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.

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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 separte giving rise to two similarly sized daughter cells and that re-initiates the morphogenetic growth cycle.

Here we have combined biophysical modelling and quantitative live cell analysis to investigate how the geometry and morphogenetic pattern of fission yeast cells result from the interplay between biochemical and mechanical regulation. We show that neither the global cell shape regulator Cdc42 and its activators Sdc1 and Sdc2 nor the major cell wall synthesis regulators Bgs1, Bgs4 and Rgf1 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 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 wall expansion profile (Fig. 2c). This canonical profile is characterized by a sharp meridional gradient in the meridional (*ε*<sub>m</sub>) and circumferential (*ε*<sub>c</sub>) 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 glucan 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 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. 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 have 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). Furthermore, using the same wall incorporation profile as before,
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...
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,j. 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 whose distribution has been shown to mirror that of Cdc42 (refs 14,15,25–27,35,36, GTP-Cdc42 cortical pattern likely does not 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 paleΔ cells. Pale1 (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 paleΔ 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 et al., 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 rga2Δ cells (Rga2 is the GTPase-activating protein (GAP) for Rho2, another GTPase involved in polarized growth12,37–40) 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\(^9,28,29,41,42\), we investigated the possible contribution to growth domain morphogenesis of the sub-pathways controlling where vesicle fusion takes place: actin cables and the exocyst\(^{1,2,13,22,30,43,44}\). We did this by using the deletion mutant for3, which lacks actin cables\(^{31,32}\). Although slightly misshapen, for3 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 for3\(\Delta\) 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 for3\(\Delta\) (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\(^{16,45,46}\) (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 GFP-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...
those in OEs (Supplementary Fig. 8), implying that there is no obvious geometrical feedback into the localization of exocyst and that it is the exocytic pattern that drives both the morphological transition from NE to OE and the stable maintenance of OE geometry throughout cell growth.

A biomechanical model of fission yeast morphogenesis. Altogether, we conclude that cell wall mechanics and the pattern of exocytosis drive growth domain morphogenesis through the entire cell cycle in this species. A schematic model summarizing our findings is shown in Fig. 6 (see also Supplementary Movie 5 for details). During division, when a fission yeast cell undergoes septation at the cell middle it generates two daughter cells, each of which initially possesses one OE-shaped cell end and one flat NE that becomes hemispherical as a result of intracellular pressure and the elastic properties of the cell wall (one daughter cell shown in Fig. 6, left). As each daughter cell starts growth, its exocytic pattern is re-established at the OE triggering monopolar cell growth (Fig. 6, middle). Subsequently, at NETO a stable exocytosis pattern is established also at the NE (Fig. 6 right), which—constrained by the mechanics of the cell wall—gradually changes shape throughout interphase until it takes the characteristic pointy shape of a growing OE, re-initiating the morphogenetic cycle.

Discussion

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 pal1Δ 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, glucansyl 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 morphogenesis 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 morphogenesis, 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 exocytosis event (a multicomponent tethering factor) with both processes considered independent and simultaneous. 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 viability. 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 internalization, 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 them. 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 polarization. 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 relocation 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 pul1Δ, rga2Δ and rga4Δ (which derived from the commercially available S. pombe Haydop Deletion Mutant Set version 2.0 strains collection (Bioneer Corporation; http://pombebioneer.com)36,37 as well as the GBP-mCherry-CaaX GFP-Syb1 strain were generated following standard crossbreeding methods36,37. 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; 11395 and Patricell Ltd; 1-1301-25). A DeltaVision system (Applied Precision/EI Healthcare) comprised of an Olympus IX81 epi-fluorescence microscope with a 40×/1.20 oil immersion lens (numerical aperture 1.4 and 1.24, respectively, Olympus) and 1.512 refractive index immersion oil (Applied Precision) was used for imaging through the proprietary software SoftWoRx.

Live calcofluor white (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 (Hux) was carried out culturing the cells during 3 h in 0.025 M of this drug in YES medium before imaging.

Qdots (Qdot 650 Streptavidin Conjugate; Life Technologies) were imaged with a standard TRITC (Tetramethylrhodamine; 555/28 617/73) filter set, whereas cells expressing GFP–, red fluorescent protein (RFP–) or mCherry–tagged proteins were imaged with fluorescein isothiocyanate and TRITC filter sets. All fluorescence imaging was performed with the DeltaVision tool ‘Optical Axis Integration’ (OAI). The bright-field images were acquired as z-stacks comprising 5.4 μm (separation between z-planes: 0.2 μm). Image processing was done using SoftWoRx 6.0. All time-lapse integration schemes (and not meant to represent the resolution of the microscope setup).

The meridional curvature was calculated as $k_m(x) = \frac{\alpha(x)}{\pi}$, where $\alpha$ is the angle measuring the amount of rotation between two successive contour segments and $l = 6.7$ nm is the length of those segments. Curve kymographies were prepared by tracing cell wall curvature maps for a time-lapse sequence as an image where pixel intensities are set by the magnitude of the curvature for each meridional and temporal positions (Supplementary Fig. 1c). The measured curvature vary temporally and is not perfectly symmetrical with respect to the pole (that is, $k_m(x,y) \neq k_m(-x)$). The growth domain geometry was determined by averaging the meridional curvature at fixed meridional distances from the pole of the cell. The curvature was also made symmetrical by averaging the ‘left’ and ‘right’ side of the cell as defined by the position of the pole. For OE growth domains, the averaged symmetrical curvature fits closely the observed curvature (Supplementary Fig. 1d).

The geometry of the NE changes abruptly after NETO and therefore could not be computed by averaging entire time-lapse sequences. Instead, a total of 35 recently divided cells were used to define canonical NE geometry.

(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 (isoelectric GS–IB4 from Griffonia simplicifolia; Invitrogen). The parameters for the Be’zier curves were then extracted from the scalable vector graphics (.svg) files and imported directly in Matlab. The intersections between a Qdot path and the cell contours were taken as the position of the Qdot and the wall element to which it is bound.

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 ($k_m$), the meridional velocity (relative displacement) of wall elements ($v_m$) and the fluorescence intensity profile of cortical markers ($f_m$), which are all functions of the meridional distance from the pole of the cell ($s$).

(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 μm were acquired each 2 min during 4 h (25 cells; Supplementary Movie 1). Subsequently, maximum intensity projections of the z-stacks were used to define one bright-field image for the cells at each time point. The outline of growing cells was then determined from these bright-field images using computer-assisted tools. The initial step requires the user to select three functions, which are all functions of the meridional distance from the pole of the cell ($s$).

In fitting, the user defines the time interval ($\Delta t$) during which the displacement was normalized to 1 at the equator ($s = \pi/2$). The intensity of all the frames was averaged and used for subsequent analysis (see Supplementary Fig. 3b for details). The averaged fluorescence profile was fitted with the function $v(s) = \sin(p (s-\pi/2) - 2\pi n(s))$, where $p$ is the amplitude of the function ($p > 1$) and $n$ is an optional phase offset. In this function, $\Delta t = 10$ min timeframe of filming 3–4 main exocytic patches were detected on average, NATURE COMMUNICATIONS | DOI: 10.1038/ncomms9400 | www.nature.com/naturecommunications 9 © 2015 Macmillan Publishers Limited. All rights reserved.
as can be observed in the S6 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 calculation of the FWHA (Supplementary Fig. 4a) and FW/5SA of the fluorescence profiles was performed using a customized Matlab routine. For the FWHA, the routine divides vertically the area under the curve of each distribution into two 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 FWHA.

For the fast phase, 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-like33).

The kinematic analysis described above yields three variables: the meridional curvature ($\kappa_m$), the areal velocity of Qdots ($v(s)$) and the fluorescence profile of cortical markers ($\gamma(s)$). From these basic variables, it is possible to compute many additional descriptors of morphogenesis.

The cell geometry was computed from the average meridional curvature ($\kappa_m$) using the equations:

$$ q(s) = \int_0^s \kappa_m(z) dz $$

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

$$ z(s) = \int_0^s \sin q(z) dz $$

where $\kappa_m$ is the circumferential curvature and $s$ is a dummy variable of integration.

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

$$ \dot{\kappa}_m = \frac{dv}{ds} = \frac{dv}{ds} \kappa_m $$

$$ \dot{\omega} = \frac{1}{r} \frac{dr}{ds} = \frac{d}{dS} $$

Two strain rates are more easily interpreted in terms of the areal strain rate $A = \dot{\kappa}_m + \dot{\omega}$ and deformation anisotropy $\gamma = (\omega-\dot{\kappa}_m)/\omega$. Here $w$ and $\omega$ represent the evolving width and meridional length of a small wall element as it is displaced away from the pole. If the wall element is found initially near the pole at position $s_p$ and has circular geometry $w_0 = l_0$, then its dimensions as it migrates along the meridian will be: $w(s) = w_0 e^{\gamma(s)/\gamma_0}$ and $\omega(s)$ such that $\gamma(s) = (\omega(s)-\dot{\kappa}_m(s))/\omega(s)$, where $\gamma(s)$ is the curvature and $\gamma(s)$ is the meridional velocity of the HU-treated cells. We conclude from this experiment that OEs geometry is not set by a precisely tuned rise and fall in growth rate.

1. If the short-term growth variations are important in controlling growth domain morphogenesis, they should make our measurements of Qdot velocities show large fluctuations in magnitude (Supplementary Fig. 2d). However, the simple step of normalizing the Qdot measurements to fall onto a unique master curve (Supplementary Fig. 2d). Thus, 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 QE geometry is not set by a precisely tuned rise and fall in growth rate.

2. As the growth fluctuations have been imputed to cycling of Cdc42 and its cofactors 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 into two groups based on the total amount of apical fluorescence (the total fluorescence intensity integrated over the 1.2-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 were highly variable in cells that showed 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 ends waxes and wanes.

(iii) Ergodicity of fluorescence profiles: We evaluated if ergodicity could be assumed for the distributions of cortical markers; that is, we checked that the canonical fluorescence profiles obtained by averaging over a population coincide with the profiles resulting from averaging single cells over time. For CRIB-3GFP, the existence of ergodicity was evidenced by filming cells expressing the marker (during 25 min every minute) and calculating the FWHA of the apical distribution profiles for each of the 25 time frames. Subsequently, we generated a histogram showing the frequencies of the FWHAs of single cells over those 2 h and another showing the population-based frequencies FWHAs ($n = 25$ cell ends) and they were similar (Supplementary Fig. 4a).

The low levels of intensity and the high levels of photobleaching of Sec6-GFP complicate tracing the distribution of that marker over long periods of time. Moreover, because of its uneven and dynamic distribution at the plasma membrane (Fig. 3b and Bendezú et al., 2011, where Sec6 is reported to have a half-time at the membrane of 5 s (ref. 30)), the information of single time-point frames—including the FWHA of the fluorescence profiles—is difficult to compare. However, we were able to use the WWPA- and GFP-expressing cells every 30 min. We observed that the average profiles were similar to the population average ($n = 29$; Supplementary Fig. 4c).

The case of Bgs4 was slightly different, as that protein is very stable at the membrane, possibly because of its large N-terminal extracellular domains (see ref. 35 and references therein, Chessel et al., manuscript in preparation). When we filmed cells expressing RFP-Bgs4 we observed that the FWHA of the distribution of that protein barely changes over time or does so very gradually (Supplementary Fig. 4d; the separation between time points is 1 min). Moreover, as Supplementary Fig. 3b shows, the variability among the stable GFP-Bgs4 apical distributions is very low (the standard deviation of the FWHA is 0.19 $\mu m$; $n = 31$). Based on those results, we concluded that the population average for Bgs4 reflects what happens cell-wise.

(iv) Fluorescence intensity is proportional to concentration: Many of the simulations we performed used as input what we call ‘canonical distribution profiles’, which resulted from averaging the apical distribution of the studied marker in: (i) a single cell over time, and/or (ii) many cells in the population in a steady-state context—for example, in growing OEs. For CRIB-3GFP, Sec6-GFP and RFP-Bgs4, which were taken as representative markers of the different components of the growth machineries, we carried out a battery of analyses to address how accurately the extracted fluorescence profiles reflect the behaviour of those markers during steady-state growth.

We checked for the saturation of the fluorescence intensity and saw no evidence for saturation for all of the markers. We assume that fluorescence intensity is proportional to concentration (and activity, as assumed in many other studies, for example, see ref. 59). We controlled for possible variations based on the type of fluorescent label (RFP versus GFP). As an internal control, we compared, using Prism (GraphPad Software Inc.), the GFP-Bgs4 with the RFP-Bgs4 data sets and found out that the differences between both are not statistically significant ($P = 0.71$).

Owing to variations in the two radii of curvature of the cell end, the fluorescence intensities captured at specific meridional locations correspond to wall elements of slightly different surface areas. To correct for these variations, we computed the surface area associated with a small neighbourhood extending to a fixed distance, $h$, from the cell contour. The area of a small wall element is $A = dsds$, where $ds$ is a small arc distance along the meridian and $d(s)$ is a small arc distance orthogonal to the meridian. Although $ds$ is kept constant in our protocol to quantify the fluorescence, $d(s)$ is not. The length element $d(s)$ can be found based on the width $h$ of the neighbourhood. We have $h = R(s) - (cos\alpha)$, where $R(s)$ is the radius of curvature orthogonal to the meridian. Approximating $cos\alpha \approx \frac{\partial{R}}{\partial{s}}$ and rearranging, we find $s = \sqrt{2hR(s)}/(\partial{R}/\partial{s})$. The length element thus is $d(s) = R(s) - (2R(s)\partial{R}/\partial{s})^{3/2}$. As $R(s)$ varies only by about 10% and it enters as the square root, we expect little curvature effect on the recorded fluorescence. This conclusion was confirmed experimentally by quantifying the fluorescence distribution of the marker CaaX-GFP, which is targeted evenly to the plasma membrane81 (Supplementary Fig. 3a).

**Comparison of fluorescence profiles and morphogenesis.** As shown in Fig. 2l), simulations of growth domain morphogenesis based on the measured cellular expansion reproduce precisely the observed OE and NE morphogenesis. In light of these results, we hypothesized that markers whose apical distribution emulates closely the general cellular expansion (i.e. are linear functions of the exocytic machinery and the cell wall regulating factors, all previously implicated in contributing to apical geometry in a variety of systems25,26,30–32,67)—would be prime candidates for regulators of morphogenesis. To test this hypothesis, we used two metrics to assess the ability of markers to predict growth domain morphogenesis. The first metric shows the topology and branch lengths of the clustering showed some slight variations depending on the metric used (Supplementary Fig. 3e), a robust consensus tree emerged from the analysis (Fig. 3c):

(i) Five markers cluster reliably closest to the areal profile. These are Tea1, the exocytic markers Sec6, Ezo70 and Syb1, and the actin cable marker For3. Both Sec6 and Tea1 have a slightly narrower distribution than the observed areal expansion. Accordingly, simulations of OE morphogenesis using these markers yield slightly narrower cells but with nearly identical shape (Fig. 3c).

However, the results regarding to Tea1 should be taken with caution, as the mean distribution of Tea1 might not be ergodic (the standard deviation is high; see Supplementary Fig. 3a,b); Tea1 is localized at the apical dome in discrete clusters with reduced motility25. Simulations using Syb1, Ezo70 and For3 were able to reproduce well the shape of the canonical OE, although they resulted in slightly wider cells, maybe because the distribution of these markers have broad shoulders but short ‘tails’, unlike the bell shape geometry of the Areal profile.

(ii) CRIB appears relatively isolated from the rest of the markers. The span of its fluorescence is comparable to the observed region of wall expansion but the shape of the distribution (and assuming the distributions of the Markov functions presenting eclipsing and non-eclipsing interference in the conversion of their fluorescence into wall expansion.

(iv) Finally, the Cdc42 GEF Scd1 and its cofactor Scd2 constitute the most distinct group. These markers display a very narrow and pointy distribution. When used in our simulations, we obtain cells with implausibly narrow cell ends. These results and the distribution of CRIB described in (ii) (and assuming what Bendezú et al., 2015, have recently shown about the correlation of the localization of CRIB and Cdc42) indicate that the meridional distribution of Cdc42-GFP is not the result only of the localization of its activators but also of other factors (localization of other GEFs, GAPS dynamics of Cdc42 and so on) that remain beyond the scope of this study31.

Taken together, these results suggest a special role for the secretion machinery in controlling growth domain morphogenesis.

**Distortion of the fluorescence profile.** The fluorescence profiles ($\gamma(s)$) recorded in our experiments pertain to proteins involved at some level in specifying where wall assembly might take place. These proteins are positioned in the cortex and membrane of the cell where cytoskeleton dynamics and membrane recycling can control their lateral diffusion. In contrast, the breaking of load-bearing bonds in the wall and the insertion of new material must be effected by molecules residing in the periplasmic space and extracellular matrix. These molecules are likely to be outside the reach of the cellular processes able to confine cortical markers within a narrow distance of the pole. Consequently, the effectors of wall incorporation must flow with the wall in the same manner that Qdots flow with expanding the wall. The implication of this flow is a potential mismatch between the fluorescence profiles extracted for cortical markers and the actual process of wall incorporation taking place outside the cell.

To account for this potential mismatch, we are considering four physical processes. First, new wall material is deposited in the periplasmic space according to the experimentally recorded fluorescence profile ($\gamma(s)$). Second, the free wall material is incorporated in the load-bearing wall at a rate proportional to the local concentration of free polymer ($k_f(s)$). Third, the free wall material is spread out by the area expansion of the wall against which it lies according to the relation: $A(s)/s$. Finally, the wall is advected meridionally $v(s)\partial{A(s)}/\partial{s}$. Note that we are not considering diffusion because the wall polymers are large molecules and are presumably entangled with the wall itself. The four physical processes lead to the following differential equation for the evolution of the free polymer concentration:

$$\frac{\partial{\gamma(s)}}{\partial{s}} = \gamma(s)\frac{\partial{v(s)}}{\partial{s}} - k_f(s)\gamma(s) - \gamma(A(s)/s) + v(s)\frac{\partial{A(s)}}{\partial{s}}$$

Here $\gamma(s)$ is the predicted, advected form of the fluorescence profile $\gamma$ recorded in the experiments. Because we have measured the velocity of wall elements with Qdots, the only degrees of liberty are the two parameters $s$ and $A$. They represent, respectively, the radius of curvature and the rate of incorporation of new wall material in the load-bearing matrix. According to the steady-state hypothesis, we will consider the steady-state solution of this equation.
which means that the left-hand term is equal to zero. The steady-state advected distribution is:

$$v(x) \frac{d^2 z}{t^2} = \gamma'(z) - (k + A(z)) z$$

Substituting the explicit form for the areal strain, the solution to this equation is:

$$\gamma(s) = \frac{1}{r(s)} \int_{r(s)}^{r(s)} \gamma'(z) e^{-k(s) - A(s)} d^2 z$$

where $z$ is a dummy variable of integration. The time of transit to point $s$ is given by the integral:

$$\tau(s) = \int_{r(s)}^{r(s)} \frac{d^2 z}{t'(z)}$$

When $k$ goes to infinity, the exponential approaches zero over the entire domain of integration except at $z = s$ 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 'smeared' laterally.

References


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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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