Roles of the fission yeast formin for3p in cell polarity, actin cable formation and symmetric cell division

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Background: Both symmetric and asymmetric cell divisions are required for the generation of appropriate cell lineages during development. Wild-type *Schizosaccharomyces pombe* cells divide in a symmetric fashion to produce two similar rod-shaped daughter cells. Formins are proteins with conserved roles in cell polarity, cytokinesis, and the regulation of actin and microtubule cytoskeletons.

Results: Here, we identify and characterize a new *S. pombe* formin, for3p. *for3*Δ mutant cells divide in an asymmetric manner; a mother cell divides medially to produce one daughter cell that develops into a monopolar cell and one daughter that develops into a bipolar cell. Both daughter cells recapitulate similar asymmetric lineages themselves. Inheritance of the bipolar pattern correlates with inheritance of the recent birth scar, not with asymmetry in the spindle pole bodies. *for3*Δ mutants lack interphase actin cables and have delocalized actin patch and myo52p (type V myosin) distributions. *for3*Δ cells have normal microtubule dynamics and cortical interactions but have defects in microtubule organization and increased numbers of microtubule bundles. for3p-GFP is localized at both cell tips in an actin-dependent manner and at the cell division site.

Conclusions: for3p is a cell polarity factor required for interphase actin cable formation and microtubule organization. The *for3*Δ phenotype suggests that cells are able to grow in a polarized manner even in the absence of functional actin cables and polarized distribution of actin patches. for3p and possibly actin cables are part of a regulatory network that ensures that cell divisions are symmetric.

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Background

Cell lineages during the development of multicellular organisms are characterized by both asymmetric and symmetric cell divisions. Although the generation of asymmetric divisions has been studied intensely in many systems, little is known about the generation of symmetric divisions. In contrast to the asymmetric budding yeast, fission yeast divide in the middle to produce two similar daughter cells. Both daughter cells grow first in a monopolar manner and then initiate bipolar growth in G2 phase, in a process known as NETO (new end take off). Analyses of proteins required for growth patterns have implicated both actin and microtubule cytoskeletons in cell polarity regulation [1].

Formins are a conserved family of proteins with roles in cytokinesis, cell polarity, and cytoskeletal organization (see reviews [2–4]). In budding yeast, *BNI1* is required for the regulation of the bipolar budding pattern [5], asymmetric localization of a partitioned mRNA [6–8], actin polarization during mating [9], and spindle positioning [10, 11]. *Drosophila cappuccino* is required for asymmetric localization of posterior determinants at the future posterior end of the oocyte [12, 13]. Some formins have been implicated as effectors for Rho and Cdc42 GTPases [9, 14–16]. Formins may regulate or associate with both the actin and microtubule cytoskeletons [17–19]. For instance, overexpression of mammalian formin mDia contributes to actin stress fiber formation and the stabilization of microtubules [14, 16, 17, 19].

In fission yeast, two formins have been previously characterized: cdc12p is an essential gene required for the assembly of the medial actin-myosin ring required for cytokinesis [20], and fus1p is required for the polarization of actin and cell fusion during mating [21]. Here, we identified a third formin gene that we named *for3* (formin 3). Our studies implicate for3p in cell polarity and in the formation of actin cables. *for3*Δ mutants had a novel phenotype in which cells divide to produce daughter cells with different growth behaviors. In addition, *for3*Δ mutants lacked functional actin cable structures. These findings suggest that for3p is part of a cellular mechanism that ensures symmetric cell divisions.

Results

*for3*Δ mutant cells divide to produce unequal daughters

We identified for3p in the *S. pombe* genome databases (Sanger Centre) on the basis of its amino acid similarity

to other formins. for3p contains domains similar to those found in other formins, including formin homology domains FH1, FH2, and FH3 and rho binding sites (see the Supplementary material available with this article online). Of the fission yeast formins, for3p is most similar to budding yeast Bni1p [5, 22].

Characterization of for3p showed that it has a primary role in cell polarity and cell morphogenesis. *for3*Δ deletion cells were generated using a PCR-based system [23]. *for3*Δ cells were viable and slow growing at 30°C (generation time of 5 hr for *for3*Δ versus 2.5 hr for wt; data not shown). In addition, cell growth was strongly inhibited by 1 M KCl (data not shown). At 30°C, *for3*Δ cells displayed a range of different cell shapes: swollen round or lemon-shaped cells, curved banana-shaped cells, tapered bottle-like cells, and normal rod-shaped cells (Figure 2b).

To determine how the different cell shapes arise, we analyzed cell growth patterns in lineages using time-lapse differential interference contrast (DIC) microscopy (Figure 1c). In wild-type cells, cell division produced two equivalent cells. Immediately after cell division, each wild-type daughter cell initiated monopolar growth from the old end (the previously growing end), and then after a period of approximately 50 min, each cell initiated bipolar growth at NETO (new end take off) [24] (Figure 1a, first column). In contrast, *for3*Δ cells produced nonequivalent daughters; one daughter grew only in a monopolar manner at the old end, while the other daughter immediately initiated growth from both ends and proceeded to grow in a bipolar manner (Figure 1a,b). Thus, one daughter (the monopolar cell) exhibited no NETO (or a greatly delayed NETO), while the other daughter (the bipolar cell) exhibited premature NETO. These patterns occurred in the large majority of cell divisions (85% of cell divisions, n = 52). Analyses of lineages over multiple generations and of lineages using morphological characterization of mothers showed that both bipolar cells and monopolar cells divided to produce one bipolar daughter and one monopolar daughter (75% of cell divisions with monopolar mothers [n = 16]; 81% of cell divisions with bipolar mothers [n = 22]).

Figure 1e,f shows quantitative differences in cell growth at the different cell ends. Although there was considerable variability of growth at cell tips in different cells, the cells consistently exhibited these growth patterns. Measurement of tip growth over time showed that both ends of the bipolar cell grew immediately after cell division without delays (Figure 1f). Most *for3*Δ cells divided medially; although, in some cells, the division plane was slightly off center (Figure 1c, second cell from the left). It is unlikely that a difference in the initial cell sizes could account for the difference in growth patterns, as many daughter cell pairs were initially the same cell size (Figure 1c, top left cell pair). Also, monopolar cells continued to grow in a monopolar manner even when they were larger than other bipolar cells. The daughter cells often septated at different times, suggesting a variability in cell cycle periods between daughter cells (Figure 1c). This difference may reflect differences in growth rates between the cells. In addition, daughter cells often adopted different cell shapes. Although the extent of these abnormal morphologies was variable among cells, bipolar cells generally grew into lemon-shaped or bottle-shaped cells, while monopolar cells were more rod-shaped or bent. Thus, *for3*Δ cells divided to produce daughter cells that were inequivalent in regard to growth pattern, cell shape, and cell cycle periods.

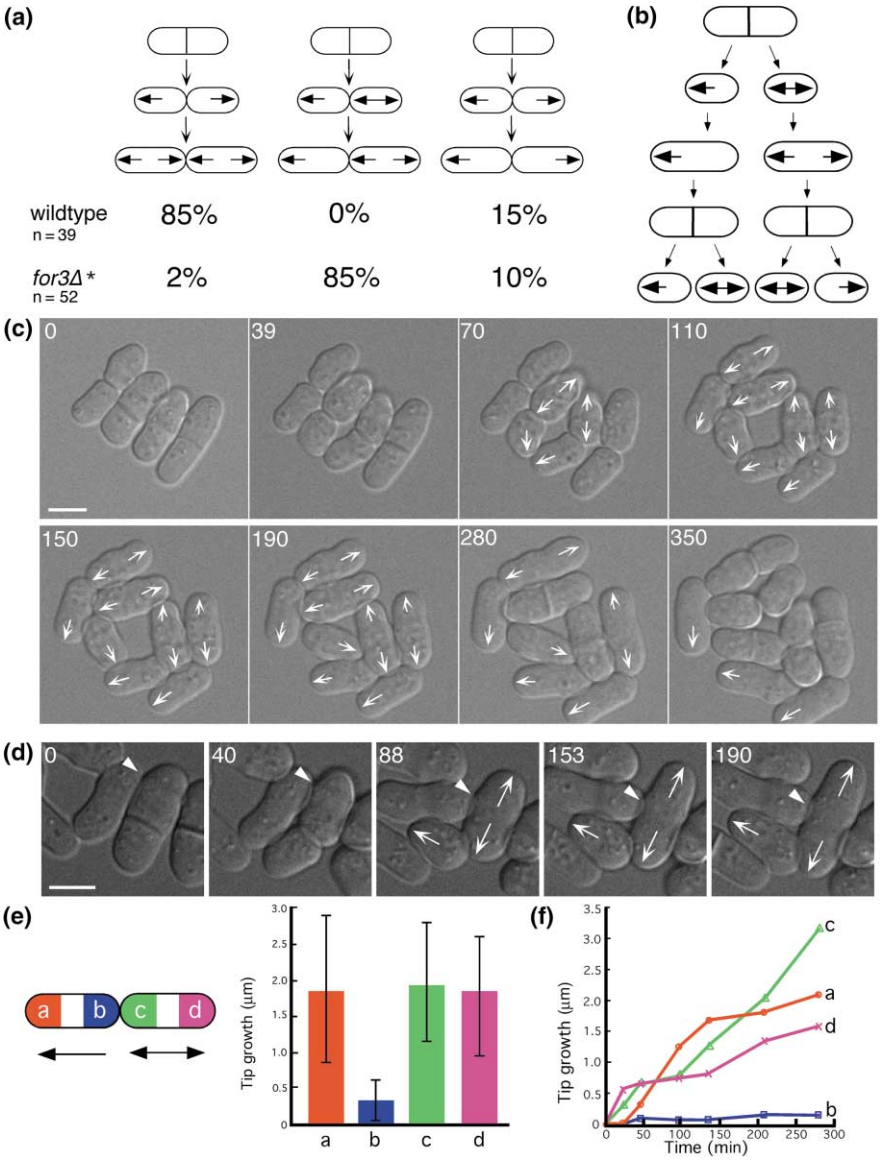
To understand how this asymmetry was generated, we examined if inheritance of a particular growth pattern correlated with the asymmetric inheritance of a cortical marker. Birth scars (sites of previous cell division) were visualized as ridges on the cell wall, which were confirmed to be birth scars by time-lapse images. Almost every cell (97% cells, n = 40 pairs) that inherited the birth scar from two cell divisions prior became the cell with a bipolar growth pattern (Figure 2a). Figure 1d shows an example of this correlation; the cell with a birth scar from the previous division (white arrowhead) grew in a bipolar pattern, whereas its sister grew in a monopolar pattern. Calcofluor staining of single cells confirmed that lemon-shaped bipolar cells had multiple birth scars, whereas monopolar cells generally had only one, from the most recent cell division (Figure 2b). This correlation of growth polarity with birth scars suggests that the history of previous cell divisions retained on the cell surface or even the age of the cell may influence the asymmetrical cell fate.

Next, we tested if asymmetry in the two daughter spindle pole bodies causes the asymmetrical cell growth patterns. cdc7p is a regulator of septation that is asymmetrically inherited on one of the daughter spindle poles [25]. In wild-type cells, inheritance of the cdc7p-GFP spindle pole body did not correlate with inheritance of the previous birth scar [25] (Figure 2c). In a similar manner, *for3*Δ cells also showed no correlation between cdc7p spindle pole body inheritance and birth scar inheritance (Figure 2c). Given the tight correlation of growth patterns and birth scars, these findings show that asymmetry in the spindle pole bodies was not responsible for *for3*Δ asymmetrical cell growth patterns.

*for3*Δ mutant cells lack actin cables

We tested the possibility that *for3*Δ cells may have defects in the organization of actin or microtubule cytoskeletons that may account for the asymmetrical growth pattern. In wild-type cells, the actin cytoskeleton is characterized by the polarized distribution of actin patch structures at sites of polarized cell growth as well as a filamentous network of actin cables [26, 27] (Figure 3a). Alexa Fluor phalloidin staining revealed that all interphase *for3*Δ cells completely

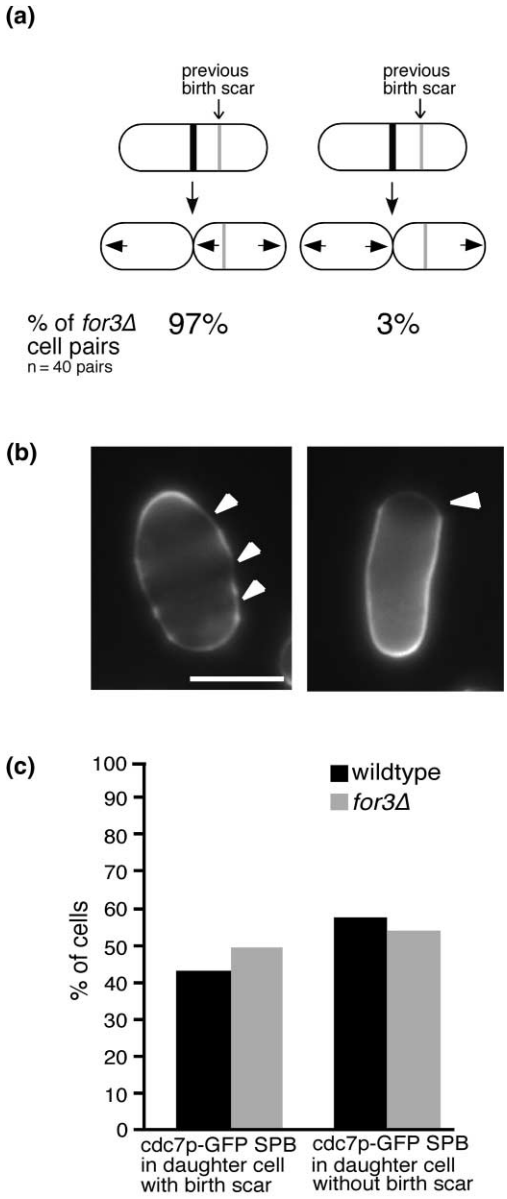
Figure 1



for3^{+} is required for symmetric division and the regulation of polarized cell growth. Growth pattern lineages of wild-type (FC421) and *for3*Δ (BFY9) cells were obtained by time-lapse DIC microscopy over 6–9 hr at 25°C–28°C. **(a)** Growth patterns of wild-type and *for3*Δ cells over a cell cycle. Arrows represent the directions of growth in pairs of daughter cells. A *for3*Δ mother cell divides medially to produce a monopolar daughter cell and a bipolar daughter cell. An asterisk indicates that 3% of *for3*Δ cell pairs showed alternate growth patterns. **(b)** The lineage pattern of *for3*Δ cells over multiple cell cycles. Note that each daughter cell gives rise to a similar pair of daughter cells with different growth patterns. **(c,d)** Time-lapse DIC images of *for3*Δ cell pairs. Arrows indicate the direction of growth. Numbers indicate minutes elapsed. The arrowhead in (d) points to a birth scar present in the bipolar daughter cell. **(e)** Measurement of total cell tip growth over a cell cycle at the different cell tips in *for3*Δ daughter cell pairs (n = 10 pairs). Cell tips labeled a, b, c, and d are as indicated. **(f)** Measurement of cell tip growth rates at different cell tips in a single representative *for3*Δ daughter cell pair. Time 0 is at the time of cell-cell separation. The scale bar represents 5 μm.

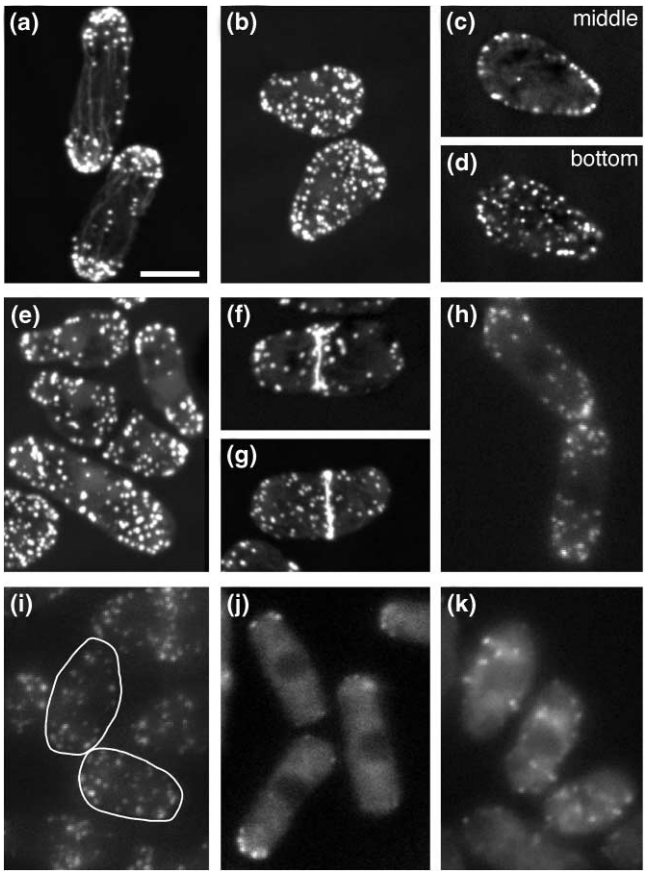
lacked detectable actin cables (Figure 3b–g) (0% of *for3*Δ cells had actin cables, n = 88 cells, versus 95% of wild-type cells had actin cables, n = 42). Actin patches were present but were depolarized in most cells (Figure 3b–g,i), although they were still polarized in more rod-shaped cells (Figure 3e, cells at top). As actin cables are thought to direct actin patch movement [27], we analyzed actin patch movement in *for3*Δ cells using the actin patch marker crn1p-GFP. Actin patches in *for3*Δ cells moved at normal rates, but only in an undirected manner (Table 1). The undirected movement and abnormal distribution of actin patches were similar to those in a tropomyosin *cdc8* mutant, which also lacks actin cables [27]. Thus, the defects in actin patch organization in *for3*Δ cells are likely due to the absence of functional actin cables. As an additional assay for actin cable function, we examined the distribution of a type V myosin, myo52p. In wild-type cells, myo52p-GFP is seen in motile dots (possible myoV-associated organelles) that move in a directional manner and are polarized at cell tips ([28, 29]; Figure 3j; Table 1). myo52p-GFP localization has been shown to be dependent on actin cables, as it is delocalized in a tropomyosin (*cdc8*) mutant and in cells treated with Latrunculin A (Lat A, an actin inhibitor) [28]. In *for3*Δ cells, myo52p-GFP dots were delocalized (Figure 3k) and exhibited no directional movement (Table 1). These effects on actin patch and myo52p localization suggest that *for3*Δ mutants lack functional actin cables, although it is possible that for3p may affect these processes more directly. Actin ring assembly, cytokinesis, and septation appeared normal (Fig-

Figure 2



Possible sources of asymmetry in *for3*Δ cells. **(a)** The *for3*Δ daughter cell that inherits a previous birth scar becomes the bipolar cell. Growth patterns (see Figure 1) and the inheritance of birth scars from a previous cell division (ridges of the cell wall; see Figure 1d) were assayed by time-lapse DIC images. Arrows indicate the direction of growth. **(b)** Calcofluor staining of *for3*Δ cells shows multiple birth scars in the lemon-shaped cell and a single birth scar in the rod-shaped cell. **(c)** Inheritance of the asymmetric spindle pole body (SPB) marker cdc7p-GFP does not correlate with inheritance of a birth-scar. Wild-type (FC855) and *for3*Δ (BFY165) cells expressing cdc7p-GFP were imaged for cdc7p-GFP SPB by GFP fluorescence and for birth scar inheritance by DIC microscopy. The scale bar represents 5 μm.

Figure 3



*for3*Δ cells lack actin cables and have defects in actin patch and myo52p-GFP organization. F-actin structures were imaged in fixed cells by staining with Alexa Fluor phalloidin in **(a)** wild-type (FC418) and **(b–g)** *for3*Δ (BFY9) cells and in **(h)** living wild-type (FC661) cells and **(i)** *for3*Δ (BFY158) cells by crn1p-GFP. Maximum projections of confocal images from multiple focal planes are shown in (a), (b), and (e–g). Two optical sections, 1 μm apart, are shown in (c) (in the middle of the cell) and (d) (the bottom of the cell). Maximum projections of wide-field images of living cells are shown in (h) and (i). *for3*Δ cells do not have (b–e) detectable interphase actin cables, but they have (f,g) normal actin contractile rings in mitosis. (b–g,i) Actin patches are depolarized in some cells, while they are still largely polarized in others. A pair of daughter cells is outlined in (i), showing no obvious differences in actin organization between daughter cells. myo52p distribution was examined using wide-field microscopy on live cells expressing myo52p-GFP (FC862, BFY163). myo52p-GFP dots are localized to the tips of **(j)** wild-type interphase cells and are delocalized in **(k)** *for3*Δ cells. The scale bar represents 5 μm.

ure 3f,g). To examine whether actin organization was asymmetric between daughter cells, we examined an actin patch marker, crn1p-GFP, in living cells. No differences in actin patch distribution were observed between daughter cells (Figure 3i).

*for3*Δ mutant cells have defects in microtubule organization

The interphase microtubule (MT) cytoskeleton in wild-type cells is composed of 3–4 microtubule bundles arranged along the long axis of the cells [30–32] (Figure 4a). The microtubule plus ends, which face cell tips, generally

Table 1

Dynamics of actin patches, microtubules, and myo52p-GFP in *for3* cells.

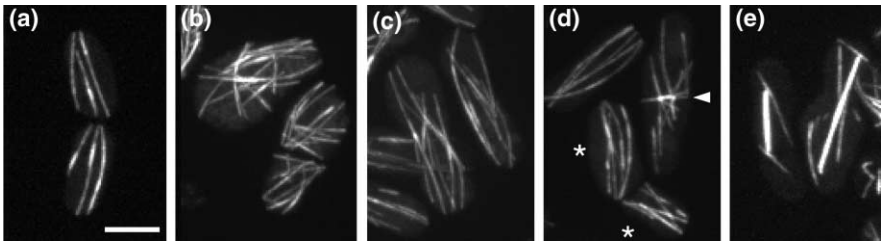
Microtubule dynamics	Wild-type	n (MTs, cells)	*for3*	n (MTs, cells)
MT growth rate (μm min^{-1})	3.29 ± 1.46	24, 20	3.73 ± 1.41	25, 20
MT shrinkage rate (μm min^{-1})	14.33 ± 4.06	30, 19	12.12 ± 4.29	30, 18
MT interphase bundles/cell	3.33 ± 0.65	140, 42	6.30 ± 2.05	296, 47
Actin patch dynamics	**Wild-type**	**n**	***for3***	**n**
Average velocities (μm s^{-1})	0.30 ± 0.04	21 patches, 15 cells	0.33 ± 0.06	20 patches, 12 cells
Percent of cells with directed actin patch movement	70%	44/63 cells	0%	0/90 cells
myo52p-GFP dynamics	**Wild-type**	**n**	***for3***	**n**
Percent of cells with directed myo52p dot movement	61%	39/64 cells	0%	0/78 cells

only exhibit catastrophe after hitting a cell tip [31–33]. Microtubules in *for3*Δ mutants were examined using immunofluorescence in fixed cells and a GFP-tubulin construct in living cells [32, 34]. *for3*Δ cells consistently had more microtubule bundles than wild-type strains (6.3 ± 2.05 MTs in *for3*Δ cells, n = 296 MTs, 47 cells, compared with an average of 3.3 ± 0.65 MTs in wild-type cells, n = 140 MTs, 42 cells) (Table 1) (Figure 4, compare [a] with [b]–[d]). The reason for the increased number of bundles is not yet clear. Microtubule growth and shrinkage rates were normal (Table 1). Like microtubules in wild-type cells, microtubules in *for3*Δ mutants still exhibited catastrophe after hitting the cortex. No abnormally stable or long microtubules were observed. In many rod-shaped cells, microtubules were arranged normally along the long axis of the cell and exhibited catastrophe only at cell tips (Figure 4c,d). However, some of the more swollen cells had more disorganized microtubules that exhibited catastrophe at cell sides and cell tips (Figure 4b). It is possible that this microtubule disorganization may be a consequence of cell polarity defects. Examination of pairs of daughter cells after cell division revealed no consistent differences in microtubule behavior between the daughter cells (Figure 4d, cells marked with asterisks). Mitotic spindles and postanaphase MT arrays appeared normal (Figure 4d,e). Thus, these analyses showed that for3p regulates both actin and microtubule cytoskeletons. Although there were no obvious cytoskeletal asymmetries that would explain the asymmetry of the daughter cells, the actin cable defect in *for3*Δ cells suggests one mechanism in which actin cables may be required for the regulation of growth patterns.

Localization of for3p

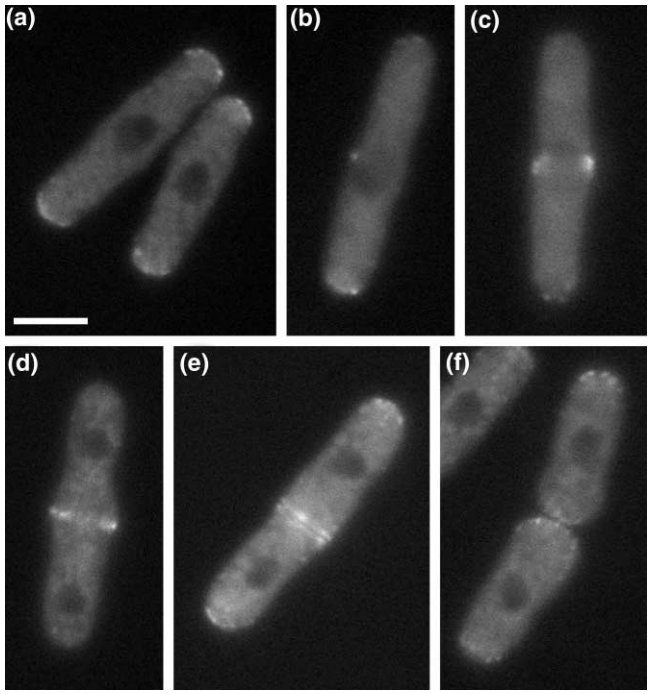
We localized for3p in the cell using a functional for3p-GFP fusion expressed at endogenous levels. During interphase, for3p-GFP was localized in cortical dots at both cell tips (Figure 5a). In early mitosis, prior to anaphase, for3p-GFP was present as a single medial spot or a medial ring, in a manner similar to cdc12p (Figure 5b,c) [20]. In late mitosis, for3p-GFP appeared as a double ring (Figure 5e), similar to the localization pattern of septins (unpublished data). During mitosis, the cell tip localization of for3p-GFP progressively decreased and was absent by anaphase (Figure 5, compare [b] and [d]). During septation, for3p-GFP was retained as a series of dots at the new ends of the daughter cells and was relocalized to the old ends, giving rise to the interphase pattern (Figure 5f). Time-lapse images showed that these for3p-GFP dots at the cell tips were highly dynamic (see Movie 1 in the Supple-

Figure 4



Microtubules in **(a)** wild-type (FC722) and **(b–e)** *for3*Δ (BFY147) strains expressing GFP-atb2p (α-tubulin) were imaged for GFP fluorescence in living cells. Maximum projections of confocal images from multiple focal planes are shown. Asterisks indicate daughter cell pairs. *for3*Δ cells have an increased number of (b–d) interphase MT bundles and sometimes show (b,c) disorganized MT bundles, but they have normal postanaphase ([d], arrowhead) MT arrays and (e) mitotic spindles. Note that there is no obvious difference in microtubule organization in the two daughter pairs. The scale bar represents 5 μm.

Figure 5



Localization of for3p-GFP to the cell tips and cytokinesis ring. Cells (BFY11) expressing a functional for3p-GFP protein fusion at endogenous levels were imaged live using wide-field microscopy in a single focal plane. Cells in different cell cycle phases are shown: **(a)** interphase cells; **(b,c)** early mitosis; **(d)** anaphase; **(e)** postanaphase or septation; **(f)** after cell-cell separation. The scale bar represents 5 μm.

mentary material available with this article online). These patterns suggested that for3p functions to regulate cortical cell polarity at the cell tips and possibly at the septum.

To determine if for3p at cell tips is associated with the F-actin or the microtubule cytoskeletons, we examined for3p-GFP localization in cells treated with 200 μM Lat A or 25 μg/ml methyl-2-benzimidazole-carbamate (MBC, a microtubule inhibitor). At these concentrations, Lat A treatment leads to complete disassembly of the F-actin cytoskeleton, while MBC treatment causes the interphase microtubules to shrink to short stubs [27, 32]. In Lat A-treated cells, for3p-GFP was localized in abnormal clusters at the cell tips (5–30 min after Lat A addition). These abnormal for3p-GFP clusters were delocalized from the cell tip or were absent at later time points (40–60 min after Lat A addition) (Figure 6a). In MBC-treated cells, no perturbation of the cell tip localization was seen after 30 min (Figure 6b) or 60 min of treatment (data not shown). DMSO alone did not perturb for3p-GFP localization. In addition, Lat A treatment (20 min) inhibited the motility of for3p-GFP dots (Movie 2). Thus, the localization and motility of for3p was dependent on F-actin.

Discussion

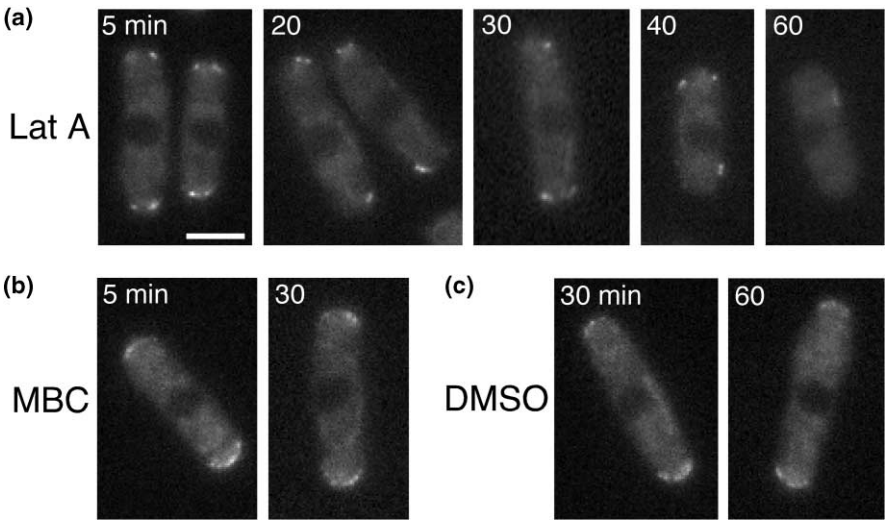
for3 is required for organization of interphase actin and microtubule cytoskeletons

Here, we show that for3p regulates cell polarity and the organization of the actin and microtubule cytoskeletons during interphase. To our knowledge, for3p is the first identified *S. pombe* factor with a specific function in actin cable assembly. Other factors, like tropomyosin, affect other actin structures, such as the contractile ring [35]. Actin cables are highly dynamic structures that may function as tracks for polarized movement of organelles and actin patches, but little is known about their organization or regulation [4]. *for3*Δ mutant cells lacked any detectable actin cable staining. Although we cannot rule out that there may be fine actin filaments in place of actin cables, some functions of actin cables were perturbed in *for3*Δ mutants, as actin patches and myosin V dots were delocalized and lacked directional movements. for3p was localized in dynamic dots at cell tips that were dependent on F-actin for localization and motility, suggesting that for3p is associated with the dynamic, dense network of actin cables and patches at the cell tip [27]. Thus, for3p may regulate the generation of actin cables from the cell tips.

Polarized cell growth is commonly believed to be dependent on the polarized distribution of actin patches and the polarized secretion directed by actin cables, but the phenotypes of *for3*Δ cells begin to show how these processes can be uncoupled. *for3*Δ cells were viable (but slow growing) and exhibited some degree of polarized growth apparently without actin cables, suggesting that actin cables are not essential for viability or polarized growth. Actin patches and polarized growth could also be uncoupled. Most *for3*Δ cells had delocalized actin patches, suggesting that polarized growth does not require polarized actin patches. These data are consistent with an observation that budding yeast cells can bud in the absence of polarized actin patches [36]. In other *for3*Δ cells (such as the more rod-shaped cells), actin patches were still polarized in the absence of actin cables, suggesting that actin cables are not always required for localized distribution of actin patches. Thus, our data suggest that actin cable structures and polarized distribution of actin patches may not be essential strictly for polarized cell growth.

The three formins in *S. pombe* regulate three different actin-based processes. cdc12p is required specifically for actin ring assembly for cytokinesis, as *cdc12* mutants lack actin ring structures but have normal distribution of interphase actin patches [20]. fus1p is required specifically for cell fusion during mating, and *fus1* mutant cells exhibit depolarized actin patches during mating [21]. No synthetically lethal interactions between *cdc12* and *for3* have been found (I. Mabuchi, personal communication). One possibility is that each of these formins may bring together different sets of actin organization proteins to build spe-

Figure 6



Localization of for3p-GFP to the cell tips is dependent on actin and not microtubules. Cells expressing for3p-GFP (BFY11) were imaged live for GFP fluorescence using wide-field microscopy. Cells were treated with **(a)** 200 μM Lat A, **(b)** 25 μg/ml MBC, or **(c)** 1% DMSO (control) for time periods indicated (min). The scale bar represents 5 μm.

cific actin structures. In budding yeast, the formins Bni1p and Bnr1p also function in actin cable assembly (A. Bretscher, D. Pellman, personal communication), cytokinesis, and cell fusion, but these formins appear to have more overlapping functions [37].

Like some other formins, for3p may also have a role in the organization of microtubules. It has been suggested that mammalian mDia can stabilize or orient interphase MTs [17, 19]. We have not detected any defects in MT stability or regulated catastrophe of MTs at the cortex in *for3*Δ mutants. However, *for3*Δ mutants did exhibit increased numbers of microtubule bundles and disorganized microtubule bundles. The orientation of microtubule bundles in longitudinal arrays may be dependent on cell shape and possibly on the polarized distribution of microtubule regulators. As microtubule orientation correlated with cell shapes, it is possible that the defects in microtubule organization were due to altered cell polarity and cell shape. As little is known about how microtubule bundles are formed or oriented, it is not clear yet how for3p or actin cables may affect the number of MT bundles or their orientation.

Role of for3p in symmetric cell divisions

Lineage analysis showed that *for3*Δ mutants have a novel cell growth pattern; after each cell division, daughter cells adopted different growth behaviors and different cell shapes. Asymmetries in wild-type *S. pombe* cells have been found previously in spindle pole bodies [25] and mating type switching [38]. This is the first report of such an asymmetry in growth patterns. The growth patterns correlated with the presence or absence of birth scars; the lineage produced one daughter cell that generally contained one or more birth scars and grew in a bipolar pattern and one daughter cell that contained no birth scars and grew in a monopolar pattern. Thus, historical markers from previous cell divisions may influence growth patterns, in a manner similar to the regulation of budding patterns in budding yeast [39, 40].

We propose that for3p and possibly actin cables play a role in ensuring symmetric division. In one model, proper symmetric growth may require the symmetric distribution of a hypothetical polarity factor in the daughter cells. In the absence of for3p, the bipolar cell may inherit too much of this factor, and the monopolar cell may inherit too little of this factor. The birth scar may be a repository of this factor or may merely be a marker for the "age" of the cell surface. for3p may directly help distribute this factor into both cells. Alternatively, for3p may function primarily to form actin cables, which are responsible for the movement and distribution of this factor to both daughter cells. The nature of this factor is not known, as actin, microtubules, and polarity factor tea1p (unpublished data) were not asymmetrically distributed in an obvious manner. Another model is that *for3*Δ mutants have a defect in establishing polarized growth at certain ends, and cells without a strong polarization site will grow using alternative polarity programs at previous sites of cell polarization or septation. Alternative cell polarity lineage patterns have been found in *bud6* mutants (NETO defect) and *tea1* mutants (one cell grows at the old end only, one at the new end only) [41], and the *for3*Δ pattern may be interpreted as a mixture of these patterns. However, in *for3*Δ lineages, bipolar cells arise both from a cell that has never grown before and from a cell that has grown at one end before, suggesting that problems with growth establishment at naive ends is not the sole defect in *for3*Δ cells. It is likely that for3p interacts and functions in association with other polarity

Table 2

***S. pombe* strains used in this study.**

Strain	Genotype	Source/reference
BFY9	*h− for3::kanMX6 ura4-D18 leu1-32 ade6*	This study
BFY11	*h+ for3-GFP:kanMX6 ura4-D18 leu1-32 ade6*	This study
BFY147	*h− for3::kanMX6 ura4-D18 leu1-32 ade6* [pDQ105; *LEU2*]	This study
BFY158	*h+ for3::kanMX6 crn1-GFP:kanMX6 ura4-D18 leu1-32 ade6*	This study
BFY162	*h+ for3::kanMX6 ura4-D18 leu1-32 ade6*	This study
BFY163	*h+ for3::kanMX6 myo52-GFP:ura4+ ura4-D18 leu1-32 ade6*	This study
BFY165	*h+ for3::kanMX6 cdc7-GFP:ura4+ ura4-D18 leu1-32 ade6*	This study
FC418	*h− ura4-D18 leu1-32 ade6*	Chang lab
FC419	*h+ ura4-D18 leu1-32 ade6*	Chang lab
FC421	*h− ura4-D18 leu1-32 ade6*	Chang lab
FC584	*h+/h− ura4-D18/ura4-D18 leu1-32/leu1-32 ade6-M210/ade6-M216*	Chang lab
FC661	*h+ crn1-GFP ura4-D18 leu1-32 ade6*	Chang lab
FC722	*h− ura4-D18 leu1-32 ade6-M210* [pDQ105; *LEU2*]	Chang lab
FC855	*h− cdc7-GFP:ura4+ ura-D18 leu1-32 ade6*	[25]
FC862	*h+ myo52-GFP:ura4+ ura4-D18 leu1-32 ade6-216*	[29]

factors, such as bud6p, profilin, and rho proteins, which have been found to interact with other formins and are also localized at the cell tips in fission yeast [4, 41–46]. Recently, for3p has been shown to interact with the small GTPases rho3p and cdc42p (I. Mabuchi, personal communication). These studies point out that although wild-type *S. pombe* cells do possess asymmetries (such as birth scars) inherent in their cell division habits, proteins such as for3p are required to ameliorate these asymmetries to produce more identical daughters. Thus, just as asymmetric cell divisions require distinct molecular mechanisms, symmetric cell divisions also require specific mechanisms to generate symmetry.

Materials and methods

Yeast strains, media, and genetic methods

S. pombe strains used in this study are listed in Table 2. Standard methods for media and genetic manipulations are described at http://www.bio.uva.nl/pombe/. for3p-GFP and *for3Δ::kanMX6* yeast strains were constructed using a polymerase chain reaction (PCR)-based approach [23]. For for3p-GFP, primers 5′-AAGAGTGCTAAGTCACTACT TGCAGAACTAACTAACGGGTCTAATGCTTCAAATCTAGTTGAAAA TGACCGCCAAAAACAACGGATCCCCGGGTTAATTAA-3′ and 5′-TTC TTTCAGACAAATCGTCAATGTATGTAATGTACAGATATACTGTTC TAAAAATCCATCCTAGAAAGAACAATGGAGCAAGAATTCGAGCT CGTTTAAAC -3′ were used to amplify GFP-kanMX from pFA6a-GFP (S65T)-kanMX6 [23]. For construction of *for3Δ*, primers 5′-CTTCCTTAC CATTTATTCCTTAATCAGCTTCGTTAGTATCTTTTTTACAACCAAA TTACCAGTTTGGTATGTTAATTCATACGGATCCCCGGGTTAAT TAA-3′ and 5′-TTCTTTCAGACAAATCGTCAATGTATGTAATGTACA GATATACTGTTCTAAAAATCCATCCTAGAAAGAACAATGGAGCAA GAATTCGAGCTCGTTTAAAC-3′ were used to amplify kanMX6 from pFA6a-kanMX6 [23]. PCR fragments were transformed into a diploid strain (FC584). Multiple independent transformants were screened by PCR and were sporulated to produce viable haploids. Cell polarity phenotypes were genetically linked with the deletion construct in genetic crosses.

Microscopy

Microscopy was carried out using a wide-field microscope station and a real-time spinning disk confocal microscope station as described [27]. Images were obtained, processed, and analyzed using Openlab 2.1.3 software (Improvision).

Analyses of growth patterns and cytoskeletal elements

Cell growth patterns and lineages were determined as follows. Cells were grown in 3-ml shaking cultures at 30°C to midexponential phase. A total of 0.5 ml cells was centrifuged at 1800 × g for 30 s and was resuspended in 50 μl media. A chamber was constructed by applying 50 μl melted 2% w/v agarose (GIBCO Ultrapure Agarose, Life Technologies) in YE5S to a glass slide (Micro Slides, Corning Glass Works). A second glass slide pretreated with Sigmacote (Sigma) was immediately placed on top to flatten the agarose into a pad. After 2 min, the top glass slide was removed, and 1 μl concentrated cells was placed on the agarose pad and covered with a glass coverslip. Finally, the chamber was sealed with VALAP (1:1:1 vaseline:lanolin:parafin). Cells were imaged at room temperature (25°C–28°C) for 6–10 hr (1–2 generations) by time-lapse DIC microscopy. Images were acquired every 10 min. Frequent refocusing was often necessary. Cell tip growth was measured from birth scars (fixed ridges on the cell surface) that served as fiduciary marks. In Figure 1a–c, a cell tip was scored as a growing tip if it grew out more than 1 μm in the cell cycle. Widening of cell tips was not considered as cell tip growth.

Actin staining and analysis of actin patch dynamics were performed as described [27]. Measurements of microtubule dynamics with GFP-tubulin were as described [32]. pDQ105 was a kind gift from D.Q. Ding and Y. Hiraoka. cdc7-GFP was introduced into a *for3Δ* strain (BFY162) by a genetic cross with strain FC855 (from V.Simanis and D. McCollum) [25]. myo52-GFP was introduced into a *for3Δ* strain (BFY 9) by a genetic cross with strain FC862 (from J. Hyams) [29]. Movements of myo52p-GFP were designated as directional if a myo52p-GFP dot moved in a linear manner over a distance of ≥ 2 μm over 5–10 time points obtained every 0.5 s.

Supplementary material

Supplementary material, which include a figure showing the amino acid sequence similarity of for3p with other formins as well as supplementary movies showing for3p-GFP motility in (Movie 1) wild-type and (Movie 2) LatA-treated cells, is available at http://images.cellpress.com/supmat/supmatin.htm.

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