Wall mechanics and exocytosis define the shape of growth domains in fission yeast

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The amazing structural variety of cells is matched only by their functional diversity, and reflects the complex interplay between biochemical and mechanical regulation. How both regulatory layers generate specifically shaped cellular domains is not fully understood. Here, we report how cell growth domains are shaped in fission yeast. Based on quantitative analysis of cell wall expansion and elasticity, we develop a model for how mechanics and cell wall assembly interact and use it to look for factors underpinning growth domain morphogenesis. Surprisingly, we find that neither the global cell shape regulators Cdc42-Scd1-Scd2 nor the major cell wall synthesis regulators Bgs1-Bgs4-Rgf1 are reliable predictors of growth domain geometry. Instead, their geometry can be defined by cell wall mechanics and the cortical localization pattern of the exocytic factors Sec6-Syb1-Exo70. Forceful re-directioning of exocytic vesicle fusion to broader cortical areas induces proportional shape changes to growth domains, demonstrating that both features are causally linked.
The regular self-assembly of viruses from protein subunits offers an interesting paradigm for how shape can be encoded at the molecular level. However, most cells are of a scale that lies above the reach of molecular self-assembly and as a consequence their shape results from a subtle interplay between biochemical regulation and mechanical constraints.

With their highly regular morphogenesis involving two opposed growth domains, the walled cells of the fission yeast *Schizosaccharomyces pombe* provide a powerful system to address this question. Following cell division, *S. pombe* cells first grow monopolarly from their ‘old end’ (OE) inherited from their mother but soon thereafter they activate their ‘new end’ (NE) derived from the site of cell septation during an event called New End Take Off (NETO). After NETO, cells grow bipolarly throughout most of the cell cycle until the next cell division, when cells septate giving rise to two similarly sized daughter cells and that re-initiates the morphogenetic growth cycle.

Here we have combined physical modelling and quantitative live cell analysis to investigate how the geometry and morphogenesis of *S. pombe* cells result from the interplay between biochemical and mechanical regulation. We show that neither the global cell shape regulator Cdc42 and its activators Scd1 and Scd2 (refs 14–17) nor the major cell wall synthesis regulators Bgs1, Bgs4 and Rgf1 (refs 18–20) are reliable predictors of the geometry of cell growth domains. Surprisingly, we instead demonstrate that their geometry can be defined by cell wall mechanics and the cortical localization pattern of the exocytic factors Sec6, Syb1 and Exo70 (refs 21,22) across a range of genotypes. By forcefully inducing the re-directioning of exocytic vesicle fusion to broader areas of the cell cortex, we further show that this induces proportional shape changes to growth domains, demonstrating that both features are causally linked. We propose that cell wall mechanics and exocytic pattern suffice to account for growth domain morphogenesis throughout the cell cycle in this species.

**Results**

**Growth domains undergo shape changes through the cell cycle.** To investigate how fission yeast cells are locally shaped, we quantitated the curvature of their growth domains, which are the areas that undergo geometrical changes through the cell cycle (Fig. 1a and Supplementary Movie 1 and Methods). Although initially flat at septation, we found that the shape of the NE (pre-NETO) becomes roughly hemispherical (Fig. 1b, red). By contrast, we found that the OE displays a much pointier, non-hemispherical shape distinct from that of the NE (Fig. 1b, green). Quantitation of end curvature through time (Fig. 1c) revealed that OE curvature does not change noticeably throughout growth, indicating that OE geometry results from a stable growth domain dynamics (Fig. 1d). On the other hand, NE geometry changes substantially following NETO and continues to change until late G2 phase, when NEs acquire an OE geometry while leaving gradually aside scars—cytokinesis-derived structural deformations of the cell wall (Fig. 1d). Thus, the morphogenesis of *S. pombe* is characterized by a simple growth domain dynamics according to which NEs transition from flat to hemispherical and then morph gradually into OEs, whereas OEs maintain their geometry that acts as a stable morphogenetic attractor (Fig. 1e).

**A mechanical model of fission yeast cell growth.** As a first step to explain the origin and maintenance of the cell ends’ geometry, we developed a technique to label cells with fluorescent quantum dots (Qdots) and use them as fiducial marks to track the cell wall deformation (Supplementary Fig. 1 and Supplementary Movies 2 and 3). We started by measuring the elastic deformation of the cell wall combining our Qdots technique with cell plasmolysis experiments, where we induced cells to lose water and turgor pressure (Supplementary Movie 4). Quantification of Qdot repositioning during plasmolysis revealed large elastic strains in the cell wall reaching as much as 30% (Fig. 2a). Moreover, the elastic stretch in the circumferential direction exceeds the meridional stretch by a factor of two (Fig. 2a). These striking elastic effects confirm that growth domain morphogenesis cannot be fully explained unless wall elasticity and mechanics are taken into consideration. We then used the Qdots technique to characterize the expansion of the cell wall during active growth, by tracking the displacement of wall elements at growing cell ends. Focusing on stably growing OEs, we found that all OEs (n = 19) share the same characteristic wall displacement field (Fig. 2b and Supplementary Fig. 2). The reproducible morphogenesis of OE allowed us to put forward a canonical expansion profile (Fig. 2c). This canonical profile is characterized by a sharp meridional gradient in the meridional (ε_M) and circumferential (ε_R) strain rates, so that more than 90% of wall expansion takes place within 3 μm of the pole. As for the reversible elastic deformation on the cell cylinder (Fig. 2a), wall expansion at the OE favours the circumferential direction (Fig. 2c). To sum up, the gluan wall of *S. pombe* experiences large elastic strains because of the internal turgor pressure, its growth is focused in a narrow area extending ~3 μm around the OE poles, and its elastic and growth deformations both favour the circumferential direction over the meridional direction (that is, the deformation is circumferentially anisotropic).

We attempted to capture these features using the simplest possible morphogenetic models (Fig. 2d–g). First, we modelled the cell shaft as a cylindrical shell with linearly elastic properties and used the results from the plasmolysis experiments (Fig. 2a) to get an initial assessment of the wall’s elastic properties (Supplementary Note). This analysis yielded a rather broad distribution of elastic parameters with a mean Young’s modulus to turgor pressure ratio (E/P) of 44 and a mean Poisson’s ratio (ν) of ~ 0.06 (Fig. 2d). Given a turgor pressure of P = 1.5 MPa (note added in proof of ref. 24), our best estimate of the Young’s modulus of the cell wall is 66 MPa. To test more precisely the validity of these material properties, we developed a model taking into account the precise cellular geometry. Based on recent models and the uniform composition of the cell wall, we simulated the cell as a thin elastic shell with homogeneous and isotropic elastic properties (Supplementary Note). Using the plasmolysed cell geometry as initial conditions, we inflated the cell and assessed which set of material properties allowed the simulated deformed shape to best fit the observed turgid cell geometry. Despite the simplicity of the model, it reproduced the deformation of plasmolysed cells accurately (Fig. 2e) while also giving robust estimates of the elastic material properties (Fig. 2d), specifically 58 for the Young’s modulus to turgor pressure ratio (E/P) and 0.03 for the Poisson’s ratio (ν). As a final test of this model, we also attempted to predict the abrupt morphogenetic transition associated with the deformation of the flat septum into the near hemispherical geometry of the NE (Figs 1e and 2f). This phase of morphogenesis presents a new challenge because the resting length of the septum is unknown, as it is first formed within the confine of the load-bearing wall of the mother cell and therefore does not experience any deformation until the daughter cells have separated. However, we found realistic NE morphologies for a broad range of resting lengths as long as these exceeded the observed septum length by ~20% (Supplementary Note). Using the same elastic model as before and a resting length of 1.3 for the septum, we were able to reproduce the morphogenetic transition precisely, including the appearance of
the division scar (Fig. 2f,g) and the characteristic meridional curvature of the NE (compare Figs 1b and 2g).

The ability of the elastic shell model to reproduce the morphological changes associated with plasmolysis and septation leaves little doubt about the importance of wall mechanics in shaping these processes. However, it could be argued that the time scale of these morphological changes is so short as to leave no room for any cellular response other than a mechanical response. In contrast, growth domain morphogenesis takes place over a few hours leaving plenty of time for active biological control over this process. We therefore asked whether mechanics remains relevant for those processes and, if so, how biochemical regulation and mechanics are integrated. We propose a model whereby wall elasticity and wall incorporation contribute in parallel to growth domain morphogenesis (Fig. 2h and Supplementary Note). Our model posits the existence of a relaxed, stress-free cell whose geometry may depart significantly from the turgid cell geometry (as seen in the plasmolysis experiments of Fig. 2e). This relaxed geometry, however, is also subjected to growth by incorporation of new wall material. Thus, the characteristic wall expansion profile made visible by the Qdots (Fig. 2b) is the by-product of a biochemical process controlling the incorporation of new wall material and a mechanical process whereby the wall is stretched elastically as it experiences the internal turgor pressure of the cell. To test how such a model can account for the measured canonical wall expansion profile, we modelled cell growth using the observed areal expansion as growth input (the areal expansion is the sum of the meridional and circumferential strain rates of Fig. 2c). Both the geometry of the OE and the anisotropy of its wall expansion were reproduced accurately with this model (Fig. 2i,j).

Figure 1 | Morphological evolution of cell growth domains in fission yeast. (a) Transmitted light images illustrating the changes in OE and NE shape in a fission yeast cell throughout interphase. Left, cell immediately before NETO. Images are maximum intensity projections of z-stacks comprising the most equatorial 2 μm of the cell. Scale bar, 5 μm. (b) Average meridional curvature of the NE (red line) and OE (green line). The coloured areas around the averages correspond to the standard deviations. n = 35 OEs and n = 35 pre-NETO NEs were averaged. (c) Cell contours extracted from the time-lapse sequence in a. (d) Curvature kymographs showing a pointy OE keeping its curvature (top) and a hemispherical NE evolving into a pointy OE (bottom). The plots, corresponding to the ends of the cell depicted in a, display the meridional curvature as a ‘heat map’ during 3 h with a 2-min resolution. One of n = 25 OEs and one of n = 18 NEs are shown. (e) Schematic illustration of the three morphogenetic transitions observed in S. pombe: (i) the deformation of the flat post-cytokinesis septum into a hemispherical NE (first arrow), (ii) the growth of the hemispherical NE into the pointy OE (second arrow) and (iii) the steady growth of the OE (third arrow).
the growth model also successfully predicted the evolution of the NE geometry observed after NETO (Fig. 2i). Thus, our morphogenetic model offers a simple, self-consistent mechanism for how wall assembly and elasticity combine to create cell end shape throughout the cell cycle in fission yeast. Finally, the growth model provides us with a third method to get at the
elastic properties of the cell wall, albeit now looking at the long-term elasticity of a growing cell wall. We therefore explored what values of the Young’s modulus to turgor pressure ratio and Poisson’s ratio permit the most accurate prediction of the expansion profile. We found a broad domain of material properties values compatible with the 95% confidence intervals observed for the wall expansion profile (Fig. 2d). Within this domain, we selected the material properties falling closest those obtained in the plasmoslysis experiment ($E/P = 40$ and $v = 0.3$).

Morphogenetic potential of cell end-distributed machineries. Because of its ability to predict OE and NE morphogenesis, we surmized that the measured areal wall expansion profile (referred to as Areal thereafter) could be a good candidate function for the distribution of a growth-controlling molecule. Any molecule presenting the same meridional distribution could be integrated in our model and predict growth domain morphogenesis to the same degree of accuracy as what was achieved in Fig. 2i). We therefore looked for such a molecule(s) by quantifying the cortical OE distribution of three different types of green fluorescent protein (GFP)-labelled machineries known to be involved in the polarized growth cascade (Fig. 3a,b and Supplementary Fig. 3a,b): (i) polarity factors, specifically the upstream polarity landmark Tea1, the global shape regulator Rho-like GTPase Cdc42 (visualized indirectly in its GTP-bound state by the localization of a fluorescently labelled CRIB domain, whose distribution has been shown to mirror that of Cdc42 (ref. 31), its guanosine exchange factor (GEF) Scd1 (ref. 16) and the Scd1 co-factor Scd2 (ref. 17); (ii) the exocytic machinery, specifically the actin cable nucleating-formin For3 (ref. 32), the actin nucleation/branching factor and Sec6 and Exo70, two subunits of the exocyst, a tethering complex essential for the exocytic vesicles docking at the plasma membrane, (iii) and the cell wall synthesis machinery, specifically the GEF Rgf1, a co-factor of the β-glucan synthase regulator Rho1 (ref. 19), and the β-glucan synthases Bgs1 (refs 2,4–7,18) and Bgs4 (refs 8–12,20).

Averaging of the cortical distribution of the factors across many cells allowed us to establish a ‘canonical’ distribution for each factor, which could then be compared among themselves (Fig. 3c middle row, and Supplementary Figs 3b and 4). A first important conclusion of this comparative analysis of marker distribution is the ‘phylogenetic’ clustering of factors belonging to the same machineries (Fig. 3c top row dendrogram, and Supplementary Fig. 3c–e). In particular, exocytosis markers and glucan synthesis markers form two non-overlapping groups in the same machineries (Fig. 3c top row dendrogram, and Supplementary Figs 3b and 4). A first comparison has led to the proposal that the extent of Cdc42-GTP localization at cell ends with the average measured across different genotypic conditions that induce changes in the distribution of markers and/or in the morphogenesis of the growth domains. A factor both necessary and sufficient for growth domain morphogenesis ought to correlate systematically with the observed cell end geometry across different genotypic conditions.

Neither Cdc42 nor cell wall synthesis reliably predict growth pattern. To ask whether the glucan synthesis machinery could underlie growth domain morphogenesis, we used pal1A cells. Pal1 (refs 8,10,13,33) is a membrane-associated protein that interacts and co-localizes with the endocytic adaptor Sla2p/End4p14–17,34 and is involved in cell morphogenesis and cell wall integrity. We found that in pal1A cells glucan synthesis factors become delocalized around the cell periphery, yet cells maintain their ability to direct exocytosis to cell ends and to grow cylindrically (Fig. 4a, Bgs4 delocalization shown; Dodgson, Chessel, manuscript in preparation). Therefore, a properly localized glucan synthesis machinery is not a necessary condition for growth domain morphogenesis, consistent with previous evidence16–20,35.

To ask whether the Cdc42 machinery could directly underpin cell end growth pattern, we quantitatively compared the average extent of its localization at cell ends with the average measured width of cell growth domains. In previous studies, this type of comparison has led to the proposal that the extent of Cdc42-GTP localization at cell ends controls global cell geometry by modulating cell end width14,15,21,22,36. To test this idea further, we did this analysis not only in wild-type cells but also in narrower rga2A cells (Rga2 is the GTPase-activating protein (GAP) for Rho2, another GTPase involved in polarized growth13,37–40) and in wider rga4A cells (Rga4 is the major Cdc42 GAP14,15,36,37,39), to explore a wider range of phenotypic variety. To our surprise, we found that the cortical extent of the Cdc42 machinery and cell width do not correlate across those morphologically diverse genotypes (Fig. 4b,c CRIB and Scd2 FWHA shown, and Supplementary Fig. 6), although there was a good correlation for the exocytic and cell wall synthesis machineries (Fig. 4b,c and Supplementary Fig. 6). Thus, despite the fact that it is well known to be a key determinant of global cell shape14,15,25–27,35,36, GTP-Cdc42 cortical pattern likely does not directly underpin the specific geometry and growth pattern of OEs.

Growth domain geometry can be defined by the pattern of exocytosis. Taken together, these observations suggested that it is
the pattern of exocytosis that defines the morphogenesis of growth domains. Given that exocytosis has been extensively shown to be a complex process composed of separable events that ultimately lead to the fusion of vesicles with the plasma membrane, we investigated the possible contribution to growth domain morphogenesis of the sub-pathways controlling where vesicle fusion takes place: actin cables and the exocyst. We did this by using the deletion mutant for $3\Delta$, which lacks actin cables. Although slightly misshapen, $3\Delta$ cells localized the exocyst subunits Exo70-GFP and Sec6-GFP and the v-SNARE Syb1 at the cell ends (Supplementary Fig. 7). Importantly, the distribution of those proteins over the OE contour was very similar for all three proteins (note that GFP-Syb1, unlike in wild-type cells, localized to the plasma membrane in exocyst-like clusters in $3\Delta$ cells; Supplementary Fig. 7). Crucially, like in wild-type cells, there was a strong correlation between the proteins’ localization profiles and the shape acquired by the OEs in $3\Delta$ (Supplementary Fig. 7). Thus, we conclude that it is the pattern of exocytosis in general, which is ultimately formed where the exocyst and the membrane fusion machineries are present and active, that might dictate growth domain shape.

**Exocytic pattern and growth domain morphogenesis are causally linked.** To test whether exocytosis is sufficient to specify cell end geometry and growth pattern, we forced the displacement of exocytosis to other areas of the cell cortex using the GFP-GBP (GFP-Binding Protein) system (Fig. 5a). In cells co-expressing GBP-CaaX-mCherry—a GBP-tagged membrane targeting CaaX domain that localizes everywhere on the cell cortex—and the v-SNARE Syb1, we found that Syb1 was redistributed along broader areas of the cell cortex, sometimes spanning the entire perimeter of the cell (Fig. 5b–d, images), indicating that Golgi-derived exocytic vesicles were fused to the plasma membrane in ectopic places. Strikingly, we found that this led to a proportionate and dramatic change both in the extent and in the shape of the growth domains (Fig. 5b–d, quantitations). This demonstrates that the pattern of exocytosis causatively determines the local geometry of growth domains.

A consequence of this conclusion is that, if the transition from NE to OE geometry following NETO is driven by exocytosis, the pattern of exocytosis should be similar in NEs and OEs. As predicted, when we measured experimentally the cortical distribution of the exocytic proteins Sec6 and Syb1 in NEs undergoing NETO, we found that they are indistinguishable from...
although with our current approach we cannot definitively assert that Cdc42 activation and glucan synthesis are not directly required for growth pattern, our data suggest that neither the Cdc42-activating machinery nor the cell wall synthesis machinery are good causative predictors of that pattern. In the case of glucan synthases, our results with pal1A cells demonstrate that they are not causative for growth domain morphogenesis. This could indicate that, although the synthesis of glucans is essential (as they are the main component of the fission yeast cell wall), the changes that the cell wall undergoes during cell growth do not only rely on that process, but also on other molecular activities that define the extent of glucan synthesis—or its remodelling—in different locations of the cell cortex and that do this in an exocytosis-dependent manner. Some candidate machineries are glucanases, glucanosyl transferases and other enzymes that modify the glucans and the rest of components of the cell wall, like mannans, which could be studied in the future if the challenge of tagging and studying the fine localization of proteins acting between the plasma membrane and the cell wall is overcome.

Surprisingly, our results also imply that, although Cdc42-GTP is known to recruit exocytic factors and to help drive exocytosis, the precise pattern of growth is defined...
by the subsequently established pattern of exocytosis, and not by the original pattern of Cdc42 activity. Thus, although Cdc42 is clearly implicated in global cellular morphogenesis14,22,50–53, its role might be primarily at the signalling level upstream of morphogenesis, whereas exocytosis might act as the downstream effector that modulates locally cell geometry. In fission yeast, it is broadly agreed that exocytosis is determined mostly by the polarized transport of vesicles from the Golgi to cell ends via actin cables and their reception by the exocyst (a multiprotein tethering factor) with both processes considered independent and simultaneous21,30,44,50. However, the contribution of each of those sub-processes in determining the overall pattern of vesicle fusion with the plasma membrane and, consequently, in the establishment and maintenance of cell geometry, is not clear. On the one hand, the exocyst is able to reach cell ends in the absence of actin cables and it is essential for fission yeast viability19,21,43,44. This could be taken to suggest that the exocyst is the major determinant of cell growth and, hence, of cell geometry. However, with the data available it cannot be concluded that the essential role of the exocyst is exclusively derived from its direct role in exocytic events. For example, recent data support a role for Sec3 as coordinator of actin cable assembly and actin patch internalization44, which would imply that the role of the exocyst as a morphogenetic factor could go beyond its tethering function. On the other hand, although the exocyst can reach the cell ends without actin cables, in wild-type cells it appears to be assembled and directed to the cell membrane through them43,44. This makes dissecting the network that leads to exocytosis very complicated. In this regard, we believe our finding that the pattern of growth is modulated by exocytosis will help shift the focus of future morphogenesis studies from the role of Cdc42 activity and the processes it directly controls, to the role of the specific processes controlling tethering and fusion of vesicles with the plasma membrane.

In our biophysical model, for simplicity and because of insufficient information in the literature about the different factors examined, we did not incorporate any information about the factors’ recycling dynamics (binding/unbinding at the plasma membrane, diffusion at the membrane and the cytoplasm, etc) or other potentially nonlinear interactions at the cell cortex, as has been done, for example, in previous modelling work focusing on the role of Cdc42 in cell polarization26,54,55. Here, we assumed that the spatial distributions observed for the different factors from populations of cells were reflective of a steady-state implicitly containing all relevant nonlinear interactions, and instead we focused on investigating whether those distributions were good predictors of growth pattern. The finding that the localization pattern of exocytic factors, but not of other factors, correlates with growth pattern across a range of genotypes and modelling parameters, and the fact that relocalization of the exocytic machinery in cells causatively relocates and alters...
growth, indicates that the simpler model recapitulates the relevant biology at play. Future biophysical and modelling efforts will be needed to address in detail the relevance of those recycling dynamics to the fine spatial and temporal modulation of cell growth, in this and other cellular systems.

**Methods**

**Strains, media and image acquisition.** The double fluorescently marked strains and the strains containing the deletions *pal1Δ, rga2Δ* and *rga4Δ* (which derived from the commercially available ‘*S. pombe*’ Haploid Deletion Mutant Set version 2.0 strains collection (Bioneer Corporation; http://pombe.bioneer.com) (36-38) as well as the GFP–mCherry–CaaX GFP–Syb1 strain were generated following standard crossing methods (39,40).** Supplementary Table 1 contains a list of all the strains used in this study. Cells were grown exponentially at 32 °C during 48 h in rich YES (yeast extract with supplements) medium before specific treatments or imaging. For this purpose, we used 35 mm glass bottom plates (MatTek Corporation) or eight-chambered coverglass Lab-Tek II plates (Nunc, Thermo Fisher Scientific Inc.) pre-coated with lectin (1 mg ml−1; Sigma; 13195 and Patricell L; 1-301-25). A DeltaVision system (Applied Precision/EI Healthcare) comprised of an Olympus IX81 epi-fluorescence microscope (Olympus) with a × 100 (NA 1.40) oil immersion lenses (numerical aperture 1.4 and 1.42, respectively, Olympus) and 1.512 reflective index immersion oil (Applied Precision) was used for imaging through the proprietary software SoftWoRx.

Live cell fluorochrome (Fluka, Sigma-Aldrich Co. LLC.) staining was performed by incubating cells in 1/100 (v/v) calcofluor white in YES. Cells were then imaged using a DAPI filter set. Treatment with hydroxyurea (HU) was carried out through the proprietary software SoftWoRx.

**Kinematic analysis.** Our empirical analysis of cell morphogenesis is articulated around three axes of quantification: (i) the change in cell geometry through the cell cycle, (ii) the deformation of wall elements during growth and (iii) the distribution of cortical markers putatively involved in controlling growth. The result of these quantification steps is three functions, which are the basis of all further analyses. These functions are: the meridional curvature (κs), the meridional velocity (relative displacement) of wall elements (v) and the fluorescence intensity profile of cortical markers (µs), which are all functions of the meridional distance from the pole of the cell. In this paper, we focus on the pole of the cell, which must correspond to the total growth incurred along the cell’s meridian, that is, the pole is simply pushed forward by the growth occurring below it. For a Qdot moving from position s to position s + Δs during the growth interval, the relative displacement is v = Δs/Δt. The relative displacement of Qdots was fitted with the function v(s) = σ [exp(a·v(s) – b·v(s))]², where v is the angle of the normal to the cell contour. In fitting, the relative displacement, the curvature and the fluorescence profiles were measured and, ultimately, averaged at fixed meridional distances (s) from the pole of the cell. On occasion, asymmetry in the cell contour led to spurious pole positioning. The misplaced pole was then shifted ‘manually’ so as to maximize the symmetry of the curvature and fluorescence profiles. The measurement of kinematic variables such as curvature was performed at a fine spatial resolution by resampling the cell contour on either side of the pole with 900 points spaced by 0.1 pixels (6.7 nm).

**Qdot microscopy.** Access to the Qdot microscopy was facilitated by use of computational tools written with Matlab (MathWorks). All programmes are freely available on request.

(i) Quantification of cell geometry: To study the evolution of the curvature of wild-type cells during the cell cycle, bright-field z-stacks encompassing a thickness of 5.4 μm were acquired using a DAPI filter set. Image processing was done using SoftWoRx.

(ii) Quantification of wall deformation using Qdots: To track the cell wall deformation during apical expansion, we tagged exponentially growing cells with streptavidin-conjugated Qdots via biotinylated lectins (isolectin GS-IB4 from Griffonia simplicifolia; Biolegend; Invitrogen) and labeled the Qdots for the open-source software Fiji (http://fiji.osuwiki/index.php/Fiji) and Matlab (MathWorks). Two-dimensional (2D) or three-dimensional deconvolution was used only for illustration purpose. Apart from very few exceptions, Qdots were not observed to detach from the cell wall throughout the times of observation.

(iii) Quantification of fluorescently tagged proteins: We studied the OE localization of different proteins—constituents of distinct intracellular machineries—that have been reported to occupy the growing cell domes: Te1Δ, For3, CRIB, Sdc1, Sdc2, Syb1, Sec6, Exo70, Rgf1, Bgp1 and Bgp4 (refs 9,15,17-20,22,30,32,37,43). To correlate the distribution profiles of these proteins at the cell ends with the canonical pattern of cell wall strains observed during growth, we imaged cells expressing GFP–Bgp4, GFP–X or both RFP–Bgp4 and X–GFP (for X, depending on the protein; ‘X’ being one of the proteins mentioned above) with the Qdot microscopy and subsequently analyzed the fluorescence profile (see Supplementary Fig. 3b for details). The averaged fluorescence profile was fitted with the smoothing spline function *sups* of Matlab using a parameter value of 0.8, which corresponds to a weak smoothing. Background subtraction and normalization were performed by subtracting the minimal intensity value of the spline and then scaling the profile so that the maximal fluorescence intensity is 1.

As an example, at the resolution at which we performed our experiments, in the 10 min timeframe of filming 3–4 main exocytic patches were detected on average.
as can be observed in the Sec6 panel of Supplementary Fig. 4. As shown in that figure, and described in the text, this was deemed sufficient to assume ergodicity, that is it was sufficient to claim that the distribution of the excotic reporter at the single-cell level was reflective of the average distribution of that reporter at the population level.

The computation of the FWH (Supplementary Fig. 4a) and FW/SA of the fluorescence profiles was performed using a customized Matlab routine. For the FWH, the routine divides vertically the area under the curve of each distribution profile in three parts: a central and two lateral parts, in a way that the sum of the two lateral parts is equal in area to the central one. Then, the width of that central part is computed as the FWH.

For the FWH, we computed the fluorescence intensity of the markers around the entire cell contour. We used only wild-type-like cells for our analysis (as reported, this mutant displays a variety of shapes: stubby, pear-like, lemon-like and wild-type-like3). The kinematic analysis described above yields three variables: the meridional curvature (κ(s)), the longitudinal velocity of Qdots (v(s)), and the fluorescence profile of cortical markers (ψ(s)). From these basic variables, it is possible to compute many additional descriptors of morphogenesis.

The cell geometry was computed from the average meridional curvature (κ(s)) using the equations:

\[ q(s) = \int_0^s \kappa(z) dz \]

\[ r(s) = \int_0^s \cos(q(z)) dz \]

\[ z(s) = \int_0^s \sin(q(z)) dz \]

\[ \kappa(s) = \sin(q/r) \]

where κ(s) is the circumferential curvature and \( z \) is a dummy variable of integration.

The local rates of wall expansion or strain rates are given by the relations:

\[ \dot{\ell}_w = \frac{d\ell_w}{dt} = \frac{d\ell_w}{d\ell} \kappa(s) \]

\[ \dot{\ell}_v = \frac{1}{r} \frac{dr}{dt} \]

\[ \dot{\ell}_s = \frac{1}{r} \frac{dz}{dt} \]

\[ \dot{s}(s) = \int_0^s \frac{1}{r} \frac{dz}{dt} \]

According to our model (see Supplementary Note for details), the areal strain of the wall incorporated strain and thus is a good target function for comparison with the distribution of various cortical markers. In contrast, the deformation anisotropy (s) is set by the balance of forces acting on the cell wall and depends on the geometry of the cell end and its material properties.

**Measurement of material properties.** The elastic properties of the fission yeast cell wall were measured using plasmolysis experiments. Cells coated with Qdots were deposited in a MatTek chamber containing 1 ml of YES medium. Drops of YES + 2M sorbitol were added each 10 s while filming until the cells stopped diminishing in size (Supplementary Movie 4). The elastic properties were inferred using two complementary approaches. First, we focused on the change in geometry in the cylindrical mid-section of the cell. The meridional strains were evaluated by selecting pairs of Qdots on opposite ends of the mid-section and measuring the distance between them in the turgid and plasmolysed states. This approach yielded values of the meridional strains. The values yielding the best fit of the experimental data (see Bending Model in the Supplementary Note). For simplicity, however, we assumed that the plasmolysed cell has no residual stresses and its material properties are homogeneous and isotropic. With these assumptions, the only two material properties to determine were the ratio of the Young’s modulus over the turgor pressure $E/P$ and the Poisson’s ratio $\nu$. These material properties were estimated following three successive steps (Fig. 2d):

(i) The cell contours before and after plasmolysis were extracted manually by fitting a Bezier curve around the cells in bright-field images.

(ii) The extracted contour was then made symmetrical.

(iii) The contour corresponding to the plasmolysed cell was then numerically inflated using specific values of $E/P$ and $\nu$. The values yielding the best fit of the turgid state were retained. This approach gave values of $E/P = 55 \pm 5$ and $\nu = 0.033 \pm 0.005$ ($n = 24$). Therefore, our two approaches give a Poisson ratio very close to zero. Such low Poisson ratios have been predicted by Boal for 2D networks under high enough tension as could the case for the cell wall of *S. pombe.*

**Validation of assumptions.** The quantification of cell morphogenesis from raw image sequences requires a number of assumptions. We regroup in this section the main assumptions used in our analysis and discuss how they were validated.

(i) Axisymmetry: An issue here is whether the morphogenesis of the cell ends is adequately captured by simply specifying the key variables along the meridian of the cell and assuming that all the variables are symmetric around the axis of the cell. This assumption would be incorrect if, for example, the cell ends curved substantially during growth. Axisymmetry is both a convenient simplification assumption for computer simulations and an effective way to reduce noise in empirical measurements by allowing us to average measurements made on either sides of the cell axis. To test this assumption, we plotted the curvature and Qdot velocity for 19 OEs. A comparison of the values obtained to the left and right of the pole show that they are identical within the precision of our measurements (Supplementary Fig. 2). Moreover, both variables were fitted by functions where axisymmetry is forced. Finally, we note that axisymmetry would fail if the axis of growth were chosen incorrectly in our image analysis protocol. Therefore, the robust symmetry observed in Supplementary Fig. 2, in particular in the curvature profile, is also a validation of our protocol to selecting the pole of the cell.

(ii) Scalable steady-state assumption: Inspection of cell morphogenesis in *S. pombe* reveals somewhat unequal growth increments at the two cell ends. Much of the variation comes from an initial increase and final decrease in cell elongation associated with the period following and preceding mitosis. However, even within the period of active end growth, variations in growth increments remain visible. These variations are often antecorrelated between the two ends of the cell. For example, relatively large growth increments at the OE are associated with relatively small growth increments at the NE. These growth fluctuations have been attributed to temporal oscillations in cortical markers such as Cdc42 and its effectors.

We must conclude from these observations that fission yeast morphogenesis does not reach a steady state at any point during the cell cycle. In light of this, it would seem important to characterize the kinematic variables and marker profiles in terms of both their spatial and temporal variation. Yet, several lines of evidence underline the minor role played by the short-term and long-term fluctuations in shaping the cell ends.

1. We first tested whether the rise and fall of the growth rate during the cell cycle is important in determining cell shape. To do so, we exposed cells to HU to inhibit DNA replication. Under such conditions, growth is prolonged allowing OEs to elongate by 3.5 μm ($n = 7$) on average, whereas the OEs of untreated cells elongate by only 2.0 μm ($n = 12$). Despite the 75% increase in cell elongation, the curvature and meridional velocity of the HU-treated cells are indistinguishable from those of untreated cells (Supplementary Fig. 2a,b). We conclude from this experiment that OE geometry is not set by a precisely tuned rise and fall in growth rate.

2. If the short-term growth variations are important in controlling growth domain morphogenesis, they should make our measurements of Qdot velocities impossible to summarize with a single steady-state function. Indeed, our raw measurements of Qdot velocities show large fluctuations in magnitude (Supplementary Fig. 2d). However, the simple step of normalizing the Qdot displacements by the size of the growth increments is sufficient for the measurements to fall onto a unique master curve (Supplementary Fig. 2c). Thus, the growth variations affect only the magnitude of the Qdot velocities but not their meridional distribution.

3. As the growth fluctuations have been imputed to cycling of Cdc42 and its effectors between the two cell ends, we tested whether the shape of the CRIB-3GFP fluorescence profile is affected by the overall intensity of CRIB-3GFP fluorescence (Supplementary Fig. 4b). To do so, we divided the frames of our CRIB-3GFP movies in two groups based on the total amount of apical fluorescence (the total fluorescence intensity integrated over the 12-μm-long curved contour spanning the pole). We observed that, after normalization, cell ends containing ‘high’ and ‘low’ levels of CRIB displayed nearly identical fluorescence profiles (Supplementary Fig. 4b).
4. Finally, we were unable to observe any repeatable pattern of fluctuation in all of the variables we quantified. In fact, the magnitude and temporal recurrence of the fluctuations highly varied from cell to cell. The first column shows the nearly identical morphogenesis. This variability excludes a direct one-to-one relationship between the growth spurs and the morphogenesis of the cell ends.

Taken together, these observations have led us to the idea of a ‘scalable’ steady state in which the spatial profile of time-dependent variables are preserved, although their absolute value may vary temporally as the growth of the cell end state in which the spatial profile of time-dependent variables are preserved.

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which means that the left-hand term is equal to zero. The steady-state advected
distribution is:
\[
\frac{\partial v(x, t)}{\partial t} = \hat{\gamma}(x) - (k + A(x))v(x)
\]  
(9)

Substituting the explicit form for the areal strain, the solution to this equation is:
\[
\hat{\gamma}(x) = \frac{1}{r(x)} \int_0^r \hat{\gamma}(\xi) \varphi(\xi) d\xi
to point \(\hat{\gamma}(x)\)
given by the integral:
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\]  
(10)

where \(\xi\) is a dummy variable of integration. The time of transit to point \(\hat{\gamma}(x)\)
given by the integral:
\[
\hat{\gamma}(x) = \frac{1}{r(x)} \int_0^r \hat{\gamma}(\xi) \varphi(\xi) d\xi
\]  
(11)

When \(k\) goes to infinity, the exponential approaches zero over the entire domain of integration except at \(x = s\) where it is equal to 1 as well as the functions that precede the exponential. Thus, for large \(k\), the advected profile is close to the observed fluorescence profile. As \(k\) decreases with respect to the time of transit, the fluorescence becomes more ‘smearred’ laterally.

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Author contributions

R.E.C.S. originally conceived/led the project, later joined by J.F.A. and J.Du. J.F.A. generated all new cell lines and carried out all experiments, helped by J.Do. and J.Di. J.F.A. and J.Du. carried out all quantitative image analysis, using image processing tools by J.Du. and with help from A.C. and J.Di. E.C. and J.Du. carried out all biophysical analysis and modelling. R.E.C.S., J.F.A. and J.Du. wrote the text with help from other co-authors.

Additional information

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