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Report

Physical Mechanisms Redirecting Cell Polarity and Cell Shape in Fission Yeast

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Summary

The cylindrical rod shape of the fission yeast *Schizosaccharomyces pombe* is organized and maintained by interactions between the microtubule, cell membrane, and actin cytoskeleton [1]. Mutations affecting any components in this pathway lead to bent, branched, or round cells [2]. In this context, the cytoskeleton controls cell polarity and thus dictates cell shape. Here, we use soft-lithography techniques to construct microfluidic channels to control cell shape. We show that when wild-type rod-shaped cells are physically forced to grow in a bent fashion, they will reorganize their cytoskeleton and redirect cell polarity to make new ectopic cell tips. Moreover, when bent or round mutant cells are physically forced to conform to the wild-type rod-shape, they will reverse their mutational phenotypes by reorganizing their cytoskeleton to maintain proper wild-type-like localization of microtubules, cell-membrane proteins, and actin. Our study provides direct evidence that the cytoskeleton controls cell polarity and cell shape and demonstrates that cell shape also controls the organization of the cytoskeleton in a feedback loop. We present a model of the feedback loop to explain how fission yeast maintain a rod shape and how perturbation of specific parameters of the loop can lead to different cell shapes.

Results and Discussion

Core mechanisms controlling cell polarity and cell shape are evolutionarily conserved [3, 4]. In general, localized dynamic interactions between the microtubule and actin cytoskeletons and the cell membrane dictate sites of polarized cell growth and thus give rise to cell polarity and cell shape. The fission yeast *Schizosaccharomyces pombe* has proven to be an excellent model organism for studying cytoskeletal organization, cell polarity, and cell shape [1]. Wild-type fission yeast cells are rod shaped, grow in a bipolar fashion by cell-tip extension, and divide by medial fission. Microscopy-based studies have revealed that microtubules are organized as several bundles along the long axis of the cell; minus ends are bundled in an antiparallel fashion at the cell center, and dynamic plus ends interact distally with the cell tips [5, 6]. Actin is organized into patches and cables that are localized to the growing cell tips [7, 8].

The current favored model suggests that microtubule plus ends deliver a group of proteins known as the +TIP complex (comprised of the conserved proteins tea1p (a kelch-repeat protein), tea2p (a kinesin-7 protein), tip1p (CLIP-170 protein) and mal3p (EB1 protein)) to the cell tip, where tea1p is docked to the membrane-bound receptor mod5p. Tea1p subsequently recruits the so-called polarisome protein complex (comprised of bud6p [a polarity protein] and for3p [a formin protein]), which nucleates the actin filaments that serve as tracks directing the growth machinery toward cell tips [1, 9]. Mutations affecting microtubule number or dynamics cause cells to grow in a bent or branched fashion [10–13]. Mutations affecting actin localization at cell tips cause cells to become round, whereas the use of actin-depolymerizing drugs inhibits cell growth [14–17]. This model implies that the actin cytoskeleton is responsible for maintaining cell polarity and cell growth per se and that the microtubule cytoskeleton is responsible for fine-tuning the axis or direction of cell growth [1, 9]. However, newly divided fission yeast cells already have a rod shape and defined cell tips, and at steady state the microtubule-membrane-actin pathway exists in a closed loop, making it difficult to determine causality. In addition, fission yeast have a rigid cell wall that is remodeled by the cytoskeleton and imparts shape. How this cell-wall-defined shape influences the underlying cytoskeleton is unknown. Here, we use fabricated micrometer-scale channels to control the shape of living yeast cells in order to investigate causal relationships between the cytoskeleton, cell polarity, and cell shape. We tested the current model, and our findings indicate that microtubules can initiate new sites of polarized cell growth while actin maintains sites of growth and that externally applied cell shape can reorganize the underlying cytoskeleton and partially reverse the mutational phenotype.

Soft lithography and patterned adhesive surfaces have been successfully used for controlling the shape of bacteria and mammalian cells [18–21]. We combined soft-lithography and microfluidics technologies to create light-microscopy-enabled, polydimethylsiloxane (PDMS) elastomer-based chambers containing micrometer-scale channels of controlled shapes and appropriate dimensions for fission yeast (Figure 1A). The cells can be syringe-pumped into these channels, in which they grow normally while conforming to specific shapes (Figure 1B), e.g., rod-shaped wild-type cells can be made to grow in a curved manner, and bent or round mutant cells can be made to grow in a straight manner. In addition, inlet and outlet holes allow for continuous flow and exchange of liquid media or drugs (Figure 1A). Cells expressing functional

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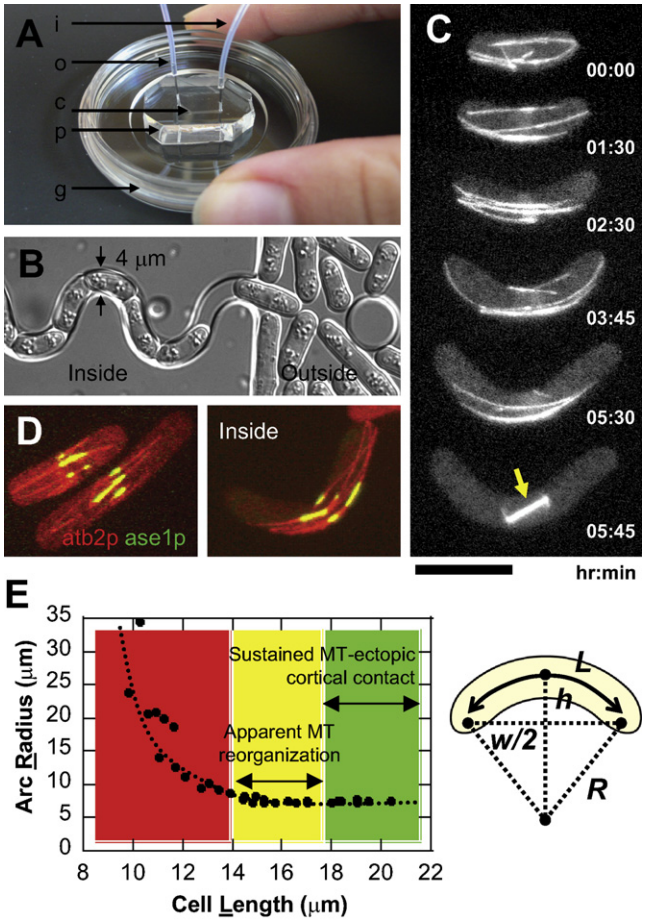

Figure 1. Microfluidic Channels Can Control the Shape of Fission Yeast Cells

(A) Microfluidic channels for constraining the shape of fission yeast cells. Shown is a typical cell chamber made by bonding a polydimethylsiloxane (PDMS) replica (p) onto a glass-bottomed dish (g). The PDMS replica contains either curved or straight microfluidic channels (c) connected to inlet (i) and outlet (o) tubings for liquid exchange.

(B) A curved microfluidic channel containing fission yeast cells. Cells growing outside the channel are free to take on their natural shape, whereas cells growing inside the channel conform to the shape of the channel.

(C) Time-lapse image of a wild-type cell expressing GFP-atb2p (tubulin) (PT.72) inside a curved channel. While growing inside the channel, the cell can progress normally through the cell cycle and form a mitotic spindle (yellow arrow) during mitosis. Time is in hr:min.

(D) Wild-type cells expressing mCherry-atb2p (tubulin) and the conserved microtubule bundler ase1p-GFP (PT.802). Microtubule bundles inside the bent cell reflect global changes in their location, not changes in the number and inherent antiparallel organization of individual microtubule bundles by ase1p (n = 8; control cells n = 14). The scale bar represents 10 μm.

(E) Plot of cell arc radius versus cell length. We calculate the arc radius as: $R = w^2/8h + h/2$ (see Appendix in the Supplemental Data). We defined three regions of interest: red zone—cells are short (<14 μm), and therefore their arc radii are large (>8 μm), and no reorganization of microtubules is apparent; yellow zone—cells are between 14.4 ± 1.7 μm and 17.7 ± 0.4 μm (n = 16) and have arc radii between 7.4 ± 0.1 μm and 7.7 ± 0.3 μm (n = 16), and unambiguous microtubule reorganization to the convex side of the cell occurs; and green zone—cells > 18 μm with average arc radii of 7.5 μm (n = 10), and frequent and sustained contact of microtubule tips with ectopic virgin cell cortex occurs. Cells in the green zone are capable of forming ectopic cell tips; 70% of cells in this zone showed unambiguous ectopic tip protrusion.

fluorescent fusion proteins were used for visualizing structures involved in the pathway leading to directed cell growth—such structures include microtubles (atb2p [22]), +TIPs (tea1p [10]), the membrane receptor mod5p [23], the polarisome complex (bud6p [24]), and actin (crn1p [8]).

First, we compared the behavior of the microtubule cytoskeleton in growing rod-shaped wild-type cells confined inside curved channels to control cells growing outside the channels by using live-cell imaging (Figure 1B). We observed that as wild-type cells grow longer inside the curved channels, their curvature increases, and their arc radii become smaller (Figures 1C and Figure 1E; Movie S1 in the Supplemental Data). Shorter cells have larger arc radii and therefore show no reorganization of the microtubules (Figure 1E, red zone). At cell lengths between 14 and 18 μm and arc radii of ~7.7 μm, microtubules unambiguously reorganize to the convex side of the cell (Figure 1E, yellow zone). This distribution is a consequence of microtubule mechanical buckling or bending due to continued elongation upon contact with the curved cell wall at or adjacent to the cell tips (Figure S1A and Movie S4). At cell lengths >18 μm and arc radii of ~7.5 μm, sustained interactions between microtubules and the ectopic cell cortex occur (Figure 1E, green zone; Figure S1B and Movie S5). In contrast, microtubules in control cells grown outside the channels remain distributed symmetrically with respect to the long axis of the cell, irrespective of cell length (Figure 1D; Movies S2 and S3). Microtubule reorganization reflects changes in the relative position of microtubule bundles within bent cells, but each microtubule bundle retains its basic structure of minus-end antiparallel bundling by the protein ase1p [25, 26] (Figure 1D). These observations indicate that when straight fission yeast cells are forced to grow bent, they will reorganize their dynamic microtubule cytoskeleton in response to their new shape.

We next examined the consequences of microtubule reorganization and contact at the ectopic cell cortex in bent wild-type cells by imaging, in combinations, proteins of the microtubule cytoskeleton, cell membrane, and/or both the polarisome and actin cytoskeleton. We chose cells that were >14 μm for analysis of cytoskeletal reorganization. In contrast to control cells, in which the actin marker crn1p preferentially localized to the growing cell tips, cells in bent channels showed diffuse localization of crn1p (Figure 2A). One-hour time-integration of microtubule and crn1p dynamics revealed that the ectopic site of microtubule-cortex interaction had begun to accumulate crn1p, indicating new actin accumulation (Figure 2A; Figure S2A). In fact, this new site had also accumulated other representative proteins of the +TIP complex, cell membrane, and polarisome cytoskeleton (Figure 2B and Figure S2A). Using high-temporal-resolution imaging, we observed individual microtubules depositing tea1p at the ectopic cell cortex in the bent cells (Figure S2B). Over time, this site initiated polarized cell growth and developed a new cell tip (Figure S2C and Movies S6–S9). We observed that ~70% of cells created unambiguous ectopic cell-tip protrusion (n > 40). No ectopic accumulation of tea1p or bud6p and no ectopic cell tips were observed inside the curved channels when cells were treated with the microtubule-depolymerization drug MBC (n > 40) (Figures S2D and S2E). Interestingly, the old cell tip that no longer received microtubule contact continued polarized cell growth (Figure 2A; Figure S2C and Movies S6–S9). These findings are a direct demonstration of the current model [9, 27]: Microtubules are required for initiating new sites of cell polarity via frequent contact with the cell membrane; however, once polarity sites have been established, polarized cell growth is accomplished and maintained by the actin cytoskeleton independently of microtubules.

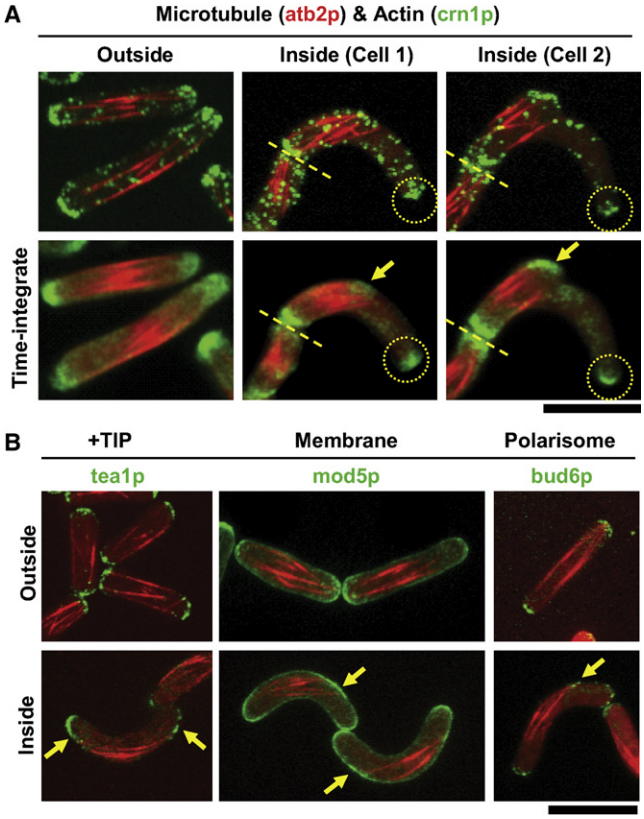

Figure 2. Cell-Shape-Induced Microtubule Reorganization Leads to New Sites of Cell Polarization

(A) Wild-type fission yeast cells expressing mCherry-atb2p (tubulin) and crn1p-GFP (a marker for actin patches) (PT.832). The top row shows single-time-point two-color merged images of cells. The second row shows two-color time-integrated images where 60 time points, representing 1 hr of cell growth, have been summed to show cumulative intensities over time. Cells inside the channel (cell 1) contain rigid microtubule bundles that can no longer extend to both cell tips. Instead, the microtubules reach one tip of the cell and make frequent and sustained contact with the cell cortex (yellow arrows) some distance away from the second tip (yellow dashed circle). Time-integration shows that actin patches accumulate at the ectopic site of microtubule and membrane contact. With longer growth times inside channels (cell 2), this site shows clearer localization of actin (yellow arrow) and the initiation of a new ectopic cell tip, making a branched wild-type cell. The old cell tip continues to be enriched in actin and grow in the absence of microtubule contact (yellow dashed circle) (n = 12; control cells n > 30).

(B) Wild-type cells expressing mCherry-atb2p (tubulin) and proteins of the +TIP (tea1p-GFP) (PT.826), cell membrane (mod5p-GFP) (PT.907), or polarisome (bud6p-GFP) cytoskeleton (PT.853). Outside channels, cell-tip proteins show clear symmetry in localization at the bipodal tips of the rod-shaped cells (n > 30). Inside curved channels, tip-protein symmetry is broken. Consistent with the reorganization of microtubules to the convex cell surface and microtubule contact at new ectopic membrane sites, all tip proteins show relocalization to the ectopic site (yellow arrows) while maintaining localization at the old cell tips (n = 19). The scale bar represents 10 μm.

Bent shape-manipulated wild-type cells that develop a new cell tip as a result of the reorganization of their cytoskeleton are reminiscent of the branched or T-shaped phenotypes exhibited by cells with genetic mutations in the microtubule cytoskeleton [10]. We therefore asked whether abnormally shaped mutant cells forced to grow inside straight channels would reorganize their microtubule and actin cytoskeletons and adopt a more wild-type-like phenotype. We first tested the round mutant *orb6^{ts}* [16, 17]. The fission yeast protein orb6p, a kinase of the conserved NDR kinase family, controls cell morphology [16, 17]. Orb6p localizes to growing cell tips; a temperature-sensitive mutation of orb6p leads to mislocalized actin patches and a consequent spherical shape [16, 17]. We pumped *orb6^{ts}* cells into straight channels at the permissive temperature (23°C) at which cells are rod shaped, then incubated the chamber for several hours at the restrictive temperature (36°C) in order to deactivate orb6p. Consistent with previous reports, at the restrictive temperature *orb6^{ts}* cells outside the channels became round, microtubules were disorganized and had no preferred axis of alignment, and tea1p, mod5p, bud6p, and crn1p were mislocalized and had no preference for cell tips (Figure 3A; Figure S3A). Remarkably, inside straight channels, *orb6^{ts}* cells contained microtubules that were aligned with the long axis of the cell, and tea1p and proteins of the membrane, polarisome, and actin cytoskeleton were localized to the cell tips in a wild-type-like fashion (Figure 3A; Figures S3A and S3B). We concluded that the disorganized cytoskeletal phenotype of round mutant *orb6^{ts}* cells can be partially rescued when cells are externally forced into a wild-type-like shape. Our results thus far suggest that there exists a positive-feedback loop between cell shape and the cytoskeleton; i.e., cell shape leads to microtubule reorganization, which leads to repositioning of polarizome complexes, which defines actin-dependent sites of polarized cell growth, which leads to new cell shape, etc. (Figure S4).

We tested our hypothesis that a feedback loop exists between the cytoskeleton and cell shape. We reasoned that even a minor shape-induced focusing of microtubules would enhance subsequent focusing of +TIP and polarisome complexes in a feedback loop. We therefore examined the distribution of tea1p and bud6p proteins in *mto1Δ* cells. Fission yeast mto1p is a conserved protein involved in microtubule organization [11]. Cells in which mto1p is deleted grow in a bent fashion and usually contain only one bundle of microtubules, located at the convex surface of the bent cell, as opposed to the multiple microtubule bundles of wild-type cells [11]. In straight channels, *mto1Δ* cells appear rod shaped but still contain a single microtubule bundle (Figure 3B). However, the distribution of microtubules, as well as tea1p and bud6p, appear more symmetrical and focused at the cell tips than in control *mto1Δ* cells outside the channels (Figures 3B and 3C; Figure S3C). In control *mto1Δ* cells, tea1p and bud6p cover 81% ± 6% (n = 10) and 82% ± 8% (n = 14) of the cell tip, respectively. In contrast, inside the straight channels, tea1p and bud6p cover only 54% ± 14% (n = 16) and 61% ± 9% (n = 6) ($p < 0.001$) of the cell tip, respectively (Figure 3B and 3C). These results are consistent with the existence of feedback loop that focuses tea1p and bud6p at the tip of the cells in a microtubule-dependent manner.

A feedback loop implies that rod-shaped cells will continue to grow straight and round cells will continue to grow round. How then can cells transition from a rod to round cell shape, as in the *orb6^{ts}* mutant? We reasoned that proper retention of either the +TIP proteins or the polarisome proteins is an important parameter of the feedback loop. We therefore measured the retention of tea1p and bud6p at the cell tips in wild-type and *orb6^{ts}* cells constrained in straight channels in the presence or absence of microtubules. Twenty minutes after MBC treatment, both tea1p and bud6p significantly delocalize from the cell tips of wild-type and *orb6^{ts}* cells. Tea1p was retained at similar levels of 53% ± 16% (n = 12) in wild-type cells and 55% ± 18% (n = 14) in *orb6^{ts}* cells ($p = 0.722$) (Figures 4A and 4B). In contrast, bud6p was retained at

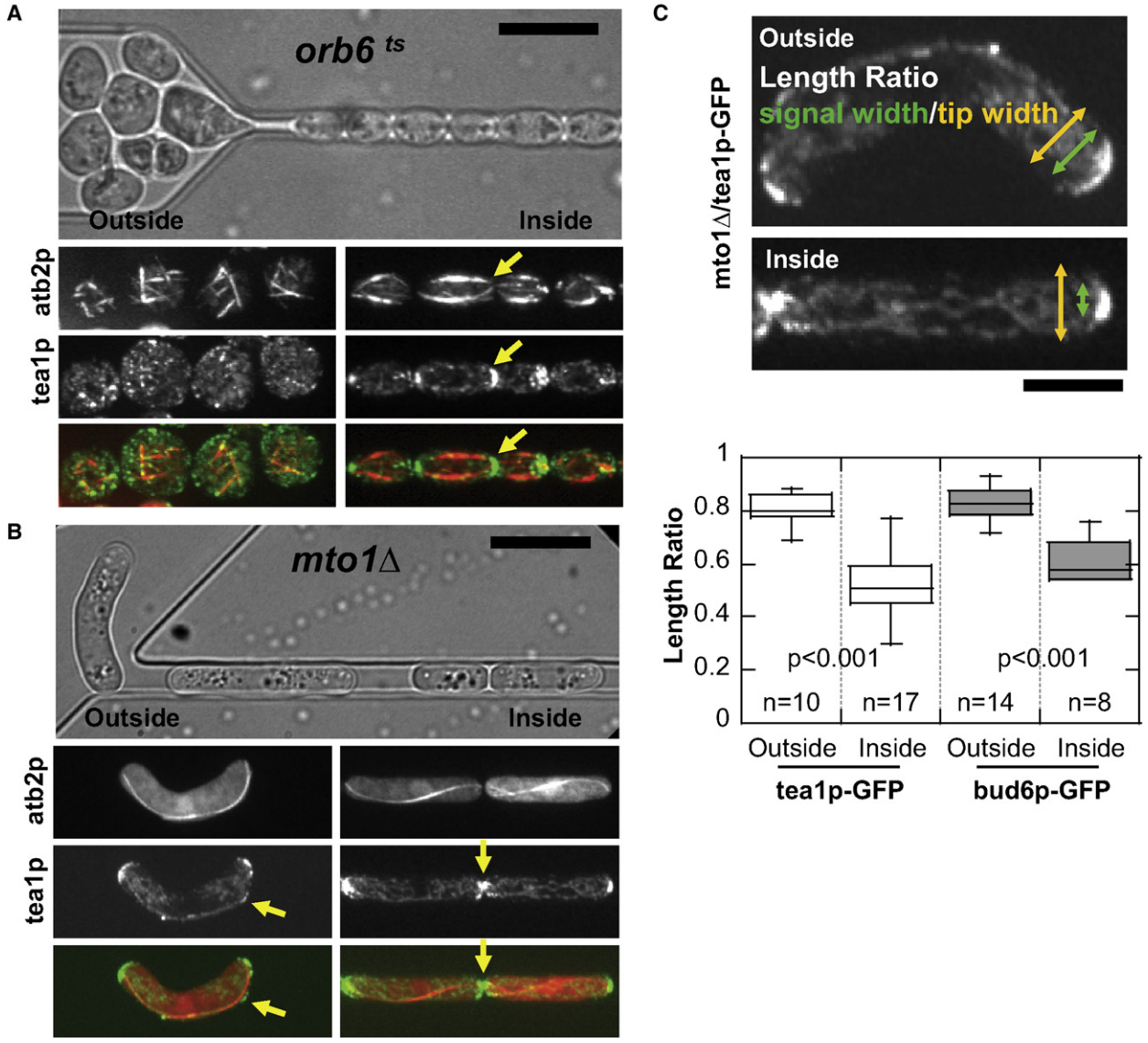

Figure 3. Round- and Bent-Shaped Mutant Cells Maintain Wild-Type-Like Cell Polarity When Constrained inside Straight Channels: A Feedback Loop

(A) Top, A straight microfluidic channel containing *orb6*^{ts} mutant cells growing at the restrictive 36°C temperature. Bottom, *orb6*^{ts} cells expressing mCherry-atb2p (tubulin) and tea1p-GFP (+TIP) (PT.893). Outside the straight channel, *orb6*^{ts} cells have lost their bipodal polarity and are round, microtubules are oriented in all directions, and +TIP protein tea1p is delocalized (observed in ~87% of control cells; n = 30). Inside the channel, *orb6*^{ts} cells are forced to maintain a rod shape, microtubules maintain their alignment along the cell's long axis, and +TIP protein tea1p is specifically localized to the growing cell tips (observed in 60% of cells; n = 15). The scale bar represents 10 μm.

(B) Top, a straight microfluidic channel containing *mto1Δ* mutant cells. Bottom, *mto1Δ* cells expressing mCherry-atb2p (tubulin) and tea1p-GFP (+TIP) (PT.910). Outside the channel, the single microtubule bundle is located at the convex surface of the bent *mto1Δ* cell. The +TIP protein tea1p shows asymmetry in its localization at the old cell tips and also begins to localize ectopically at a new cortical site (yellow arrow). Inside the channel, the microtubule bundle of *mto1Δ* cells shows no preference for a particular cell surface but instead interacts with the bipodal cell tips. As a consequence, cell-tip proteins show symmetrical localization at the cell tips and not at other ectopic sites (observed in ~88% of cells, n = 17; control cells n = 17). The scale bar represents 10 μm.

(C) Top, *mto1Δ* cells from (B). We defined the length ratio as the ratio of the width of the tea1-GFP (or bud6-GFP) signal divided by the width of the cell tip. Bottom, comparison plots of length ratios of tea1-GFP and bud6-GFP in *mto1Δ* cells growing outside versus inside the straight channels.

78% ± 16% (n = 14) in wild-type cells but only at 46% ± 16% (n = 13) in the *orb6*^{ts} cells ($p < 0.001$) (Figures 4A and 4B). This indicates that microtubule-dependent tea1p delivery and retention at cell tips is not affected in *orb6*^{ts} cells, whereas bud6p retention at the cell tips is orb6p dependent. We found that *orb6*^{ts} cells transititon from straight to round by slowly decreasing their lengths and increasing their widths through successive cell cycles, so that the aspect ratio of the cell tends toward unity after 2–4 generations (Figures 4C and 4D). These data are also consistent with our feedback-loop model, where different mutant cell shapes can evolve from an initial rod-shaped cell (Figure 4E; Figure S4).

Our current work shows that *S. pombe* morphogenesis is governed by a feedback loop between the cytoskeleton and cell shape. Two key steps in this loop allow cells to maintain a rod shape: (1) Focusing of microtubules by cell shape induces

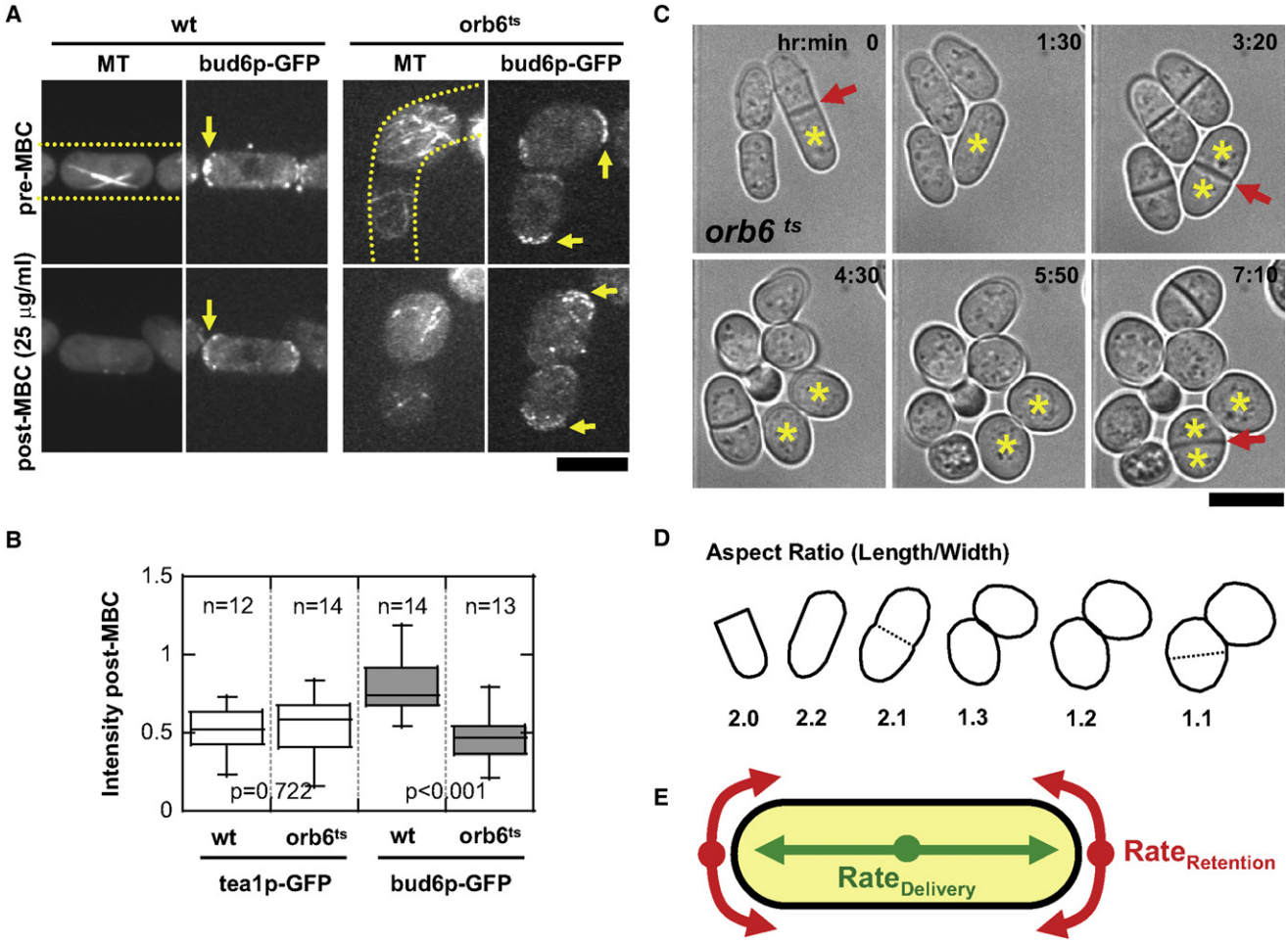

Figure 4. A Mechanism for the Cell Shape-Cytoskeleton Feedback Loop: bud6p Retention at Cell Tips Is Important for Shape Changes

(A) Straight microfluidic channels containing wild-type and *orb6*^{ts} mutant cells growing at the restrictive 36°C temperature (channels are indicated by dashed yellow lines). Inside the channel, *orb6*^{ts} cells are constrained and maintain a somewhat elongated rod shape. Wild-type and *orb6*^{ts} cells are expressing mCherry-atb2p (tubulin) and bud6p-GFP (polarisome) (PT.924 and PT.894, respectively). During imaging, 25 μg/ml of MBC (microtubule-depolymerizing drug) was perfused through the channels. Approximately 20 min after MBC treatment, no microtubules were present, and a significant fraction of bud6p-GFP no longer localized to the cell tips (yellow arrows). This experiment was also performed for wild-type and *orb6*^{ts} mutant cells expressing mCherry-atb2p (tubulin) and tea1p-GFP (+TIP) (PT.922 and PT.893, respectively).

(B) Normalized intensity plots comparing the retention of tea1p-GFP and bud6p-GFP at the cell tips before (pre-MBC) and 20 min after (post-MBC) microtubule depolymerization with 25 μg/ml of MBC.

(C) Bright-field time-lapse images of *orb6*^{ts} cells growing at the restrictive 36°C temperature. Cells transition from a straight to a round shape by decreasing their lengths and increasing their widths during successive cell cycles (septa are indicated by red arrows).

(D) Aspect ratio of *orb6*^{ts} cells through successive cell cycles. Tracing of cells from (C). The aspect ratio is defined as length/width.

(E) A model for cell shape: a positive feedback loop. At steady state, cells maintain a rate of delivery (R_D) of proteins such as tea1p to the cell tips and a rate of retention (R_R) of proteins such as bud6p at the cell tips. When $R_D > R_R$, cells maintain a straight shape. When $R_D < R_R$, cells evolve into a round shape after successive cell cycles.

focusing of polarisome deposition at cell tips; and (2) retention of a focused polarisome is necessary for maintaining straight cell growth at cell tips. It will be interesting to test the robustness, evolutionary conservation, and/or disease-reversal implications of this proposed cytoskeleton and cell-shape feedback loop. For example, will cells maintain their newly acquired cytoskeleton and shape phenotypes when removed from the confinement of microfluidic channels either immediately or after a certain number of generations? Studies in bacteria have indicated that shape can be maintained once cells are freed from external constraints [20]. Second, which aspects of the feedback loop are conserved? One key parameter of the feedback loop determined by this study is the proper retention of the polarisome protein bud6p at the cell tips. This may be analogous to the mechanism for forming the polar cap in budding yeast [28]. Finally, can externally imposed shape such as that found in multicellular organisms compensate for or reverse the cytoskeletal phenotypes of a mutant cell located inside a tissue composed mainly of wild-type cells? There are indications that cancer cells can be made to form morphologically normal structures and are more resistant to apoptosis when grown in certain three-dimensional contexts [29, 30].

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, an appendix, four figures, one table, and nine movies and are available with this article online at http://www.current-biology.com/supplemental/S0960-9822(08)01277-3.

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