresses, combined with a growing appreciation of the importance of physical considerations in biology, may begin to challenge and refine some basic paradigms in the morphogenesis of walled cells.

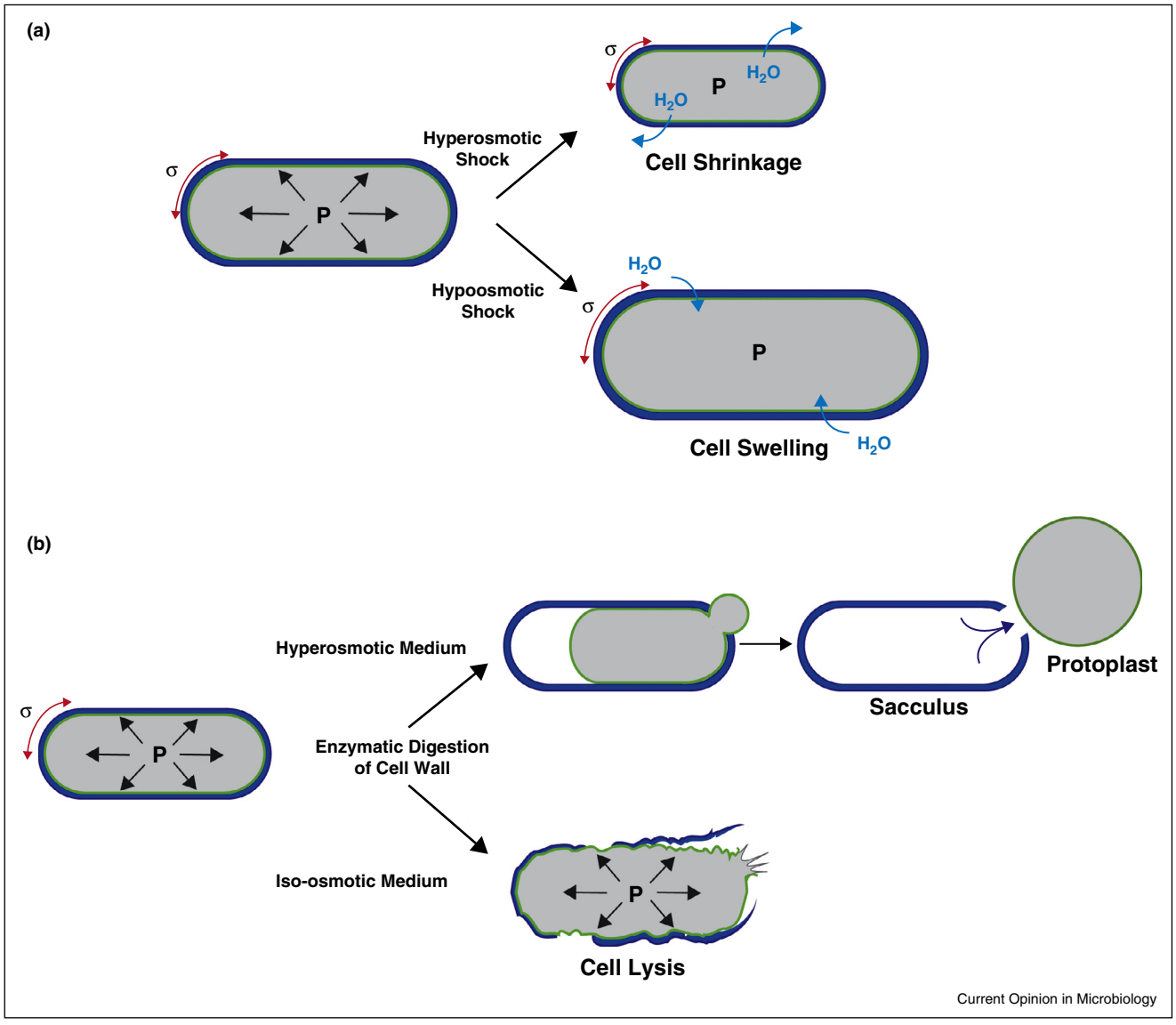
Here we focus on recent progress in the mechanics and morphogenesis of fission yeast cells. These rod-shape cells exhibit stereotypical tip growth and elongate from 7 to 14 μm in length during interphase, with a near constant diameter of 4 μm [14,15]. Growth ceases at mitosis and cells divide in their exact middle [16]. For tip elongation, polarity machineries organized around active GTP-Cdc42 and actin, are targeted to cell tips to restrict membrane and cell wall addition there [14]. During cell division, similar modules are re-targeted to the cell middle to drive the assembly of a cytokinetic ring and septum needed for division [16]. To date, this system has been mostly used as a model to dissect the basics of cell-cycle regulation and cytoskeletal assembly which are conserved from yeast to humans, and their contribution to growth, size, polarized behavior and cell shape [17]. The influence of cell mechanics however, is only beginning to be appreciated. We will first introduce the physical parameters characterizing fission yeast mechanics, the molecular elements that control these mechanical properties, and then discuss how they may influence processes such as cell shape, growth, division and polarization.

Main text

Mechanical properties of fission yeast cells

The balance between mechanical stress in the cell wall and turgor pressure contributes to define fission yeast cell shape (Figure 1). If turgor is reduced by submitting cells to high osmolarity medium (by adding sorbitol), cells shrink in a dose-dependent manner, and cease growth temporarily until turgor has adapted (Figure 1a) [8•,13••]. Second, if the cell wall is digested, cells rapidly burst; and if wall digestion is done in high osmolarity medium, to reduce turgor, they form round protoplasts which, in certain conditions can leave behind cell wall remnants (sacculus) with near intact rod-shapes (Figure 1b) [18]. These extreme experimental evidences, which apply to most walled cell, suggest that the values of mechanical

Figure 1



Cell mechanics and cell shape in fission yeast. **(a)** Cell shape changes upon hyper- (top panel) or hypo- (bottom panel) osmotic shock. Water permeates the cell, to balance the difference in osmolytes concentration, causing drastic changes in turgor pressure and cell wall stress. As a result, cells rapidly change shape, by shrinking (hyperosmosis) or swelling (hypoosmosis). **(b)** Cell shape changes after enzymatic digestion of the cell wall in hyperosmotic (top) or isoosmotic (bottom) media. In hyperosmotic medium, the cell wall opens, but turgor is low, which yields to the slow exit of the cell membrane and cytoplasm into a round protoplast leaving behind cell wall remnants, that may maintain near intact rod-shape in certain conditions. In isoosmotic medium, turgor is high and an opening in the cell wall yields immediate cell lysis since turgor is not balanced anymore by cell wall stress.

parameters characterizing turgor and cell wall are important elements for morphogenesis. How can they be measured?

On time scales on the order of few minutes, the fission yeast cell wall may behave as an elastic material ('a spring'). This has been evidenced in experiments in which cells were pushed into bent shapes into microfabricated wells [8•,19,20]. In this assay, cells occasionally popped out of microwells and restored their straight rod-shapes in seconds, suggesting the wall is elastic. On longer time-scales (tens of minutes to hours), however, the wall may also incorporate irreversible deformations and behave more as a plastic material; but relevant measurements are still lacking to document these aspects.

The bulk elastic modulus, or Young's modulus of an elastic material, has units of pressure, and characterizes

its intensive properties, independently of its geometry. For the cell wall, it can be seen as a physical measure of the composition and/or crosslinking of sugars chains. The surface modulus, which is the product of the bulk modulus with wall thickness, which has been estimated to be around 200 nm from transmission electron microscopy images [21,22], has units of tension, and provides the most relevant parameter to understand the contribution of wall mechanics to cell shape and growth [8•,23]. A method often used to compute surface mechanics in various cell types is atomic force microscopy (AFM) [24–26]. However, a complication of using this approach in walled cells, is to separate the contribution of wall mechanical properties and turgor pressure [9,27,28]. A rather simpler method, consists of using microfabricated wells made of elastomers with controlled elastic properties, as single cell force sensors. When fission yeast cells are pushed into these wells, they will buckle and deform the chamber at the same time. The balance between the buckling force and chamber deformation yields an estimate of the wall surface modulus of 6.5 N m^{-1}, typically 3–4 orders of magnitude higher than measured surface tension in animal cells [29], which corresponds to a bulk elastic modulus of about 30 MPa, similar to that of rubber [8•].

Estimating the values of internal turgor pressure in small cells such as yeast, can be challenging, as it involves impaling cells with pressure probes [6]. Here again cell-scale microchambers, have been used to estimate turgor values, by measuring how cells deform an elastic microchambers, to derive force–velocity relationships for single growing cells. Based on a minimal model for growth, these data yield estimates of the maximal force that yeast cells exert as they grow, to be on the order of 11 μN, which corresponds to a turgor pressure of approximately 0.85 MPa [8•].

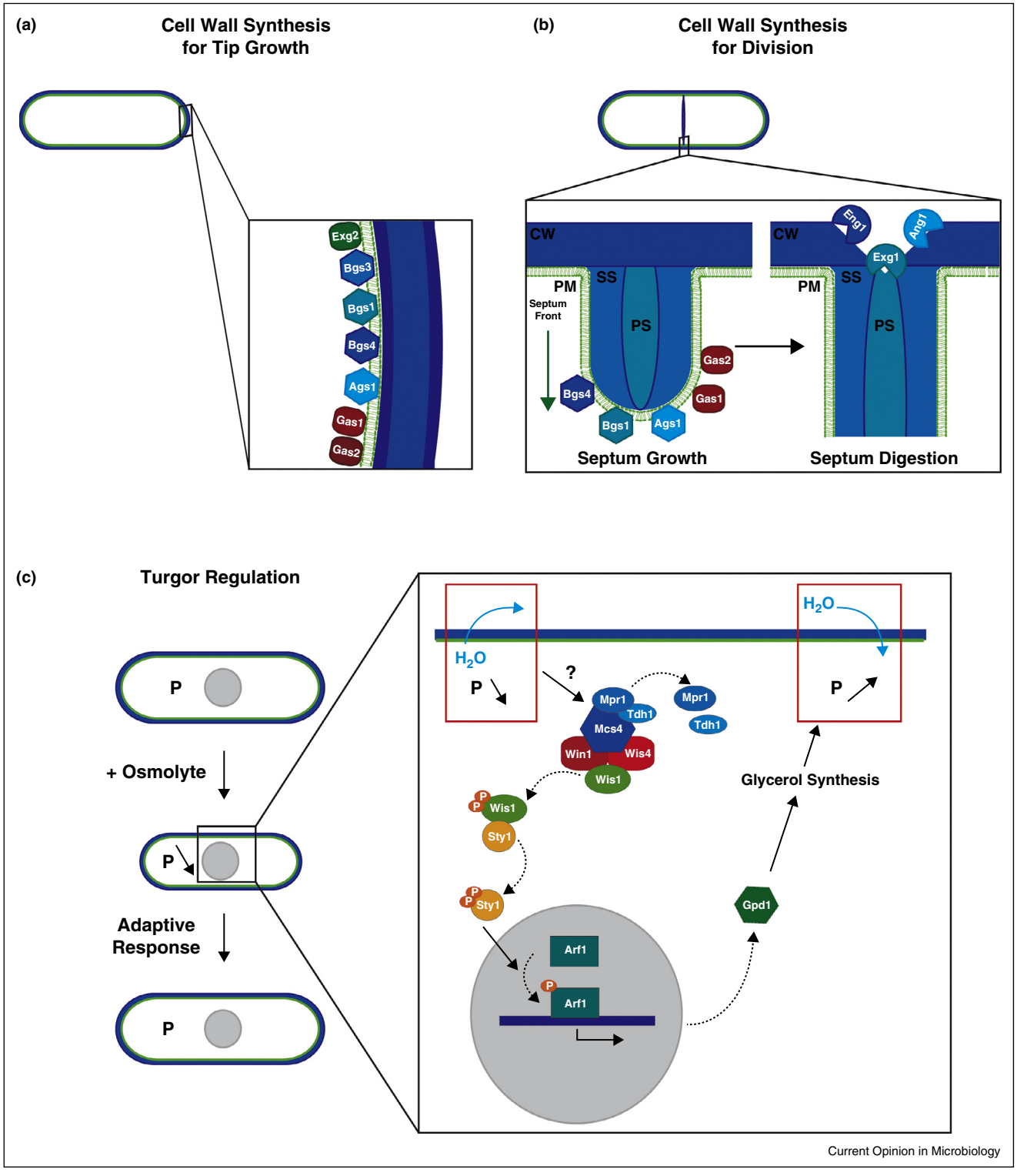
These measurements, which fall within the same range as for other walled cell such as bacteria, plants and other fungi [6,9,26,30,31]; provide a basic mechanical picture of fission yeast cells, similar to an inflated tire of a racing bicycle. They are key to support quantitative descriptions and modeling, and to test basic concepts in other mechanically-driven processes like endocytosis and cytokinesis (see hereafter). These mean values are however not sufficient to explain the asymmetric morphology of fission yeast cells [12]. In rod-shaped bacteria, for instance, anisotropies in the elasticity of the cell wall plausibly created by a circumferential orientation of wall fibers, are thought to contribute to define a rod [31,32]. In other tip-growing cells, like plant pollen tubes, local variations in wall mechanical properties along the cell perimeter, associated with spatial pattern of wall composition have been proposed to guide tip growth [33]. Future work in fission yeast will reveal which spatial mechanical anisotropies may guide rod-shape establishment, and more importantly how these anisotropies relate to intracellular distribution of polarity machineries.

The fission yeast cell wall

The mechanical properties of the cell wall are regulated by its biochemical composition, but the links between mechanics and biochemistry remain poorly described. The fission yeast cell wall is a polymer network made of two major polysaccharides: β(1,3) glucan chains with β(1,6) branches and α(1,3) glucan chains attached to short chains of α(1,4)glucan; two minor polysaccharides: linear β(1,3) glucan chains and β(1,6) glucan chains with a high amount of β(1,3) branches; and glycoproteins (α-galactomannan). Glucan synthases promote the synthesis of sugar chains. Endoglucanases digest and remodel the cell wall by shortening the chains, and glucanosyl-transferases may promote chain elongations and control crosslinking [34].

The architecture, synthesis and mechanics of the cell wall vary in different life stages of *Schizosaccharomyces pombe*. In interphase, new cell wall is synthesized at growing cell tips through the localization and activation of the β(1,3) glucan synthase Bgs4 and the putative α-glucan synthase Ags1/Mok1 [35–37]. Bgs1 and Bgs3, two other putative β-glucan synthases, are also recruited to cell tips, but their function there remains to be clarified [38,39]. One glucanase, Exg2, and two β(1,3)-glucanosyltransferases Gas1 and Gas2 are also localized to cell tips and could potentially influence wall synthesis or crosslinking [40,41] (Figure 2a). For cytokinesis, the septum is assembled in a centripetal manner outside the ring in the cell middle, and is composed of a central primary septum, flanked by two secondary septa. Bgs1 may be predominantly involved in assembling the primary septum [42], while Bgs4 and Ags1 may function in the synthesis of both primary and secondary septa [35,43,44]. After septum has finished ingression, it is digested in its middle by the endoglucanases Eng1, Exg1 and Agn1 to complete cell separation [40,45,46] (Figure 2b). During mating, and cell–cell fusion, a local degradation and remodeling of the cell wall is necessary to allow the fusion of the two plasma membranes of mating partners. This event is mechanically challenging because an opening of the walls before fusion would yield cell lysis; and involves a specialized focused actin structure which ensures precise spatio-temporal patterning of endoglucanases and glucan synthases around mating tips [47••]. Ascospores, which are products of meiosis, possess a particular cell wall composed of an inner spore wall surrounded by a thin outer spore wall, that confers resistance to spores [13••,48]. Although the composition of the spore wall remain understudied, it may involve in addition to other aforementioned enzymes a specific set of factors, such as the β-glucan synthase Bgs2, the α-glucan synthase Mok12, Mok13 and Mok14, [48–50] and the glucanosyl-transferase Gas4 for elongation and crosslinks [51]. In addition, the chitin synthase Chs1 may promote chitin or

Figure 2



Molecular regulation of fission yeast mechanics. **(a)** Cell wall synthesis at cell tips during interphase. α and β glucan synthases (Ags1, Bgs4) at the plasma membrane catalyze the synthesis of sugar chains in the cell wall. Other putative β glucan synthases (Bgs1 and 3) are also rectruited there, but their role remains uncharacterized. Gas1,2 are glucanosyltransferases that may influence sugar chain elongation and crosslinks. Exg2 is a predicted glucanase that could remodel or digest the wall. The cell wall is a three layered structure with an inner and outer electron dense layers and a less dense middle layer. **(b)** Septum synthesis and degradation during cytokinesis and cell division. Bottom left panel: During cytokinesis α and β glucan synthases and glucanosyltransferases are recruited at the cell middle to synthesize the septum (PS: primary septum, SS: secondary

chitosan synthesis, a component absent from vegetative walls [52]. The outer spore wall features extreme mechanical properties, and has been suggested to have a Young's elastic modulus 30 times higher than the vegetative cell wall [13••].

Synthesis and remodeling of the cell wall is regulated in space and time by the Rho GTPases Rho1, Rho2 and Rho1-homologue Rho5 [53–58]. Rho1 regulates the activity of β-glucans synthases both directly and/or through the protein kinases C, Pck1 and Pck2, while Rho2, may regulate α-glucans synthases through Pck2 [57,58]. Damage in the cell wall stimulates the cell wall integrity pathway mediated by the MAPK cascade Mkh1/Pek1/Pmk1 [59], which results in the activation of Pck1 and Pck2 by Rho1 and Rho2, for cell wall repair [60,61]. Recent work in budding yeast suggests that cell wall damage may also be linked to polarity machineries to ensure the very local recruitment of repairing cell wall factories [62••].

Overall, the links between wall assembly/composition, mechanics and morphogenesis remain poorly established at a quantitative level. Many mutants in synthases and Rho GTPases activation display globally thinner or thicker walls, or dramatic changes in wall composition [36,42,63–67]; while others may have more localized defects, at cell tips or septum [40,43]. These defects have striking consequences on cell shapes and growth patterns; which support the broad concept that wall properties, and likely mechanics, is key to control cell shape. Integration of biochemical tools with mechanical measurements will be necessary to rigorously establish those links.

Turgor pressure regulation

Turgor pressure in walled cells is osmotically generated, and maintained through rapid and efficient homeostatic systems [68]. In fission yeast, a hyperosmotic stress leads to the intracellular accumulation of glycerol [69] (Figure 2c). This mechanism is regulated by the stress activated pathway through the MAPK Sty1, a homolog of Hog1 in budding yeast and p38 in mammalian cells [70–72]. This cascade is coordinated by Mcs4 which forms a complex with the two MAPKKK, Win1 and Wis4 [73–76]. In hyperosmotic conditions, the MAPKK Wis1 becomes phosphorylated and unbinds from this complex, to phosphorylate the MAPK Sty1, which enters the nucleus to activate the transcription factor Atf1 [71,77]. Atf1 then regulates the transcription of 13 core genes [78]. This includes an increase in the expression of Gpd1 and Gpd2, glycerol-3-phosphate dehydrogenases, which promote glycerol synthesis; and the repression of genes involved in the degradation and translocation of sugars to compensate osmotic imbalance and restore turgor [69,79]. To fine tune pressure adaptation, Atf1 also promotes a negative feedback loop, through the transcription of *psy1* and *psy2*, that encode for phosphatases that inhibit Sty1 [71,80]. In budding yeast, several trans-membrane proteins have been proposed as upstream 'osmosensors' to trigger Hog1-dependent adaptation [81–83], whether similar systems exist or function in *S. pombe* remains to be explored.

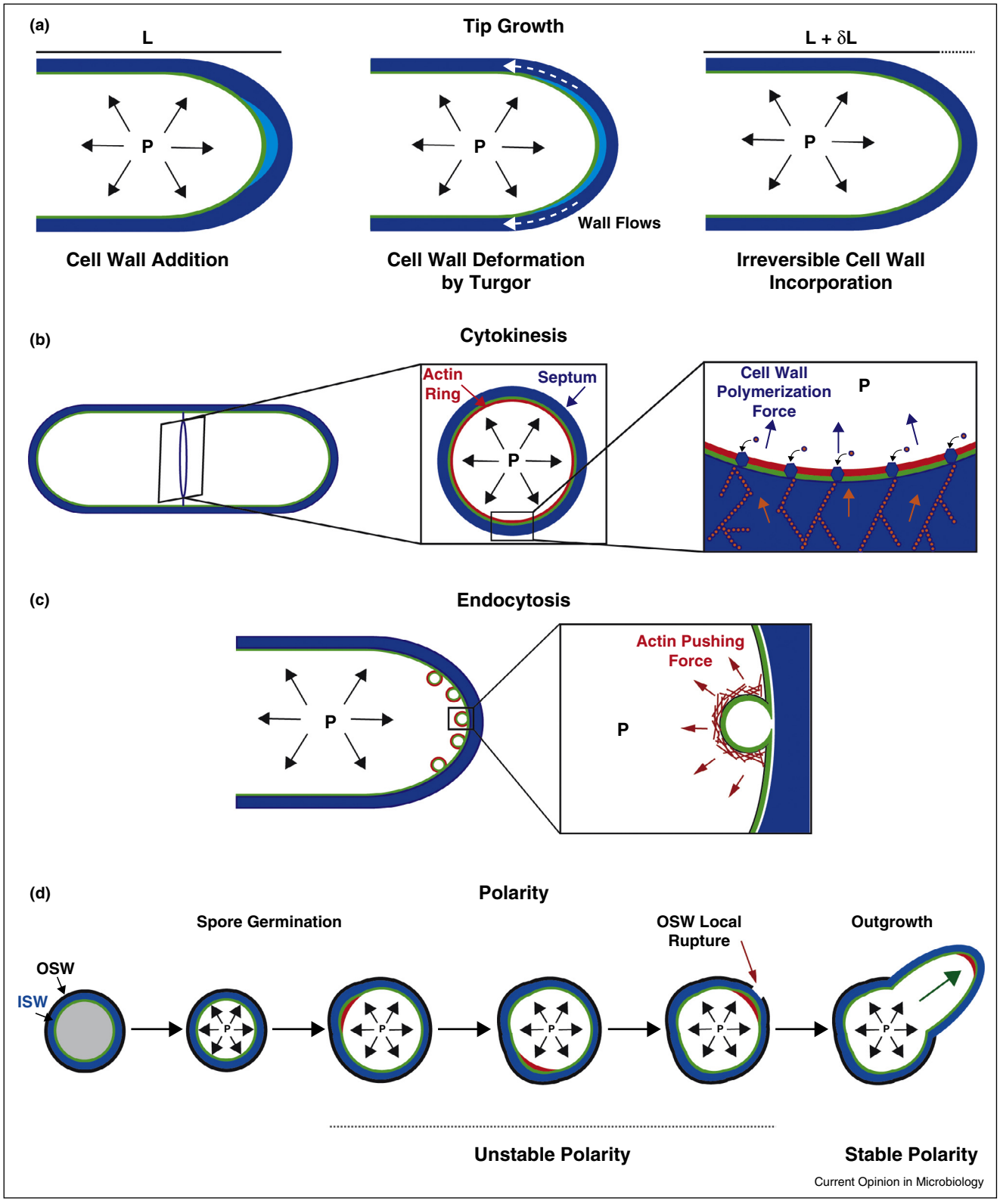
The response to hypoosmotic stress is markedly different, and admittedly less understood. It leads to cell swelling and intracellular Ca^{2+} increase [84]. Two homologues of the *E. coli* mechanosensitive channels MscS [85] have been recently proposed to influence this response: Msy1 and Msy2. Both are ER associated proteins, which become overexpressed in hypoosmotic conditions to support cell viability, but mechanistic details remain to be established [86,87]. Both hyper-osmotic and hypo-osmotic stress also promote the activation of Pmk1 and the cell wall integrity pathway, suggesting links between regulatory systems of cell mechanical properties [88,89].

Cell mechanics and cell growth

How do walled cell grow is not fully understood. Both material synthesis (membrane and cell wall) and turgor pressure are required for growth, yet, how these parameters contribute to define elongation rates is not known [8•]. Without turgor, deposition of new material, is predicted to yield a thicker wall with no growth, and turgor alone would only yield thinning of the wall [90]. Those important questions have long motivated theoretician, and several models for walled cell growth have been proposed over the years [90,91,23,8•,92,13••,106]. Experimental tests for these models are still sparse. A commonly used modeling framework, is to represent growth as a viscoplastic process. In that view the elastic cell wall freshly deposited at cell tips, is deformed by the stress exerted by internal turgor and this deformation becomes irreversible (plastic) passed a certain deformation threshold. The details for how cell wall remodeling may relate to viscoplasticity remain however poorly documented [91]. Conceptually, this amounts to an 'ageing' picture of the wall: as new wall is being incorporated at the tip it becomes irreversibly stretched with a certain time scale. Old cell wall may then flow along cell sides during cell elongation to maintain a constant wall thickness (Figure 3a).

(Figure 2 Legend Continued) septum, CW: cell wall, PM: plasma membrane). Bottom right panel, glucanases are then targeted to the sides of the septum to degrade the PS and cell wall edges for cell separations. **(c)** Osmoadaptation to hyperosmotic shocks. (Left) Upon hyperosmotic treatments cells first shrink and activate the stress pathway to increase internal turgor and recover their initial length and diameter. (Right) Details of signaling pathways involved in turgor maintenance upon hyperosmosis. Hyperosmotic stress causes an immediate loss of water from the cytosol, and consequent decrease in turgor pressure. This activates a MAPK cascade that leads to the expression of several genes, such as gpd1 which promotes glycerol synthesis to balance osmotic differences and restore turgor.

Figure 3



Influence of cell mechanics on fission yeast growth, division, endocytosis and polarization. **(a)** During tip elongation, cell wall is added at cell tip, and strained by the work of turgor and becomes irreversibly deformed for cell length addition. Maintenance of a constant cell wall thickness implies cell wall flows from the tip to cell sides. **(b)** Mechanical considerations in fission yeast cytokinesis. During cytokinesis, the acto-myosin ring

These mathematical models can make interesting predictions on cell diameters, exact tip shapes, or the dependence of elongation rates on mechanical values. For instance, a simple scaling model can recapitulate the variation of cell size observed among many walled cells based solely on their mechanical properties [23]. The probably most complete model adapted to fission yeast tip growth incorporates a spatial distribution of wall synthesis directly correlated with GTP-Cdc42 concentration at cell tips. This model makes important tests on the stability conditions required to maintain a straight rod-shape axis and a fixed diameter over generations of dividing cells, and predicts the existence of complex feedbacks between cell geometry and Cdc42-based polarity [93••].

Role of cell mechanics in endocytosis and cytokinesis

How might other mechanically-driven process, like cytokinesis and endocytosis may adapt to the particular mechanical properties of fission yeast or other walled cells? During cytokinesis, for instance, a conserved acto-myosin contractile ring attached beneath the plasma membrane has long been thought to provide the mechanical force to drive membrane ingression [94,95]. However, simple calculations suggest that the mechanical work exerted by the ring can only account for a small fraction (around 1%) of the work needed to overcome internal turgor pressure [96•]. Recent studies indeed indicate that reducing turgor yields faster constriction rates, and that once cytokinesis has initiated, the ring may become dispensable for membrane ingression and septum completion [43,96•]. This work suggests that most of the mechanical work may be generated by septum assembly, rather than the ring, shifting an important paradigm for cell division in yeast (Figure 3b). The function of the ring, could be instead to keep an homogenous septum assembly around the cell equator, a process plausibly regulated by a curvature-dependent septum assembly mechanism [97••].

Endocytosis, also involves local inward deformation of the plasma membrane to invaginate vesicles with a typical size of tens of nm. This process requires the subsequent assembly of various coat proteins, such as clathrin, myosin and BAR-proteins [98]. Although actin is dispensable for endocytosis in animal cells, it is strictly required in yeast [99]. Two recent studies, one performed in budding yeast [99] and one in fission yeast [100•], suggest that endocytosis also works against turgor, and that actin polymerization around endocytic vesicles generate the necessary forces to overcome turgor (Figure 3c). One set of striking evidence supporting these claims is to show that a reduction in turgor can rescue the defects of endocytic mutants and the requirement for actin. Altogether these data suggest that the regulation of these essential processes may have evolved along with extreme variations in cellular mechanics.

Cell mechanics and polarity

Cell mechanics may also influence cellular spatial organization. Recent studies in migrating animal cells have for instance suggested that surface mechanics, regulated by changes in the actin cortex or membrane tension, could contribute to cell polarization and directed migration [101,102]. Similarly, polarized walled cells such as fungi or plant roots can exhibit thigmotropism, a process during which polarity reorients as a consequence of mechanical contact with a physical barrier [103,104]. In fission yeast, a recent study investigating how initially round spores define their very first polar growth axis provides initial evidence that similar mechanical-polarity crosstalk may exist in these cells [13••]. A very intriguing observation of this work was to find that single polarity domains, built around active Cdc42, spontaneously polarize in germinating spores, but first exhibit a long unstable phase of successive assembly and disassembly, to eventually stabilize to promote polar tip extension at outgrowth. Interestingly, this switch in polarized behavior was found to be concomitant with the opening of the outer spore wall at the site of outgrowth (Figure 3d). By combining mathematical models and laser ablation of the spore wall, this study demonstrates that the spore wall has destabilizing effects on polarity. When the rigid spore wall is intact it acts as a barrier that hinders growth and destabilizes polarity, and opening of the spore wall (either naturally or with a laser) is sufficient to stabilize polarity. These data suggest the existence of feedback systems between wall mechanics and polarity machineries. By considering and testing different hypothesis of feedback (surface curvature, stress in the wall, among others), the authors propose that a positive feedback between growth and polarity, in which polarity localizes growth and more surface growth tend to maintain polarity in place, can explain polarity stabilization in outgrowing spores. Future work should reveal how these feedbacks may be regulated, and if they have relevance to other cellular states and cell types.

(Figure 3 Legend Continued) is surrounded by the septum. Forces generated by cell wall assembly (orange arrows) in the septum may support ring progression against high turgor pressure (adapted from [96•]). **(c)** Mechanical considerations in fission yeast endocytosis. (Left) Endocytic vesicles at cell tips; (Right) Close up on a single vesicle: invagination of the plasma membrane may be driven by a branched actin network that polymerizes and pushes against the membrane and cell wall (red arrows) to overcome turgor pressure (adapted from [100•]). **(d)** Cell mechanics and polarization in fission yeast spores. In spores the inner wall (ISW) is surrounded by a particular rigid outer spore wall (OSW) which may mechanically confine spores. Upon germination single polarity domains (red patch) assemble and disassemble around the spore surface promoting local growth sites that fail to progress. When spores have grown enough, the OSW ruptures at the site of the polarity domain; and this opening releases the hindering effect of the OSW, and polarity becomes stable for outgrowth and polar tip extension (adapted from [13••]).

Conclusion

The contribution of cell mechanics to morphogenesis, is becoming more and more appreciated in different fields, from bacterial growth to embryonic development and tissue homeostasis [1,105]. In fission yeast, which is probably the most established system to link gene function and cell shape, the role of turgor and cell wall mechanics remain surprisingly understudied. This is in contrast with the literature in plants, bacteria and other fungal cells, which have long focused on the mechanics of the wall and turgor for describing morphogenesis. A key endeavor of future studies in fission yeast, will thus be to document how cytoskeletal organization, and more generally gene function, ultimately contribute to pattern and regulate cellular mechanics. Given the genetic power of this system, and its quantitative growth and shape habits, we foresee that it could serve as a novel standard for establishing the biomechanical rules controlling the morphogenesis of single walled cells.

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