Oscillatory AAA⁺ ATPase Knk1 constitutes a novel morphogenetic pathway in fission yeast

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Edited by Thomas D. Pollard, Yale University, New Haven, CT, and approved November 3, 2014 (received for review April 19, 2014)

Cellular morphogenesis relies partly on cell polarization by the cytoskeleton. In the fission yeast *Schizosaccharomyces pombe*, it is well established that microtubules (MTs) deliver the spatial cue Tea1, a kelch repeat protein, to the tip regions to direct the growth machinery at the cell tips driving the linear extension of the rod-shaped organism to maintain a straight long axis. Here, we report the characterization of Knk1 (kink), a previously unidentified member of the superfamily of ATPases associated with various cellular activities (AAA⁺), whose deletion causes a unique morphological defect characterized by the formation of kinks close to cell tips. Through genetic analysis, we place Knk1 into a novel pathway controlling cell shape independently of MTs and Tea1. Knk1 localizes at cell tips. Its localization is mediated by the Knk1 N terminus and is enhanced upon ATP binding to the C-terminal ATPase domain. Furthermore, Knk1 tip recruitment is regulated by SRC-like adaptor 2 (Sla2) and cell division cycle 42 (Cdc42) independently of Sla2's role in endocytosis. Finally, we discovered that Knk1 shows an anticorrelated oscillatory behavior between the two cell tips at a periodicity that is different from the reported oscillatory Cdc42 dynamics.

oscillation | morphogenesis | kink

Wild-type fission yeast forms rod-shaped cells 4 μ m in diameter that grow linearly from the cell tips and divide medially. After division, growth resumes in a monopolar fashion before switching to a bipolar growth during NETO (new end take off) (1). Its morphogenesis is regulated by both, the microtubule (MT) and actin cytoskeletons, and defects in these respective cytoskeletons lead to characteristic shape changes. Mutants affecting actin-based structures usually partially fail to polarize growth at cell tips, resulting in plump cells; in contrast interfering with MT-related functions leads to the loss of a straight axis of growth, resulting in bent or T-shaped cells (Fig. 1A).

In interphase, actin forms two distinct structures: actin cables delivering, via myosin V, secretory vesicles and actin patches representing sites of endocytosis (2). Briefly, the activity of the small GTPase cell division cycle 42 (Cdc42), constituting the core of the polarization machinery, is confined to the cell poles (3). Otherwise cells become roundish because of isotropic growth as observed upon inactivation of the NDR kinase Orb6 (4). Cdc42 activates formin For3, the formin responsible for actin cable nucleation (5). *for3* Δ cells lacking actin cables partially lose their polarized state and become bulbous or swollen. Disruption in the endocytic pathway, such as deletion of the endocytic adaptor protein Sla2, results in a similar shape phenotype (6, 7).

MTs contribute to cell shape by marking cell tips as sites of growth through the deposition of a landmark protein complex, including the kelch repeat protein, Tea1 (8, 9). Interphase MTs organized in three to five bundles parallel to the cell's long axis are anchored to the nucleus with their minus ends. Their plus ends show dynamic behavior, extending toward cell poles and contacting the cortex for about 50–80 s. Simultaneously, the Tea1 protein complex is carried by MT plus ends in a plus tip microtubule aberrant length protein 3 (Mal3; EB1 homolog)-dependent manner toward the cell tips, where Tea1, in turn, indirectly contributes to the activation of For3 (10). Upon loss of

Tea1, cells fail to maintain a straight axis and become bent (11). Similarly, an aberrant MT cytoskeleton leads to bent cells resulting from Tea1 complex mispositioning (12, 13). For example, the deletion of either Mal3, which results in very short MTs that do not reach cell ends (14, 15), or of the MT nucleator Mto1 (pericentrin homolog), which reduces the number of MT bundles, causes a bent shape (16).

In this study, we describe a novel gene, *knk1* (kink, encoded by SPBC947.01/alf1), which plays a role in morphogenesis and founds a new class of shape mutants forming kinks. Knk1 belongs to the superfamily of ATPases associated with various cellular activities (AAA⁺) found in all organisms and implicated in various cellular processes such as protein degradation and membrane trafficking (17). Despite their diversity in function, they share a common core mechanism by assembling into ring-shaped hexamers. Interestingly, Knk1 shows a fascinating oscillatory behavior between the two cell tips.

Results

The *knk1* Δ Mutant Displays a Novel Kinked Shape Phenotype. We identified *knk1* in a visual screen for new shape mutants using the genomewide library of haploid fission yeast deletion mutants (18). Intriguingly, cells carrying a deletion of *knk1* displayed a kink close to cell tips (Fig. 1A and B). Kinks were observed, on average, in 8.2% of cells at 25 °C and in up to 20.7% of cells after 3 h at 36 °C (Fig. 1C), usually at only one pole but rarely at both (~4% of kinked cells at 36 °C). Although wild-type cells always displayed a straight long axis, kinked mutant cells were characterized by an angle (θ) between the tip growth axis and the otherwise straight long cell axis (Fig. 1E). The average angle of kinks was $25.6 \pm 7.7^\circ$ at 25 °C (Fig. 1D).

Significance

We describe a novel pathway controlling fission yeast cell morphogenesis depending on Knk1, a member of the conserved ATPases associated with various cellular activities (AAA⁺) family regulating important cellular processes in all organisms and often functioning in unfolding, degradation, or dissociation of multi-meric complexes. Deletion of *knk1* (kink, encoded by SPBC947.01/alf1) founds a new class of morphogenesis mutants exhibiting sudden changes in directional growth. Knk1 performs oscillations between the two cell ends at a periodicity different from the reported cell division cycle 42 oscillations. We conclude that at least two oscillatory systems may coexist in fission yeast to control cell morphogenesis. We speculate that oscillatory properties could represent a key feature of morphogenetic factors to control the growth machinery in a dynamic manner and achieve an accurate definition of cell shape.

Author contributions: K.S. designed research; K.S. performed research; P.R. contributed new reagents/analytic tools; K.S. and P.R. analyzed data; K.S., A.P., and P.T.T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1407226111/-DCSupplemental.

Fig. 1. *knk1Δ* has a novel kinked shape phenotype. (A) Differential interference contrast (DIC) images of shape mutants showing the normal rod shape of wild-type cells and the aberrant round (*orb6-25*, 7 h at 36 °C), plump (*for3Δ* and *sla2Δ*), bent (*mal3Δ* and *tea1Δ*), T-shaped (*tea1Δ* after starvation), and kinked (*knk1Δ*) shapes of morphology mutants (at 25 °C if not stated otherwise). (B) Kinked and bent cell morphology in *cdc25-22 knk1Δ* and *cdc25-22 tea1Δ* strains after 3 h at 36 °C. (C) Percentage of wild-type and *knk1Δ* cells displaying kinks at 25 °C or after 3 h at 36 °C. Bars represent means from three independent experiments ($n > 300$ cells per experiment). Error bars indicate SD. (D) Distribution of angles θ in *knk1Δ* cells after 3 h at 25 °C; $n = 261$. The dotted line indicates the mean angle of $25.6 \pm 7.7^\circ$. (E) Schematic highlighting the characteristics of a bent, T-shaped, or kinked morphology. θ is the angle between the growth direction (defined as the tip orientation) and the cell's long axis. (F) Time-lapse images of a *knk1Δ* cells by brightfield microscopy at 36 °C. Arrowheads indicate the direction of growth. (Scale bars, 5 μ m.)

To understand the origin of kinks, we followed their formation by time-lapse recording at 36 °C. *knk1Δ* cells first grew linearly; then growth was reoriented toward another direction (Fig. 1F). After this event, the direction of tip growth remained straight. Kinks formed more frequently at old ends (70% kinks, $n = 60$ cells) at 5–150 min after cell separation and at a cell length of 7.6–13.6 μ m but also could appear at new ends (30% kinks), at 30–105 min after cell separation and at a cell length of 8.4–12.7 μ m (Fig. S1B). In a few kinked cells (6%), kinks formed at both cell tips; this event could be observed more frequently (34%) in a *cdc25-22* background at 36 °C in which the growth phase is extended (Fig. S1A). Taken together, these results suggest that kinks are not produced when growth resumes at the old end after sister cell separation nor at the new end after NETO but arise at low frequency at any time point of the growth phase.

Overexpression of Knk1-YFP in *knk1Δ* cells led to a gradual disappearance of kinks, demonstrating that Knk1 expression was not able to correct established kinks but did prevent the formation of new kinks (Fig. S1C). Old kinks were lost after several rounds of division.

Importantly, the kinked phenotype clearly differed from the bent phenotype, which is characterized by a gradual directional deflection of growth resulting in a curvature (Fig. 1D and Fig. S1D) evidenced in a *cdc25-22* background (Fig. 1B). Bent cells frequently are observed in *tea1Δ* cells (18.2%) or MT-defective mutants such as *mal3Δ* (19.4%) (Fig. 2A and B) in which Tea1 is dispersed along the cortex as shown for *tip1* (12), both resulting in mispositioning of the growth machinery away from cell tips. Indeed, the majority of *tea1Δ* and *mal3Δ* cells with morphological defects displayed a gradual directional deflection of growth. Kinked cells could be observed only at low frequency (2.7% or 3.7%) in these mutants, as could T-shapes (0.5% or 0%) (Fig. 2

A and B) which exhibit a medial outgrowth approximately perpendicular to the main long axis (Fig. 1D and Fig. S1E). Thus, the *tea1Δ* and MT mutants differ significantly from the *knk1Δ* mutant that displays a very low frequency (2%) of bent cells and a complete absence of T-shapes (Fig. 2A and B). In addition, unlike Tea1, Knk1 is not necessary for the initiation of bipolar growth during NETO (Fig. S1F) (19).

We conclude that the kink phenotype defines a novel class of shape mutants in fission yeast.

Aggravation of Kinked Phenotype by MT Defects. The new shape phenotype of the *knk1Δ* mutant suggested that Knk1 may control cell morphogenesis by orientating growth direction independently of the MT/Tea1 pathway. Accordingly, the MT cytoskeleton labeled with GFP-Atb2 (alpha tubulin) appeared indistinguishable from the wild-type MT cytoskeleton in straight or kinked *knk1Δ* cells (Fig. S2A). We observed no significant changes in MT dynamics (Fig. 2C), demonstrating that Knk1 is not involved in MT regulation and that the kinks present in *knk1Δ* cells are not a consequence of an abnormal MT behavior.

Fig. 2. Knk1 supports straight growth independently of MTs and Tea1. (A) Percentage of kinked, bent, and T-shaped cells and cells with ambiguous “kink-like” morphology, including cells with angles close to the middle of the cell and cells in which one half is bent and one half is kinked, in wild-type and mutant cells at 25 °C. Bars represent means from three independent experiments ($n > 300$ cells per experiment). Error bars indicate SD. (B) DIC images of the mutants shown in A. Dotted lines indicate midlong axis of cells with abnormal shapes. Yellow represents kinked cells, blue represents bent cells, and orange represents ambiguous cells. (C) MT dynamic parameters in wild-type and *knk1Δ* cells (mean $\pm$ SD; $n = 50$ for each value). (D) Tea1-GFP localization in wild-type and *knk1Δ* cells. The yellow asterisk marks a kinked tip with asymmetrical distribution of Tea1. (E) Mean angles at 25 °C: *knk1Δ* ($25.6 \pm 7.7^\circ$), *knk1Δ tea1Δ* ($27.3 \pm 9.8^\circ$, $P = 0.01$), *knk1Δ mal3Δ* ($38.3 \pm 13.6^\circ$, $P < 10^{-37}$), and *knk1Δ mal3Δ tea1Δ* ($31.4 \pm 11.8^\circ$, $P < 10^{-8}$). $n \geq 197$. (Scale bars, 5 μ m.)

wild-type Knk1 (Fig. 3 *D* and *E*), implying that ATP hydrolysis is not required for Knk1 localization.

Taken together, these data indicate that Knk1 acts as an ATPase at the cell tip. Furthermore, recruitment to the tip is controlled by the N terminus and is enhanced upon ATP binding, possibly through the formation of Knk1 hexamers, which may allow the cooperation of several N termini.

Knk1 Localization at the Cell Tip Is Independent of MTs and Actin Cables.

Next, we examined whether Knk1 recruitment to cell tips is dependent on the actin or MT cytoskeleton. When cells were treated with 50 μ M carbendazim (MBC) to depolymerize MTs, Knk1 localization was not affected (Fig. 4*A*). Knk1 also stained growing cell tips in mutants of the MT–Teal1 pathway, *mal3 Δ* or *tea1 Δ* , and even after 4 h MBC treatment (Fig. S4*A*).

In contrast, Knk1 was no longer detected at cell tips upon treatment with 100 μ M latrunculin A (LatA), an inhibitor of F-actin (Fig. 4*B*), leading to disassembly of F-actin patches marked by coronin-like protein 1 (Crn1)-GFP (Fig. 4*B*). Interestingly, although Crn1 patches were restored 20 min after washout of LatA, Knk1 tip localization was restored only after 90 min, suggesting that Knk1 localization was not controlled simply by the presence of F-actin but rather by F-actin-based processes that are required for the establishment and maintenance of Knk1 at cell tips. Alternatively, Knk1 degradation might be induced by LatA, resulting in long recovery periods after drug washout.

Importantly, Knk1 levels at cell tips in a *for3 Δ* mutant that lacks F-actin cables were comparable to levels in wild-type cells (Fig. 4 *C* and *E*) (25), demonstrating that Knk1 tip localization relies on F-actin patches rather than on F-actin cables.

Sla2 and Cdc42 Regulate Knk1 Localization. Because F-actin patches correspond to sites of endocytosis, we next tested the impact of

endocytosis defects on Knk1 distribution. In the *sla2 Δ* mutant, in which endocytosis is blocked before membrane ingression by the uncoupling of membrane and actin dynamics (26), Knk1 levels at cell tips were two- to threefold higher than in wild-type cells (Fig. 4 *C* and *E*), but global protein levels were not altered, as shown by Western blot (Fig. S4*E*). Line scans along the long axis confirmed increased tip but lowered cytoplasmic levels (Fig. 4*D*), demonstrating that Knk1 was more bound to cell tips.

The observed accumulation at tips in the *sla2 Δ* mutant suggested that Knk1 may be internalized by endocytosis. We therefore tested Knk1 distribution in two other endocytic mutants, class I myosin (*myo1 Δ*) and Wasp homolog (*wsp1 Δ*) mutants, which fail to activate F-actin nucleation by Arp2/3 (27–29) and lead to the accumulation of Sla2 at cell tips (Fig. S4*D*). Surprisingly, Knk1 tip levels were not increased significantly in either mutant (Fig. 4 *C* and *E* and Fig. S4*C*), demonstrating that Knk1 accumulation in *sla2 Δ* cells is not caused by endocytosis defects. Consistently, costaining of Sla2-TagRFP and Knk1-2xGFP revealed only a partial overlap (Fig. S4*F*). As demonstrated by a line scan along the cell tip (asterisk in Fig. S4*F*), Sla2 formed patches at the tip that moved inwards (arrow in Fig. S4*F*), but Knk1 stained tips more uniformly and never was detected in patches distant from the cell tip cortex. This observation suggests Knk1 is not directly associated with F-actin patches, although we cannot exclude the possibility that there may be a minor pool of Knk1 molecules at endocytic sites.

We next wondered whether Knk1 might function in endocytosis, which can be monitored by the uptake of the lipophilic dye FM4-64. The control *sla2 Δ* cells showed a significant disruption in FM4-64 uptake, but the *knk1 Δ* cells did not (Fig. S4*G*). Thus, Knk1 is not required for proper endocytosis.

As mentioned above, during cell division Knk1 forms a contractile ring similar to that formed by Cdc42 GEFs (21). We therefore wondered whether Cdc42 activity might impact Knk1 distribution. To test this notion, we used the temperature-sensitive allele *cdc42-L160S* (30–32) that exhibits no defects in the early steps of endocytosis but reduced intensity of active Cdc42 at cell tips monitored by Cdc42/Rac interactive binding (CRIB)-GFP (33) at 25 °C. Intriguingly, Knk1 accumulated to high levels at tips in these conditions (Fig. 4 *C* and *E*), similar to *sla2 Δ* . However, Sla2 localized to actin patches in *cdc42-L160S* cells (Fig. S4*D*), demonstrating that increased Knk1 tip intensity was not caused simply by the absence of Sla2. Furthermore, a double *sla2 Δ cdc42-L160S* mutant did not differ significantly from a single *sla2 Δ* mutant (Fig. 4 *C* and *E*), indicating that Sla2 and Cdc42 function in the same pathway to regulate Knk1 recruitment to cell tips in an endocytosis-independent manner.

Knk1 Exhibits Novel Oscillatory Dynamics at Cell Tips. When we visualized Knk1-2xGFP in *sla2 Δ* cells at 1-min intervals, we observed that Knk1 was highly dynamic at cell tips, continuously undergoing cycles of binding and detachment (Fig. 5 *A* and *B*). Strikingly, the behavior of the two cell tips appeared anti-correlated, and this observation was confirmed using a cross-correlation function (cross-correlation coefficient $r = -0.39 \pm 0.24$). Furthermore, in ~60% of cells, these oscillations appeared to be periodic with an average periodicity of 13.9 min (frequency = $0.072 \cdot \text{min}^{-1}$) obtained by Fast Fourier Transform (FFT) analysis on all cells and all recorded intervals, whether or not the oscillatory behavior was detected by eye (Fig. 5*C*). A similar periodicity of 13.5 min was detected when FFT was performed for a subgroup of cells exhibiting oscillations noticeable by eye (Fig. S5*A*), demonstrating the robustness of the FFT analysis.

When we followed tip intensities of Knk1-2xGFP in wild-type cells, no striking oscillatory behavior could be observed by eye (Fig. S5 *B* and *C*), nor was a specific frequency detected by FFT analysis (Fig. S5*D*). Nevertheless, Knk1 often showed anti-correlated fluctuations (marked by arrows in Fig. S5*C*) with a cross-correlation coefficient $r = -0.16 \pm 0.25$, in contrast to uncorrelated fluctuations seen for Tea1-GFP with $r = 0.04 \pm 0.34$. This result indicates that the two cell tips compete for Knk1

Fig. 4. Sla2 and Cdc42 regulate Knk1 cell tip localization. (*A* and *B*) Cells expressing Knk1-2xGFP mCherry-Atb2 (*A*) or Knk1-2xGFP (yellow asterisks) or Crn1-GFP (actin patch, blue asterisks) (*B*) were treated with 50 μ M MBC (*A*) or 100 μ M LatA (*B*) for 20 min. Drug was washed out, and images recorded at the indicated time points. (*C*) Knk1 localization in wild-type and mutant strains. (*D*) Line scan of Knk1-2xGFP fluorescence intensity along the long axis of 12 individual wild-type and *sla2 Δ* cells of comparable length. Intensities were measured over the full cell width at each point along the cell axis. a.u., arbitrary units. (*E*) Knk1 intensity (a.u.) averaged over individual tips in wild-type cells (70.4 ± 46.9 a.u.) and in the mutants *for3 Δ* (73.9 ± 70.3 , $P = 0.68$), *sla2 Δ* (150.3 ± 119.9 , $*P < 10^{-8}$), *myo1 Δ* (71.6 ± 59.5 , $P = 0.87$), *cdc42-L160S* (142.4 ± 119.9 , $*P < 10^{-6}$), and *sla2 Δ cdc42-L160S* (197.7 ± 225.3 , $P < 10^{-6}$ relative to wild type, $P = 0.07$ relative to *sla2 Δ*). $n = 100$. (Scale bars, 5 μ m.) n.s., not significant.

Fig. 5. Oscillations and fluctuations of Knk1-2xGFP at cell tips. (A) Time-lapse images of Knk1-2xGFP in an *sla2Δ* strain at 25 °C. (Scale bars, 5 μm .) (B) Knk1-2xGFP tip intensities at 1-min intervals in the *sla2Δ* cell shown in A. The period is defined as the average time span between two subsequent maxima of intensity (amplitude). Periodicity = $1/\text{frequency}$. (C) Frequency spectrum of Knk1-2xGFP tip intensities analyzed by FFT analysis using all recorded intervals in *sla2Δ*; $n = 25$. The red line represents a Gaussian function of intervals of frequencies with higher amplitudes indicating average frequencies of 0.072-min^{-1} for *sla2Δ*. (D) Frequency spectrum of CRIB-GFP in selected intervals (with periodic anticorrelated dynamics detected by eye) in wild-type cells ($n = 14$; 0.19-min^{-1}) and in the mutants *knk1Δ* ($n = 13$, 0.21-min^{-1}) and *sla2Δ* [$n = 8$; not determined; the arrow indicates potential frequency at 0.18-min^{-1} (5.6 min periodicity)].

and that the detachment of Knk1 from one tip allows the accumulation of Knk1 at the second competing tip. Because we failed to detect an oscillatory behavior for Knk1 in wild-type cells, the periodic oscillations might be triggered by the deletion or inactivation of *sla2* and *cdc42*. Alternatively, Knk1 also might undergo a periodic oscillation in wild-type cells, but the dynamics of tip-bound Knk1 might not be measured reliably enough to detect them because of the strong fluctuations in cytoplasmic intensity (Fig. S5E).

A recent study using CRIB-GFP as a reporter for active Cdc42 evidenced anticorrelated oscillations of Cdc42 at cell tips with an average period of 5 min (34). To determine whether Knk1 and Cdc42 dynamics were linked, we analyzed the dynamics of Knk1 in the *cdc42-L160S* mutant. Knk1 oscillated with a period of 14.8 min in an anticorrelated fashion ($r = -0.3 \pm 0.31$) (Fig. S5F–H), similar to oscillations seen in *sla2Δ*. CRIB-GFP dynamics could not be determined in this mutant, because levels were dramatically decreased (30). However, the detection of Knk1 oscillation despite a reduction of active Cdc42 in cell tips strongly suggests that Knk1 oscillations are not driven by Cdc42 dynamics. Conversely, we also tested whether Cdc42 oscillations were influenced by the deletion of *knk1*. In wild-type cells, CRIB-GFP oscillations had an average periodicity at 5.3 min (Fig. 5D), consistent with a previous report (34). CRIB-GFP distribution and oscillation were unaffected in *knk1Δ* cells with an average period of 4.8 min (Fig. 5D and Fig. S5J). Finally, we studied CRIB dynamics in *sla2Δ* cells (Fig. S5I), in which Knk1 exhibited anticorrelated oscillations at 13.9-min intervals. Although less clearly than in wild-type cells, we observed a peak at a frequency of 0.18-min^{-1} (5.6 min), which is similar to observations in wild-type and *knk1Δ* cells (Fig. 5D), implying that Knk1 and Cdc42 oscillate at different frequencies in *sla2Δ*.

Finally, to determine if the Knk1 oscillation is linked to its turnover at cell tips, we performed fluorescence recovery after photobleaching (FRAP) experiments of cell tips. Knk1 intensity recovered partially, about 30% in wild-type and 15% in *sla2Δ*

cells, with a half-time ($\tau_{1/2}$) of ~ 6 s for both (Fig. S5J), suggesting that a large fraction of Knk1 is immobile at this timescale. At a longer timescale after FRAP, Knk1 levels were largely restored (over 60% compared with original levels) after varying time periods (Fig. S5K). However, once maximal Knk1 levels were reached, no plateau phase of the Knk1 intensity was detected. Instead, the Knk1 level dropped again, probably controlled by its oscillatory properties.

Discussion

We identified a novel component of fission morphogenesis, the AAA⁺ ATPase Knk1, supporting linear growth in an MT/Tea1-independent pathway.

Consistent with its role in morphogenesis, Knk1 localizes to cell tips and the septum region. Tip intensity is enhanced in the endocytic defective *sla2* mutant and in *cdc42-L160S* mutant cells that appear functional in early endocytic steps (31), independent of Sla2's role in endocytosis. In addition, double *sla2 cdc42* mutants mimicked single mutants, indicating that Cdc42 and Sla2 act in the same pathway. No direct link between these proteins has been reported in fission yeast, but in budding yeast the Cdc42 system is known to interact with the key endocytic proteins—epsins that function cooperatively with Sla2 to couple plasma membrane and actin cytoskeleton dynamics in early steps of endocytosis (35). Mutants in the epsin N-terminal homology (ENTH) domain displayed polarity defects resulting from decreased levels of active Cdc42, but endocytosis was unaffected. Because our study in fission yeast also suggests a link between the early endocytic adaptor protein Sla2 and Cdc42 in Knk1 localization, there may be a conserved connection between endocytic processes and the Cdc42 pathway linking endocytosis and cell polarization machinery to regulate downstream growth factors. Nevertheless, the exact mechanisms regulating Knk1 localization remain elusive at this point.

We detected an oscillatory behavior for Knk1 in *sla2Δ* and *cdc42-L160S* mutants that, at least in *sla2Δ* cells, does not seem to coincide with Cdc42 oscillations. Thus, our work suggests the coexistence of two oscillatory systems with distinct periodicities, for Cdc42 at 5 min and Knk1 at 15 min. Future work will be required to test whether these systems are coupled. We did not detect clear periodic Knk1 fluctuations in wild-type cells. Here, cytoplasmic Knk1 intensity fluctuations are strong and might artifactually influence the analysis of the dynamics of tip intensities. Second, FRAP experiments suggest a fraction of highly dynamic Knk1 at cell tips, 30% in wild-type and 15% in *sla2Δ* cells, whereas the turnover of more stably bound Knk1 molecules might be controlled by its oscillatory properties. This relatively large fraction of rapidly overturning Knk1 in wild-type cells may obscure its oscillatory signal. We therefore speculate that Knk1 oscillation also might occur at specific periods in wild-type cells. However, we cannot exclude the possibility that the periodic oscillations might be triggered by cellular alterations in *sla2Δ* and *cdc42-L160S*, for example by limiting the amount of available Knk1 resulting in the competition of the two cell tips for Knk1. In fact, the enhanced Knk1 levels in *sla2Δ* and *cdc42-L160S* cells indicate that Cdc42 and Sla2 might be part of the negative feedback mechanism leading to cell tip detachment of Knk1. Under wild-type conditions, such striking anticorrelated periodic oscillations might not be observed; for example, regulatory proteins usually might be present in sufficient quantities, and thus the two cell tips would not compete for Knk1. Furthermore, it remains to be addressed whether Knk1's oscillatory behavior is required for its function in morphogenesis.

Interestingly, a simulation based on the Turing reaction-diffusion model, using MT-Tea1 reinforcement of growth zone and Cdc42 growth zone signaling, generated sharply bent fission yeast cells reminiscent of kinks (36). However, this model cannot be a mechanism for kink formation in the absence of Knk1, because *knk1Δ* cells generate kinks independent of MT-Tea1.

The kinks seen in *knk1Δ* cells are reminiscent of the behavior of fission yeast subjected to an electrical field, where cell growth is reoriented orthogonally to the field (37). This report shows

that, in an electrical field, β -glucan synthases accumulate abnormally at one side of cell tips, leading to asymmetric cell wall synthesis. The *knk1 Δ* phenotype might be caused by a similar asymmetrical distribution of certain polarity factors. However, no apparent cell wall abnormalities were observed with staining with the cell wall marker Calcofluor (Fig. S6B). Interestingly, 1,3- β -glucan synthase subunit 4 (Bgs4) (Fig. S6C), CRIB-GFP (Fig. S5F), and the actin cytoskeleton labeled by GFP-CDH (calponin homology domain) (Fig. S4B) in straight *knk1 Δ* cells appeared undistinguishable from those in wild-type cells and also were symmetrically distributed along a kinked cell tip, suggesting that these components of the growth machinery have followed the new growth direction. Possibly, these components might be transiently relocalized during straight growth, resulting in kink formation. It remains elusive, whether Knk1 controls the distribution pattern of certain growth factors or whether Knk1 controls other processes at cell tips that in turn indirectly affect the localization of these proteins.

At this point, we can only speculate on how Knk1 may influence cell shape. AAA⁺ ATPases are implicated in various cellular processes (17), e.g., membrane trafficking. However, we did not detect any endocytic (via FM4-64 uptake) or exocytic (via secretion of acid phosphatase; Fig. S6D) defects, suggesting that Knk1 is not essential for these processes.

Alternatively, Knk1 might regulate the turnover of some cell-tip proteins via protein folding/unfolding, degradation, or protein complex disassembly, because many AAA⁺ proteins are implicated in such processes (17). Potentially, by periodically removing components of the cell growth machinery, Knk1 might prevent the amplification of random deflection in growth

direction that otherwise is stabilized by positive feedback loops reinforcing the growth machinery locally (38).

Our study suggests that oscillatory properties represent a key feature in controlling cell shape. A first description of oscillatory dynamics emerged from studies in bacteria (39), where the MinCDE system undergoes oscillations to prevent division away from the cell middle. Remarkably, these oscillations are driven by the activity of the ATPase MinD. Future work may show that oscillatory dynamics are used commonly among ATPases or other NTPases to control various cellular processes.

Experimental Procedures

Standard fission yeast media and techniques were used as described (40). Live cell imaging was performed by spinning disk confocal microscopy as previously described (41). Frequency/periodicity of oscillations was obtained by FFT analysis using Matlab. Detailed descriptions are supplied in *SI Experimental Procedures*. *S. pombe* strains used in this study are listed in Table S1. Knk1-2xGFP and CRIB-GFP dynamics in wild-type and mutant cells are quantified in Table S2.

ACKNOWLEDGMENTS. We thank the laboratories of J.-C. Ribas (University of Salamanca), P. Perez (University of Salamanca), and T. Pollard (Yale University) and the Japan National BioResource Project for generously providing reagents and Sergio Rincon for helpful technical advice and discussion. Imaging was performed at the Biomaging Cell and Tissue Core Facility of the Institut Curie, a member of the France-Bioimaging National Research Infrastructure. K.S. is supported by a PhD fellowship from Complexité du Vivant-Université Pierre et Marie Curie and Fondation ARC pour la Recherche sur le Cancer. This work was supported by grants from the National Institutes of Health, the Agence Nationale de la Recherche, Institut National du Cancer, and Fondation ARC pour la Recherche sur le Cancer.

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