Cell, Vol. 89, 939–949, June 13, 1997, Copyright ©1997 by Cell Press

tea1 and the Microtubular Cytoskeleton Are Important for Generating Global Spatial Order within the Fission Yeast Cell

Juan Mata and Paul Nurse
Cell Cycle Laboratory
Imperial Cancer Research Fund
44 Lincoln's Inn Fields
London WC2A 3PX
United Kingdom

Summary

Fission yeast cells identify and maintain growing regions exactly opposed at the ends of a cylindrical cell. *tea1* mutants disrupt this organization, producing bent and T-shaped cells. We have cloned *tea1* and shown that tea1 is located at the cell poles. Microtubules are continuously required to transfer tea1 to the cell ends, and tea1 is located at the ends of microtubules growing toward the cell poles. We suggest that tea1 acts as an end marker, directing the growth machinery to the cell poles. tea1 is down-regulated in cells treated with pheromone that grow toward a mating partner and no longer maintain their ends exactly opposed. tea1 may also influence microtubular organization, affecting the maintenance of a single central axis.

Introduction

An important problem for a cell is how it organizes itself in space. This organization requires that different components or points located at a distance within the cell are correctly positioned with respect to each other. The problem is similar in principle to a developmental field establishing positional information during embryogenesis but acts at the level of a single cell. There are many examples of this phenomenon: epithelial cells have opposed apical and basal faces with different properties (Eaton and Simons, 1995); Drosophila oocytes contain determinants localized to discrete regions of the cytoplasm (Lehmann, 1995); and bipolar budding yeast cells bud at the opposite end of the cell to the previous bud (Roemer et al., 1996). In the case of the fission yeast Schizosaccharomyces pombe, two growing tips located at the ends of the cylindrical cell grow apart in a precisely opposed manner (Snell and Nurse, 1993). This is a simple case of spatial organization in which a cell identifies and maintains domains at its extremities that are directly opposite each other. Two points are said to be antipodal when they are located at the extreme opposite ends of an object, and we shall use this term to describe the organization of tip growth in fission yeast, where intracellular domains are directly opposed to each other at the extremities of the cell along a single central axis.

Immediately after cell division, a newly born fission yeast cell starts growth in a unipolar mode, growing only at the old tip that already existed in the mother cell. After a certain period of the cell cycle, the cell switches to a bipolar mode, with both old and new tips undergoing growth. This transition is called new-end take off, or NETO. In both unipolar and bipolar growth modes, the cell elongates while keeping its ends precisely opposed. Upon onset of mitosis, all tip growth ceases and resumes again only after cytokinesis and cell division are complete; this occurs at the two old tips, now present in two different cells (Mitchison and Nurse, 1985). The precise opposition of growing tips is maintained throughout vegetative growth but is not found in cells undergoing conjugation, in which the rigid relationship between the tips needs to be uncoupled to allow a growing tip to seek out a cell of opposite mating type (Fukui et al., 1986; Leupold, 1987).

In order to understand how a fission yeast cell can identify its ends and maintain them growing in an antipodal fashion, we have isolated mutants in which the tips do not grow away from each other in a precisely opposed manner (Snell and Nurse, 1994; Verde et al., 1995). When tips are offset by a small angle, bent cells are generated, but when the angle subtended is increased, a new third tip growing out of the side of the cell body is made, producing T-shaped cells. Mutants behaving in this manner define two genes, *tea1* and *tea2* (for tip elongation aberrant), which we have hypothesized may define gene products acting as markers of the extreme opposite ends of the fission yeast cell (Verde et al., 1995). In this paper, we describe the cloning of *tea1* and characterize its encoded protein, tea1. The protein is found to be tightly located at the ends of the cell, consistent with a role as an end marker, and the microtubular cytoskeleton is shown to be required to maintain the location of tea1 at the cell tips. From these results, we propose that tea1 is constantly transferred to the extremities of the cell by the microtubules, producing a dynamic mechanism whereby the space of the cell is explored, and the antipodal positions of the growing tips are identified and maintained, thus contributing to global spatial order within the cell.

Results

Cloning and Deleting the *tea1* Gene

Mutants in the *tea1* gene are viable, and their bent and T-shaped phenotype can only be identified by microscopic inspection of the cells within individual colonies. It proved impossible to clone *tea1* by complementation because of plasmid loss, so we used positional cloning as an alternative method. *tea1* was mapped to within 1.3 cM of the *nmt1* gene and was located within an ordered cosmid library of the S. pombe genome (Hoheisel et al., 1993). Cosmids were selected that spanned the region around the *nmt1* gene and were individually transformed into a *tea1–3* mutant strain. One cosmid completely rescued the mutation, and after subcloning a 4.3 kb fragment from this cosmid was found to contain the rescuing activity.

Sequencing of the 4.3 kb fragment identified a 3441 bp ORF of 1147 amino acids and a molecular mass of 127 kDa (Figure 1A). Analysis of the sequence revealed six internal repeats at the N-terminal half of the protein (amino acids 82–394) belonging to the kelch family (Xue

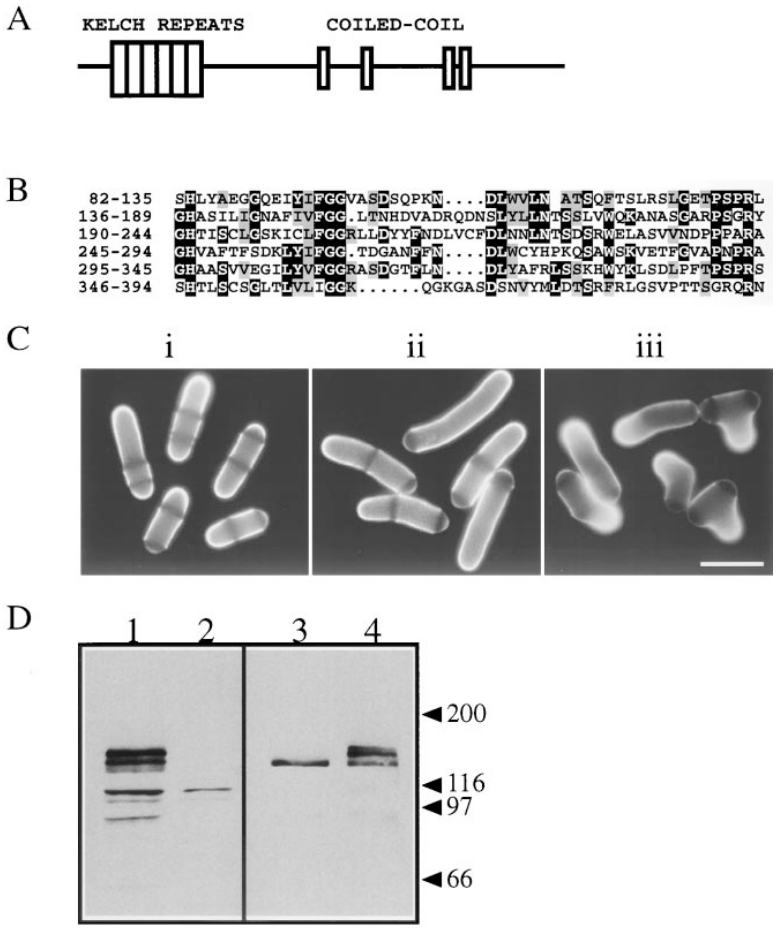

Figure 1. Molecular Analysis and Deletion of the *tea1* Gene

(A) General structure of the predicted tea1 protein. The position of the six kelch internal repeats is shown by the boxes on the left. Regions predicted to form coiled–coils ($p > 0.85$) are shown by the boxes on the right.

(B) Alignment of the kelch repeats. Identical residues on at least three repeats are shown in closed boxes, and conserved residues in shaded boxes.

(C) Morphological phenotype of a *tea1*Δ strain. All cells are stained with the cell wall–binding compound calcofluor. (i) Wild-type cells grown at 25°C; (ii) *tea1*Δ cells grown at 25°C; (iii) *tea1*Δ cells grown at 25°C and transferred to 36°C for 4 hr.

(D) Characterization of antibodies against tea1. Affinity-purified antibodies raised against the C-terminal half of tea1 were used to probe Western blotted extracts from wild-type cells (lane 1), *tea1*Δ cells (lane 2), *tea1*Δ cells transformed with plasmid pR2 containing the *tea1* gene lacking the first ATG (lane 3), or *tea1*Δ cells transformed with plasmid pR1 containing the whole *tea1* gene (lane 4).

Scale bar = 5 μm.

and Cooley, 1993; Bork and Doolittle, 1994). An alignment of the repeats is shown in Figure 1B. Comparison of the repeats with proteins in the PIR and Swissprot databases revealed that the most similar repeats were in two hypothetical proteins from Saccharomyces cerevisiae (PIR accession numbers S46769 and S57704), in host-cell factor (Wilson et al., 1993), and in actin-fragmin kinase (Eichinger et al., 1996). Kelch repeats are thought to be structural repeats (Bork and Doolittle, 1994) and are present in several proteins known or suspected to bind actin (Xue and Cooley, 1993; Varkey et al., 1995; Way et al., 1995; Eichinger et al., 1996). Several regions of the C-terminus showed a high probability of forming coiled–coil interactions (Figure 1A), as predicted by the Lupas algorithm (Lupas et al., 1991). The two S. cerevisiae ORFs that showed homology to tea1 in the N-terminus also had a similar coiled–coil structure.

We constructed a deletion of the *tea1* gene in a diploid strain by replacing most of the ORF with the *ura4*$^+$ gene. Sporulation of the resulting diploids produced four viable spores, which grew with normal generation times at 18°C, 25°C, 32°C, and 36°C. Examination of the cells under the microscope revealed that cells deleted for the *tea1* gene had a defect in cell shape similar to the original *tea1* mutants (Snell and Nurse, 1994). At 25°C, about 30% of the cells can be seen to be bent (Figure 1C, [ii]; compare with wild type in [i]), and at 36°C the phenotype becomes stronger, with 35% of the cells obviously bending and 20% forming T shapes (Figure 1C, [iii]). Because scoring bent cells is difficult in short, normally growing cells, we produced elongated cells by using a temperature-sensitive *cdc25* mutant. This mutation causes slight elongation at 25°C and blocks the cell in G2 at 36°C, making it very elongated. This allows the bent phenotype to be more easily scored. With these conditions, the phenotype exhibited a high penetrance, with a total of 70% of cells bent at 25°C when cells are only slightly elongated and 95% bent or T shaped after 3 hr at 36°C. We conclude that the *tea1* gene is necessary to maintain the growing tips precisely opposed to each other and so plays a role in maintaining antipodal growth in the fission yeast cell.

Localization of tea1

To investigate the intracellular localization of tea1, we raised polyclonal antibodies against bacterially produced fragments of the tea1 protein fused to a 6His tag. After affinity purification, the antibodies against the C-terminal half of the protein recognized two bands of 130 and 135 kDa in extracts from wild-type cells (Figure 1D, lane 1), which were absent in *tea1*Δ extracts (Figure 1D, lane 2). Lower molecular weight bands probably represented degradation products. Affinity-purified antibodies produced against the N-terminal half of the protein did not detect the tea1 protein in wild-type cells, but bands of 130 and 135 kDa were obtained with this

antibody in extracts derived from cells overexpressing *tea1* (data not shown). The *tea1* ORF has a second ATG at position 135, and a *tea1* genomic clone lacking the first ATG (pR2) was able to rescue a *tea1*Δ, showing that the first 45 amino acids were not essential for the protein function. When the tea1 protein was examined in a *tea1*Δ strain containing this plasmid, a single band was detected of the same size as the lower band (130 kDa) in wild-type cells (Figure 1D, lane 3). In contrast, extracts from *tea1*Δ cells with a plasmid containing the full ORF (pR1) had both bands (Figure 1D, lane 4). The presence of the two bands could be explained either by alternative use of the first or second ATG of the ORF, given that the predicted size difference between the two products is 4.6 kDa (similar to that observed), or alternatively, by the N-terminus being processed in some manner.

We used the antibodies to determine the intracellular localization of the tea1 protein by indirect immunofluorescence. The majority of tea1 was localized to a small region at both poles of wild-type cells with some dots elsewhere in apparently random positions within the cell (Figure 2A). The specificity of the staining was confirmed by the fact that antibodies raised against nonoverlapping regions of the protein produced similar results and by the absence of any signal when *tea1*Δ cells were stained (Figure 2A).

During the early part of the cell cycle, the cell grows in a unipolar manner, while during mitosis neither tip undergoes growth (Mitchison and Nurse, 1985). In order to establish whether tea1 marks growing tips or just tips, we monitored tea1 localization in cells at different stages of the cell cycle. Double staining of tea1 and tubulin was used, allowing cells at certain stages of the cell cycle to be identified (Hagan and Hyams, 1988); during mitosis there is a mitotic spindle, during cytokinesis microtubules form a postanaphase structure, and during interphase cells have cytoplasmic microtubules extending along the cell. It is not known whether the cytoplasmic microtubules observed by immunofluorescence are single microtubules or microtubule bundles. In interphase cells, tea1 staining was found at both ends of the cells (Figure 2B, [i]). In mitotic cells with an intranuclear spindle (Figure 2B, [ii]), tea1 remained at the tips, but the intensity of the staining was lower than in interphase cells. In cells just completing division, tea1 could still be seen at both of the old ends, but was also just visible at the newly formed ends in the region of the septum (Figure 2B, [iii]). In recently separated cells, tea1 was present at both tips, but the staining was asymmetric (Figure 2B, [iv]), being weaker at the new end that was recognized by its flat shape. This experiment shows that tea1 is present at both poles of the cells throughout the cell cycle whether they are growing or not.

To confirm that both tips contain tea1 whether they are growing or not, we blocked cells both early and late in the cell cycle, when growth was either monopolar or bipolar (Mitchison and Nurse, 1985). We used a *cdc10*^{ts} mutant that blocks at late G1, before activation of bipolar growth, and a *cdc25*^{ts} mutant, which blocks entry into mitosis after activation of bipolar growth. After 4 hr at the restrictive temperature of 36°C, tea1 was present at

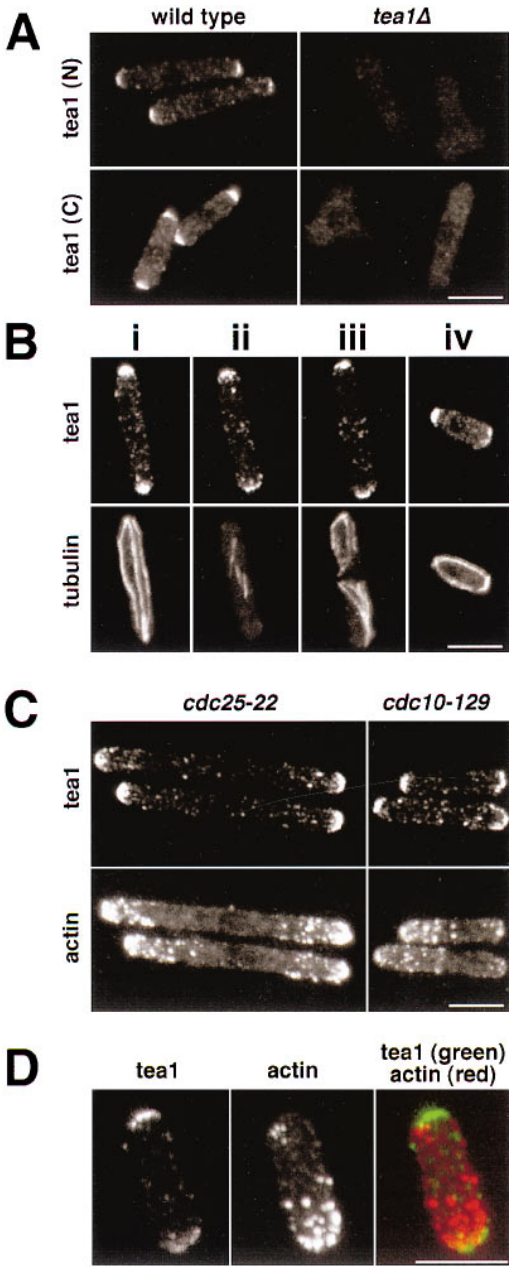

Figure 2. Immunolocalization of tea1

(A) Specificity of the staining. Wild type or *tea1*Δ cells were fixed and stained with antibodies raised against the N-terminal half of tea1 (tea1 [N]) or the C-terminal half (tea1 [C]).

(B) tea1 localization in the cell cycle. Wild-type cells grown at 25°C were fixed and double stained with antibodies against tea1 (top) or tubulin (lower panels). (i) Interphase; (ii) Mitosis; (iii) Septation; (iv) Early interphase.

(C) tea1 localization in cell-cycle mutants. *cdc25–22* cells or *cdc10–129* cells were grown at 25°C, transferred to 36°C for 4 hr, fixed, and double stained with antibodies against tea1 (top) or actin (lower panels).

(D) tea1 localization in *orb2* mutants. *orb2–34* mutants were grown at 25°C, fixed, and double stained with antibodies against tea1 or actin. The overlaid stainings are shown on the right, with tea1 in green and actin in red.

Scale bars = 5 μm.

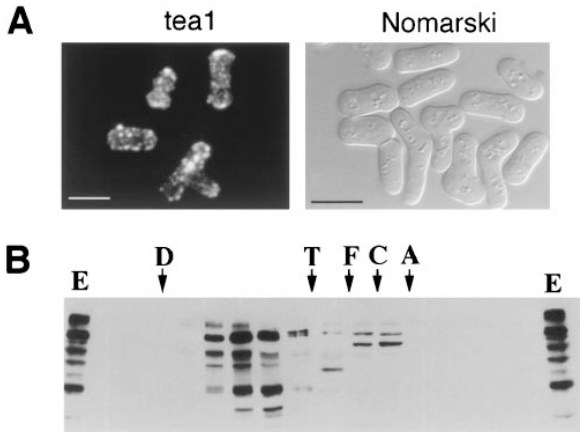

Figure 3. tea1 Is Part of a High Molecular Weight Complex and Effects of tea1 Overexpression

(A) Overexpression of *tea1* causes cell shape defects. Cells carrying an integrated copy of *tea1* driven by the *nmt1* promoter were grown in minimal medium containing thiamine. Thiamine was removed from the medium to induce the promoter. After 16 hr at 36°C, the cells were fixed and stained with antibodies against tea1 (left panel) or viewed using Nomarski optics (right panel). Scale bars = 5 μm.

(B) tea1 is present in a high molecular mass complex. Fission yeast protein extracts were prepared and fractionated by gel filtration as described in Experimental Procedures. The fractions were Western blotted and probed with antibodies against tea1. tea1 is very degraded after the fractionation procedure. The positions of the total extracts (E) and the molecular mass markers are shown: Dextran blue (D, 2000 kDa), thyroglobulin (T, 700 kDa), ferritin (F, 440 kDa), catalase (C, 230 kDa), and albumin (A, 67 kDa).

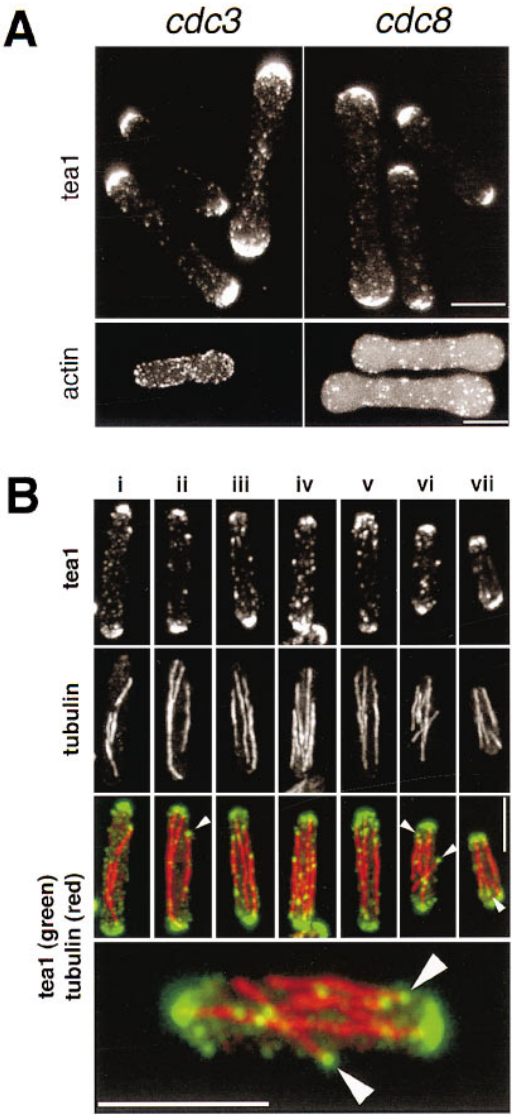

Figure 4. Localization of tea1 and the Cytoskeleton

(A) Localization of tea1 in mutants in actin-binding proteins. *cdc3–313* (left) and *cdc8–382* (right) mutants were incubated at 36°C for 4 hr, fixed, and stained with anti-tea1 antibodies (top panels) or with rhodamine-phalloidin to show the actin cytoskeleton (lower panels).

(B) tea1 colocalizes with the ends of cytoplasmic microtubules. Wild-type cells were double stained with antibodies against tea1 (top) and tubulin (middle). An overlay of both stains is shown in the lower panels, with tea1 in green and tubulin in red. The arrowheads show accumulation of tea1 at only one end of microtubules. The cell from (vi) is shown at higher magnification.

Scale bars = 5 μm.

both poles in both the *cdc25*^{ts} and *cdc10*^{ts} cells (Figure 2C). In these experiments, the cells were also double stained for actin, which is located in growing tips of fission yeast (Marks et al., 1986). As expected, actin was located at both tips in the *cdc25*^{ts} mutant and mostly at a single tip in the *cdc10*^{ts} mutant (Figure 2C), and it was also clear that tea1 was more tightly concentrated at the tips than actin. This was confirmed with an *orb2*^{ts} mutant that grows only at one end (Verde et al., 1995). In this mutant, tea1 was located tightly at both ends of the cell, while actin was located in a more diffuse manner at the single growing end (Figure 2D). Although the distribution of tea1 sometimes appears asymmetric in *orb2* mutants (Figure 2D), there is no correlation between the presence of actin and high or low levels of tea1 at the same pole.

We conclude that tea1 acts as an end marker, regardless of whether the ends are actively growing or not. This location is consistent with a role for tea1 in identifying the poles of the cell and maintaining a single central axis. If tea1 acted as a focus for orientating the growing tip machinery, then by marking the cell ends tea1 would restrict growth to the regions necessary to establish and maintain antipodal growth. To investigate this further, *tea1* was expressed under the control of the *nmt1* thiamine-repressible promotor (Maundrell, 1990). After removing thiamine, tea1 levels increased about 50-fold (data not shown), and tea1 was located at random positions throughout the cell (Figure 3A, left panel). This caused the formation of bent and T-shaped cells (Figure 3A, right panel). This defect could be due to the mislocalization of tea1. Alternatively, high levels of tea1 could titrate away other essential proteins. It is likely that tea1 does interact with other proteins, given that it migrates as part of a high molecular mass complex of around 1000 kDa after gel filtration. (Figure 3B).

Role of the Cytoskeleton in Locating tea1

We next investigated whether the actin or tubulin components of the cytoskeleton were involved in locating tea1 at cell poles. To test if actin has a such role, we

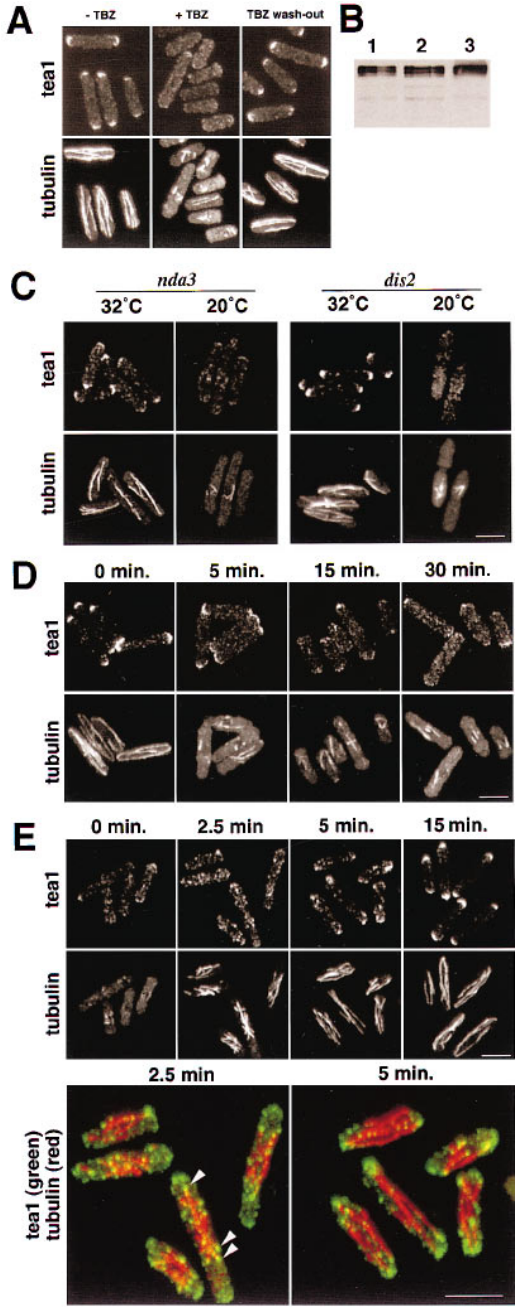

Figure 5. Role of the Microtubular Cytoskeleton in tea1 Localization

(A) Effect of TBZ on the localization of tea1. Wild-type cells were grown at 25°C, incubated with TBZ at a concentration of 100 μg/ml for 30 min, washed with medium free of TBZ, and incubated at 25°C for a further 10 min. Samples were taken before addition of TBZ (−TBZ), after 30 min in TBZ (+TBZ), and 10 min after the wash (TBZ wash-out). Cells were fixed and immunostained with antibodies against tea1 (top) and tubulin (lower panels).

(B) tea1 levels are not affected by TBZ. TBZ was added to cells growing at 25°C to a concentration of 100 μg/ml. Cells were collected 0, 15, and 30 min after addition of the drug, processed for Western blotting, and probed with anti-tea1 antibodies.

(C) Localization of tea1 in *nda3* (β-tubulin) and *dis2* mutants. *nda3-KM311* cells (left panels) were grown at 32°C and transferred to 20°C for 1 hr, fixed, and double stained with antibodies against tea1 (top) or tubulin (lower panels). *dis2–1* mutants (right panels) were grown at 32°C, transferred to 20°C for 6 hr, and processed as described for *nda3*.

(D) Kinetics of tea1 delocalization after TBZ treatment. TBZ was added to cells growing at 25°C, and cells were fixed after 0, 5, 15, and 30 min and immunostained with anti-tea1 (top) and anti-tubulin antibodies (lower panels).

(E) Kinetics of relocalization of tea1 after cold shock. Wild-type cells were incubated on ice for 30 min and transferred to a 25°C water bath. Samples were taken after 0, 2.5, 5, and 15 min at 25°C. Cells were stained with antibodies against tea1 (top) or tubulin (middle). An overlay of the stainings at times 2.5 and 5 min is shown in the lower panels, with tea1 in green and tubulin in red. The arrowheads show clear examples of colocalization of tea1 and microtubules. Scale bars = 5 μm.

examined temperature-sensitive mutants in *cdc3* (encoding profilin) and *cdc8* (encoding tropomyosin), which disrupt the actin cytoskeleton and fail to organize actin into the tips at 36°C (Balasubramanian et al., 1992, 1994; Chang et al., 1996). In both mutant strains, tea1 was still correctly located to the ends (Figure 4A, top panels), even though cortical actin patches were delocalized (Figure 4A, lower panels). These observations indicate that a normally organized actin cytoskeleton is not required to locate tea1 at the poles.

Although most of tea1 is found at the cell tips, some tea1 can be seen as dots in random locations throughout the cell. After double staining for tea1 and tubulin, 60% of these dots were found clearly to colocalize with the ends of cytoplasmic microtubules (Figure 4B). In most cases where both ends of a single microtubule could be distinguished, tea1 dots were observed at only one end of the microtubule. The arrowheads mark clear examples (Figure 4B, [ii], [vi], and [vii]).

To examine further the role of microtubules in the intracellular localization of tea1, we depolymerized microtubules using the tubulin-binding drug thiabendazole (TBZ) (Umesono et al., 1983). Before treatment with the drug, tea1 and tubulin showed normal patterns of distribution. Addition of TBZ caused microtubules to become very short, and at the same time tea1 disappeared from the ends of the cell, becoming located randomly throughout the cell (Figure 5A). When TBZ was washed out, microtubules and tea1 returned to their usual location (Figure 5A). Addition of TBZ did not affect tea1 levels (Figure 5B), indicating that TBZ did not change protein stability. To confirm that delocalization of tea1 was caused by disorganization of the microtubules and not by some other nonspecific effect of TBZ, we disrupted microtubules using a cold-sensitive mutant in the *nda3* gene (Umesono et al., 1983; Hiraoka et al., 1984), which encodes β-tubulin (Figure 5C). At the permissive temperature of 32°C, cytoplasmic microtubules in the *nda3-KM311* mutant were similar to those of wild-type cells, although slightly shorter. At this temperature, tea1 was found to be present at both poles of the cells. After 1 hr at the restrictive temperature of 20°C, cytoplasmic microtubules had almost disappeared, and tea1 became completely delocalized. A similar temperature shift in a control wild-type strain did not produce any effect on either microtubules or tea1 distribution (data not shown).

tea1 is reduced in level at cell ends during mitosis, which could be explained by the absence of cytoplasmic microtubules. This explanation predicts that tea1 should

become more delocalized in cells blocked in mitosis without cytoplasmic microtubules. To test this possibility, we used cold-sensitive mutants in *dis2* (Ohkura et al., 1989). At 32°C, the mutants had normal tea1 and microtubule distributions. After 6 hr at 20°C, cells arrested in mitosis and tea1 was almost completely delocalized (Figure 5C).

We next followed the kinetics of delocalization of tea1, after treatment of the cells with TBZ, and of relocalization of tea1, when microtubules were allowed to regrow after previous depolymerization. Cells were treated with TBZ, and the localization of tea1 and microtubules monitored by indirect immunofluorescence (Figure 5D). Microtubules were already disorganized within 5 min of drug addition; at this point, tea1 was still largely localized at the poles, although the end staining was reduced and had become more patchy. After 15 min, microtubules were observed to be very short, and tea1 was almost completely delocalized. To follow the relocalization of tea1, we depolymerized microtubules using a cold-shock treatment (Figure 5E). This treatment causes disorganization of microtubules, which is rapidly reversed by transferring the cells into warm medium (J. Hyams, V. Snell, J. Cope, and P. N., unpublished data). Incubation of cells on ice for 30 min depolymerized the microtubules and led to delocalization of tea1. After 2.5 min in warm medium, short microtubules became visible in all cells, and microtubules were longer after 5 min and looked completely normal after 15 min. tea1 was observed as bright dots dispersed throughout the cell after 2.5 min, some staining was detected at cell tips after 5 min, and tip staining was predominant after 15 min. Examination of the overlaid tea1 and tubulin distributions revealed that 80% of the dispersed tea1 spots colocalized with the ends of the regrowing microtubules (Figure 5E, lower panels; the arrowheads show clear examples).

We conclude that localization of tea1 at the ends of the cells is dynamic and can only be maintained for a short time (5–15 min) in the absence of intact microtubules. In addition, tea1 is found on the tips of the growing microtubules recovering after disruption and becomes located at the ends of the cell when microtubules extend along the whole cell. These results indicate that microtubules transfer tea1 to the ends of the cell and thus identify microtubules as playing an important role in establishing and maintaining antipodal growth.

Loss of Antipodal Behavior during Conjugation

During conjugation, tip growth is not maintained in an antipodal manner because it is necessary for a tip to grow more flexibly so it can grow toward a cell of opposite mating type (Fukui et al., 1986; Leupold, 1987). Therefore, we examined tea1 behavior in cells exposed to the pheromone P factor. We used the P factor–responsive strain s*xa2*Δ*cyr1*Δ*h*$^-$ (Maeda et al., 1990; Imai and Yamamoto, 1992), which is slightly shorter than wild-type cells. Addition of pheromone caused the production of thin and bent mating projections (Figure 6A). The tea1 levels were found to be reduced 3- to 4-fold by 5 hr after addition of pheromone, as determined by both Western blot (Figure 6B) and immunofluorescence

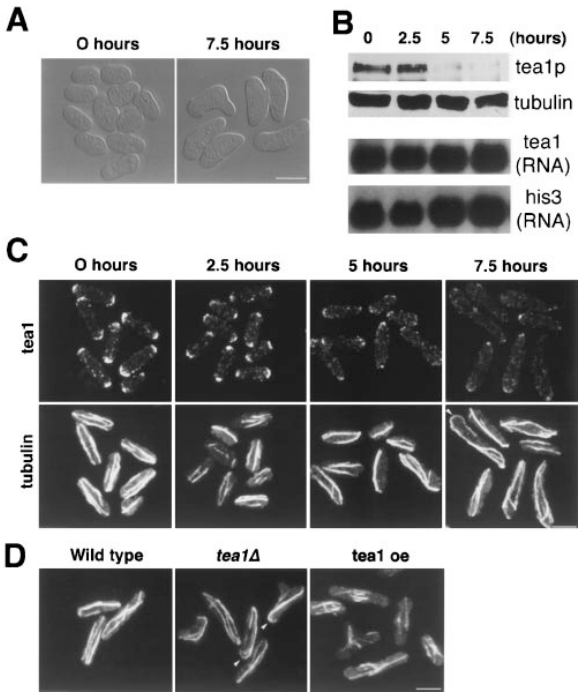

Figure 6. Effects of Pheromone on tea1 and Effects of tea1 on Microtubules

(A–C) Effect of pheromone on tea1. P factor was added to *sxa2*Δ*::ura4*$^+$ *cyr1*Δ*::LEU2 h*$^-$ cells to a final concentration of 1.6 μg/ml, and the cells were incubated at 25°C. Samples were taken after 0, 2.5, 5, and 7.5 hr in pheromone. (A) Effect of pheromone on fission yeast morphology as seen by Nomarski. (B) Extracts were processed for Western blotting and probed with antibodies against tea1 and tubulin, or for Northern blotting, and hybridized with *tea1* or *his3* probes. The band corresponding to *tea1* mRNA is not detected in a *tea1*Δ (data not shown). (C) Cells were immunostained with antibodies against tea1 (top) and tubulin (lower panels).

(D) Effects of tea1 on microtubules. Wild type (left) or *tea1*Δ cells (middle) were grown at 25°C, shifted to 36°C for 4 hr, fixed, and stained with an antibody against tubulin. Cells overexpressing *tea1* at 36°C for 16 hr were processed similarly (right). The arrowheads in (C) and (D) show abnormal microtubules.

Scale bars = 5 μm.

(Figure 6C). In contrast, *tea1* messenger RNA levels were not reduced (Figure 6B), showing that the regulation is posttranscriptional. Thus, in a physiological situation in which the controls that maintain antipodal growth need to be overridden, tea1 is down-regulated.

Effects of tea1 on Microtubules

During the course of these experiments, we noted some effects of tea1 on microtubules (Figure 6D). In wild-type cells, cytoplasmic microtubules run along the main axis of the cell to the poles of the cell, but do not normally extend any further. In *tea1*Δ cells, we found that in about 10%–15% of the cells microtubules curled round the ends of the cell (Figure 6D, arrowheads), a situation that was very rare in wild type-cells (<0.5%). Furthermore, in pheromone-treated cells that have reduced levels of tea1, microtubules were also found to curl round the ends of the cells (arrowhead in Figure 6C). Overexpression of wild-type tea1 also caused defects in cytoplasmic microtubules, which were found to be much

shorter than normal. These results suggest that, as well as being transferred by microtubules, tea1 may also affect the organization of the microtubular cytoskeleton. tea1 may have some inhibitory effect on microtubular growth, such that absence of tea1 allows microtubules to grow around the ends of the cells, while high levels of tea1 cause microtubules to be shorter than normal. Thus, tea1 could inhibit microtubular growth in the tips of the cell and thus contribute to the organization of microtubules necessary to maintain a single central axis.

Discussion

In this paper, we have shown that tea1 plays an important role in the spatial organization of a fission yeast cell. Our major observations and conclusions are as follows: (i) the *tea1* gene is required to maintain the growing tips in an antipodal manner, keeping the two tips growing in a precisely opposed fashion; (ii) tea1 acts as a tightly localized end marker, regardless of whether the tips are growing; (iii) the microtubular cytoskeleton is required for locating and maintaining tea1 at the cell tips; (iv) tea1 level is down-regulated by pheromone; and (v) tea1 affects the organization of the microtubular cytoskeleton in a way that may contribute to the maintenance of antipodal growth behavior.

When tea1 is deleted, cells fail to grow with their tips precisely opposed. The tip centers are not located at the ends of a single axis running through the middle of the cell, as is the case in a wild-type cell, but instead the centers become offset at an angle from each other. If the angle is small, a bent cell is produced, but if the angle becomes greater, an extra tip is generated that grows out of the side of the cell body, producing a T-shaped cell. Given that tea1 is located at the ends, we propose that tea1 acts as an end marker, defining where the cell-growth zones should be organized and thus the direction of growth. It is important to understand that tea1 is not required for a growth zone to become organized; it is only required to place the growth zone in the correct place. The components making up the growth machinery can form a growth zone in the absence of tea1, but tea1 is necessary to locate the growth zone in the correct place at the cell tips, presumably by interacting with components making up the growth machinery.

The *tea1* gene is required to maintain antipodal growth throughout the cell cycle and not only when polarity is reestablished after mitosis. This is shown by the behavior of *cdc2*^{ts}-*tea1*^{ts} double-mutant cells that form some branches and become highly bent even though they fail to enter mitosis. However, if repeated cycles of mitosis are allowed in the absence of cytokinesis using a septation mutant, more branched cells are formed, suggesting that tea1 is particularly important during polarity reestablishment after mitosis (Verde et al., 1995; see Figure 4).

Microtubules are required for tea1 to be located at the cellular ends. Within 15 min of disrupting microtubules, tea1 becomes delocalized throughout the cell. Several experiments support the conclusion that proper tea1 localization is a consequence of the microtubular cytoskeleton being used for tea1 transference to cell ends. First, when microtubules grow back after having been disrupted, tea1 is initially observed at the ends of the regrowing microtubules. As the microtubules lengthen, tea1 is moved toward the tips of the cell and becomes deposited there after the microtubules have attained full length. Therefore, the ends of the cell are identified by a dynamic process requiring tea1 to be transferred to the cell ends by the microtubular cytoskeleton. Second, mutants in genes such as *nda3* (β-tubulin) (Umesono et al., 1983; Hiraoka et al., 1984) and *atb2*/*ban5* (α_2-tubulin) (Umesono et al., 1983; Toda et al., 1984; Verde et al., 1995; Yaffe et al., 1996) or treatment with drugs such as TBZ (Umesono et al., 1983), all of which disrupt microtubules, generate bent or T-shaped cells. In very elongated *cdc* cells, antipodal behavior is lost and cells bend and occasionally form branches (J. M. and P. N., unpublished data). In the conditions used in the experiments with the *cdc* mutants in this paper, microtubules extended along the length of the cells, and tea1 was localized at the poles. However, it is possible that bending and branching in very elongated cells is caused by microtubules failing to reach the cell ends, leading to incorrect deposition of tea1. We suggest that in normal cells, the microtubules explore the space of the cell and usually become distributed along the long axis of the cell, finishing in the region of the cell poles. As a consequence, tea1 is placed at the cellular tips, leading to a proper localization of the growth machinery and then to antipodal growth. With this view, it is tea1 localized at the cell ends that carries out tea1 function, by acting as a marker for the growth machinery. However, we cannot exclude the possibility that tea1 carries out its function by influencing microtubule distribution, which in turns determines the direction of growth. In this view, it is the tea1 bound to microtubule ends that is active, while the accumulation of tea1 at the poles of the cells is a by-product of the distribution of microtubules.

Because tea1 is usually only detected at one end of a microtubule, the transference to the cell poles is likely to be polarized. The polarity of microtubules in a fission yeast cell has not yet been determined, but because after disruption they grow back in all directions from the middle of a cell (J. Hyams, V. Snell, J. Cope, and P. N., unpublished data), it is probable that microtubules of opposite polarity are distributed along the length of the cell. This means that in the tip regions, there will be both (+) and (−) ends, with tea1 being delivered by only those microtubules of the appropriate polarity. tea1 could be transferred to cell ends by microtubules in two different ways. First, tea1 could bind to the microtubules at any point along their length and then be transported toward the end, presumably by a motor protein. If this is correct, then the terminal location of tea1 in regrowing microtubules implies that the rate of transport must exceed the rate of microtubular growth. Second, tea1 may become localized at the ends of growing microtubules and be moved toward the cell tips simply by microtubular elongation. Since tea1 accumulates at the ends of growing microtubules and at the tips of the cell when the microtubules reach the cell end, there must be some mechanism that ensures that tea1 is transferred from the microtubule end to the cell tip. This could be due to different affinities of tea1 for the motor or microtubule ends and

some component located in the region of the cell surface, such as the plasma membrane or the cell cortex, or due to a changed affinity of tea1 for the motor or the microtubule end when the microtubule stops growing. It is also possible that tea1 is associated with vesicles during microtubule transport, which might facilitate transference to the plasma membrane by membrane fusion.

For tea1 to be correctly located at the cellular ends, the microtubules must run along the long axis of the cell, finishing at the poles and depositing tea1 at the tips of the cell. We shall discuss three models that could bring this about. In the first model, some as-yet-unidentified marker is already located at the cellular ends. This marker stabilizes the microtubules such that they run along the long axis of the cell, ensuring that tea1 is delivered to the ends. Therefore, the localization of this marker gives rise to a distribution of microtubules, such that tea1 is delivered to the tips, and marks the position of the end to the growth machinery. Growth machinery present in the middle of the dividing cell at the time of septation could be responsible for locating this unknown marker. This creates a cycle of contiguous markers in which the localization of a component provides the spatial information to position the next one. When *tea1* is deleted, the cell loses the connection between this marker and the growth machinery, leading to growth at incorrect places and to the formation of bent and T-shaped cells.

In the second model, microtubules form along the long axis of the cell, finishing at the poles because this is the energetically most favorable mode of microtubular growth. If microtubules are sufficiently stiff that they do not bend easily within the cell, then they will extend throughout the cellular space until they run along the long axis and extend between the two poles of the cell. As a consequence, tea1 will be delivered to exactly the correct position in the cell to organize antipodal growth. In this view, the growth properties of microtubules result in them searching the space inside the cell, continuously finding the ends of the cell that are then marked by deposition of tea1. This model does not rely on a cycle of contiguous markers to identify the position of the ends, but on the ability of microtubules to explore and recognize the geometry of the cell (Kirschner and Mitchison, 1986). The third model is an elaboration of the second, incorporating the effects tea1 has on microtubular growth. As before, the microtubules search the space of the cell, and their properties ensure that the ends of growing microtubules come into the vicinity of the cellular tips. When tea1 is overexpressed, the high levels of tea1 lead to shortened microtubules, while deletion of *tea1* or pheromone addition allows microtubules in some cells to curve around the ends of some cells, growing more than usual. Therefore, an accumulation of tea1 in a specific region of the cell could reduce or stop microtubular growth, forcing the microtubule ends to remain within that region. This provides a positive feedback whereby microtubule ends that have identified the cellular poles by their growth properties become stabilized in this region by the effect of tea1. As a consequence, more tea1 is delivered, leading to greater stability. The interest of the second and third models is that they provide some explanation of how spatial order acting over a distance can be generated within a cell.

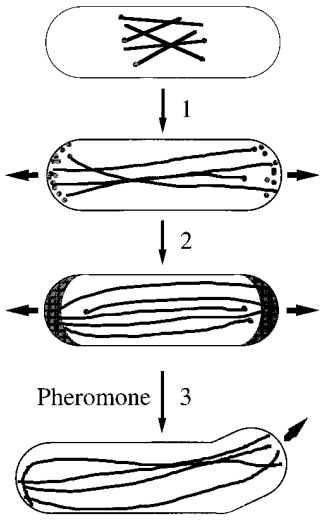

Figure 7. A Model for tea1 Role in Maintaining Antipodal Growth

Microtubules are represented by dark lines inside of the cell; tea1 is represented by stippled dots and stippled regions at cell ends. (1) Microtubules explore the cell and find the cell poles as a consequence of their growth properties. (2) tea1 is transferred by the microtubules to the ends of the cell, where tea1 acts as an end marker that directs the growth machinery to the correct place, ensuring that ends are kept exactly opposed to each other. (3) When cells are treated with mating pheromone, tea1 is down-regulated, allowing growth to be directed toward a mating partner.

Immediately after mitosis and cell division, fission yeast cells initiate polarized growth from the old tip, the end of the cell inherited from the mother cell. They then elongate unipolarly until NETO, when they activate growth at the new end, leading to bipolar growth (Mitchison and Nurse, 1985). At mitosis, the cytoplasmic microtubular cytoskeleton is disrupted and reestablished about the time of cell division (Hagan and Hyams, 1988). During mitosis, tea1 staining at the old cellular tips is somewhat reduced, presumably owing to the transient disappearance of cytoplasmic microtubules, but rapidly increases again after cell division. In the newly born cell, the old tip is marked with tea1 more heavily than the newly formed tip. This may provide some explanation for why growth in the next cell cycle is usually activated at the old end (Mitchison and Nurse, 1985). A prediction of this model is that cells released from a mitotic block during which tea1 has been delocalized would randomly select between the old and the new tip as the site for the initiation of growth after mitosis.

The models described above generate spatial order without recourse to external cues. However, when a growing tip is seeking out another cell for mating, the fixed relationship between the two cellular tips must be relaxed, allowing growth toward a mating partner (Fukui et al., 1986; Leupold, 1987). In these circumstances, tea1 levels are depressed, uncoupling growth-zone positioning from the tea1 control system. The positioning of the growth zone will respond to the external cues provided by pheromone gradients outside the cell and is no longer subject to the internal organizing mechanism.

Thus, the control allows a shift between order generated from within the cell to order imposed from without. A model of how tea1 could perform its function is presented in Figure 7.

The localization of tea1 to the ends of the cell by microtubules in the manner described in models 2 and 3 above would generate positional information within the cell. In other organisms, localized deposition of proteins or RNAs inside the cell by the cytoskeleton is also used as a mechanism to produce positional information. This localization then gives rise to localized growth or to differences in cell fate after division. A well-studied system is the axial budding pattern observed in haploid budding yeast cells, where new buds appear adjacent to the position of old buds. It is thought that the old bud position is first marked by a ring of septins, which is then used to position a cortical tag requiring the *BUD3*, *BUD4*, and *BUD10/AXL2* genes that in turn determines the position of a new bud and septin ring (for review, see Roemer et al., 1996). This cycle of contiguous markers, locally assembling the cortical tag that locates the next cycle of septins, is formally similar to the first model described above. Addition of pheromone changes the axial growth pattern, such that the growth of mating projections occurs toward a mating partner and causes the down-regulation of *BUD4* and *AXL2* (reviewed in Roemer et al., 1996). Thus, the shift in the control of growth from internal cues to external cues could work in a similar way in both yeasts, by down-regulation of genes required for the internal cues. However, this control system differs from the one we have described because it is based on the history of the cell, in the sense that an earlier event localized in space, such as the septin ring–cortical tag, makes a mark at that location that is used by a later event. In fission yeast, the tea1–microtubular system is dynamic, with the cellular space being reexplored and reestablished regularly after each cell division. This allows any earlier mistakes to be corrected and also provides an explanation for how location within a cell can be determined across space at a position distant from the original location, which is not easily possible with a historical model.

The tea1–microtubular system in fission yeast we have described here can generate a cellular field whereby global spatial order is imparted to a cell. The microtubules explore the space of the cell, identifying the cell ends, and the delivery of tea1 marks the ends for locating the growth zones and may also stabilize the microtubular pattern to generate a more robust positioning system. Further analysis of this system will be useful to understand the way in which the cytoskeleton and interacting proteins can lead to global cellular organization in fission yeast. This should also be of relevance to understanding related problems in other eukaryotes.

Experimental Procedures

S. pombe Methods

Standard methods were used for growth, transformation, and genetic manipulation of S. pombe. All experiments were carried out in EMM2 minimal medium. Plasmids used were pIRT2 and pREP3X (Moreno et al., 1991). Pheromone experiments were carried out as described (Stern and Nurse, 1997).

Mapping of the *tea1* Gene

Genetic mapping was carried out in three steps. First, *tea1* was shown to map to chromosome III by using mitotic haploidization as described (Alfa et al., 1993). Second, the position of *tea1* on the chromosome was determined by long-range mapping, measuring genetic linkage in a *swi5* mutant background (Schmidt, 1993) (*swi5* strains were a gift of H. Schmidt). A *tea1–3 swi5–39 ura4–294 tps14–5* h^+ strain was constructed and crossed to an *ade5–36 swi5–39* h^- strain. Tetrad analysis of this cross showed that *tea1* is localized to the right arm of chromosome III, between the *tps14* and the *ade5* genes. Third, the *tea1* gene was mapped by linkage analysis in a *swi5*$^+$ background. A *tea1–3 ura4-D18* strain was crossed to mutants in genes that map to this chromosome arm (*tps28–9*, *arg1–230*, and *nmt1::ura4*). Tetrad analysis of the crosses was performed, and *tea1* was found to be closely linked to the *nmt1* gene (1.3 cM, 116 tetrads analyzed).

Tagging and Transformation of Cosmids

Cosmids in the *nmt1* region (provided by E. Maier) were tagged with a randomly inserted transposon Tn1000 containing the *his7*$^+$ gene (Morgan et al., 1996) (gift of S. Sedgwick), allowing selection of cosmids after transformation. Tagged cosmids were individually transformed into a *tea1–3 his7–366* strain and screened for complementation of the cell-shape defect. Cosmid 25D6 (Hoheisel et al., 1993) completely rescued the mutant phenotype.

Subcloning, Sequencing, and Deletion of *tea1*

Cosmid 25D6 was partially digested with Sau3A, and fragments of about 6 kb were gel purified and cloned into the BamHI site of pIRT2. All clones were pooled and transformed into *tea1–3 leu1–32*, and the transformants screened for rescue of the tea phenotype. Five complementing plasmids were isolated. Four of them contained a 4.3 kb BamHI fragment. The other one (pR2) contained 3.9 kb of the same 4.3 kb fragment, lacking the first 10 codons of the *tea1* ORF.

Sequencing was carried out using an ALF system from Pharmacia. The sequence was analyzed using the Genetics Computer Group package. Prediction of coiled–coil regions was done using the Lupas algorithm (Lupas et al., 1991).

To delete *tea1*, a 4.3 kb BamHI fragment containing the *tea1* gene and flanking regions was digested with HindIII, removing a 2.5 kb fragment containing 72% of the ORF (codons 68–895). This fragment was replaced with the *ura4*$^+$ gene and transformed into a *ura4-D18* diploid strain. Stable transformants were isolated, and deletion of one copy of *tea1* was confirmed by Southern blotting.

Production of tea1 Antibodies and tea1 Analysis

A SacI-BglII fragment from pR2 containing most of the 5′ half of *tea1* and a BglII-BamHI fragment containing the 3′ half were cloned into the bacterial pQE30 expression vector (Quiagen), producing 6His-tagged versions of fragments of tea1 containing amino acids 10–553 and amino acids 554–1147, respectively. The proteins were purified using Ni^{2+}-NTP resin under denaturing conditions and subsequently by SDS–PAGE. The gel slices containing the protein with Freund's adjuvant were used to inoculate rabbits. The antibodies were affinity purified from nitrocellulose strips containing the corresponding His-tagged protein as described (Pringle et al., 1991).

Preparation of total boiled protein extracts, Western blot analysis, and detection of antibodies were done as described (Grallert and Nurse, 1996). The antibodies used were affinity-purified anti-tea1-N-terminus (1:100 dilution), anti-tea1-C-terminus (1:2000), and anti-α-tubulin monoclonal antibody (1:5000; Sigma T-5168). Extracts for gel filtration were prepared by protoplasting cells as described (Grallert and Nurse, 1996), centrifuged at 100,000 × g for 60 min, and loaded onto a Superose-6 column.

Immunofluorescence Techniques

Cells were fixed by adding formaldehyde to 4% final concentration to the medium for 30 min, or collected by filtration and fixed with −20°C methanol, and processed as described (Alfa et al., 1993). Similar results were obtained with both fixations. Cell stainings are of cells fixed with methanol except for Figure 2D and for the actin

staining in Figure 4A, for which formaldehyde was used. Affinity-purified antibodies against the N-terminal half of tea1 were used at 1:20 dilution, and antibodies against the C-terminal half at 1:60. For immunofluorescence of tubulin, TAT1 monoclonal antibody was used (Woods et al., 1989) (gift of K. Gull) at 1:15 dilution and for actin N350 (Amersham) at 1:150. Fluorescein-linked anti-rabbit (Amersham) at 1:150 dilution and Cy3-linked anti-mouse (Sigma) at 1:2000 were used as secondary antibodies. Rhodamine-phalloidin stainings of actin were done as described (Alfa et al., 1993). Photographs were taken using a Bio–Rad MRC600 confocal microscope and analyzed with Bio–Rad software.

Overexpression of tea1

The *tea1* gene was overexpressed using the thiamine-repressible *nmt1* promotor (Maundrell, 1990). A 4.3 kb fragment BamHI containing tea1 full ORF was cloned into the BamHI site of pREP3X (Forsburg, 1993).

Acknowledgments

We thank P. Jordan for help with confocal microscopy, C. Lehane for technical assistance, and all members of the Cell Cycle Lab, particularly K. Sawin, for help and discussions. We would also like to thank B. Baum, N. Le Bot, D. Brunner, J. Hayles, K. Sawin, B. Stern, G. Schiavo, G. Warren, and anonymous reviewers for comments on the manuscript. We thank the Ministerio Español de Educación y Ciencia and the European Union for support to J. M.

Received March 18, 1997; revised May 7, 1997.

References

Alfa, C.E., Gallagher, I.M., and Hyams, J.S. (1993). Antigen localization in fission yeast. Meth. Cell Biol. *37*, 201–222.

Balasubramanian, M.K., Helfman, D.M., and Hemmingsen, S.M. (1992). A new tropomyosin essential for cytokinesis in the fission yeast *S. pombe*. Nature *360*, 84–87.

Balasubramanian, M.K., Hirani, B.R., Burke, J.D., and Gould, K.L. (1994). The *Schizosaccharomyces pombe cdc3*$^{+}$ gene encodes a profilin essential for cytokinesis. J. Cell Biol. *125*, 1289–1301.

Bork, P., and Doolittle, R.F. (1994). Drosophila kelch motif is derived from a common enzyme fold. J. Mol. Biol. *236*, 1277–1282.

Chang, F., Woollard, A., and Nurse, P. (1996). Isolation and characterization of fission yeast mutants defective in the assembly and placement of the contractile actin ring. J. Cell Sci. *109*, 131–142.

Eaton, S., and Simons, K. (1995). Apical, basal, and lateral cues for epithelial polarization. Cell *82*, 5–8.

Eichinger, L., Bomblies, L., Vandekerckhove, J., Schleicher, M., and Gettemans, J. (1996). A novel type of protein kinase phosphorylates actin in the actin-fragmin complex. EMBO J. *15*, 5547–5556.

Forsburg, S.L. (1993). Comparison of *Schizosaccharomyces pombe* expression systems. Nucleic Acids Res. *21*, 2955–2956.

Fukui, Y., Kaziro, Y., and Yamamoto, M. (1986). Mating pheromone-like diffusible factor released by *Schizosaccharomyces pombe*. EMBO J. *5*, 1991–1993.

Grallert, B., and Nurse, P. (1996). The ORC1 homologue orp1 in fission yeast plays a key role in regulating onset of S phase. Genes Dev. *10*, 2644–2654.

Hagan, I.M., and Hyams, J.S. (1988). The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast *Schizosaccharomyces pombe*. J. Cell Sci. *89*, 343–357.

Hiraoka, Y., Toda, T., and Yanagida, M. (1984). The *NDA3* gene of fission yeast encodes β-tubulin: a cold-sensitive *nda3* mutation reversibly blocks spindle formation and chromosome movement in mitosis. Cell *39*, 349–358.

Hoheisel, J.D., Maier, E., Mott, R., McCarthy, L., Grigoriev, A.V., Schalkwyk, L.C., Nizetic, D., Francis, F., and Lehrach, H. (1993). High resolution cosmid and P1 maps spanning the 14 Mb genome of the fission yeast S. pombe. Cell *73*, 109–120.

Imai, Y., and Yamamoto, M. (1992). *Schizosaccharomyces pombe* *sxa1*$^{+}$ and *sxa2*$^{+}$ encode putative proteases involved in the mating response. Mol. Cell. Biol. *12*, 1827–1834.

Kirschner, M., and Mitchison, T. (1986). Beyond self-assembly: from microtubules to morphogenesis. Cell *45*, 329–342.

Lehmann, R. (1995). Cell–cell signaling, microtubules, and the loss of symmetry in the Drosophila oocyte. Cell *83*, 353–356.

Leupold, U. (1987). Sex appeal in fission yeast. Curr. Genet. *12*, 543–545.

Lupas, A., Van, D.M., and Stock, J. (1991). Predicting coiled coils from protein sequences. Science *252*, 1162–1164.

Maeda, T., Mochizuki, N., and Yamamoto, M. (1990). Adenylyl cyclase is dispensable for vegetative cell growth in the fission yeast *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. USA *87*, 7814–7818.

Marks, J., Hagan, I.M., and Hyams, J.S. (1986). Growth polarity and cytokinesis in fission yeast: the role of the cytoskeleton. J. Cell Sci. (Suppl.) *5*, 229–241.

Maundrell, K. (1990). *nmt1* of fission yeast. A highly transcribed gene completely repressed by thiamine. J. Biol. Chem. *265*, 10857–10864.

Mitchison, J.M., and Nurse, P. (1985). Growth in cell length in the fission yeast *Schizosaccharomyces pombe*. J. Cell Sci. *75*, 357–376.

Moreno, S., Klar, A., and Nurse, P. (1991). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Meth. Enzymol. *194*, 795–823.

Morgan, B.A., Conlon, F.L., Manzanares, M., Millar, J.B., Kanuga, N., Sharpe, J., Krumlauf, R., Smith, J.C., and Sedgwick, S.G. (1996). Transposon tools for recombinant DNA manipulation: Characterization of transcriptional regulators from yeast, Xenopus, and mouse. Proc. Natl. Acad. Sci. USA *93*, 2801–2806.

Ohkura, H., Kinoshita, N., Miyatani, S., Toda, T., and Yanagida, M. (1989). The fission yeast *dis2*$^{+}$ gene required for chromosome disjoining encodes one of two putative type 1 protein phosphatases. Cell *57*, 997–1007.

Pringle, J.R., Adams, A.E., Drubin, D.G., and Haarer, B.K. (1991). Immunofluorescence methods for yeast. Meth. Enzymol. *194*, 565–602.

Roemer, T., Vallier, L.G., and Snyder, M. (1996). Selection of polarized growth sites in yeast. Trends Cell Biol. *6*, 434–442.

Schmidt, H. (1993). Effective long range mapping in *Schizosaccharomyces pombe* with the help of *swi5*. Curr. Genet. *24*, 271–273.

Snell, V., and Nurse, P. (1993). Investigations into the control of cell form and polarity: the use of morphological mutants in fission yeast. J. Cell Sci. Dev. (Suppl.) *1993*, 289–299.

Snell, V., and Nurse, P. (1994). Genetic analysis of cell morphogenesis in fission yeast—a role for casein kinase II in the establishment of polarized growth. EMBO J. *13*, 2066–2074.

Stern, B., and Nurse, P. (1997). Fission yeast pheromone blocks S-phase by inhibiting the G1 cyclin B-p34^{cdc2} kinase. EMBO J. *16*, 534–544.

Toda, T., Adachi, Y., Hiraoka, Y., and Yanagida, M. (1984). Identification of the pleiotropic cell division cycle gene *NDA2* as one of two different α-tubulin genes in Schizosaccharomyces pombe. Cell *37*, 233–242.

Umesono, K., Toda, T., Hayashi, S., and Yanagida, M. (1983). Cell division cycle genes *nda2* and *nda3* of the fission yeast *Schizosaccharomyces pombe* control microtubular organization and sensitivity to anti-mitotic benzimidazole compounds. J. Mol. Biol. *168*, 271–284.

Varkey, J.P., Muhlrad, P.J., Minniti, A.N., Do, B., and Ward, S. (1995). The Caenorhabditis elegans spe-26 gene is necessary to form spermatids and encodes a protein similar to the actin-associated proteins kelch and scruin. Genes Dev. *9*, 1074–1086.

Verde, F., Mata, J., and Nurse, P. (1995). Fission yeast cell morphogenesis: identification of new genes and analysis of their role during the cell cycle. J. Cell Biol. *6*, 1529–1538.

Way, M., Sanders, M., Garcia, C., Sakai, J., and Matsudaira, P. (1995). Sequence and domain organization of scruin, an actin cross-linking protein in the acrosomal process of Limulus sperm. J. Cell Biol. *128*, 51–60.

Wilson, A.C., LaMarco, K., Peterson, M.G., and Herr, W. (1993). The VP16 accessory protein HCF is a family of polypeptides processed from a large precursor protein. Cell *74*, 115–125.

Woods, A., Sherwin, T., Sasse, R., MacRae, T.H., Baines, A.J., and Gull, K. (1989). Definition of individual components within the cytoskeleton of *Trypanosoma brucei* by a library of monoclonal antibodies. J. Cell Sci. *93*, 491–500.

Xue, F., and Cooley, L. (1993). *kelch* encodes a component of intercellular bridges in Drosophila egg chambers. Cell *72*, 681–693.

Yaffe, M.P., Hirata, D., Verde, F., Eddison, M., Toda, T., and Nurse, P. (1996). Microtubules mediate mitochondrial distribution in fission yeast. Proc. Natl. Acad. Sci. USA *93*, 11664–11668.

EMBL Nucleotide Sequence Database Accession Number

The EMBL Nucleotide Sequence Database accession number for the *tea1* gene sequence described in this paper is Y12709.