Trends in Microbiology

CellPress

Review

Cells under pressure: how yeast cells respond to mechanical forces

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In their natural habitats, unicellular fungal microbes are exposed to a myriad of mechanical cues such as shear forces from fluid flow, osmotic changes, and contact forces arising from microbial expansion in confined niches. While the rigidity of the cell wall is critical to withstand such external forces and balance high internal turgor pressure, it poses mechanical challenges during physiological processes such as cell growth, division, and mating that require cell wall remodeling. Thus, even organisms as simple as yeast have evolved complex signaling networks to sense and respond to intrinsic and extrinsic mechanical forces. In this review, we summarize the type and origin of mechanical forces experienced by unicellular yeast and discuss how these forces reorganize cell polarity and how pathogenic fungi exploit polarized assemblies to track weak spots in host tissues for successful penetration. We then describe mechanisms of force-sensing by conserved sets of mechanosensors. Finally, we elaborate downstream mechanotransduction mechanisms that orchestrate appropriate cellular responses, leading to improved mechanical fitness.

Highlights

In their natural habitat, unicellular fungi are exposed to a myriad of physical forces, including compressive, tensile, and shear forces.

Fungi are protected from mechanical stress by a rigid cell wall. During polarized growth, turgor-derived intrinsic forces press outward to deform weaker cell wall regions.

When yeast cells encounter an external force or rigid surface, the polarized growth machinery is destabilized and reorganized away from the obstacle. Fungal pathogens exploit this mechanism to invade host tissues through weaker regions and turgor-derived forces for penetration.

Yeast cells use multiple mechanosensors with nano-spring-like extracellular domains, stretch-activated calcium channels, and specific membrane structures to convert external mechanical forces into intracellular biochemical signals.

MAP kinase and calcium signaling pathways conserved across unicellular fungi and higher eukaryotes are crucial to orchestrate cellular mechano-stress.

Introduction

Organisms as simple as microbes or as complex as mammals are continually exposed to a myriad of biochemical or physical signals originating from within the cell or the environment. To orchestrate appropriate cellular responses they have evolved specific receptors to sense different signals and transduce the information through an interlinked signaling network to effector proteins. While biochemical signaling has long been investigated, the wide-spread importance of mechanical forces in cell biology has only recently been appreciated. Indeed, the cellular response to physical stimuli in a microenvironment underlies physiological processes from development to pathology in multicellular organisms. Physical cues determine cell shape, functions, and motility, and drive developmental programs such as embryogenesis. When perturbed, aberrant mechanical feedback mechanisms have been linked to several pathological conditions, including muscular dystrophy, hearing disorder, polycystic kidney disease, and cancer progression (reviewed in [1]).

Unlike mammalian cells, many microbes and plant cells have a stiff cell wall, in addition to the plasma membrane, to opposes physical forces. The cell wall is thought to protect cells by bearing mechanical stresses derived from internal turgor pressure that pushes the plasma membrane outwards and by passively absorbing many external physical forces, including shear, tensile, and compressive forces (Box 1). During physiological processes, such as polarized growth, dynamic adaptation of the rigid cell wall requires carefully orchestrated spatiotemporal remodeling. Thus, even unicellular organisms protected by a cell wall require dedicated mechanoresponsive mechanisms to balance internal and external forces and prevent lysis. Genetically amenable microbes can therefore serve as valuable model systems to investigate fundamental principles of mechanotransduction. Indeed, recent discoveries in the yeasts *Saccharomyces cerevisiae* and

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Box 1. Understanding forces at the surface of yeast and fungal cells

Compressive stress

This is a force per unit area applied orthogonal to the cell wall. It will cause cells to flatten, and cell walls to thin, as well as become tense along certain axes of the surface plane. This stress may be caused by an external object that moves onto cells (a rock, or a PDMS roof), or more naturally as cells grow onto rigid obstacles, so that the stress originates from internal turgor pressure as well as from neighboring cells.

Tensile stress

This stress is most commonly associated with turgor pressure that pushes outward against the cell wall. It puts the wall under lateral tension and may also cause the wall to thin locally or globally. Expansion of a substratum on which yeast/fungi adhere could also create tensional stress by stretching cells.

Shear stress

This stress may be caused by fluid flows on the surface of cells that apply a force on the surface parallel to the flow. Since the cell is adhered to the substratum, its cell wall is sheared. This stress will be essentially proportional to the viscosity of the fluid and the fluid flow speed at the cell surface divided by the cell height. Solid shear stress may also arise in colonies from cells sticking onto each other and expanding or rearranging at varying rates in different parts of the colony.

Hooke's law

The simplest form of Hooke's law describes the linear relationship between force, F, and deformation, ΔL, for a linear elastic spring of stiffness k: $F = k\,\Delta L$. For an elastic material of more complex geometry it is generalized as $\sigma = Y\varepsilon$, with σ the stress (force per unit surface), ε the elastic strain which is a normalized measurement of material deformation, and Y the Young's modulus of the material. Young's modulus has the same units as pressure (Pa) and represents a stiffness per unit area for the bulk of the elastic material. Stresses and strains depend on the geometry/thickness of the material and may have different values along different axes. For example, for a tube-like cell, such as *S. pombe*, the stress and strains of the cell wall will have different values along the long axis of the cell vs along the radial direction, vs across the cell surface.

Schizosaccharomyces pombe identified a family of mechanosensors – remarkably conserved even among nonwalled mammalian cells – and revealed molecular insight into their downstream signaling networks. Together, these findings help to explain how cells coordinate surface growth and cell wall synthesis and how they balance internal and external forces in changing environments. The emerging mechanisms have implications to understand filamentous growth and pathogen invasion, and they may reveal concepts relevant for multicellular organisms such as plants and animals. Here, we describe the different forces experienced by fungal microbes in their natural habitat. We then summarize how mechanosensitive feedback mechanisms crosstalk with cell division and polarized growth and describe mechanical regulation in fungal pathogenicity. Finally, we highlight the underlying mechanotransduction pathways and cellular stress responses.

Mechanical forces in the natural habitat of unicellular fungi

In their natural habitat, unicellular fungi grow as clusters adhering to biotic surfaces (e.g., plant or animal hosts) or abiotic surfaces (rocks, medical devices, etc.), thus forming a functional community (Figure 1A). *S. cerevisiae*, the simplest fungi, can initiate biofilm formation when grown in low glucose concentrations on a plastic surface or on semisolid agar [2]. Cells in such biofilms are surrounded by a hydrogel formed by complex polymers and water, which is further confined by physical boundaries, leading to growth-induced compression at contact points. Moreover, the local geometry generates a complex network of contact forces among neighboring cells. Tensile forces can arise if the substratum is stretched, for instance by heat-mediated contraction or expansion, leading to lateral cellular expansion and compression in the perpendicular direction. Finally, cells are often exposed to tangential forces called shear stress, generated by flow of water or physiological fluids, including blood or urine, across the biofilm or invading pathogens. Thus, cells need to appropriately respond to combined physical forces to grow and survive in complex microenvironments.

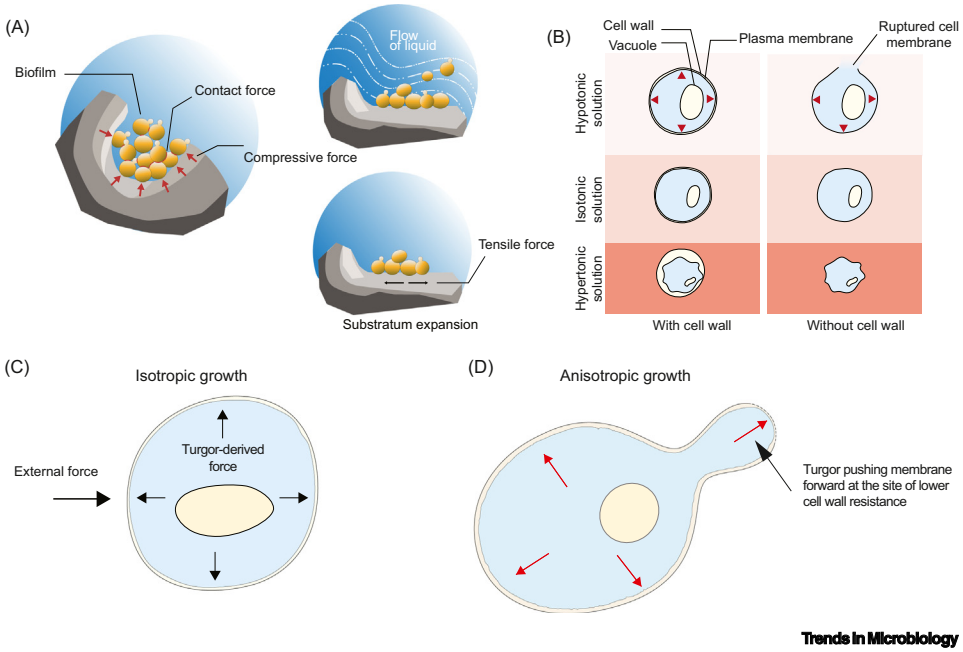

Figure 1. Origin of mechanical forces in unicellular fungi. (A) Mechanical forces in the natural habitat of unicellular fungi. Mechanical forces experienced by cells can be grouped as compressive, shear, and tensile forces. The growth of fungal biofilms in confined spaces, such as rocks, gives rise to compressive forces exerted at the boundary–cell interface and at cell–cell contacts. The flow of fluids across a biofilm exposes cells to shear stress. Tensile forces can arise when a substratum – such as body surfaces, rocks, and medical devices on which microbial biofilms grow – expand acutely. (B) Generation of turgor pressure. Turgor is generated as an inward osmotic flow of water expands the cell volume, generating outward forces against the plasma membrane. When cells are in a hypotonic solution, this influx causes swelling of cells, ultimately leading to cell bursting (top right). However, the cell wall stiffness provides resistance, enabling cells to better withstand outward turgor-derived stresses (top left). In an isotonic solution with no net influx of water, or a hypertonic solution with water efflux from cells, internal pressure becomes equal to, or less than, the external water potential, respectively. (C) Forces experienced by a cell that is undergoing isotropic growth include external mechanical forces and internal turgor-derived forces. While external forces can act inward (e.g., compressive forces) or pull the cell outward (tensile forces), turgor-derived forces act outward from the cell. Thus, net force, and the resulting mechanotransduction, is a combination of turgor-derived forces and components of compressive, tensile, or shear forces acting on the cell. (D) Turgor pressure drives anisotropic polarized growth in yeast. Degradation or weakening of the cell wall at the growth site results in local net expansion of the membrane and cell wall.

One well-studied source of external mechanical stress emerges from cell growth against rigid obstacles, or from neighboring cells in a packed colony or biofilm. For instance, when grown in microfabricated wells (Box 2) rod-shaped fission or filamentous yeast cells can buckle under the force at their tips pushing against the chamber wall [3,4]. Colonies of budding yeast cells grown to high density in deformable polydimethylsiloxane (PDMS) bioreactors (Box 2) develop stresses of up to 0.7 ± 0.1 MPa, which corresponds to 7 atmospheric pressures, similar to the pressure in a champagne bottle. Such physical forces propagate within the colony, stretching cell contacts and creating large tensile stresses on cell walls [5]. Similarly, forces resulting from a moving rigid boundary onto a cell, such as a PDMS roof, flatten cells and expose lateral cell walls to tensile stresses [6]. Other physical stress conditions in the natural habitats of fungi are associated with osmotic changes, for example during drought or humidification of the environment. Osmotic forces cause cells to shrink or stretch, thereby yielding large surface stress. Thus, fungal cells may be exposed to external forces that cause dramatic changes in cell shape and large mechanical stress at the cell surface.

Box 2. Innovative technologies for studying yeast mechanobiology

Microfabrication using polydimethylsiloxane (PDMS) has revolutionized the study of yeast mechanobiology. PDMS is a bioinert and transparent soft polymer that allows the building of devices that can be used for live-cell imaging. Moreover, the stiffness of PDMS structures can be altered by combining elastomer and curing agent in different ratios, enabling a variety of microfluidic strategies to study different aspects of yeast mechanobiology. In the last decade, numerous PDMS-based microfluidic devices have been developed to directly apply pressure to yeast cells, confine them in desired geometries, create obstacles that cells have to contour or penetrate, and study the downstream mechanotransduction and mechanoresponses. In addition to microfluidic technologies, the AFM, laser, and micropipettes have been used to apply localized physical stress to the yeast cell surface. Some examples of innovative technologies by which extrinsic mechanical forces can be applied to yeast cells to study different aspects of mechanobiology are summarized in Table I.

Table I. Examples of technologies used to trigger physical stress in yeast cells

Refs	Technology	Stress type	Organism
[115]	Optic fiber probe	Compression	*S. cerevisiae*
[116]	AFM	Nanoindentation of cell wall in hyphal or spore forms	*Aspergillus nidulans*
[3,117]	Cylindrical PDMS microchambers	Reactionary force from walls of the chambers	*S. pombe*
[70,118]	AFM	Single molecular stretching	*S. cerevisiae*
[104]	Micropipette	Plasma membrane tension	*S. cerevisiae*
[53]	Localized laser ablation	Cell wall damage	*S. cerevisiae*
[54]	Nano-fabricated obstacles	Contact forces	*C. albicans*
[5,119]	Microfluidic bioreactor	Growth-induced compressive stress	*S. cerevisiae*
[120]	Integrated elastomeric micropillar array	Protrusive forces in hyphal invasion	*Achlya bisexualis*
[6,51]	Microfluidic growth chambers with compressing pillars	Compressive stress	*S. cerevisiae*
[8,13,55]	Microfabricated channels	Confinement and contact forces	*S. pombe*
[4]	Cylindrical PDMS microchambers	Contact forces	*C. albicans*

The cell wall and turgor pressure protect yeast cells by opposing external mechanical forces

To withstand external mechanical stress, fungi are equipped with two mechanical systems: a cell wall and an inflated cytoplasm which features an unusually large pressure called turgor [7]. The plasma membrane of a turgid cell is protected much like an inflated inner air tube of a bicycle tire, with the cell wall analogous to the elastic outer rubber tire limiting deformation in response to extrinsic compressive, tensile, or shear forces.

The fungal cell wall is a stiff elastic multilayered thin shell encasing the plasma membrane. Its thickness typically varies between 50 and 500 nm, and its bulk elastic modulus – Young's modulus – is equivalent to that of rubber, ranging from 10 to 100 MPa [8,9]. The strength and elasticity of the cell wall is associated with the presence of glucan chains and chitin, linked by hydrogen bonds (reviewed in [10–12]). This glucan layer is connected to an outer layer of mannoproteins, which promote cell–cell recognition and sense diverse extracellular signals. Synthesis of cell wall polysaccharides and glycoproteins occurs in the Golgi where the products accumulate in the lumen before being transported to the cell surface on secretory vesicles. Cell walls exhibit large variations in composition and architecture among species, in different conditions or life stages, or even in different parts of the same cell [13–17]. These modulations adapt to changing environments and are needed to accommodate properties such as growth, morphogenesis, and polarity. For instance, cell walls can thicken and stiffen to better protect cells against drastic

physical insults such as temperature or osmotic changes during prolonged nutrient starvation or spore preservation [18,19]. Major cell wall modifications are also hallmarks of fungal infections as cells move within the different chemical environments of their hosts [14].

Turgor pressure values range around 0.5–2 MPa, with some unusually inflated compartments of fungal pathogens, called appressoria, which can build pressures of up to 8–10 MPa [20]. Typical estimates of turgor pressure in proliferating yeast cells are about 0.5–1.5 MPa [3,21,22], resulting from water influx driven along an osmotic gradient, associated with higher solute and ion concentrations in the cytoplasm compared to the extracellular milieu (Figure 1B). Turgor pushes the plasma membrane against the cell wall and deforms it elastically, providing a mechanical engine for cell growth and invasion and to resist external forces. Turgor is vital for fungal cell physiology, and cells have evolved robust homeostatic systems to maintain it even against drastic osmotic variations in the environment (Figure 1B). When cells are exposed to high osmolarity, turgor pressure rapidly decreases as the efflux of water across the semipermeable plasma membrane leads to shrinkage of the cytoplasm. As a result, the plasma membrane disconnects from the cell wall, leading to activation of the high osmolarity glycerol (HOG)-signaling cascade, which, in turn, closes glycerol channels and triggers synthesis of intracellular glycerol to restore osmotic balance and reinflate the cell [23–25]. Conversely, when cells are exposed to hypotonic solutions, water influx inflates the cytoplasm and causes cell expansion. Adaptation to hypo-osmotic shocks is poorly understood but may involve mechanosensitive channels that open to release water from the cytoplasm [26,27]. As cells expand, the increased surface is provided by flattening membrane reservoirs called eisosomes [28].

Turgor pressure generates intrinsic mechanical stress on the cell wall

The primary mechanical role of the cell wall is to balance the tensional stress generated by turgor pressure (Figure 1C). For a typical yeast cell of radius R = 2 μm, the tension T created by a turgor pressure of P = 1 MPa will be of T = PR/2 = 1 N/m. This is three orders of magnitude higher than the tension needed to lyse the plasma membrane [29], highlighting the vital role of the cell wall in ensuring surface integrity. Accordingly, digesting the cell wall with lytic enzymes, or piercing it with a laser, can cause rupture of the membrane and cell death. The cell wall is thus put under tension and strained by turgor pressure. Its elastic polysaccharide bonds resist this tensional stress resulting to a force balance that contributes to cell shapes and sizes. The wall of a rounded cell of radius R bears a tensional stress (the force per unit area along the plane of the wall) $\sigma = PR/2h$, with h the thickness of the wall. The Hooke's law (Box 1) for elastic materials gives $\sigma = Y\varepsilon$ with Y the Young's modulus of the cell wall and ε the elastic strain in the wall, yielding $PR = 2hY\varepsilon$. Estimates of typical elastic strains in yeast are around 20–30%, meaning that cells will reduce their radius by 20–30% when turgor is brought to 0 [8]. Elastic failure, at which the cell wall may start to rupture, has been estimated at ~45% in *S. cerevisiae* [30]. Thus, turgor-derived stress entails significant risk of cell wall lysis. An interesting feature of this force balance is that stress increases with cell size. Fungal cells ranging in size over two orders of magnitude resist extreme tension on the wall as they grow. Cells may thicken or stiffen their cell walls by modulating elastic bonds or adding extra layers, allowing elastic strains to remain below failures [8].

An important function of cell wall stress generated by turgor is to promote cell growth [31] (Figure 1D). Yeast and fungal cells with reduced pressure almost always slow or even halt growth [3,31]. Growth has been modeled by assuming that the cell wall can undergo plastic, irreversible deformations above a stress threshold, and exhibit viscous behavior so that the rates of deformations increase with stress [32]. Thus, in order to grow, cells may transiently thin, soften, or fluidize their cell wall, which entails risks of failure, highlighting how growth is a life-threatening process for walled cells. One common strategy to cope with this challenge is

to compartmentalize cell wall properties and restrict wall remodeling to one location in the generic process of anisotropic growth, typically referred to as polarized growth. Polarized growth occurs in budding yeast during three physiological processes: budding, mating, and filamentous growth [33–35]. Different physiological signals initiate these processes, including sufficient nutrition for budding, exposure to a pheromone gradient [36], and starvation to trigger filamentous growth to escape scarce nutrient conditions [37]. Tip growth is also the main growth mode of both fission and filamentous yeasts, including *S. pombe*, *Candida albicans*, and most fungal hyphae.

Cyclin-dependent kinase 1 (Cdk1) in complex with the G1 cyclins (Cln1/2/3) drives polarized growth in budding yeast (Figure 2) [38,39]. Cdk1 phosphorylates Cdc24 [39], a GTP exchange factor, which in turn activates the small GTPase Cdc42, a conserved master regulator of cell polarity [40–42] (for reviews see [35,43]). Cdc42 recruits the polarisome complex, which uses its combined activity of actin filament nucleation and exocytosis to deliver cargo required for

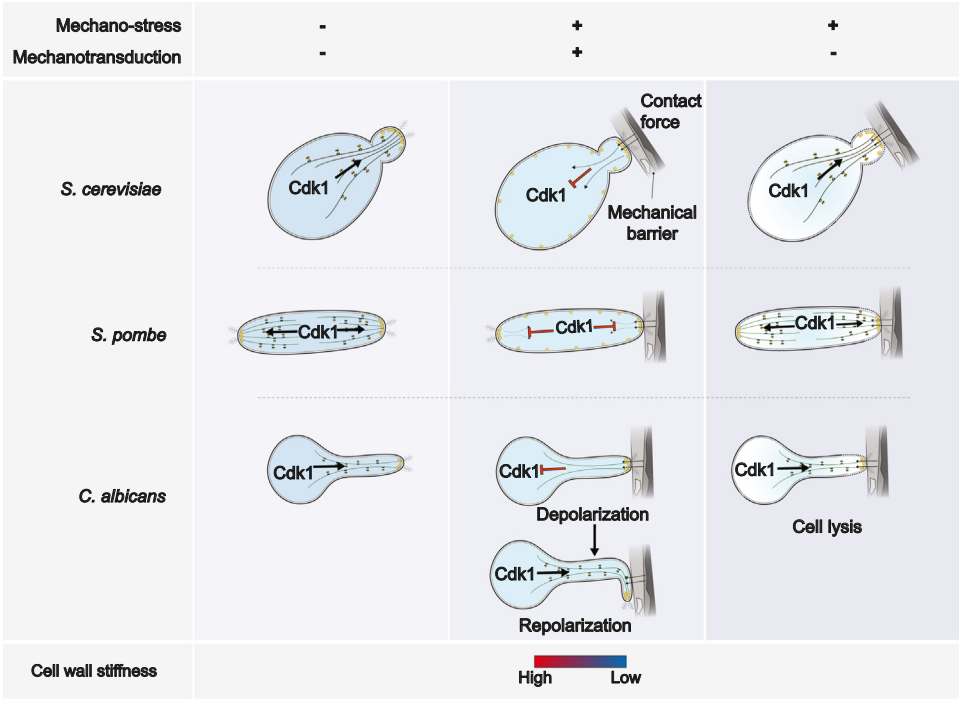

Figure 2. Reorganization of cell polarity is essential to survive external mechanical forces. Unicellular fungi, including *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Candida albicans*, undergo different modes of polarized growth in a context-dependent manner. As a part of cell division, *S. cerevisiae* and *C. albicans* undergo budding, while *S. pombe* undergoes fission. Similarly, polarized growth occurs when these organisms respond to pheromones, forming a polarized protrusion, called a 'shmoo', or to starvation, forming hyphae. Polarized growth is driven by cyclin-dependent kinases (Cdk1) and involves directed delivery of cell-growth components towards the sites of growth (left column). It further requires regulated degradation of the cell wall at sites of growth, pushing of membrane forward using internal turgor pressure, and resynthesis of the cell wall at the new position. As a result, a gradient in cell wall mechanics is formed, with softer regions at sites of polarized growth. Given the mechanosensitivity of the process, cells have to finely regulate cell polarization. When subjected to mechanical forces, cells activate a signaling network to rapidly depolarize their actin cytoskeleton and halt their cell cycle by inhibiting Cdk1. After stress release, when the net force equalizes, cells repolarize and restart the cell cycle (middle column). Cells lacking such mechanosensitive mechanisms fail to depolarize or repolarize towards less stiff sites and, as a result, continue polarized growth in the presence of external forces, leading to cell lysis, predominantly at sites of polarized growth (right column).

polarized growth [44,45], including the components of the cell wall synthesis machinery [46,47]. As a result of polarized cell growth, cell tips become thinner or softer, and wall segments transfer or flow from the apex to the side, likely thickening and stiffening the cell wall away from the tips [13]. Recent studies have modeled the process of tip growth using different frameworks and hypotheses [48,49]. Some of these models can remarkably account for important aspects such as variations in tip shape or growth speed [50]. However, experimental validation is limited by the difficulty of properly quantifying the time-evolution of local mechanical parameters in the cell wall.

Mechanical feedback mechanisms for polar growth and infections

Reorganization of cell polarity by external forces

Since growth is a vulnerable process, prone to cause lysis, fungal cells have evolved mechanotransduction mechanisms to reorganize polarity and wall synthesis when they encounter external forces. Yeast cells respond to cell wall damage and external mechanical signals by rapid growth arrest and depolarization due to the inactivation of Cdk1 (Figure 2) [6,51,52]. Depolarization involves disruption of actin cables and destabilization of the polarisome complex at growth sites. For instance, compressive stress applied to *S. cerevisiae* using a flattening microfabricated roof (Box 2) disperses Bni1 and other polarisome components, leading to uniform synthesis and strengthening of the stretched cell wall, thereby preventing cell lysis [6]. However, when a transient and/or local external mechanical pressure is applied, the original polarity site is rapidly abandoned and a new site is assembled at a different location. Conversely, when the cell wall is locally damaged, by use of a laser beam for instance, the polarisome complex rapidly relocates to the damaged site, directing actin polarization and targeted delivery of cell wall synthesis enzymes to repair the damage [53].

Conceptually equivalent behavior is observed when filamentous fungi encounter a physical barrier, and thus compress their cell wall from the force of their own growth. In such cases, the Spitzenkörper – a structure akin to the polarisome complex that contains secretory vesicles – will abandon the contact site where force is applied to rapidly reform at a new site away from the obstacle [54]. In fission yeast, when an active growth zone encounters a physical barrier, the polarity complex dissociates from the tip and wanders along the cell cortex until it is stabilized at a new mechanically favorable site to restart polarization [55]. Such depolarization/repolarization processes may be widespread in fungi, and not only allow navigating away from obstacles, a process called thigmotropism, but are likely also relevant to support several modes of fungal infection.

Stabilizing the polarity axis is crucial for fungal invasion

There are other instances in which growth direction and polarization must be stabilized for successful invasion of host tissues by fungal pathogens (Figure 3). One example is the formation of penetration pegs that act as micrometric piercing needles to breach the stiff plant cuticle in many plant pathogens. These pegs form at the contact between a fungal specialized structure, called an appressorium, and the plant cell wall. The appressorium is a dome-shaped specialized cell, which firmly adheres to the cell surface. It is formed when the cell at the tip of the germ tube ceases polarized growth, possibly by encountering a rigid surface. It then inflates by enhanced glycerol synthesis to increase turgor pressure, which, in the appressorium, can be as high as 8–10 MPa (80–100 atmospheric pressure) [20]. The cell wall of the appressorium is melanized to sustain the pressure and limit glycerol efflux, thus acting as a sealing cage [56,57]. The penetration peg grows from the appressorium into the plant cell surface, fracturing the plant cell wall to allow the fungus to invade the host tissue. In the pathogen *Magnaportheoryzae,* which causes rice blast disease, firm stabilization of the polarity machinery is achieved by the assembly of a septin ring that acts as a diffusion barrier to channel the polar growth machinery at the exact point of penetration

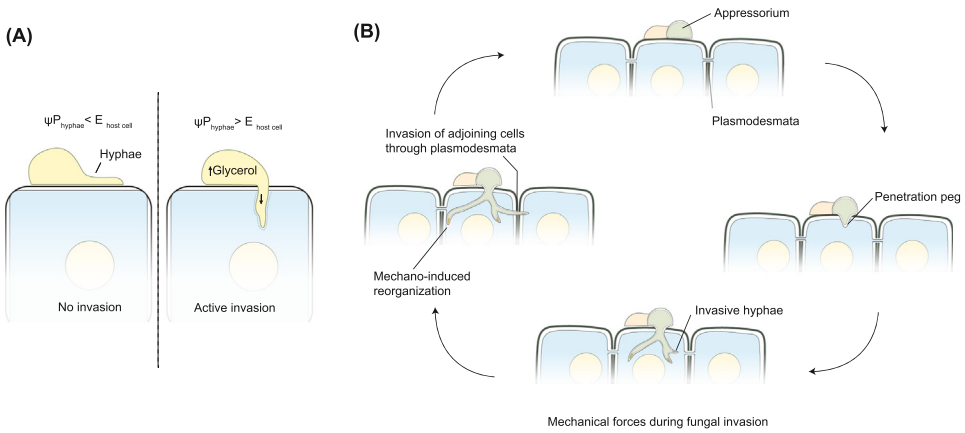

Figure 3. Fungal pathogens enhance the turgor pressure to breach stiffer host tissues. (A) Fungal pathogens are able to invade host tissue when their turgor pressure becomes higher than the surface stiffness and/or pressure of the host cell. The turgor pressure in the pathogen cell is often increased by boosting the cytosolic concentration of solutes such as glycerol. (B) The mechanism of host cell penetration in the rice fungal pathogen, *Magnaporthe oryzae*. The pathogen, *M. oryzae*, penetrates a host cell by stabilizing the polarity complex and generating high turgor pressure that exceeds the stiffness of the rice plant cuticle. Generating high turgor pressure is achieved in a dome-shaped appressorium via increased glycerol production and preventing glycerol efflux by melanization. After the pathogen enters the host cell the hyphae depolarize, when blocked by a stiffer cell structure, and re-establish polarized growth in a different direction to bypass the obstacle. The hyphae explore the cytoplasm until they find a weaker spot, such as the cell-wall-free plasmodesmata, allowing the pathogen to penetrate and colonize the neighboring cell.

[56]. Thus, invasion requires high turgor pressure and channeling in one focused direction, thereby generating a highly localized force to breach occlusions. Once the pathogen is inside a plant cell, it invades neighboring cells though plasmodesmata [58,59]. During this process, the polarity complex on hyphal tips continuously reorients until it finds weaker plasmodesmata regions. Persistent localization of the polarity complex and stabilization of hyphal tips using mechanical feedback mechanisms may lead to development of a narrower infection peg, called a transpressorium, which *M. oryzae* use to transverse neighboring cells through plasmodesmata [59]. Animal pathogens, such as *C. albicans*, similarly depend on physical penetration mechanisms to infect host tissues without rigid cell walls. However, they are not known to form an appressorium, although some species are able to breach medical devices. In the case of the human pathogen *Wangiella dermatitidis*, the invasive hyphal growth depends on melanin biosynthesis [60], suggesting that the disruptive physical forces may use similar mechanisms to increase turgor pressure.

How pathogens sense and orchestrate the underlying processes required for invasion remains unclear. We speculate that, when pathogens encounter rigid host tissue, they activate a signaling network that triggers enhancement of turgor pressure. Cells must have evolved mechanisms to sense whether they have reached a critical turgor threshold sufficient to penetrate their hosts. Indeed, a recent study found that Sln1, a histidine-aspartate kinase, localizes at the appressorium pore of *M.* oryzae in a turgor-dependent manner and contributes to sensing the turgor threshold required for breaching the rice leaf cuticle [61]. If the turgor pressure can be maintained, or is greater than the resistance from the host, there is less external force acting on the hyphae. Thus, below a threshold of stress, cells continue polarized growth and manage to breach the host tissue. This turgor threshold may vary in different pathogens, contributing to their capacity to invade specific host tissues. However, if the turgor pressure is lower than the Young's modulus of the host tissue, polar growth is halted, destabilizing the polarisome complex at growth sites (Figure 3A). The polarisome complex migrates and reassembles in an adjacent region with

lower external force, thereby reorienting growing tips. Through this repolarization process, filamentous growth turns away from rigid, impenetrable regions and instead searches for softer sites. Indeed, both plant and animal pathogens were shown to invade their host tissue at softer points [4,58,62]. Taken together, it is plausible that fungal pathogens use the polarisome complex as a molecular probe to scan the stiffness of the host tissue and find weaker/softer regions where they can sustain polarized growth for successful invasion.

Surface mechanosensors can convert physical forces into biochemical signals

Yeast cells achieve appropriate mechanoresponses, including cell-cycle arrest, depolarization, and cell-wall strengthening, by converting physical forces into biochemical signals. The net resultant forces are detected by cell-surface proteins called mechanosensors and cause phosphorylation changes in signaling proteins and/or an increase in the concentration of cytoplasmic calcium ions. One important class of mechanosensors in yeasts are the single-pass cell-surface proteins that have a short cytoplasmic tail and a highly glycosylated extracellular domain that functions as a nanospring (Figure 4). Another class consists of stretch-activated ion channels. Mechanical forces may also be sensed by topological changes that occur in cell membranes, releasing and activating signaling proteins. In the following text, we describe how mechanosensors convert external mechanical forces acting on the cell surface into intracellular biochemical signals.

A conserved family of mechanosensors containing nanospring-like domains

Wsc1 is among the best described mechanosensors and coordinates mechanotransduction and cell wall integrity in budding yeast [47,63–65]. This transmembrane protein accumulates at sites of polarized growth and functions as an upstream regulator of the Cell Wall Integrity (CWI) pathway that adjusts cell wall synthesis in response to chemical or heat stress [65–67]. Wsc1 is conserved across fungi, and live cell analysis of cell wall thickness in *S. pombe* suggests that Wsc1 functions as a surface sensor to promote homeostasis and safeguard cell wall integrity at growing tips [13,63]. Wsc1 is one of five putative cell wall surface sensors in *S. cerevisiae* (Figure 4A), which belong to either the Wsc or Mid2/Mtl1 family. These mechanosensors share an extracellular serine/threonine-rich (STR) domain (reviewed in [68]), with more than 50% serine or threonine residues. Transfer of mannose groups to these residues yields highly mannosylated transmembrane proteins [69], converting their folded structure into a linear rod that projects into the cell wall matrix [68]. The functional relevance of mannosylation is suggested by the mechanosensitive phenotype of cells lacking the enzymes required for this post-translational modification [67].

Direct evidence that STR cell-surface proteins sense external mechanical forces is provided by atomic force microscopy (AFM) [70]. AFM was used to apply force to a chimeric Wsc1 fused with the STR domain of Mid2, revealing a Hookean spring behavior, in which the chimera extends linearly with force. This nanospring-like property of Wsc1 was validated *in vivo*, where compressed Wsc1 was visualized at thinner cell walls in growth regions. Wsc1 harbors a short cytoplasmic tail which is constitutively phosphorylated on multiple sites [67]. It is plausible that unbalanced turgor and extracellular forces at growth sites squeeze the cell wall, driving compression of the spring-like STR domain of Wsc1 (Figure 4A), which, in turn, leads to dephosphorylation of those cytoplasmic sites, eliciting downstream signaling to locally increase cell wall synthesis. Once the cell wall is repaired and stress lowered, the STR domain may extend back, halting the process. Thus, Wsc1 provides an appealing module to detect local mechanical stress impinging on the cell wall and activate compensatory cell wall synthesis enzymes to strengthen its integrity.

In addition to Wsc1, recent evidence implicated Mating-induced death 2 (Mid2) as a mechanoreceptor activated by compressive stress [6]. Mid2 was originally identified in a screen for genes that protect cells from prolonged pheromone exposure, as *mid2Δ* cells lyse during shmoo

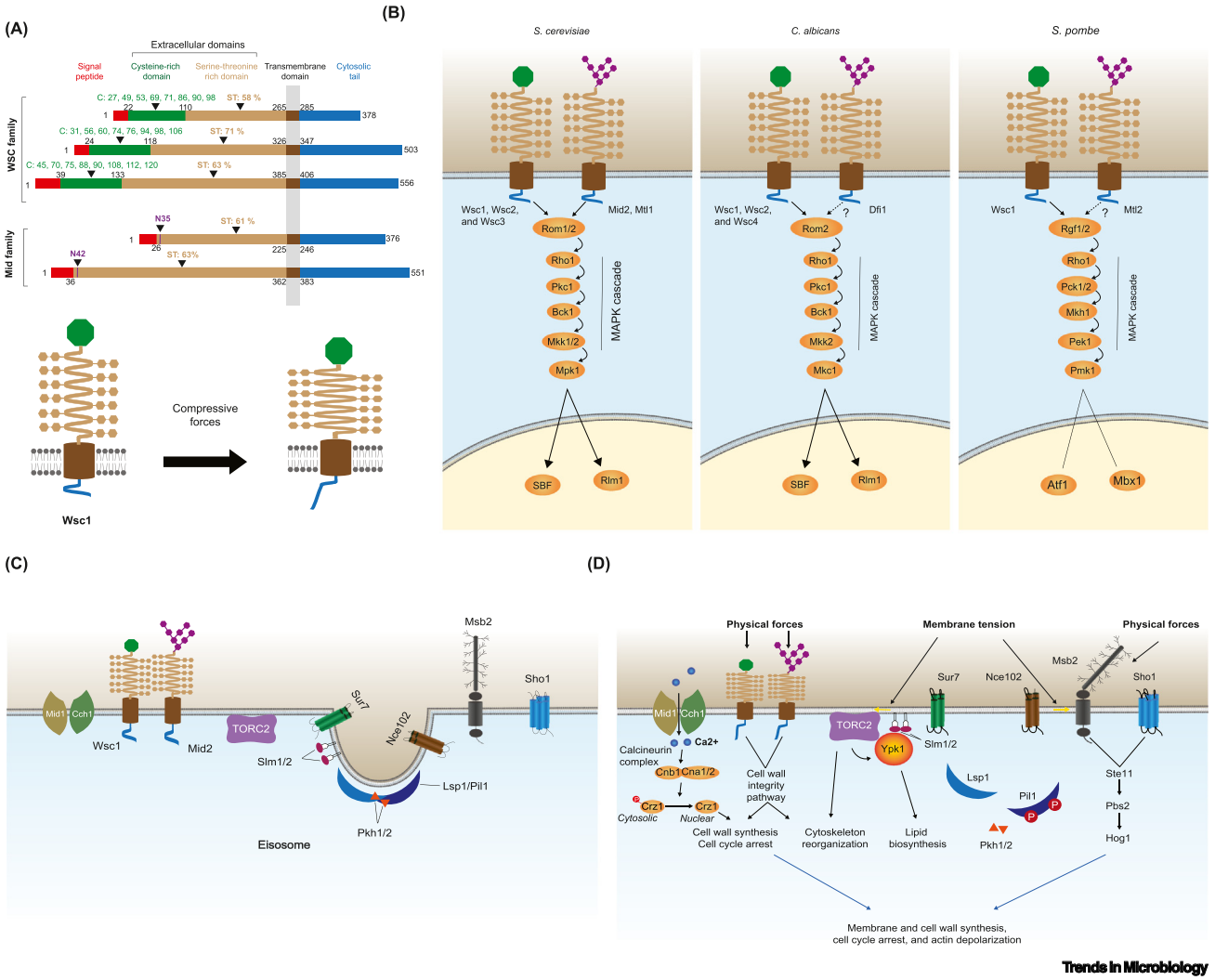

Figure 4. Mechanosensors translate mechanical forces into biochemical signaling pathways to orchestrate a coordinated mechano-stress response. (A) A conserved family of mechanosensors containing nanospring-like domains. In budding yeast, there are five putative cell-surface sensors containing a serine/threonine-rich (STR) domain in the extracellular cell wall region. This extracellular nanospring is compressed by mechanical force, and this conformational change is then converted into intracellular biochemical stimuli by the cytosolic tail. To date, only Wsc1 and Mid2 were shown to respond directly to mechanical forces. CT, cytoplasmic tail; TMD, transmembrane domain; PM, plasma membrane; CRD, cysteine-rich domain. The positions of a cysteine (C) residue within the CRD domain, the percentage of serine threonine (ST) residues within the STR domain, and the residue that is N-glycosylated in Mid2 and Mtl1 are indicated in the figure. (B) Conservation of mechanotransduction pathways across *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Candida albicans*. Compressed STR mechanosensors activate the conserved cell wall integrity (CWI) MAP kinase pathway, which, in turn, confers cell survival by blocking cell-cycle progression and depolarizing the actin cytoskeleton to prevent polarized growth. Moreover, activated MAP-kinase phosphorylates the transcriptional regulators Swi4/6 cell cycle box-binding factor (SBF) and Rim1 to allow cell wall remodeling. The mechano-stress responses, pathway topology, and signaling proteins are highly conserved across fungi. (C,D) Multiple intracellular and extracellular mechanosensors coordinately trigger appropriate cellular responses to various physical insults. Depicted is the yeast cell surface and various intracellular responses in the absence (C) or presence (D) of mechanical stress. In addition to the STR receptors activating the CWI pathway, yeast cells contain stretch-activated calcium channels, composed of Mid1 and Cch1, which sense multiple mechanical signals impinging on the cell wall and plasma membrane. Ca^{2+} influx activates a myriad of targets, among them cytoskeletal components and the calcineurin complex. Calcineurin also triggers nuclear translocation of the transcription factor Crz1. The Msb2/Sho1 module not only senses changes in osmolarity but may also activate Mpk1 and other MAP kinases in response to compressive mechanical stress. Finally, membrane tension is sensed by flattening of eisosomes, which results in activation of TORC2 (target of the rapamycin complex 2) after release of Slm1/Slm2. TORC1 and its downstream kinase, Ypk1, trigger cytoskeletal reorganization and sphingolipid biosynthesis. Together with the STR receptors, spatial and temporal activation of this mechanotransduction network preserves mechanical integrity by strengthening the cell wall and cellular membranes, and by regulating turgor pressure, cell cycle arrest, and polarized growth.

formation [71]. Likewise, *mid2Δ* cells burst at bud emergence when exposed to compressive stress applied by a pressure-controlled microfluidic device (Box 2; [6]). Indeed, upon compression, Mid2 activates a signaling response that depolarizes the actin cytoskeleton, thereby inhibiting polarized growth. The STR domain of Mid2 is essential for this function [6], indicating that compressive physical stress is directly sensed by its nanospring domain.

We speculate that STR domains may generally function as nanosprings that respond to mechanical forces. Although individual STR domains may respond differently to force intensity or type, they also sense mechanical stress at distinct cellular compartments. Indeed, Wsc1 accumulates at sites of polarized growth, while Mid2 localizes uniformly to the cell cortex [6,65]. Mid2 contains a glycosylated asparagine at the N terminus, while Wsc-family sensors encompass a conserved lectin-like domain that was proposed to promote binding to newly synthesized cell wall structures at growth sites [72]. Indeed, recent data in fission yeast suggest that this lectin-like domain relocates and clusters Wsc1 to sites where forces are locally applied onto the cell wall [73]. Moreover, Wsc1 localization to polarized sites requires its cytoplasmic tail, which stimulates endocytic trafficking of the mechanoreceptor [74].

Like Wsc1, Wsc2 and its paralog Wsc3 have been implicated in maintaining cell wall integrity and recovery from heat shock, while Mtl1 preserves cell integrity during glucose starvation and oxidative stress [75]. Further work is required to study the phenotypes of cells deleted for these mechanosensors, and in particular compare their expression and subcellular localization in response to different stress conditions.

STR mechanosensors activate the CWI MAP kinase pathway

The CWI pathway emerged as a core signaling pathway that mediates cellular responses to intrinsic and extrinsic cell wall threats downstream of STR mechanosensors (Figure 4B). Its components are highly conserved across budding, fission, and filamentous fungi, suggesting that the type of mechanical cues and downstream responses are comparable (reviewed in [68,76,77]). CWI proteins accumulate at sites of polarized growth where they monitor and adapt cell wall synthesis to local mechanical needs. Moreover, they are recruited to damaged cell wall structures to reinforce integrity [53,68]. Activated STR mechanosensors recruit the exchange factors Rom1 or Rom2 (Rom1/2) by binding to their intracellular tails and activate a dedicated MAPK module via Pkc1 (reviewed in [78]). In addition, Pkc1 phosphorylates the signaling scaffolds Far1 and Ste5, thereby preventing pheromone signaling and mating responses in the presence of cell wall stress [51,79]. A similar crosstalk mechanism may inhibit pheromone signaling in the presence of oxidative stress [80]. Activated Mpk1 triggers nuclear translocation of Rlm1 and the SCB binding factor (SBF) complex to induce a transcriptional program, including upregulation of cell wall synthesis genes [81,82]. Moreover, Mpk1 arrests the cell cycle by phosphorylating the Cdk1 inhibitor Sic1 and regulates unknown substrates that disrupt polarized actin cables and thereby counteract polarized growth [83,84].

Intracellular and extracellular mechanosensors without STR domains

While STR domains may provide force-sensing capacities to a family of membrane proteins, other fungal mechanoresponsive components do not contain such nanosprings. For example, Mid1 and Cch1 form a complex that allows rapid influx of calcium ions into the cytosol upon cell wall damage, increased mechanical stress and/or membrane tension [6,85,86] (Figure 4C,D). The molecular mechanisms opening Mid1/Cch1 channels upon mechanical stress remains unclear. The calcineurin phosphatase complex is among the major targets of intracellular Ca^{2+}, and cells lacking calcineurin subunits are sensitive to several environmental stress conditions [87–93]. Activated calcineurin dephosphorylates the transcription factor Crz1, resulting in its

rapid nuclear translocation [95–97], orchestrating a transcriptional program to strengthen the cell wall [68]. Mechanically gated calcium channels are present in cells ranging from bacteria to human cells (reviewed in [98]), suggesting that Ca^{2+} signaling is a conserved intracellular response to mechanical stress. Piezo1 and Piezo2 are mechanosensors that translate forces on the plasma membrane into cation influx into the cytosol in animal cells [99], while piezo homologs in plants localize to the vacuole and regulate its morphology during tip growth, possibly by sensing vacuolar turgor [100].

Using a microfluidic bioreactor (Box 2), it was found that yeast cells also sense compressive stress through a module consisting of the mucin Msb2 and the plasma membrane sensor Sho1 (Figure 4C,D). Indeed, cells lacking Msb2 or Sho1 often lyse when they reach high density upon expansion in confined microenvironments [52]. Moreover, Msb2 and Sho1 protect cells from high osmolarity [101] by increasing turgor pressure through blocking water efflux and increasing glycerol synthesis. It is thus possible that Msb2/Sho1 sense mechanical stresses associated with hyperosmotic conditions. Interestingly, Msb2 harbors a highly glycosylated extracellular domain with an autoinhibitory function [102]. It is likely that mechanical forces acting on the cell surface release this autoinhibitory function, thereby converting mechanical cues into intracellular biochemical signals. Release of autoinhibition is mediated by cleavage of this extracellular domain by the aspartic protease Yps1, which is attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor [102]. Further work is required to elucidate the mechanism activating Yps1 cleavage in response to contact forces.

Finally, the target of the rapamycin complex 2 (TORC2) responds to mechanical cues generated on the yeast cell membrane (Figure 4C,D). This evolutionarily conserved signaling complex regulates actin remodeling [103] and modulates lipid synthesis to balance membrane tension and internal turgor pressure [104]. TORC2 localizes as puncta at the plasma membrane, adjacent to eisosomes [105]. When membrane tension increases through raising turgor pressure, changes in lipid composition, or physical stretching, eisosomes flatten and release the Slm1 and Slm2 paralogs, which, in turn, activate TORC2 signaling [104]. Conversely, loss of membrane tension clusters TORC2 into PtdIns(4,5)P2-enriched plasma membrane (PM) domains and inactivates it [106], implying that TORC2 uses distinct mechanisms to sense increased and decreased membrane tension to maintain membrane homeostasis.

Taken together, available results show that different mechanosensors respond to multiple intracellular and extracellular mechanical cues impinging on the cell wall or plasma membrane (Figure 4C,D) and cooperate to accomplish intracellular responses that adapt cells to different types and magnitudes of physical force. Interestingly, genetic experiments suggest that Ca^{2+} signaling and CWI activation synergize to respond to mechanical stress. Indeed, lysis of cells exposed to compressive stress is strongly increased if cells lack both Mpk1 and Cnb1 compared to either single mutant [6]. Similarly, the 'SMuSh' pathway downstream of the Msb2/Sho1 sensors synergizes with the CWI pathway to respond to mechanical pressure in confined environments [107]. Further studies are warranted to understand how these mechanoresponsive pathways are spatiotemporally regulated to restore cell integrity and survival under mechanical stress conditions.

Concluding remarks

The ultimate goal of the mechanotransduction network is to strengthen the cell wall and plasma membrane in response to diverse physical perturbations while maintaining mechanical integrity and physiological function (Figure 4D). Mechanical insults can act either on the cell surface or internal structures and may occur in parallel or sequentially. In turn, mechanotransduction

Outstanding questions

How do cells regulate mechanosensitive intrinsic processes when exposed to external mechanical forces? What are the signaling networks preserving cellular integrity under these circumstances?

How is cell wall remodeling regulated and coordinated with polarized growth?

Plant pathogens use specialized cells generating high turgor pressure to penetrate rigid structures. What are the molecular mechanisms orchestrating this complex process? Do animal pathogens use similar mechanisms to invade stiffer host tissues? Can the underlying mechanisms be targeted to develop antifungal agents?

Pathogens need to stabilize the polarization machinery and sustain tip growth to breach host tissues. While recent studies revealed how yeast cells reorient their polarity axis when encountering rigid surfaces, the underlying signaling mechanism in pathogens remains unknown.

How do cell wall surface sensors, including those with STR domains, translate mechanical forces into biochemical signals? Although the extracellular STR domain functions as a nanospring it remains unclear how these conformational changes translate into intracellular recruitment of the downstream components such as Rom2.

Fungi harbor multiple cell surface sensors with STR domains, but it is yet unknown how these sensors achieve specificity towards mechanical forces and subcellular locations.

Appropriate mechano-stress responses are orchestrated by a conserved signaling network, including MAPK modules (CWI and SMuSh), TORC2, and calcium signaling. How are these pathways spatially and temporally regulated, what are the underlying positive and negative feedback mechanisms, and how do the individual components synergize and crosstalk?

Can the cellular mechanisms activated by physical forces in unicellular

systems serve as a paradigm to study mechanical stress signals in health and disease?

pathways function in an adaptive or sustained manner and are activated around the cell or at localized sites. For instance, calcineurin- and Pkc1-activated pathways cooperate to antagonize polarized cell growth and ensure cell survival upon compressive mechanical stress [6]. While early responses depend on adaptive Ca^{2+} signaling and transient activation of the general stress response, Mpk1 induces sustained cellular responses. Similarly, the SMuSh and CWI pathways cooperatively confer adaptation to persistent mechanical pressure [52]. While TORC2 responds to cell membrane tension, its involvement in other types of mechanical stress remains unclear. Thus, a combinatorial analysis of mechanosensitive signaling pathways is needed to understand how individual components synergize and crosstalk with each other to orchestrate the complex cellular responses to physical stress (see Outstanding questions).

Although mammalian cells lack a rigid cell wall and feature much lower cytoplasmic pressure, they share interesting parallels with respect to mechanosensing and downstream responses. Mammalian cells express mechanosensitive proteins such as ion channels, G-protein-coupled receptors, integrins, and cadherins that sense different types of mechanical cues such as shear stress, stretch forces, and osmotic changes (reviewed in [108]). Similar to yeasts [6], they also express mechanosensors which convert compressive forces into intracellular signals. For example, human polycystin-1 (PC-1)/polycystin-2 (PC-2) form heteromeric calcium channels, similar to Mid1 and Cch1, but use WSC domains to respond to shear force in kidney tubules [109,110]. Likewise, ERK5, the closest mammalian Mpk1 homolog, is activated by physical stimuli, including osmotic and shear stresses [111–113], and ERK5-deficient endothelial cells are susceptible to shear stress [111]. Interestingly, ERK5 regulates the epithelial–mesenchymal transition (EMT) [114] which involves reorganization of the actin cytoskeleton and loss of cell polarity. ERK5 may respond to compressive forces arising during tumor expansion in confined microenvironments, possibly contributing to EMT and cancer progression. Thus, organisms as simple as yeast have evolved conserved mechanisms to respond to mechanical forces, which may have implications for an understanding of mechanotransduction in health and disease of complex organisms.

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Declaration of interests

No interests are declared.

References

1. Jaalouk, D.E. and Lammerding, J. (2009) Mechanotransduction gone awry. *Nat. Rev. Mol. Cell Biol.* 10, 63–73
2. Reynolds, T.B. and Fink, G.R. (2001) Bakers' yeast, a model for fungal biofilm formation. *Science* 291, 878–881
3. Minc, N. *et al.* (2009) Mechanical forces of fission yeast growth. *Curr. Biol.* 19, 1096–1101
4. Puerner, C. *et al.* (2020) Mechanical force-induced morphology changes in a human fungal pathogen. *BMC Biol.* 18, 122
5. Delarue, M. *et al.* (2016) Self-driven jamming in growing microbial populations. *Nat. Phys.* 12, 762–766
6. Mishra, R. *et al.* (2017) Protein kinase C and calcineurin cooperatively mediate cell survival under compressive mechanical stress. *Proc. Natl. Acad. Sci. U. S. A.* 114, 13471–13476
7. Harold, F.M. (1990) To shape a cell: an inquiry into the causes of morphogenesis of microorganisms. *Microbiol. Rev.* 54, 381–431
8. Davi, V. *et al.* (2019) Systematic mapping of cell wall mechanics in the regulation of cell morphogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 116, 13833–13838
9. Ma, H. *et al.* (2005) Surface ultrastructure and elasticity in growing tips and mature regions of *Aspergillus* hyphae describe wall maturation. *Microbiology* 151, 3679–3688
10. Klis, F.M. *et al.* (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* 26, 239–256
11. Lesage, G. and Bussey, H. (2006) Cell wall assembly in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 70, 317–343
12. Orlean, P. (2012) Architecture and biosynthesis of the *Saccharomyces cerevisiae* cell wall. *Genetics* 192, 775–818
13. Davi, V. *et al.* (2018) Mechanosensation dynamically coordinates polar growth and cell wall assembly to promote cell survival. *Dev. Cell* 45, 170–182 e7

14. Garcia-Rubio, R. *et al.* (2020) The fungal cell wall: *Candida*, *Cryptococcus*, and *Aspergillus* Species. *Front. Microbiol.* 10, 2993
15. Hopke, A. *et al.* (2018) Dynamic fungal cell wall architecture in stress adaptation and immune evasion. *Trends Microbiol.* 26, 284–295
16. Ikezaki, S. *et al.* (2019) Mild heat stress affects on the cell wall structure in *Candida albicans* biofilm. *Med. Mycol. J.* 60, 29–37
17. Patel, P.K. and Free, S.J. (2019) The genetics and biochemistry of cell wall structure and synthesis in *Neurospora crassa*, a model filamentous fungus. *Front. Microbiol.* 10, 2294
18. Bonazzi, D. *et al.* (2014) Symmetry breaking in spore germination relies on an interplay between polar cap stability and spore wall mechanics. *Dev. Cell* 28, 534–546
19. Ene, I.V. *et al.* (2015) Cell wall remodeling enzymes modulate fungal cell wall elasticity and osmotic stress resistance. *mBio* 6, e00986
20. Bastmeyer, M. *et al.* (2002) Force exertion in fungal infection. *Annu. Rev. Biophys. Biomol. Struct.* 31, 321–341
21. Atilgan, E. *et al.* (2015) Morphogenesis of the fission yeast cell through cell wall expansion. *Curr. Biol.* 25, 2150–2157
22. Schaber, J. *et al.* (2010) Biophysical properties of *Saccharomyces cerevisiae* and their relationship with HOG pathway activation. *Eur. Biophys. J.* 39, 1547–1556
23. Miermont, A. *et al.* (2011) The dynamical systems properties of the HOG signaling cascade. *J. Signal Transduct.* 2011, 930940
24. Brewster, J.L. *et al.* (1993) An osmosensing signal transduction pathway in yeast. *Science* 259, 1760–1763
25. Brewster, J.L. *et al.* (1993) An osmosensing signal transduction pathway in yeast. *Science* 259, 1760–1763
26. Saxena, A. and Sitaraman, R. (2016) Osmoregulation in *Saccharomyces cerevisiae* via mechanisms other than the high-osmolarity glycerol pathway. *Microbiology (Reading)* 162, 1511–1526
27. Nakayama, Y. *et al.* (2012) Organellar mechanosensitive channels in fission yeast regulate the hypo-osmotic shock response. *Nat. Commun.* 3, 1020
28. Kabeche, R. *et al.* (2015) Eisosomes provide membrane reservoirs for rapid expansion of the yeast plasma membrane. *J. Cell Sci.* 128, 4057–4062
29. Le Roux, A.L. *et al.* (2019) The plasma membrane as a mechanochemical transducer. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 374, 20180221
30. Stenson, J.D. *et al.* (2011) Determining the mechanical properties of yeast cell walls. *Biotechnol. Prog.* 27, 505–512
31. Lew, R.R. (2011) How does a hypha grow? The biophysics of pressurized growth in fungi. *Nat. Rev. Microbiol.* 9, 509–518
32. Julien, J.D. and Boudaoud, A. (2018) Elongation and shape changes in organisms with cell walls: A dialogue between experiments and models. *Cell Surf.* 1, 34–42
33. Pruyne, D. and Bretscher, A. (2000) Polarization of cell growth in yeast I. Establishment and maintenance of polarity states. *J. Cell Sci.* 113, 365–375
34. Madden, K. and Snyder, M. (1998) Cell polarity and morphogenesis in budding yeast. *Annu. Rev. Microbiol.* 52, 687–744
35. Bi, E. and Park, H.O. (2012) Cell polarization and cytokinesis in budding yeast. *Genetics* 191, 347–387
36. Arkowitz, R.A. (2009) Chemical gradients and chemotropism in yeast. *Cold Spring Harb. Perspect. Biol.* 1, a001958
37. Dickinson, J.R. (2008) Filament formation in *Saccharomyces cerevisiae* – a review. *Folia Microbiol. (Praha)* 53, 3–14
38. Lew, D.J. and Reed, S.I. (1993) Morphogenesis in the yeast cell cycle: regulation by Cdc28 and cyclins. *J. Cell Biol.* 120, 1305–1320
39. McCusker, D. *et al.* (2007) Cdk1 coordinates cell-surface growth with the cell cycle. *Nat. Cell Biol.* 9, 506–515
40. Adams, A.E. *et al.* (1990) CDC42 and CDC43, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* 111, 131–142
41. Johnson, D.I. and Pringle, J.R. (1990) Molecular characterization of CDC42, a *Saccharomyces cerevisiae* gene involved in the development of cell polarity. *J. Cell Biol.* 111, 143–152
42. Zheng, Y. *et al.* (1994) Control of the yeast bud-site assembly GTPase Cdc42. Catalysis of guanine nucleotide exchange by Cdc24 and stimulation of GTPase activity by Bem3. *J. Biol. Chem.* 269, 2369–2372
43. Johnson, D.I. (1999) Cdc42: an essential Rho-type GTPase controlling eukaryotic cell polarity. *Microbiol. Mol. Biol. Rev.* 63, 54–105
44. Ziman, M. *et al.* (1993) Subcellular localization of Cdc42p, a *Saccharomyces cerevisiae* GTP-binding protein involved in the control of cell polarity. *Mol. Biol. Cell* 4, 1307–1316
45. Tcheperegine, S.E. *et al.* (2005) Regulation of cell polarity by interactions of Msb3 and Msb4 with Cdc42 and polarisome components. *Mol. Cell. Biol.* 25, 8567–8580
46. Delley, P.A. and Hall, M.N. (1999) Cell wall stress depolarizes cell growth via hyperactivation of RHO1. *J. Cell Biol.* 147, 163–174
47. Banavar, S.P. *et al.* (2018) Mechanical feedback coordinates cell wall expansion and assembly in yeast mating morphogenesis. *PLoS Comput. Biol.* 14, e1005940
48. Abenza, J.F. *et al.* (2015) Wall mechanics and exocytosis define the shape of growth domains in fission yeast. *Nat. Commun.* 6, 8400
49. Drake, T. and Vavylonis, D. (2013) Model of fission yeast cell shape driven by membrane-bound growth factors and the cytoskeleton. *PLoS Comput. Biol.* 9, e1003287
50. Campas, O. *et al.* (2012) Strategies for cell shape control in tip-growing cells. *Am. J. Bot.* 99, 1577–1582
51. van Drogen, F. *et al.* (2019) Mechanical stress impairs pheromone signaling via Pkc1-mediated regulation of the MAPK scaffold Ste5. *J. Cell Biol.* 218, 3117–3133
52. Delarue, M. *et al.* (2017) The SMuSh pathway is essential for survival during growth-induced compressive mechanical stress. *Proc. Natl. Acad. Sci. U. S. A.* 114, 13465–13470
53. Kono, K. *et al.* (2012) Proteasomal degradation resolves competition between cell polarization and cellular wound healing. *Cell* 150, 151–164
54. Thomson, D.D. *et al.* (2015) Contact-induced apical asymmetry drives the thigmotropic responses of *Candida albicans* hyphae. *Cell. Microbiol.* 17, 342–354
55. Haupt, A. *et al.* (2018) A positive feedback between growth and polarity provides directional persistency and flexibility to the process of tip growth. *Curr. Biol.* 28, 3342–3351 e3
56. Dagdas, Y.F. *et al.* (2012) Septin-mediated plant cell invasion by the rice blast fungus, *Magnaporthe oryzae*. *Science* 336, 1590–1595
57. Veneault-Fourrey, C. *et al.* (2006) Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science* 312, 580–583
58. Sakulkoo, W. *et al.* (2018) A single fungal MAP kinase controls plant cell-to-cell invasion by the rice blast fungus. *Science* 359, 1399–1403
59. Cruz-Mireles, N. *et al.* (2021) From appressorium to transpressorium – defining the morphogenetic basis of host cell invasion by the rice blast fungus. *PLoS Pathog.* 17, e1009779
60. Brush, L. and Money, N.P. (1999) Invasive hyphal growth in *Wangiella dermatitidis* is induced by stab inoculation and shows dependence upon melanin biosynthesis – PubMed. pdf>. *Fungal Genet. Biol.* 28, 190–200
61. Ryder, L.S. *et al.* (2019) A sensor kinase controls turgor-driven plant infection by the rice blast fungus. *Nature* 574, 423–427
62. Wachtler, B. *et al.* (2012) *Candida albicans*–epithelial interactions: dissecting the roles of active penetration, induced endocytosis and host factors on the infection process. *PLoS One* 7, e36952
63. Cruz, S. *et al.* (2013) The fission yeast cell wall stress sensor-like proteins Mtl2 and Wsc1 act by turning on the GTPase Rho1p but act independently of the cell wall integrity pathway. *Microbiologyopen* 2, 778–794
64. Straede, A. and Heinisch, J.J. (2007) Functional analyses of the extra- and intracellular domains of the yeast cell wall integrity sensors Mid2 and Wsc1. *FEBS Lett.* 581, 4495–4500
65. Kock, C. *et al.* (2016) Yeast cell wall integrity sensors form specific plasma membrane microdomains important for signalling. *Cell. Microbiol.* 18, 1251–1267
66. Verna, J. *et al.* (1997) A family of genes required for maintenance of cell wall integrity and for the stress response in

Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13804–13809
67. Lodder, A.L. *et al.* (1999) Characterization of the Wsc1 protein, a putative receptor in the stress response of *Saccharomyces cerevisiae*. *Genetics* 152, 1487–1499
68. Levin, D.E. (2011) Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway. *Genetics* 189, 1145–1175
69. Lommel, M. *et al.* (2004) Aberrant processing of the WSC family and Mid2p cell surface sensors results in cell death of *Saccharomyces cerevisiae* O-mannosylation mutants. *Mol. Cell. Biol.* 24, 46–57
70. Dupres, V. *et al.* (2009) The yeast Wsc1 cell surface sensor behaves like a nanospring in vivo. *Nat. Chem. Biol.* 5, 857–862
71. Ono, T. *et al.* (1994) The MID2 gene encodes a putative integral membrane protein with a Ca(2+)-binding domain and shows mating pheromone-stimulated expression in *Saccharomyces cerevisiae*. *Gene* 151, 203–208
72. Heinisch, J.J. *et al.* (2010) Single-molecule atomic force microscopy reveals clustering of the yeast plasma-membrane sensor Wsc1. *PLoS One* 5, e11104
73. Neeli-Venkata, R. *et al.* (2021) Detection of surface forces by the cell-wall mechanosensor Wsc1 in yeast. *Dev. Cell* 56, 2856–2870 e7
74. Piao, H.L. *et al.* (2007) NPFXD-mediated endocytosis is required for polarity and function of a yeast cell wall stress sensor. *Mol. Biol. Cell* 18, 57–65
75. Petkova, M.I. *et al.* (2010) Mtl1 is required to activate general stress response through Tor1 and Ras2 inhibition under conditions of glucose starvation and oxidative stress. *J. Biol. Chem.* 285, 19521–19531
76. Perez, P. *et al.* (2018) Fission yeast cell wall biosynthesis and cell integrity signalling. *Cell Surf.* 4, 1–9
77. Dichtl, K. *et al.* (2016) Cell wall integrity signalling in human pathogenic fungi. *Cell. Microbiol.* 18, 1228–1238
78. Vay, H.A. *et al.* (2004) Mutational analysis of the cytoplasmic domain of the Wsc1 cell wall stress sensor. *Microbiology (Reading)* 150, 3281–3288
79. Van Drogen, F. *et al.* (2020) Crosstalk and spatiotemporal regulation between stress-induced MAP kinase pathways and pheromone signaling in budding yeast. *Cell Cycle* 19, 1707–1715
80. Lee, B. *et al.* (2020) Quantitative analysis of yeast MAPK signaling networks and crosstalk using a microfluidic device. *Lab Chip* 20, 2646–2655
81. Truman, A.W. *et al.* (2009) Mechanism of Mpk1 mitogen-activated protein kinase binding to the Swi4 transcription factor and its regulation by a novel caffeine-induced phosphorylation. *Mol. Cell. Biol.* 29, 6449–6461
82. Jung, U.S. *et al.* (2002) Regulation of the yeast Rlm1 transcription factor by the Mpk1 cell wall integrity MAP kinase. *Mol. Microbiol.* 46, 781–789
83. Moreno-Torres, M. *et al.* (2015) TORC1 controls G1-S cell cycle transition in yeast via Mpk1 and the greatwall kinase pathway. *Nat. Commun.* 6, 8256
84. Schwob, E. *et al.* (1994) The B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S transition in *S. cerevisiae*. *Cell* 79, 233–244
85. Kanzaki, M. *et al.* (1999) Molecular identification of a eukaryotic, stretch-activated nonselective cation channel. *Science* 285, 882–886
86. Fischer, M. *et al.* (1997) The *Saccharomyces cerevisiae* CCH1 gene is involved in calcium influx and mating. *FEBS Lett.* 419, 259–262
87. Arsenault, H.E. *et al.* (2015) Hcm1 integrates signals from Cdk1 and calcineurin to control cell proliferation. *Mol. Cell. Biol.* 26, 3570–3577
88. Bultynck, G. *et al.* (2006) Slm1 and Slm2 are novel substrates of the calcineurin phosphatase required for heat stress-induced endocytosis of the yeast uracil permease. *Mol. Cell. Biol.* 26, 4729–4745
89. Cyert, M.S. (2003) Calcineurin signaling in *Saccharomyces cerevisiae*: how yeast go crazy in response to stress. *Biochem. Biophys. Res. Commun.* 311, 1143–1150
90. Garrett-Engele, P. *et al.* (1995) Calcineurin, the Ca2+/calmodulin-dependent protein phosphatase, is essential in yeast mutants with cell integrity defects and in mutants that lack a functional vacuolar H(+)-ATPase. *Mol. Cell. Biol.* 15, 4103–4114
91. Guiney, E.L. *et al.* (2015) Calcineurin regulates the yeast synaptojanin Inp53/Sjl3 during membrane stress. *Mol. Biol. Cell* 26, 769–785
92. Kafadar, K.A. and Cyert, M.S. (2004) Integration of stress responses: Modulation of calcineurin signaling in *Saccharomyces cerevisiae* by protein kinase A. *Eukaryot. Cell* 3, 1147–1153
93. Ly, N. and Cyert, M.S. (2017) Calcineurin, the Ca2+-dependent phosphatase, regulates Rga2, a Cdc42 GTPase-activating protein, to modulate pheromone signaling. *Mol. Biol. Cell* 28, 576–586
94. Cai, L. *et al.* (2008) Frequency-modulated nuclear localization bursts coordinate gene regulation. *Nature* 455, 485–490
95. Polizotto, R.S. (2001) Calcineurin-dependent nuclear import of the transcription factor Crz1p requires Nmd5p. *J. Cell Biol.* 154 (5), 951–960. https://doi.org/10.1083/jcb.200104078
96. Stathopoulos-Gerontides, A. *et al.* (1999) Yeast calcineurin regulates nuclear localization of the Crz1p transcription factor through dephosphorylation. *Genes Dev.* 13, 798–803
97. Tisi, R. *et al.* (2016) Calcium homeostasis and signaling in fungi and their relevance for pathogenicity of yeasts and filamentous fungi. *AIMS Mol. Sci.* 3, 505–549
98. Coste, B. *et al.* (2010) Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science* 330, 55–60
99. Radin, I. *et al.* (2021) Plant PIEZO homologs modulate vacuole morphology during tip growth. *Science* 373, 586–590
100. O'Rourke, S.M. and Herskowitz, I. (2002) A third osmosensing branch in *Saccharomyces cerevisiae* requires the Msb2 protein and functions in parallel with the Sho1 branch. *Mol. Cell. Biol.* 22, 4739–4749
101. Vadaie, N. *et al.* (2008) Cleavage of the signaling mucin Msb2 by the aspartyl protease Yps1 is required for MAPK activation in yeast. *J. Cell Biol.* 181, 1073–1081
102. Cybulski, N. and Hall, M.N. (2009) TOR complex 2: a signaling pathway of its own. *Trends Biochem. Sci.* 34, 620–627
103. Berchtold, D. *et al.* (2012) Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis. *Nat. Cell Biol.* 14, 542–547
104. Gaubitz, C. *et al.* (2016) TORC2 Structure and function. *Trends Biochem. Sci.* 41, 532–545
105. Riggi, M. *et al.* (2018) Decrease in plasma membrane tension triggers PtdIns(4,5)P2 phase separation to inactivate TORC2. *Nat. Cell Biol.* 20, 1043–1051
106. Chen, R.E. and Thorner, J. (2007) Function and regulation in MAPK signaling pathways: lessons learned from the yeast *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 1773, 1311–1340
107. Gasparski, A.N. and Beningo, K.A. (2015) Mechanoreception at the cell membrane: More than the integrins. *Arch. Biochem. Biophys.* 586, 20–26
108. Nauli, S.M. *et al.* (2003) Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat. Genet.* 33, 129–137
109. Delmas, P. *et al.* (2004) Polycystins, calcium signaling, and human diseases. *Biochem. Biophys. Res. Commun.* 322, 1374–1383
110. Yan, C. *et al.* (1999) Fuid shear stress stimulates big mitogen-activated protein kinase 1 (BMK1) activity in endothelial cells. Dependence on tyrosine kinases and intracellular calcium. *J. Biol. Chem.* 274, 143–150
111. Abe, J. *et al.* (1996) Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. *J. Biol. Chem.* 271, 16586–16590
112. Sohn, S.J. *et al.* (2002) ERK5 MAPK regulates embryonic angiogenesis and acts as a hypoxia-sensitive repressor of vascular endothelial growth factor expression. *J. Biol. Chem.* 277, 43344–43351
113. Pavan, S. *et al.* (2018) A kinome-wide high-content siRNA screen identifies MEK5-ERK5 signaling as critical for breast cancer cell EMT and metastasis. *Oncogene* 37, 4197–4213

114. Smith, A.E. *et al.* (2000) The mechanical properties of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 9871–9874
115. Zhao, L. *et al.* (2005) Elastic properties of the cell wall of *Aspergillus nidulans* studied with atomic force microscopy. *Biotechnol. Prog.* 21, 292–299
116. Minc, N. (2014) Microfabricated chambers as force sensors for probing forces of fungal growth. *Methods Cell Biol.* 120, 215–226
117. Heinisch, J.J. *et al.* (2010) Measurement of the mechanical behavior of yeast membrane sensors using single-molecule atomic force microscopy. *Nat. Protoc.* 5, 670–677
118. Delarue, M. *et al.* (2017) SCWISh network is essential for survival under mechanical pressure. *Proc. Natl. Acad. Sci. U. S. A.* 114, 13465–13470
119. Tayagui, A. *et al.* (2017) An elastomeric micropillar platform for the study of protrusive forces in hyphal invasion. *Lab Chip* 17, 3643–3653