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Cells under pressure: how yeast cells respond to mechanical forces

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Abstract

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 yeasts, 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 mechano-sensors and elaborate on downstream mechanotransduction mechanisms, which orchestrate appropriate cellular responses, leading to improved mechanical fitness. Finally, we highlight the conservation of signaling pathways in the fungal kingdom and mammalian systems, comparing mechano-transduction during microbial expansion to tumor growth in confined spaces.

Introduction

In their natural habitat, organisms are continually exposed to a myriad of biochemical or physical signals originating from within the cell or from the environment. Organisms as simple as microbes or as complex as mammals have evolved specific receptors to sense different signals and transduce the information through an interlinked signaling network to effector proteins which ultimately orchestrate an appropriate cellular response. 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 and polycystic kidney disease (reviewed in [1]). Likewise, mechanical forces that result from tumor expansion in confined niches are implicated in driving cancer progression, for example by activating the β -catenin pathway [2, 3]. It is possible that mechanical forces induce an epithelial-mesenchymal transition, thereby not only contributing to embryogenesis of *X. laevis* but also to the progression of solid tumors.

Unlike mammalian cells, many microbes and plant cells have a stiff cell wall in addition to the plasma membrane to oppose 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**). Although rigid, the cell wall can undergo dynamic adaptation during physiological process such as polarized growth that require carefully orchestrated spatiotemporal remodeling. Thus, even unicellular organisms protected by a cell wall may require dedicated mechano-responsive mechanisms to balance internal and external forces and prevent lysis. Although the origin of mechanical forces may be different, genetically-amenable microbes can therefore serve as simple yet valuable model systems to investigate fundamental principles of mechano-transduction. Indeed, recent discoveries in the yeasts *S. cerevisiae* and *S. pombe* identified a family of mechano-sensors – remarkably conserved even between non-walled mammalian cells - and revealed molecular insight into their downstream signaling pathways, which together help to explain how cells coordinate surface growth and cell wall synthesis, and

also how cells balance internal and external forces in changing environments. The emerging mechanisms have implications to understand filamentous growth and pathogen invasion, and may also reveal concepts relevant for multicellular organisms such as plants and animals. In this review, we will first describe the different forces experienced by fungal microbes in their natural habitat. We will then summarize how mechano-sensitive feedback mechanisms crosstalk with cell division and polarized growth, and describe mechanical regulation in fungal pathogenicity. Finally, we will highlight the underlying mechano-transduction pathways and cellular stress responses, and relate yeast findings to mammalian cells.

Mechanical forces in the natural habitat of unicellular fungi

In their natural habitat, unicellular fungi grow attached to each other and adhered onto biotic (e.g. plant or animal hosts) or abiotic (rocks, medical devices, etc.) surfaces, thus forming a coordinated functional community called biofilms (**Fig. 1A**). In biofilms, cells are surrounded by complex polymer and water, forming a hydrogel, which is further confined by physical boundaries, leading to growth-induced compression at contact points. Moreover, the local geometry of the biofilm itself can generate a complex network of contact forces among neighboring cells. In addition, tensile forces can arise if the substratum is stretched, for instance by heat-mediated contraction or expansion. Similar to compression, tensile stress leads 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 and protect themselves against these combined physical forces to grow and survive in complex microenvironments.

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 rod-shaped single fission yeast cells, or filamentous yeast, can buckle under the force developed at their tips that pushes against the wall of the chamber [4, 5]. Multicellular colonies of the rounded budding yeast in deformable PDMS bioreactors grown to high density close to jamming transitions can develop increased stresses of up to 0.7 ± 0.1 MPa, which corresponds to 7 atmospheric pressure, similar to the pressure in a bottle of champagne.

Such stresses will propagate within the colony, stretching cell contacts and creating large tensile stresses on cell walls [6]. Similarly, stresses resulting from a moving rigid boundary onto a cell, such as a PDMS actuated roof, can cause cells to flatten and expose lateral cell walls to tensile stresses [7]. Other typical sources of physical stress in the natural habitats of yeasts and fungi are those associated with osmotic changes, during drought or humidification of the environment. Osmotic forces can be very large causing cells to shrink or stretch, thereby yielding to large surface stress. Thus, fungal cells may be exposed to external forces that cause them to undergo dramatic changes in cell shape and experience large mechanical stress at the cell surface.

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

To withstand external mechanical stress, yeast and fungal cells are equipped with two mechanical systems: a stiff elastic cell wall and an inflated cytoplasm which features an unusually large pressure called turgor [8]. In analogy with an inflated inner air tube (the turgid cell and its plasma membrane) encased by an elastic rubber-made tire (the cell wall), these elements will limit cell 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 50-500 nm, and its bulk elastic modulus (Young's modulus) is equivalent to that of rubber, ranging from 10-100 MPa [9, 10]. The strength and elasticity of the cell wall are associated with the presence of glucan chains and chitin, which form hydrogen bonds with each other (reviewed in [11-13]). The glucan layer in the cell wall is primarily composed of β -1,3-glucan chains that are branched with β -1,6 linkages. In *S. cerevisiae*, the β -1,3- and β -1,6-glucan polymers and chitin make up 50-55%, 5-10% and 1-2% of dry cell wall mass, respectively. This glucan layer is connected to an outer layer of mannoproteins, which constitute about 35-40% of the cell wall dry mass. These mannoproteins emanate from the cell surface and serve to promote cell-cell recognition and the sensing of 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 different species, in different conditions, at different life stages of a given species, or even in different parts of the same cell [14-18]. These modulations

are needed to accommodate properties such as growth, morphogenesis and polarity, and they are also largely adapted to changing environments. 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 [19, 20]. Major cell wall modifications are also a hallmark of fungal infections as cells move within the different chemical environments of their hosts [15].

Turgor pressure values, on the other hand, 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 [21]. Typical estimates of turgor pressure values in proliferating yeast cells are about 0.5-1.5 MPa [4, 22, 23]. Turgor results from water influx driven along an osmotic gradient, associated with the higher solute and ion concentrations in the cytoplasm as compared to that in the extracellular milieu (**Fig. 1B**). Turgor pushes the plasma membrane onto the cell wall, and deforms it elastically, providing a mechanical engine that powers cell growth and invasion and also resists external forces. Turgor is vital for fungal cell physiology, and these cells have evolved robust homeostatic systems to maintain it even against drastic osmotic variations in the environment (**Fig. 1B**). When cells are exposed to high osmolarity conditions, turgor pressure rapidly decreases, as efflux of water across the semi-permeable plasma membrane leads to cytoplasm shrinkage. As a result, the plasma membrane disconnects from the cell wall, leading to the activation of the HOG-signaling cascade, which in turn closes glycerol channels and also triggers synthesis of intracellular glycerol to restore the osmotic balance, and re-inflate the cell [24-26]. Conversely, when cells are exposed to hypotonic solutions, water flows inside the cells, inflating the cytoplasm and causing cells to expand. Adaptation to hypoosmotic shocks is less well understood, but may involve mechanosensitive channels that open to release water from the cytoplasm [27, 28]. As cells expand, the necessary membrane surface increase is provided by flattening membrane reservoirs called eisosomes [29].

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 (**Fig. 1C**). For a typical yeast cell of radius $R=2\ \mu\text{m}$, the tension T created by a turgor pressure of $P=1\ \text{MPa}$ will be of $T=PR/2 = 1\ \text{N/m}$. This is typically three orders of magnitude

higher than the tension needed to lyse the plasma membrane [30], 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 yielding to a force balance that contributes to set 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 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 [9]. Elastic failure at which the cell wall may start to rupture have been estimated to be around ~45% in *S. cerevisiae* [31]. 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. Because fungal cells come with a large range of sizes spread over 2 orders of magnitudes, this could create overly large tension in the wall and cause it to break. As such, cells may thicken or stiffen their cell walls by modulating elastic bonds or adding extra layers, as they grow to larger sizes, allowing elastic strains to remain below failures [9].

A critically important function of cell wall stress generated by turgor is to promote cell growth [32] (**Fig. 1D**). Yeast and fungal cells with reduced pressure almost always slow or even halt growth [4, 32]. Growth has been typically modeled by assuming that the cell wall can undergo plastic, irreversible deformations above a stress threshold, and can also exhibit a viscous behavior so that the rates of deformations increase with stress [33]. Thus, in order to grow, cells may transiently thin, soften or fluidize their cell wall, which entails risks of failure, highlighting how growth has potential to be 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 at polarized tips, typically referred to as polarized growth. In budding yeast, polarized growth occurs during three physiological processes: budding, mating and filamentous growth [34-36]. Different physiological signals initiate these processes, including sufficient nutrition for budding, exposure to a pheromone gradient[37], and starvation to trigger filamentous growth to escape scarce nutrient conditions [38]. Tip growth is also the main growth mode of 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) is involved in driving polarized growth in budding yeast (**Fig. 2**) [39, 40]. Cdk1 phosphorylates Cdc24 [40], a GTP exchange factor, which in turn activates the small GTPase Cdc42, a conserved master regulator of cell polarity [41-43] (for reviews see [36, 44]). In brief, Cdc42 recruits the polarisome complex, which uses its combined activity of actin filament nucleation and exocytosis to achieve spatiotemporal control of vesicle transport and fusion [45, 46]. In budding yeast, the polarisome complex is composed of the scaffolds Pea2 and Spa2, the formin Bni1, the actin nucleator Bud6, and the GAPs for the Rab GTPase Msb3 and Msb4 (reviewed in [36, 44]). Components of the cell wall synthesis machinery are among the major cargos transported by myosin along actin cables to the polarization site [47, 48]. For example, the cell wall β -1,3-glucan synthesizing enzymes Fks1/2 are localized uniformly during isotropic growth, but delivered by secretory vesicles to growing tips, where their activity is stimulated by the GTPase Rho1 [48-51]. This mechanism ensures that glucan chains and chitin are produced at sites of polarized growth, while the mannoproteins are directly delivered via the secretory pathway. Similar concepts and homologous regulators of cell wall synthesis and remodeling are generally present in other yeasts and fungi (reviewed in [34, 52-54]).

Polarized cell growth results in the formation of thinner or softer cell tips, and wall segments transfer or flow from the apex to the cell sides, likely thickening and stiffening the cell wall away from the tips [14]. Recent studies have modeled the process of tip growth using different frameworks and hypothesis [55, 56]. Some of these models can remarkably account for important aspects such as variations in tip shape or growth speed [57]. To date, however, the difficulty of properly quantifying the time-evolution of local mechanical parameters in the cell wall has limited experimental validation of these models.

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 machineries when they encounter external mechanical forces. Faced with cell wall damage and external mechanical

signals, yeast cells respond by rapid growth arrest and depolarization due to the inactivation of Cdk1 (**Fig. 2**) [7, 58, 59]. Depolarization involves disruption of actin cables and destabilization of the polarisome complex at growth sites. For instance, in *S. cerevisiae*, if a compressive stress is applied uniformly using a flattening microfabricated roof (**Box 2**), Bni1 and other polarisome components relocalize and become dispersed around the cell surface, leading to uniform synthesis and strengthening of the stretched cell wall, thereby preventing cell lysis [7]. 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 the 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 [60].

Conceptually equivalent behavior has been 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 site of contact where force is applied, to rapidly reform at a new site in a direction away from the obstacle [61]. 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 [62]. Such depolarization/re-polarization processes may be wide spread in yeast and 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, described next.

Stabilizing the polarity axis is crucial for fungal invasion

While local depolarization in response to mechanical stress can allow cells to change their growth axis, there are other instances in which growth direction and polarization must be stabilized for successful invasion of host tissues by fungal pathogens (**Fig. 3**). An important example of such a process 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 on 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 gets inflated by enhanced glycerol synthesis to increase turgor pressure, which in the appressorium can be as high as 8-10 MPa (80-100 atmospheric pressure) [21]. The cell wall of the appressorium is melanized to sustain the pressure and limit glycerol efflux, thus acting as a sealing cage [63, 64]. The penetration peg grows from the appressorium into the plant cell surface, generating finely localized stresses that fracture the plant cell wall and allow the fungus to invade the host tissue. In the pathogen *M. oryzae*, 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 [63]. Thus, invasion requires generation of 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 colonizes neighboring cells by invading through plasmodesmata [65]. During this process, the polarity complex on hyphal tips continuously reorients until it finds weaker plasmodesmata regions where the polarity complex remains persistent, allowing penetration of neighboring cells using mechanical feedback mechanisms to stabilize growth tips, as discussed above. Animal pathogens such as *C. albicans* similarly depend on physical penetration mechanisms to infect the softer 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 *W. dermatitidis*, the invasive hyphal growth depends on melanin biosynthesis [66], 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 signal transduction 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 sense the turgor threshold required for breaching of rice leaf cuticle [67]. 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 their unidirectional 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 the

Young's modulus of the host tissue, polar growth is halted, destabilizing the polarisome complex at growth sites (**Fig. 3A**). The polarisome complex migrates and then reassembles in an adjacent region with lower external force, and finally reorients the 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 [5, 65, 68]. 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 thereby find weaker/softer regions where they can sustain polarized growth for successful invasion.

Surface mechano-sensors can convert physical forces into biochemical signals

Yeast cells achieve appropriate mechano-responses 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 mechano-sensors and cause phosphorylation changes in signaling proteins and/or increase in concentration of cytoplasmic calcium ions. One important class of mechano-sensors in yeasts are single-pass cell surface proteins that possess a short cytoplasmic tail and a highly glycosylated extracellular domain that functions as a nanospring. Another class consists of stretch-activated ion channels. Lastly, mechanical forces may also be sensed by topological changes that occur in cell membranes, releasing and activating signaling proteins. Below we will describe how mechano-sensors convert external mechanical forces acting on the surface of yeast into intracellular biochemical signals.

A conserved family of mechano-sensors containing nano-spring-like domains

Wsc1 is among the best described mechano-sensors and coordinates mechano-transduction and cell wall integrity in budding yeast [48, 69-71]. This transmembrane protein accumulates predominantly at sites of polarized growth and functions as an upstream regulator of the Cell Wall Integrity pathway (CWI) that adjusts cell wall synthesis in response to chemical or heat stress [71-73]. Indeed, *wsc1Δ* cells are sensitive to cell wall damaging agents [72] and often lyse during polarized growth in processes such as budding and shmooing. Wsc1 is conserved across fungi including budding-, fission-, and filamentous fungi, and live cell analysis of cell wall thickness in

S. pombe cells suggest that Wsc1 also functions as a surface sensor to promote homeostasis and safeguard cell wall integrity at growing tips [14, 69]. Wsc1 is one of five putative cell wall surface sensors in *S. cerevisiae* (**Fig. 4A**), which belong to either the Wsc- or Mid2/Mtl1-family. These mechano-sensors share an extracellular Serine/Threonine-rich (STR) domain (reviewed in [74]), with about 50% serine or threonine residues. Transfer of mannose-groups to these residues yields highly mannosylated transmembrane proteins [75], resulting in a significantly higher molecular weight in western blot analysis compared to the unmodified protein. Upon mannosylation, the typical folded structure is converted into a linear rod that projects into the cell wall matrix [74]. The functional relevance of mannosylation is suggested by the mechano-sensitive phenotype of cells lacking the enzymes required for this post-translational modification [73]. Likewise, although the STR domain is extracellular, it is suggested that the cytosolic tail is constitutively phosphorylated on multiple sites. Interestingly, these sites are dephosphorylated upon mechanical stress, and this mechanism was proposed to affect mechano-transduction[73].

Importantly, direct evidence that STR-containing cell surface proteins function as mechano-sensors was provided by Atomic Force Microscopy (AFM) [76]. 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. Taken together, available data suggests that unbalanced turgor and extracellular forces at growth sites squeeze the cell wall driving compression of the spring-like STR domain of Wsc1 (**Fig. 4A**), which in turn signals downstream to locally increase cell wall synthesis to prevent cell wall rupture and lysis. 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 onto 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 mechano-receptor activated by compressive stress. As indicated by its name, Mid2 was originally identified in a screen for genes that protect cells from prolonged pheromone exposure, as *mid2Δ* cells lyse during shmoo formation [77]. Likewise, *mid2Δ* cells burst at bud emergence when exposed to compressive stress applied by a pressure-controlled microfluidic device (**Box 1**, [7]). 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 [7], indicating that compressive physical stress is directly sensed by its nano-spring domain.

Based on these results, we speculate that STR-domains may generally function as nano-springs that respond to mechanical forces. Although it is possible that individual STR domains respond differently to force intensity or type, they may also sense mechanical stress at distinct cellular compartments. Indeed, Wsc1 accumulates at sites of polarized growth, while Mid2 localizes uniformly to the cell cortex [7, 71]. Mid2 contains a glycosylated asparagine at the N-terminus, while Wsc-family sensors encompass a conserved lectin-like domain that was proposed to promote clustering and binding to newly synthesized cell wall structures at growth sites [78]. Indeed, recent data in fission yeast suggest this lectin-like domain relocates and clusters Wsc1 to sites where forces are locally applied onto the cell wall [79]. Moreover, Wsc1 localization to polarized sites requires its cytoplasmic tail, which stimulates endocytic trafficking of the mechano-receptor [80].

Much less is known about the function and regulation of the other *S. cerevisiae* STR-containing receptors. Like Wsc1, Wsc2 and its paralogue Wsc3 have been implicated in maintaining cell wall integrity and recovery from heat shock, while Mtl1 may help to preserve cell integrity during glucose starvation and oxidative stress [81]. Further work is required to study the phenotypes of cells deleted for these mechano-sensors, and in particular compare their expression and subcellular localization in response to force, growth and stress-conditions.

STR-containing mechano-sensors activate the CWI MAP kinase pathway

The cell wall integrity (CWI) pathway emerged as a core signaling pathway that mediates cellular responses to intrinsic and extrinsic cell wall threats downstream of STR-containing mechano-sensors (**Fig. 4B**). Its components are highly conserved across budding, fission, and filamentous fungi, suggesting that the type of mechanical cues and downstream responses are comparable [74, 82, 83]. CWI proteins accumulate at sites of polarized growth, where they monitor and adapt wall synthesis to local mechanical needs. Moreover, they are recruited to damaged cell wall structures to reinforce integrity [60, 74]. Activated STR-containing mechano-sensors recruit the exchange factors Rom1 or Rom2 (Rom1/2) by binding to their intracellular tails. How

compression of the extracellular nanospring is transmitted across the transmembrane segment to the intracellular tail remains to be elucidated. While the cytosolic tail domains of STR receptors vary in length and sequence (**Fig. 4A**), they are often dephosphorylated in response to cell wall damage, thereby promoting interaction with Rom1/2 [84]. Membrane-associated Rom1/Rom2 then binds the small GTPase Rho1, and catalyzes exchange of GDP to GTP. In turn Rho1-GTP activates Protein Kinase C 1 (Pkc1), which phosphorylates the downstream MAPK module culminating in Mpk1/Stl2 activation (reviewed in [74]). In addition, Pkc1 also phosphorylates the signaling scaffolds Far1 and Ste5, thereby preventing pheromone signaling and mating responses in the presence of cell wall stress [58]. Activated Mpk1 triggers nuclear translocation of Rlm1 and the SBF complex to induce a transcriptional program including upregulation of cell wall synthesis genes [85, 86]. Moreover, Mpk1 phosphorylates Sic1, an inhibitor of Cdk1 activity, arresting the cell cycle in G1, and regulates unknown substrates to disrupt polarized actin cables and thereby counteracts polarized growth [87, 88].

Intra- and extracellular mechano-sensors without STR domains

While STR-domains may provide force-sensing capacities to a family of membrane proteins, other fungal mechano-responsive components do not contain such nano-springs. 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 [7, 89, 90] (**Fig. 5**). These proteins and their functions are conserved across fungal microbes [91], suggesting that Ca²⁺ signaling is part of the intracellular response to mechanical stress (see below). Although the Mid1/Cch1 complex has not formally been demonstrated to act as a calcium channel due to technical challenges with patch-clamping across the yeast plasma membrane, co-expression of budding yeast or *Cryptococcus neoformans* Mid1 and Cch1 in mammalian cells revealed stretch-activated cation channel activity [92] [93]. However, the molecular mechanism for how Mid1 and/or Cch1 sense mechanical forces impacting cell wall and plasma membrane distortions remains elusive. Experiments with the Ca²⁺-chelator EGTA suggest that the major source of calcium originates from outside the cell, but mobilization of internal Ca²⁺ reservoirs such as vacuoles cannot be excluded [94, 95]. Thus, developing genetic tools in which mobilization of

external or internal calcium sources can be switched on or off would be valuable to decouple the role of Ca^{2+} reservoirs in mechano-stress responses.

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 (**Fig. 5**). Indeed, cells lacking Msb2 or Sho1 often lyse when they reach high density upon expansion in this confined microenvironment [59]. Previous work established that these proteins comprise a sensor module to protect cells from high osmolarity [96] by increasing turgor pressure through blocking water efflux and increasing glycerol synthesis. It is thus possible that Msb2/Sho1 responds to mechanical stresses associated with hyperosmotic conditions. Interestingly, Msb2 harbors a highly glycosylated extracellular domain with an autoinhibitory function, which projects from the cell surface [97]. It is likely that mechanical forces acting on the cell surface release this auto-inhibitory function, thereby converting mechanical cues into intracellular biochemical signals. Release of this autoinhibition is mediated by the cleavage of this extracellular domain by the aspartic protease Yps1, which is attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor [97]. Further work will be required to elucidate the mechanism activating Yps1-cleavage in response to contact forces.

Finally, target of rapamycin complex 2 (TORC2) was found to respond to mechanical cues generated on the yeast cell membrane (**Fig. 5**). This evolutionarily conserved signaling complex is involved in actin remodeling [98], and modulates lipid synthesis to balance membrane tension and internal turgor pressure [99]. TORC2 localizes as puncta at the plasma membrane, adjacent to eisosomes [100]. When the membrane is exposed to increased tension for example through raising turgor pressure upon hypotonic stress or changes in lipid composition, eisosomes flatten and release the Slm1 and Slm2 paralogues, which in turn activate TORC2 signaling [101].

Taken together, these results show that different mechano-sensors respond to multiple intra- and extracellular mechanical cues impinging on the cell wall or plasma membrane (**Fig. 5**), and cooperate to accomplish an intracellular response that properly adapts cells to different types and magnitudes of physical forces.

Temporal-spatial regulation and synergies among cellular mechano-transduction pathways

Surprisingly, the Msb2/Sho1 compressive stress sensors also operate, at least in part, via the Mpk1 MAP-kinase module. Instead of Pkc1, Sho1 stimulates the MAPKKK kinase Ste11, which in turn activates the MAP kinases Mpk1, Hog1 and Kss1 [102]. While further work is required to understand how Ste11 activates Mpk1, this “SmuSh” pathway affects multiple cellular effectors involved in cell wall integrity, filamentous growth and osmoadaptation. Moreover, TORC2 activates Pkc1 and the Mpk1 MAP-kinase module when cells are exposed to the carbonyl-compound methylglyoxal [103]. TORC2 also phosphorylates the AGC kinase Ypk1 thereby modulating lipid synthesis and regulation of membrane tension and internal turgor pressure [101]. Together, these results suggest that different cell wall- and plasma membrane stress sensors converge on the CWI pathway to restore cell integrity and confer cell survival.

Interestingly, genetic experiments suggest that Ca^{2+} signaling and activation of the CWI pathway 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 [7]. Likewise, an earlier study reported that both Pkc1, the upstream kinase of Mpk1, and calcineurin are required for heat stress responses involving expression of Fks2 [104]. The molecular explanation for this additive effect remains to be elucidated. Cells express many Ca^{2+} -binding proteins, among them Pkc-isoforms and proteins regulating cytoskeletal dynamics. However, 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 [105-112]. Interestingly, activated calcineurin dephosphorylates the transcription factor Crz1, resulting in rapid nuclear translocation of Crz1 [113-115], orchestrating a transcriptional program to strengthen the cell wall [74]. Nuclear accumulation of Crz1 follows a fluctuation pattern, but the underlying regulator for such pulsing activation remains to be elucidated. Unlike cells lacking the calcineurin subunits Cna1 or Cnb1, *crz1Δ* mutants are not mechano-sensitive [7], implying that calcineurin must have other targets relevant for cell survival upon mechano-stress. Indeed, comprehensive studies identified many calcineurin substrates [116], and it will be interesting to examine which ones are relevant for cellular responses to mechanical stress.

The ultimate goal of the mechano-transduction network is to strengthen the cell wall and plasma membrane in response to diverse physical perturbations while maintaining mechanical integrity and physiological function (**Fig. 5**). The mechanical input can act either on the cell surface or internal structures, and may occur in parallel or by sequential activation to account for short-,

medium- and long-term responses. For instance, calcineurin and Pkc1-activated pathways cooperate to antagonize polarized cell growth and ensure cell survival upon compressive mechanical stress [7]. Similarly, when both the SmuSh and the CWI pathways are deleted, cells fail to adapt to compressive stress, and all cells lyse at relatively low pressure when grown in confinement [59]. The TORC2 pathway responds to cell membrane tension, but its involvement in other types of mechanical stress remains to be studied. Given that TORC2 activity was linked to cytoskeletal dynamics, it will be interesting to explore whether TORC2 synergizes with other mechano-stress pathways in actin depolarization and polarized growth inhibition. Mechano-transduction can either be adaptive or sustained. For instance, temporal and spatial mechanisms strengthen the cell wall at growth sites, while sustained cell wall thickening occurs during prolonged starvation [117]. Moreover, while early responses depend on adaptive Ca^{2+} signaling and transient activation of the general stress-transcription factor Msn2, Mpk1 activation executes a sustained cellular response to mechano-stress. Likewise, transient Hog1 activation allows cells to rapidly adapt to high osmolarity conditions [26, 118, 119] and possibly other mechanical stress [59]. Thus, a combinatorial analysis of mechano-sensitive signaling pathways in different stress conditions will shed light on spatio-temporal regulation, positive and negative feedback mechanisms, and reveal how individual components synergize and crosstalk with each other to orchestrate the complex array of cellular responses to physical stress.

Conservation of signaling beyond the fungal kingdom

Although mammalian cells lack a rigid cell wall, and feature much lower cytoplasm pressure, their surface mechano-sensing modules may share interesting parallels to those found in yeast and other fungi. Mammalian cells possess 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 [120]). Furthermore, they likely express compression-specific mechano-sensors on their cell surface similar to budding yeasts [7], which convert external compressive forces into biochemical signals that elicit appropriate responses. Similar to yeast, mechanical stress in mammalian cells also triggers Ca^{2+} influx and activation of the calcineurin complex. In humans, polycystin-1 (PC-1)/Polycystin-2 (PC-2) ion channels sense shear forces in renal tubule epithelial cells. Similar to Mid1 and Cch1,

PC-1 and PC-2 form a heteromeric calcium channel complex that allows calcium influx in response to fluid drag force [94, 121]. Moreover, PC-1 possesses a WSC domain, a cysteine-rich domain found in STR-family members in yeast [72, 122]. Thus, the mechanistic elucidation of Mid1/Cch1 and STR family sensors may enhance our understanding of PKD pathogenesis, which is generally caused by loss or disruption of PC-1/PC-2 functions, leading to renal epithelial cells unable to sense mechanical cues that normally regulates kidney morphogenesis [94].

In fungi, mechano-sensing is mediated by STR-containing family members, which in turn activate CWI pathway comprising the MAP kinase Mpk1. Although an correlation of these sensors with integrins has been suggested based on their linear extracellular domains protruding into the cell wall and their clustering under force [123], there are no known homologs in mammalian cells containing such extracellular nanospring domains rich in highly mannosylated serine and threonine residues. However, there are several proteins such as MUC1 and CD44 which share functional similarities. MUC1 is a cell surface transmembrane protein whose extracellular domain is heavily glycosylated [124-126], and its short cytoplasmic tail is regulated by protein kinase C delta [127]. Moreover, MUC1 regulates external forces and matrix stiffness and has been shown to cluster integrins leading to the formation of focal adhesions [128]. Thus, we speculate that MUC1 may directly sense mechanical forces and mediate an integrin-mediated signaling response. As the mammalian genome encodes more than 1000 surface glycoproteins [129], functional assays will be required to screen for possible mechano-sensors.

In contrast to mechano-sensors, the closest mammalian homologue of Mpk1 is the MAP kinase ERK5. Interestingly, ERK5 is activated by multiple stress conditions including osmotic, shear, oxidative and hypoxic stress in addition to growth factors and cytokines [130-132]. ERK5-null mice exhibit a cardiovascular defect [133], and endothelial cells are susceptible to shear stress [130]. A recent study using a kinome-wide screen demonstrated that ERK5 and its upstream kinase MEK5 regulate the epithelial-mesenchymal transition (EMT) [134], an epigenetic program leading to loss of cell-cell junctions and reorganization of the cytoskeleton. Loss of ERK5 activity in MCF7 cells confirmed suppression of EMT markers including SNAIL and ZEB1, and upregulation of E-cadherin [135]. Taken together, these results suggest that ERK5 may respond to mechanical stress, such as compressive forces that arise due to expansion of tumors in limited space and possibly regulate EMT for cancer progression. Thus, organisms as simple as yeast have evolved

conserved mechanisms to respond to mechanical forces, which may have implications to understand mechano-transduction in health and disease of complex organisms.

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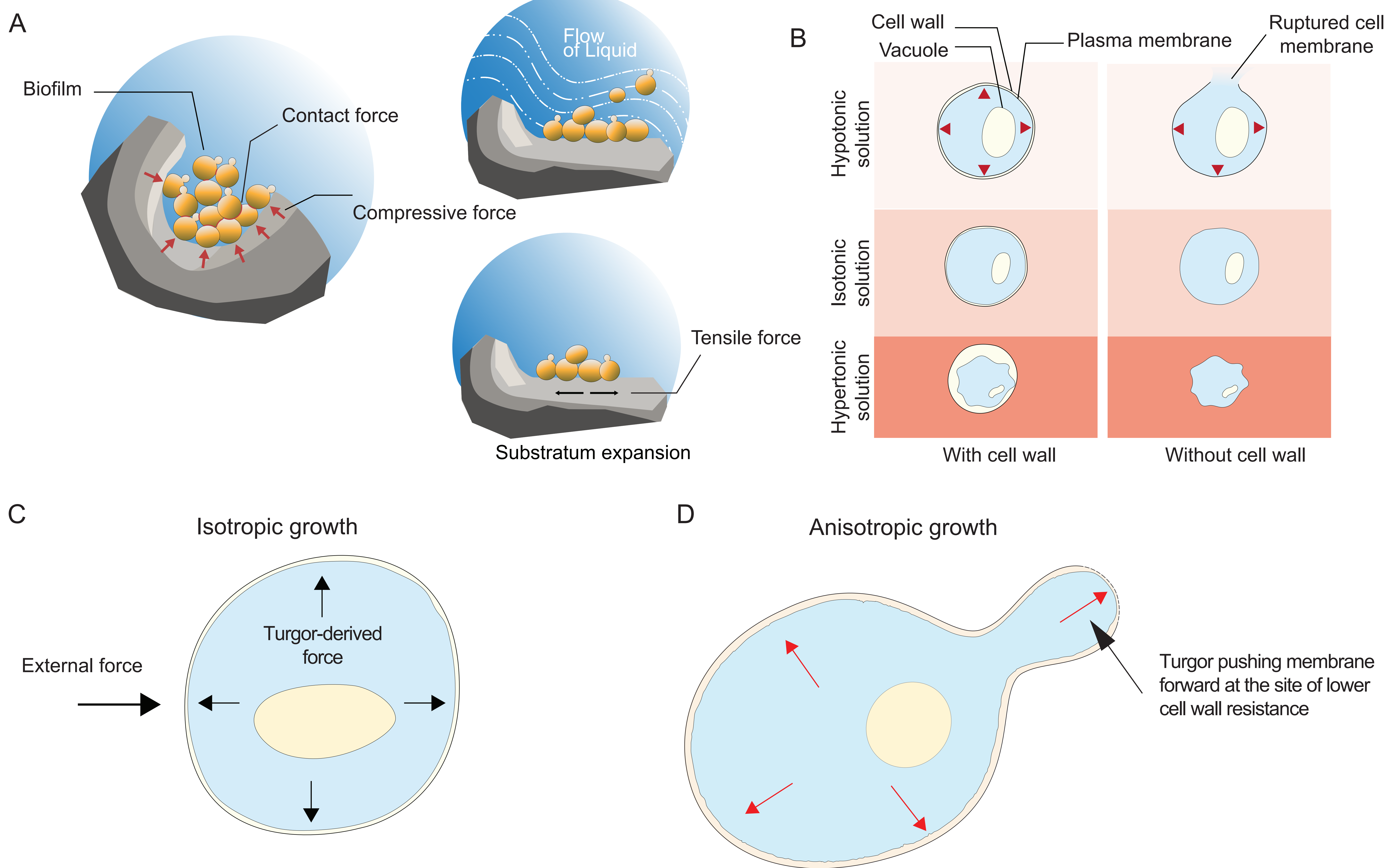


Figure 1

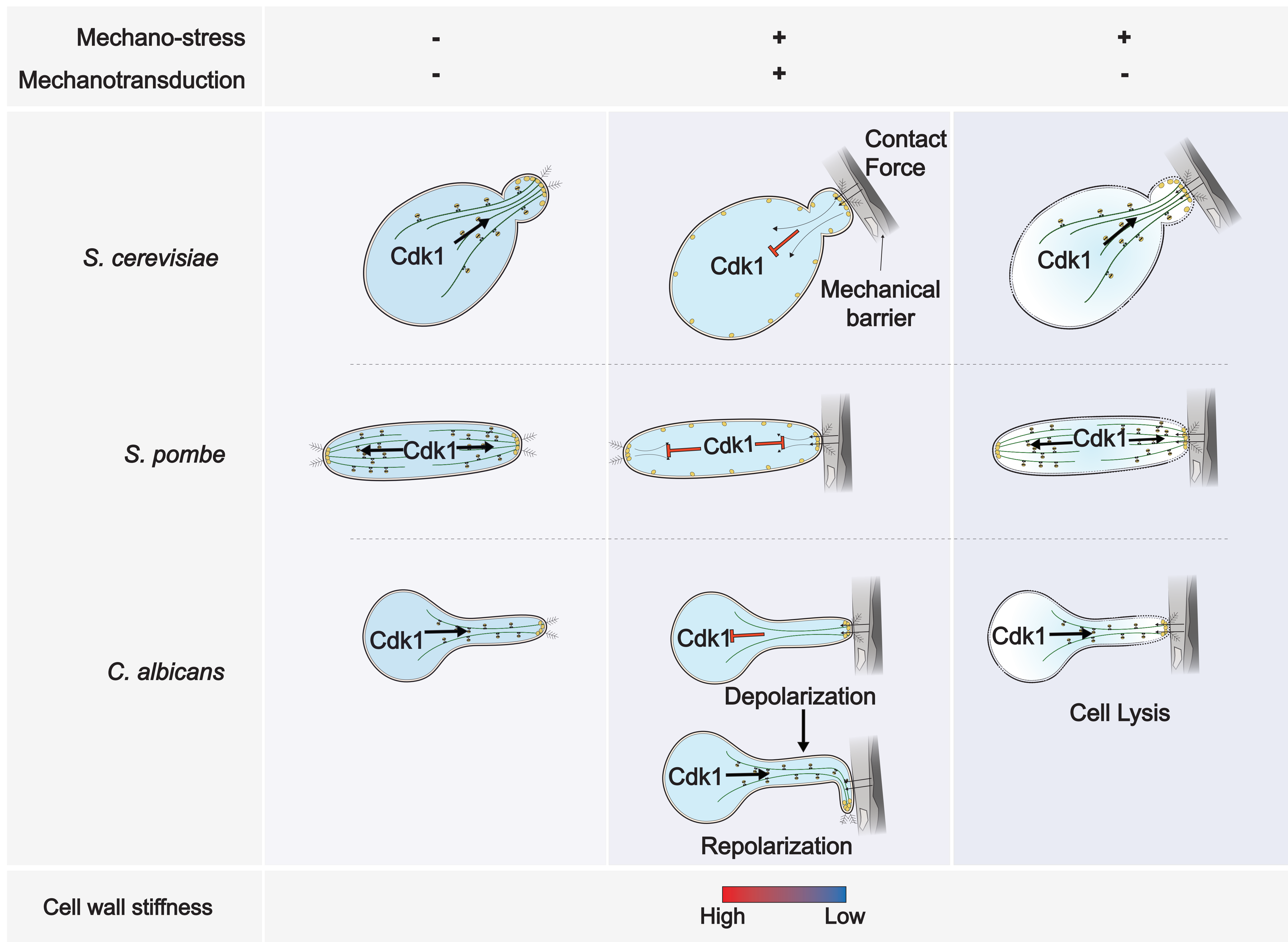


Figure 2

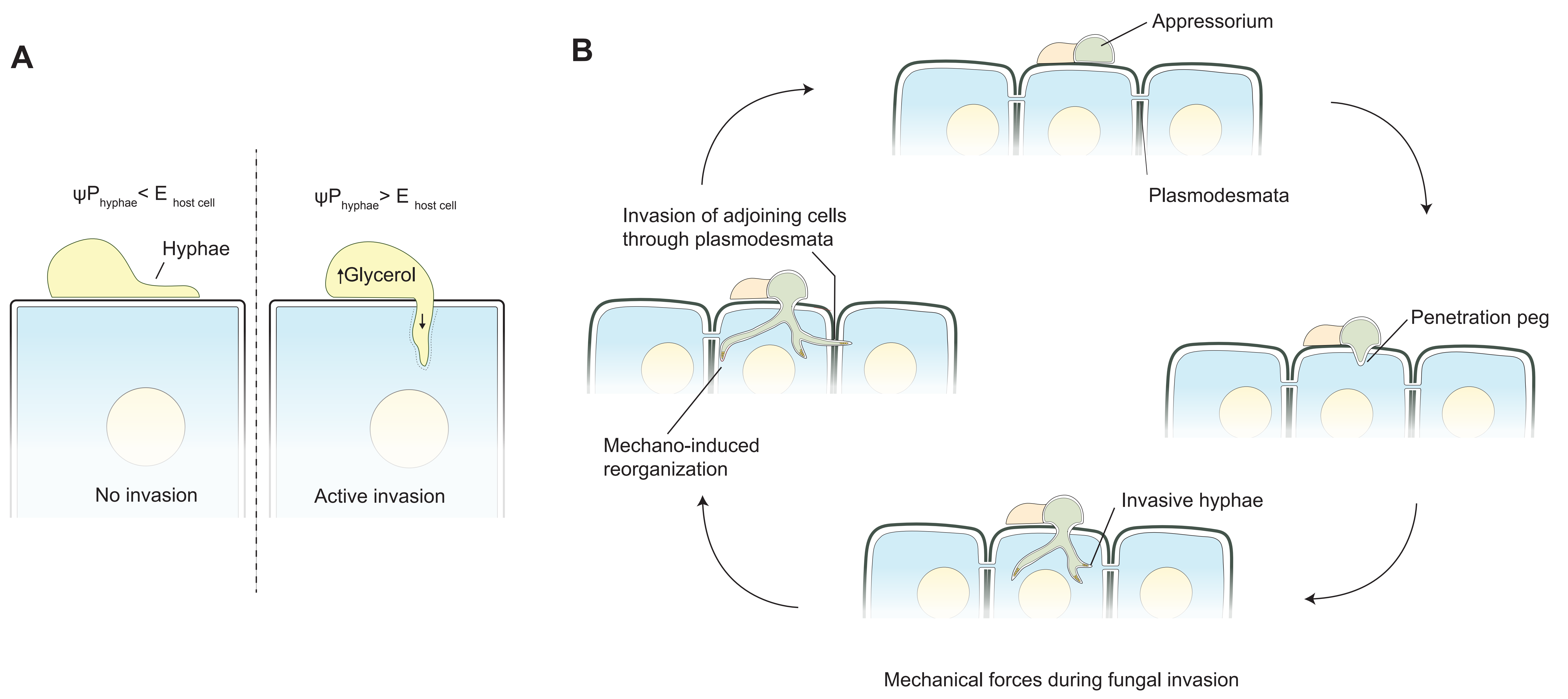


Figure 3

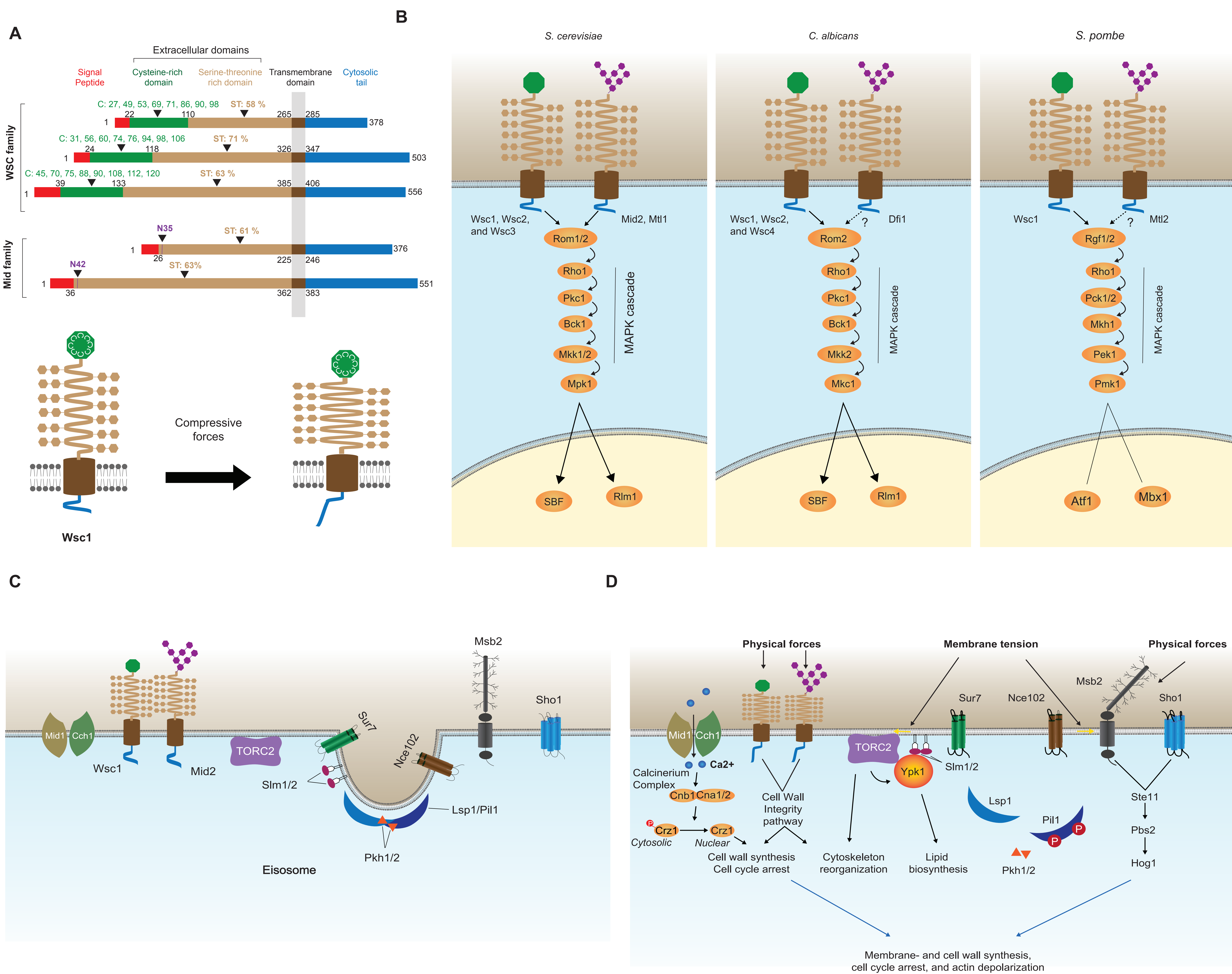


Figure 4