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Roles of fission yeast tea1p in the localization of polarity factors and in organizing the microtubular cytoskeleton

Ralf Behrens and Paul Nurse

Cancer Research UK, Cell Cycle Laboratory, London WC2A 3PX, United Kingdom

The cylindrical shape of the fission yeast cell is generated by linear polarized growth from its cell ends. Using immunofluorescence and live imaging microscopy, we have investigated the roles of the cell end marker tea1p in generating linear polarized growth. We found that tea1p is primarily transported on plus ends of microtubules from the vicinity of the nucleus to the cell ends, and that its movement near the nucleus is independent of the kinesin tea2p. Deletion analysis identified a coiled-coil domain in tea1p essential for its retention at cell ends, and demonstrated that tea1p exerts different functions dependent on its location. On the tips of microtubules, tea1p prevents the curling of microtubules around the cell ends, whereas it is required for maintaining linear cell growth and for retention of polarity factors such as the Dyrk kinase pom1p, the CLIP170-like tip1p, and tea2p at the cell ends. We propose that tea1p has roles in organizing the microtubule cytoskeleton on the tips of microtubules, and in the retention of factors at the cell ends necessary for the cell to grow in a straight line.

Introduction

An important biological question is how eukaryotic cells become spatially organized to create specific cell shapes. Particular shapes are generated by limiting growth to restricted areas of the cell. This requires the proper positioning of polarized structures to direct growth within the cell. Examples in budding yeast are the cortical landmarks used for bud site selection and the mating type receptors required for mating projections (Chant, 1999; Dohlman and Thorner, 2001), and in *Drosophila* the polarized structures that generate asymmetry in neuroblasts and apical/basal polarity in epithelial cells (Doe and Bowerman, 2001). The unicellular fission yeast *Schizosaccharomyces pombe* provides another simple eukaryotic model system to study spatial organization of cells (Nurse, 1994; Hayles and Nurse, 2001). Fission yeast is a cylindrical cell formed by highly polarized growth zones located precisely opposed to each other at the ends of the cell (Snell and Nurse, 1993). The mechanisms involved in ensuring this spatial organization have been investigated using morphological mutants, which can generate growth zones but fail to locate them properly so that the cell no longer grows in a straight line and becomes bent and branched (Snell and Nurse, 1994; Verde et al., 1995; Brunner and Nurse, 2000a). These mutants define three genes: *tea1, tea2,* and *tip1*. In this paper we have investigated the function of tea1p in living cells, focusing on its transport, location at the polarized cell ends, and its role in microtubular and cellular organization.

Tea1p is important for maintaining linear growth because cells become bent and branched in its absence. Tea1p localizes tightly to the cell ends and is also present on the tips of microtubules (Mata and Nurse, 1997). Experiments with microtubular disrupting drugs have indicated that an intact microtubule cytoskeleton is required for tea1p to be efficiently localized to the cell ends. The interphase microtubules are organized in bundles of 3–4 microtubules that extend along the long axis of the cell (Hagan, 1998). The plus ends of each bundle grow towards the cell poles and their minus ends overlap in the cell center in an antiparallel configuration (Drummond and Cross, 2000; Tran et al., 2001). Microtubules are first nucleated in the vicinity of the nucleus and then extend until thsey reach the cell ends where they stop growing and undergo catastrophe. In the absence of tea1p, some microtubules curl around the cell ends such that tea1p may influence microtubular organization (Mata and Nurse, 1997). The CLIP170-like protein tip1p and the kinesin-like protein tea2p are microtubule-associated morphogenetic factors which are required for tea1p delivery to the cell ends (Browning et al., 2000; Brunner and Nurse, 2000a). In

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Address correspondence to Paul Nurse, Cancer Research UK, Cell Cycle Laboratory, 44 Lincoln's Inn Fields, London WC2A 3PX, UK. Tel.: 44-20-7269-3276. Fax: 44-20-7269-3610. E-mail: p.nurse@icrf.icnet.uk

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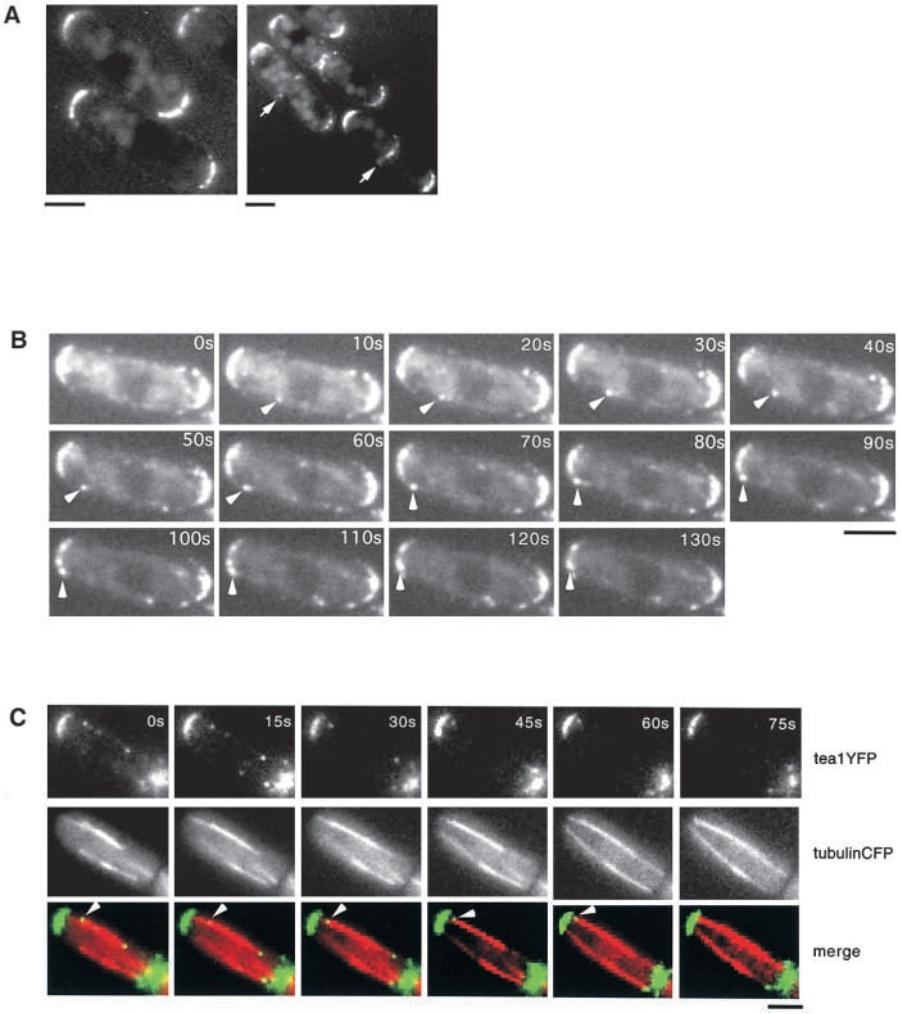

Figure 1. **Localization and movement of tea1GFP/YFP in vivo.** (A) Living cells expressing the endogenous *tea1* gene tagged with GFP (strain RB1056). Arrowheads show tea1GFP localizing to the division septum. (B) Live-time imaging of tea1GFP movement. Arrowheads follow the movement of a selected tea1GFP dot, and time is shown in seconds (s). (C) Two-color live imaging of tea1YFP and tubulinCFP (strain RB134). Arrowheads follow a selected tea1YFP dot colocalizing with the tip of a polymerizing microtubule. Bars, 3 μm.

tip1Δ and *tea2Δ* mutants, tea1p is localized to short microtubules and is absent from the cell ends. Tip1p regulates microtubule catastrophe, ensuring that microtubules find the cell end and deliver tea1p to these locations. Tea2p may function as a tea1p transporter or influence microtubular dynamics to allow microtubules to reach the cell ends. Tip1p and tea2p also localize to the cortex at the cell end, and this localization is dependent on tea1p. Tea1p is also necessary for the localization of bud6p, pom1p, and rho1p to the cell ends (Arellano et al., 1997; Bahler and Pringle, 1998; Glynn et al., 2001). Bud6p is required for the switch from monopolar to bipolar growth in early G2, called new-end takeoff (Mitchison and Nurse, 1985), and interacts physically with tea1p, whereas pom1p is a member of the Dyrk family of protein kinases and functions in the switch to bipolar growth and in the proper selection of the cell division site. The rho1p GTPase is required for proper actin localization and activates β-1,3-glucan synthase involved in cell wall synthesis.

Tea1p has six Kelch repeats and several coiled-coil domains at its COOH terminus (Mata and Nurse, 1997; Adams et al., 2000; Burkhard et al., 2001). It has been reported to share this structure with Kel1 in budding yeast, a protein which is involved in cell morphology and cell fusion during mating (Philips and Herskowitz, 1998). Tea1p also has some weak homology to the ezrin/moesin/radixin (ERM)* family of cytoskeletal linker proteins (Vega and Solomon, 1997; Mangeat et al., 1999).

Using immunofluorescence and live imaging microscopy, we show here that (a) tea1p is transported at the plus ends of polymerizing microtubules from the vicinity of the nucleus to the cell ends; (b) tea2p is not required for movement of tea1p on the tips of polymerizing microtubules near to the nucleus but is required for tea1p to reach the cell ends; (c) Part of the coiled-coil region is necessary to retain tea1p at the cell ends; and (d) The cell end localization of tea1p is not required to maintain proper microtubular organization but is required to maintain growth in a straight line and to retain tea2p, tip1p, and pom1p at the cell ends.

Results

Tea1p is transported from the nucleus to the cell ends on the tips of microtubules

To investigate the transport of tea1p through the cell, we tagged the chromosomal *tea1* gene with green fluorescence

*Abbreviations used in this paper: CFP, cyan fluorescent protein; ERM, ezrin/moesin/radixin; GFP, green fluorescent protein; YFP, yellow fluorescent protein.

Table I. **Parameters of interphase microtubules**

Strain	Growth rate	Shrinkage rate	Dwelling time[a]
	μm/min	*μm/min*	*s*
tea1+	3.87 ± 0.72 (23, 10)	12.13 ± 3.91 (48, 15)	100 ± 54 (53, 20)
tea1(Δ200)	5.02 ± 1.06 (49, 16)	14.38 ± 3.27 (45, 16)	117 ± 44 (25, 10)
tea1Δ	5.15 ± 1.07 (45, 15)	14.41 ± 4.28 (56, 20)	94 ± 50 (39, 13)

The number of analyzed microtubules and cells is given in parentheses (microtubules, cells).
[a]Defined as the time a microtubule remains in contact with the cell end cortex before undergoing catastrophe.

protein (GFP). The GFP tag was fused to the COOH terminus of the protein, with the fused gene regulated by the endogenous *tea1* promotor resulting in the fused protein being expressed at wild-type levels. The tea1GFP cells had an identical cell morphology to wild-type cells, establishing that tea1GFP could fully substitute for tea1p. Immunofluorescence using α-tea1 antibodies revealed that tea1GFP had a similar cellular localization to tea1p, being localized both to the cell ends and to the tips of microtubules (unpublished data; see Figs. 3, B and C, and 5, A and B). These experiments demonstrate that tea1GFP can be used as valid model to investigate the transport of tea1p in living cells.

Examination of live cells revealed that tea1GFP was localized primarily at the cell ends with occasional dots in the cytoplasm, a distribution similar to fixed cells investigated by immunofluorescence (Fig. 1 A). Additionally, in live cells, tea1GFP was observed associated with the septum, a localization not previously reported in immunofluorescence studies (Mata and Nurse, 1997), suggesting that the association of tea1p with the septum may be lost during fixation. Tea1GFP movement was followed in living cells using time-lapse microscopy and an example of a typical time course is shown in Fig. 1 B (Video 1, available at http//:www.jcb.org/cgi/content/full/jcb.200112027/DC1). In all cases, tea1-GFP dots first appeared in the vicinity of the nucleus. In this region, the direction of the tea1GFP dots changed frequently, but once the dots moved out of the vicinity of the nucleus, they moved processively towards the cell ends. Only 5% of the dots ($n = 92$) reversed direction once they were beyond the nucleus. Such dots were distinguishable from dots on shrinking microtubules (see below) because they moved at a slower speed. We conclude that tea1p is loaded on microtubules in the vicinity of the nucleus, that changes in the direction of movement can occur within the loading region, and that once tea1p has moved beyond that region, it generally moves processively toward the cell ends.

To investigate the behavior of tea1p on the microtubules in live cells, we tagged the *tea1* gene with yellow fluorescence protein (YFP) and performed two-color imaging with tubulin cyan fluorescence protein (CFP) expressed from a plasmid (Glynn et al., 2001). In the vicinity of the nucleus, changes in the direction of tea1YFP movement were usually confined to bright regions of the microtubules which have been reported to contain overlapping ends of antiparallel microtubule bundles (Drummond and Cross, 2000; Tran et al., 2001). Tea1YFP dots were found to be present on the tips of microtubules and the dots remained colocalized with the tips of polymerizing microtubules while moving from the nucleus to the cell ends (Fig. 1 C). The speed at which tea1GFP dots moved toward the cell ends was determined to be 3.6 ± 1.0 μm/min ($n = 37$ dots), similar to the rate of polymerization of GFP-labeled microtubules (Table I). Large dots were sometimes followed by smaller dots that moved at a similar speed. The frequency with which tea1GFP dots were delivered to cell ends was also measured and found to be 0.86 ± 0.63 min^{-1} end^{-1} ($n = 86$ dots). This means that during the duration of an average interphase (at 23–25°C in minimal medium, ∼250 min [Moreno et al., 1991]), at least 215 (250 × 0.86) tea1GFP dots are delivered to each cell end. The frequency of tea1GFP delivery to the cell ends is in the same order as we observed for GFP-labeled microtubules at 0.50 ± 0.24 min^{-1} end^{-1} ($n = 169$ microtubules). This suggests that most microtubule bundles are carrying tea1p at their tips to the cell ends.

The majority of tea1YFP was deposited at the cell ends, but some dots were found to reverse direction once they had reached the cell ends and to move backwards toward the cell center remaining colocalized with the tips of the shrinking microtubules. We analyzed the kinetics of the backward movement of tea1GFP dots and found that they moved smoothly with an average speed of 17.0 ± 2.0 μm/min ($n = 14$ dots), usually becoming progressively fainter and disappearing after retreating for 2–3 μm. This speed of backward movement was similar to that of GFP-labeled microtubules undergoing catastrophe (Table I). The frequency with which tea1GFP dots retreated from the cell ends was determined to be 0.06 ± 0.16 min^{-1} end^{-1} ($n = 20$ dots), whereas the frequency of GFP-labeled microtubules retreating from the cell ends was 0.55 ± 0.26 min^{-1} end^{-1} ($n = 167$ microtubules). This indicates that ∼11% of the dots that reach the cell ends on the tips of microtubular bundles leave the cell ends on shrinking microtubules. We conclude that a small fraction of tea1p fails to be deposited at the cell ends and remains associated with the tips of microtubules undergoing shrinkage away from the ends.

The tea2p kinesin is not required for tea1p movement near the nucleus

In cells lacking the tea2p kinesin, there is a major reduction of tea1p at the cell ends, indicating that tea2p is required for tea1p localization at the cell ends (Browning et al., 2000). In >95% of the *tea2Δ* cells, tea1GFP was absent from both cell ends or was present at one end but at significantly reduced levels (Fig. 2 A). To investigate whether the tea2p kinesin is required for the movement of tea1p, we determined the speed of tea1GFP dot movement in *tea2Δ* cells. A typical time course is shown in Fig. 2 B (Video 2, available at http//:www.jcb.org/cgi/content/full/jcb.200112027/DC1). Tea1GFP speed of movement was found to be 3.0 ± 0.8

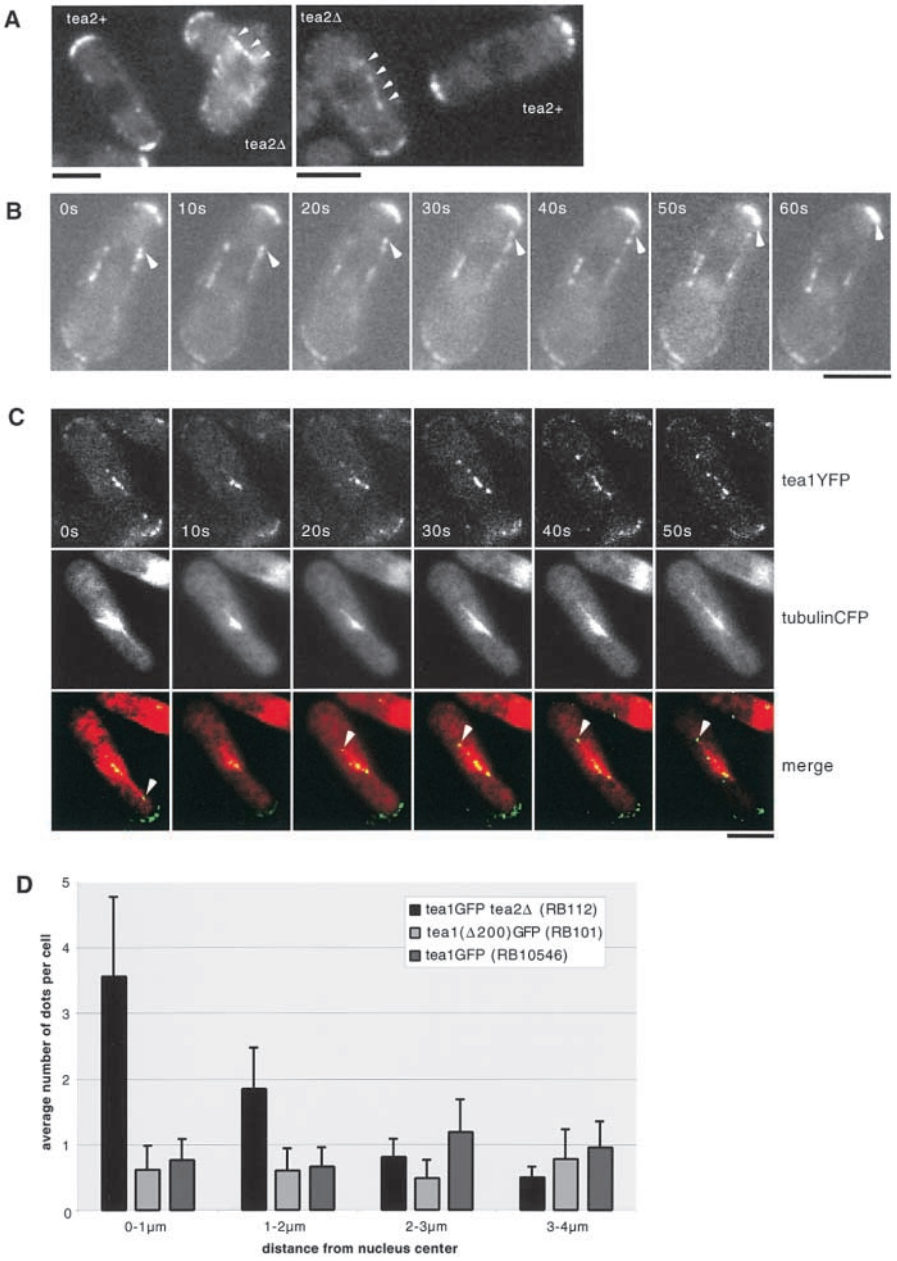

Figure 2. **Dynamics of tea1GFP/YFP in *tea2Δ* cells.** (A) Comparison of fluorescence level and localization of tea1GFP in *tea2+* and *tea2Δ* cells. Strain RB10546 (tea2+) and strain RB112 (tea2Δ) were grown under identical conditions, mixed and tea1GFP visualized by fluorescence microscopy. The arrowheads indicate lines of tea1GFP dots. Bars, 4 μm. (B) Live-time imaging of tea1GFP movement in a *tea2Δ* cell. Arrowheads follow the movement of a selected tea1GFP dot, and time is shown in seconds (s). Bar, 4 μm. (C) Two-color live imaging of tea1YFP and tubulinCFP in *tea2Δ* cells (strain RB136). Arrowheads show tea1YFP dots colocalizing with the tips of polymerizing microtubules. Bar, 4 μm. (D) Statistics of cytoplasmic tea1GFP distribution in *tea2Δ* and *tea2+* cells, and of tea1(Δ200)GFP distribution. The average number of dots per cell was determined, and the distribution of dots within 4 μm distance from the nucleus center analyzed. Error bars give the standard deviation.

μm/min ($n = 36$ dots), a rate very similar to the 3.6 ± 1.0 μm/min observed in wild-type cells ($P > 0.05$, two-tailed t test). Colocalization studies of YFP-tagged tea1p and CFP-labeled tubulin in live *tea2Δ* cells showed that tea1YFP moves on the tips of polymerizing microtubules (Fig. 2 C). In *tea2Δ* cells, microtubules are significantly shorter than in wild-type cells (Verde et al., 1995; Browning et al., 2000). Consistent with this observation, we observed that tea1GFP dots often failed to move away from the vicinity of the nucleus in *tea2Δ* cells, with >80% of the dots clustered within 2 μm of the nucleus center. In wild-type cells, tea1GFP dots were distributed more evenly throughout the cell (Fig. 2 D). Generally, only short lines of tea1GFP dots were observed in *tea2Δ* cells, and the dots frequently changed their direction of movement and often disappeared. The protein levels were similar in *tea2Δ* and wild-type cells (unpublished data), but significantly more dots were observed in *tea2Δ* cells (6.7 ± 2.3 dots/cell, $n = 30$ cells) than in wild-type cells (3.8 ± 1.5 dots/cell, $n = 21$ cells), yielding $P < 0.01$ in a two-tailed t test. We conclude that in the absence of the tea2p kinesin, tea1p associates normally with the tips of polymerizing microtubules and can move with a rate similar to that observed in wild-type cells, but that this movement is confined primarily to the central region of the cell.

Tea1p retention at the cell ends requires a COOH terminal coiled-coil region

Tea1p is retained efficiently at cell ends, and to define regions of the protein required for this retention, deletion mutants were constructed that lacked the COOH terminus of the protein. We constructed three deletion strains lacking 100 amino acids (*tea1[Δ100]*), 150 amino acids (*tea1[Δ150]*), and 200 amino acids (*tea1[Δ200]*) of the COOH terminus (Fig. 3 A). All the proteins were expressed

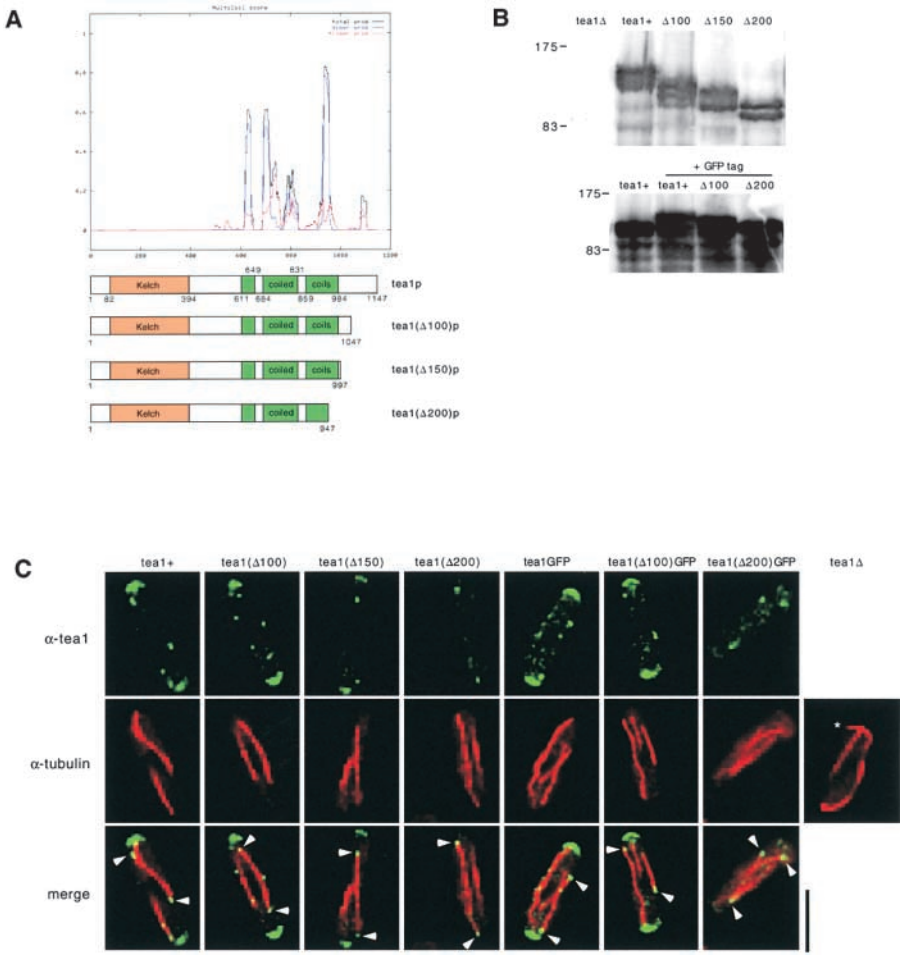

Figure 3. **Analysis of expression level and localization of tea1p mutants.** (A) Tea1p deletion mutants. The graph gives the probabilities of predicted coiled-coil motifs implicated in dimeric (blue) and trimeric (red) interactions. The position of predicted coiled-coil (green) motifs and of six Kelch repeats (orange) in tea1p are shown. The amino acid position of the NH_2- and COOH-terminal borders for each motif are given. (B) Western blot analysis of untagged and tagged *tea1* mutants. Immunoblotting was performed with α-tea1 antibodies against boiled protein extracts prepared from wild-type and *tea1* mutant strains. Ponceau staining was used after blotting to verify that equal amounts of total protein were loaded in each lane (unpublished data). The molecular mass markers are shown left of the gel in kD. (C) Single optical sections (0.45 μm) of mutant cells stained for tea1p (green) and microtubules (red) were obtained by confocal microscopy. Arrowheads show tea1p dots colocalizing with the tips of microtubules. The asterisk (*) indicates a microtubule curling around the end of a *tea1Δ* cell. Bar, 5 μm. Untagged strains: tea1+ (RB10), tea1Δ (RB14), tea1(Δ100) (RB116), tea1(Δ150) (RB106), tea1(Δ200) (RB120); GFP-tagged strains: tea1GFP (RB10546), tea1(Δ100)GFP (RB100), tea1(Δ200)GFP (RB101).

at levels similar to that observed in wild-type cells (Fig. 3 B). The localization of tea1p was monitored in the three deletion strains. Immunofluorescence studies showed that *tea1(Δ100)* cells had a tea1p localization pattern that was indistinguishable from wild-type, whereas *tea1(Δ150)* cells were almost like wild-type, although there was a slight reduction in amount of tea1(Δ100)p at the cell ends (Fig. 3 C). However, tea1(Δ200)p was almost completely eliminated from the cell ends with only a few dots being present (Fig. 3 C). Tea1(Δ200)p was still associated with the tips of microtubules and the few tea1(Δ200)p dots present at the cell ends were located at the tips of microtubules. GFP-tagged proteins were constructed for *tea1(Δ100)* and *tea1(Δ200)* and had the same localization pattern as the untagged proteins (Fig. 3 C); they were expressed at a similar level to tea1p in wild-type cells (Fig. 3 B).

The elimination of tea1(Δ200)p from the cell ends could be due to inefficient transport through the cell or inefficient retention at the cell ends. To investigate the first possibility, we carried out time-lapse live imaging experiments with the *tea1(Δ200)GFP* cells. Tea1(Δ200)GFP dots appeared first in the vicinity of the nucleus and were efficiently transported to the cell ends (Fig. 4 A; Video 3, available at http//:www.jcb.org/cgi/content/full/jcb.200112027/DC1). The movement was highly processive with a speed of 4.2 ± 1.1 μm/min (n = 23 dots), similar to the 3.6 ± 1.0 μm/min observed for tea1GFP. The rate of delivery of dots to the cell ends at 0.84 ± 0.50 dots min^{-1} end^{-1} (n = 107 dots) was also similar to tea1GFP (0.86 ± 0.63 dots min^{-1} end^{-1}), as was the average number of dots per cell, 3.5 ± 2.3 dots/cell (n = 37 cells), compared with 3.8 ± 1.5 dots/cell for tea1GFP. The distribution of tea1GFP and tea1(Δ200)GFP dots throughout the cell was also similar (Fig. 2 D). Colocalization experiments in live cells with YFP-tagged tea1(Δ200)p and CFP-labeled microtubules showed that tea1(Δ200)YFP moves in a similar manner as the wild-type protein on the tips of polymerizing microtubules to the cell ends (Fig. 4 B). Therefore, the transport of tea1(Δ200)p through the cell appears to be similar to that of intact tea1p.

Given that tea1(Δ200)p is transported normally through the cell, its failure to accumulate properly at the cell ends could be due to its inability either to disengage from the microtubular tips or to be retained at the cell end cortex after disengagement from the microtubular tips. To try and distinguish between these two possibilities, the behavior of CFP-labeled microtubules and of tea1(Δ200)YFP was analyzed at the cell ends by time-lapse microscopy. Typically, tea1(Δ200)YFP dots at the tips of microtubules reached the cell ends and then stopped, remaining associated with the paused microtubular tips. When microtubules underwent catastrophe, most of the dots (89%, n = 35 total) disappeared from the cell ends at the same time as the microtubules (Fig. 4 B, a). Around 20% of these dots were found on the shrinking microtubules. However, in some cases (11%),

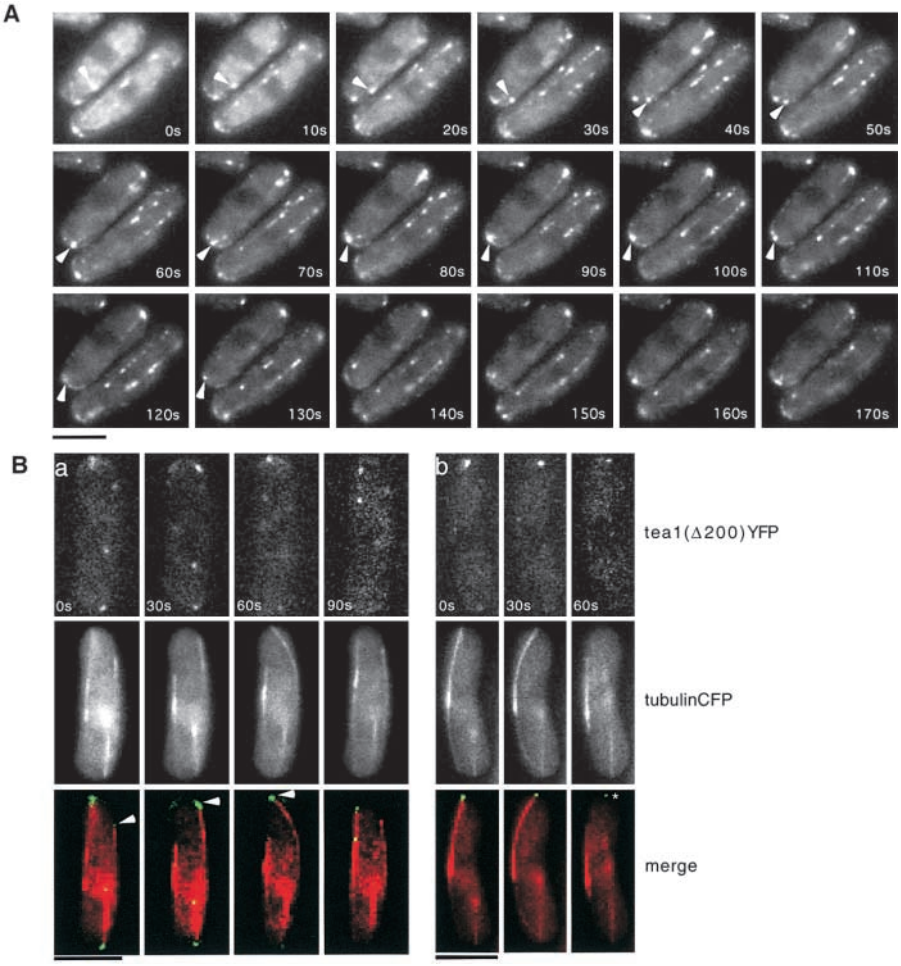

Figure 4. **Movement of tea1(Δ200)GFP/YFP in living cells.** (A) Live-time imaging of tea1(Δ200)GFP movement (strain RB101). Arrowheads follow the movement of a selected tea1(Δ200)GFP dot, and time is shown in seconds (s). (B) Two-color live imaging of tea1(Δ200)YFP and tubulinCFP (strain RB135). (B a) Arrowheads follow a tea1(Δ200)YFP dot colocalizing with the tip of a polymerizing microtubule. (B b) The asterisk (*) highlights a tea1(Δ200)YFP dot present at the cell ends after a microtubule has undergone catastrophe. Bars, 5 μm.

a tea1(Δ200)YFP dot remained visible at the cell end for the duration of one movie frame (30 s) after the microtubule had undergone catastrophe and then the dot disappeared from the cortex (Fig. 4 B, b). These observations suggest that tea1(Δ200)p generally disengages from the microtubular tips but either fails to become associated with the cell end cortex or persists there for only a short time.

Our results indicate that tea1(Δ200)p is efficiently transported to the cell ends but cannot be retained there. Tea1(Δ200)p is missing part of a coiled-coil region at the COOH terminus, whereas tea1(Δ150)p, which behaves essentially like tea1p, has that region intact (Fig. 3 A), suggesting that the coiled-coil region is needed for retention of tea1p at the cortex of the cell ends.

Tea1p influences the organization of the microtubular cytoskeleton on the tips of microtubules and maintains linear cell growth at the cell ends

In the absence of tea1p, cells become bent and branched, and a fraction of the microtubules fail to terminate properly at the cell ends and curl around the ends toward the sides the of the cell (Fig. 3 C) (Mata and Nurse, 1997). The behavior of tea1(Δ200)p allows us to investigate whether tea1p has to be located at the tips of the microtubules or at the cell ends to ensure that cells do not become bent or branched and microtubules terminate in the cell ends. As already shown, tea1(Δ200)p is located on microtubular tips but cannot be retained at the cellular ends. Microtubules in *tea1(Δ200)* and *tea1(Δ200)GFP* cells did not curl around the ends of the cells (Fig. 5 A). This means that the presence of tea1p at the microtubular tips is sufficient for normal microtubular organization, and that tea1p does not have to be properly retained at the cell ends to ensure the efficient termination of microtubular growth in this region of the cell. The effect of tea1p on this aspect of microtubular organization is likely to be due to the presence of tea1p on the microtubular tips, although it is possible that low amounts of tea1(Δ200)p on the cortex may be sufficient to terminate microtubular growth. We also found that microtubule growth and shrinkage rates as well as microtubule dwelling times at the cell ends were similar in wild-type, *tea1(Δ200)*, and *tea1Δ* cells (Table I), as determined using GFP-labeled tubulin.

In contrast, the morphology of *tea1(Δ200)* and *tea1 (Δ200)GFP* cells was defective, with cells becoming bent and branched to an extent similar to *tea1Δ* cells (Fig. 5 B). We conclude that it is insufficient to have tea1p located at the microtubular tips to bring about normal linear cell growth, and that it is probably the location of tea1p at the cell ends which is to bring about proper cell organization.

In *tea1Δ* cells, the morphogenetic factors tip1p, tea2p, and pom1p are reduced in levels at the cell ends (Bahler and Pringle, 1998; Browning et al., 2000; Brunner and Nurse, 2000a). We used the *tea1(Δ200)* strain to investigate

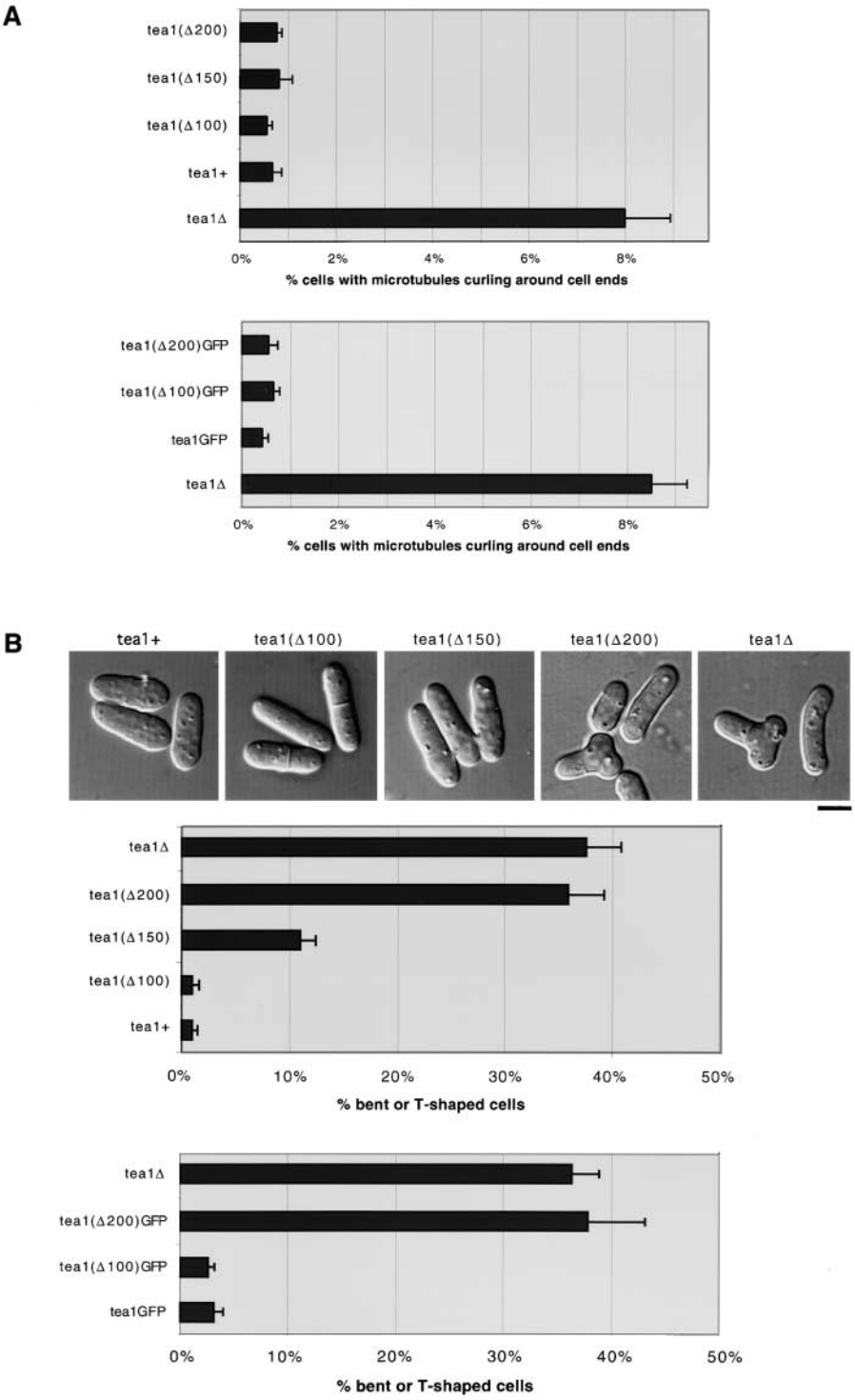

Figure 5. **Organization of the microtubular cytoskeleton and cell morphology of *tea1* mutant strains.** (A) Quantification of microtubule curling around cell ends in *tea1* mutant strains. Fixed cells were stained with α-tubulin antibodies and microtubules were analyzed by fluorescence microscopy. (B) Differential interference contrast micrographs of *tea1* mutant cells and quantification of bending and branching of untagged and tagged *tea1* mutant strains. Error bars give the standard deviation. Untagged strains: tea1+ (RB10), tea1Δ (RB14), tea1(Δ100) (RB116), tea1(Δ150) (RB106), tea1(Δ200) (RB120); GFP-tagged strains: tea1GFP (RB10546), tea1(Δ100)GFP (RB100), tea1(Δ200)GFP (RB101).

whether tea1p has to be located at the tips of the microtubules or at the cell ends to ensure the proper cellular organization of these factors. In wild-type and *tea1(Δ100)* cells, tea2GFP, tip1GFP, and pom1GFP were all localized normally (Fig. 6, A–C). However, in *tea1(Δ200)* cells, all three proteins were found to be absent from the cell ends as in *tea1Δ* cells (Fig. 6, A–C). Live time imaging of tea2GFP and tip1GFP showed that both proteins moved normally on the microtubules to the cell ends but were not retained there (unpublished data). We conclude that proper retention at the cell ends of tip1p, tea2p, and pom1p all require the presence of tea1p at the cell ends.

Discussion

The work in this paper has led to the following conclusions: (a) The transport of the morphogenetic factor tea1p in fission yeast occurs at the tips of polymerizing microtubules, moving processively from the vicinity of the nucleus towards the ends of the cell; (b) The tea2p kinesin is not required for

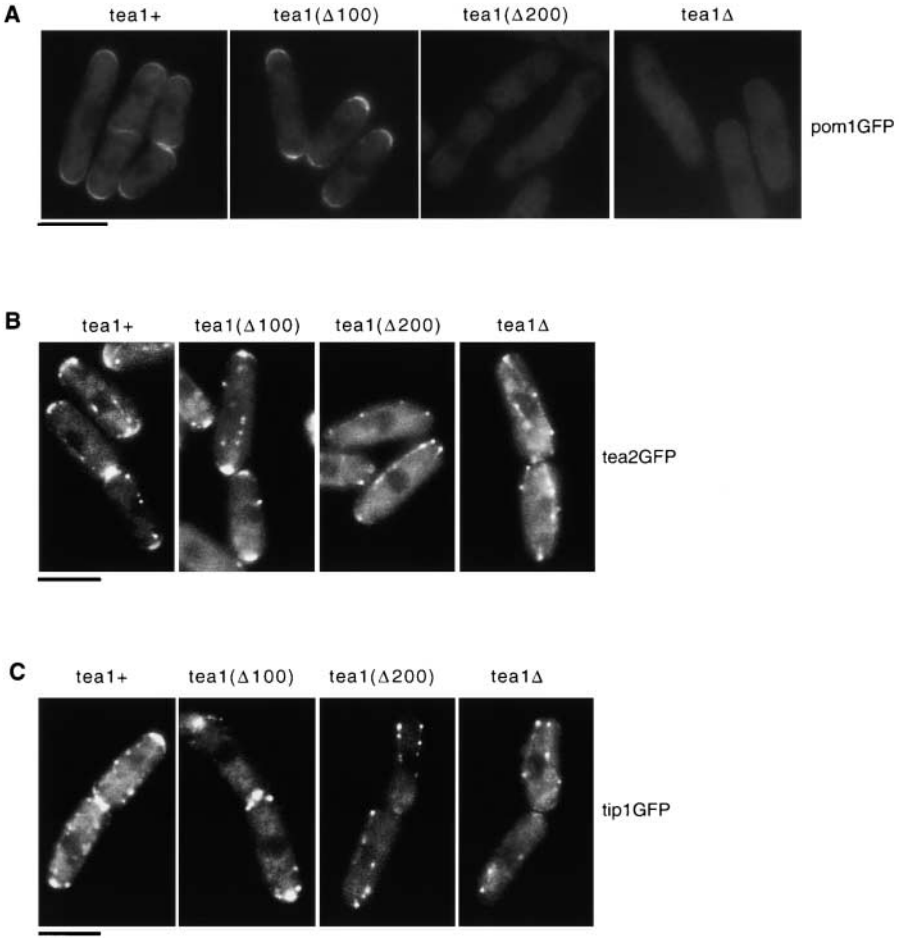

Figure 6. **Localization of pom1GFP, tip1GFP, and tea2GFP in tea1 mutant strains.** (A) The in vivo localization of pom1GFP, (B) tea2GFP, and (C) tip1GFP in *tea1* mutant strains was visualized by fluorescence microscopy. Bars, 6 μm. Strains shown are (from left to right for each panel): (A) RB3141, RB125, RB126, RB3172 ; (B) RB2435, RB129, RB130, RB2500; and (C)RB3643, RB131, RB132, RB133.

tea1p movement on the tips of polymerizing microtubules, but in the absence of tea2p moving tea1p is confined to the vicinity of the nucleus; (c) A COOH-terminal coiled-coil region of tea1p is required for the efficient retention of the protein at the cortex of the cell ends; and (d) The localization of tea1p at the cell ends is not required for the termination of microtubular growth at the cell ends, but is required for the retention of the morphogenetic factors tea2p, tip1p, and pom1p at the cell ends, and for proper cell morphogenesis.

Dots of tea1p appear in the vicinity of the nucleus, and in this region of the cell dots frequently change their direction of movement. Microtubules are organized as antiparallel bundles around the nucleus (Drummond and Cross, 2000; Tran et al., 2001), and we propose that tea1p is loaded onto the microtubules within these structures. Once the tea1p dots have moved out on microtubules beyond the nuclear region, they continue to move processively toward the cell ends without changing direction at a rate similar to the growth rate of polymerizing microtubules. Because most tea1p is located at the tips of microtubules, we conclude that the majority of tea1p is transported to the cell ends at the plus ends of polymerizing microtubules, although some may be moved along the polymerized microtubules. During an interphase period, at least 215 tea1GFP dots are delivered to each cell end. The number of tea1GFP molecules in each of these dots is unknown, and it is possible that they may be contained in some sort of vesicle. In the absence of the kinesin tea2p, which has been proposed as a transporter of tea1p (Browning et al., 2000; Hayles and Nurse, 2001), movement of tea1p on the tips of polymerizing microtubules continues to occur in the vicinity of the nucleus. However, in *tea2Δ* cells, tea1p fails to accumulate efficiently at the cell ends, and the most likely explanation for this is the fact that in *tea2Δ* cells only a few microtubules extend throughout the length of the cell (Browning et al., 2000). Another less likely possibility is that tea1p movement on the microtubules is independent of tea2p near the nucleus but requires tea2p for movement further away from the nucleus. It is also possible that tea2p is the tea1p transporter but can be substituted by another kinesin in *tea2Δ* cells.

When tea1p reaches the end of the cell, it is mostly transferred from the tip of the paused microtubules to the cortex at the cell end. The majority (~90%) of shrinking microtubules have no tea1p at their tips, indicating that generally tea1p is transferred off the paused microtubule tip before it undergoes catastrophe. Efficient retention of tea1p at the cell end requires an intact coiled-coil region at the COOH terminus of tea1p, which contains no significant similarities with other protein motifs or phosphorylation sites. This region may act as a retention signal or anchor for tea1p at the cortex in the cell end. Tea1(Δ200)p, which would lack such a retention signal, is transported normally by the microtubules to the ends of the cell, but is not retained there. These cells have a normal microtubular

cytoskeleton, whereas in *tea1Δ* cells, ~10% of microtubules curl around the cell ends (this study; Mata and Nurse, 1997). This indicates that the failure of these microtubules to terminate their growth at the cell ends is not due to tea1p being absent from the cell ends (Mata and Nurse, 1997; Brunner and Nurse, 2000b), but rather is due to tea1p being absent from the microtubule tips. We suggest that the presence of tea1p at the growing microtubule tips is required for the tips to efficiently recognize the cell ends as the correct place to terminate microtubular growth and that this function of tea1p is important for the cell to grow in a straight line. If this is the case, tea1p can only be part of this mechanism because many microtubules terminate their growth correctly at the cell ends in *tea1Δ* cells. In *tea1(Δ200)* cells, the morphogenetic factors tea2p, tip1p, and pom1p are not found at the cell ends and yet microtubules terminate growth normally, suggesting that the cell end localization of these factors is not required for proper microtubular organization. These observations also suggest that the presence of tea1p at the cell ends is required for the retention of tip1p, tea2p, and pom1p at the cell ends. We propose that tea1p may act as a scaffold at the cell ends for the retention of other morphogenetic factors including tip1p, tea2p, pom1p, and possibly also bud6p and rho1p (Arellano et al., 1997; Glynn et al., 2001), and that this cell end location for tea1p is required for the fission yeast cell to be properly organized to grow in a straight line.

Our functional analysis of tea1p indicates that it plays a major role in fission yeast cell morphogenesis. It contributes to the overall organization of the microtubular cytoskeleton, a role it appears to play when located at the plus ends of microtubules. When located at the cell ends, it is necessary to retain other morphogenetic factors at the cell ends and to ensure that the cell grows in a straight line. The tea1p homologue in budding yeast Kel1 also localizes to zones of polar cell growth, and in its absence cells show heterogeneous cell shape defects suggesting that Kel1 has a general role in the formation of polar growth zones rather than in the correct positioning of these zones (Philips and Herskowitz, 1998). Kel1 is also important for cell fusion during mating, a function not performed by tea1p in fission yeast, suggesting that tea1p and Kel1 perform different functions in the two yeasts. Tea1p has been reported to have weak homology to members of the ERM family (Vega and Solomon, 1997) which function as structural linkers between the plasma membrane and the actin cytoskeleton (for review see Mangeat et al., 1999), and thus might share some of the characteristics of a scaffold with tea1p.

Several other proteins have been identified in other eukaryotes which function at the cell cortex in a manner related to what we have reported for tea1p. In budding yeast treated with pheromone the Gβγ heterodimer acts through the Far1–Cdc24–Cdc42 complex to recruit the growth machinery to the cortex resulting in the formation of mating projections (for review see Dohlman and Thorner, 2001). Also, the selection of bud sites in *S. cerevisiae* is determined by the location of cortical landmarks which direct the growth machinery towards the bud site using the Bud1 GTPase and the Cdc42 GTPase (for review see Chant, 1999). In *Drosophila*, three proteins, Baz, Par-6, and aPKC, form an apical complex that polarizes protein localization to the opposite sides of neuroblasts and epithelial cells (Wodarz et al., 2000; Petronczki and Knoblich, 2001). These three proteins perform a similar function in the *C. elegans* zygote (Rose and Kemphues, 1998; Hung and Kemphues, 1999), and in mammals where they are localized to apical tight junctions in epithelia (Joberty et al., 2000; Kim, 2000). These factors/protein complexes are present in different cell types and organisms but like tea1p all direct polarized growth at the cell cortex.

Other factors have functions on the microtubules related to those we have reported here for tea1p. In budding yeast, Kar9 interacting with the EB-1 homologue Bim1 located at the tips of astral microtubules, ensures the proper orientation of the microtubules for nuclear migration (Korinek et al., 2000; Lee et al., 2000; Miller et al., 2000). In mammalian cells adenomatous polyposis coli protein located at the tips of microtubules stabilizes microtubules in areas of cell migration (Nathke et al., 1996; Mimori-Kiyosue et al., 2000; Zumbrunn et al., 2001), and CLIP-associated proteins also at the ends of microtubules, are associated with microtubular stabilization in wounded regions of the cell (Akhmanova et al., 2001). Like tea1p, all of these are located at the plus ends of microtubules, are associated with specific regions of the cellular cortex, and have effects on microtubular dynamics. The general principle that emerges is that the location of regulatory proteins at microtubular tips have roles in modifying microtubular dynamics at specialized regions of the cellular cortex, and that these properties contribute to the overall positional information within the cell. Microtubules are important for positional information because they provide a means to explore the cell, allowing continual evaluation of the location of particular regions within the cell. This may be an important common mechanism for ensuring the proper spatial organization of eukaryotic cells.

Materials and methods

Strains, media, and genetic methods

Strains used in this study are listed in Table II. YE5S and EMM2 medium were used and standard methods were followed as described (Moreno et al., 1991).

Construction of tagged and deletion strains

The *tea1* gene tagged with GFP/YFP at the COOH terminus and untagged and tagged COOH-terminal deletions of the *tea1* gene were constructed at its genomic site by a PCR-based gene targeting method (Bahler et al., 1998). Western blot analysis was carried out as described previously (Hayles et al., 1994) to verify expression of all constructs using anti-tea1 antibodies (1:4,000 dilution) (Mata and Nurse, 1997). All experiments in this paper were carried out with several independent clones to avoid artifacts due to PCR-induced mutations.

Indirect immunofluorescence microscopy

Standard methods were used (Mata and Nurse, 1997; Sawin and Nurse, 1998). Cells were fixed with –70°C methanol and primary antibodies (anti-tubulin [TAT-1] 1:50 dilution) (Woods et al., 1989), a gift of K. Gull (University of Manchester, Manchester, UK); anti-tea1 1:1,000 dilution) were applied, followed by Alexa 488 goat anti–rabbit or Alexa 546 anti–mouse secondary antibodies (Molecular Probes). Immunofluorescence images were acquired with a chilled video CCD camera (model C5985; Hamamatsu) connected to a computer (Apple Power Macintosh G3/400) or with a laser scanning confocal microscope LSM510 (Zeiss Co.), and were processed with Adobe® Photoshop (v. 5.5).

Table II. ***Schizosaccharomyces pombe*** **strains used in this study**

Strain	Genotype	Reference
RB10	*ade6-M210 ura4-D18 h-*	Our stock
RB22	*leu1-32 h-*	Our stock
RB14	*tea1Δ::ura4+ ura4-D18 ade6-M210 h-*	Mata and Nurse, 1997
RB10546	*tea1GFP:kanR ura4-D18 ade6-M210 h-*	This study
RB134	*tea1YFP:kanR leu1-32 ade6-M210 ura4-D18 h-*	This study
RB100	*tea1(Δ100)GFP:kanR ade6-M216 ura4-D18 h-*	This study
RB101	*tea1(Δ200)GFP:kanR ade6-M216 ura4-D18 h-*	This study
RB135	*tea1(Δ200)YFP:kanR leu1-32 ade6-M210 ura4-D18 h-*	This study
RB116	*tea1(Δ100):kanR leu1-32 ade6-M210 ura4-D18 h-*	This study
RB106	*tea1(Δ150):kanR ade6-M210 leu1-32 ura4-D18 h-*	This study
RB120	*tea1(Δ200):kanR leu1-32 ade6-M216 ura4-D18 h-*	This study
RB3141	*pom1GFP:kanMX6 h-*	Bahler and Pringle, 1998
RB125	*pom1GFP:kanR tea1(Δ100):kanR ade6-210 h-*	This study
RB126	*pom1GFP:kanR tea1(Δ200):kanR leu1-32 ade6-210 ura4D18 h-*	This study
RB3172	*pom1GFP:kanMX6 tea1Δ::ura4 ura4-D18 h-*	Bahler and Pringle, 1998
RB112	*tea1GFP:kanR tea2Δ::his3+ his3-D1 ura4-D18 ade6-M210 h-*	This study
RB136	*tea1YFP:kanR tea2Δ::his3+ leu1-32 ade6-M210 ura4-D18 h-*	This study
RB2435	*tea2GFP:kanR ade6-M210 leu1-32 ura4-D18 h-*	Browning et al., 2000
RB129	*tea2GFP:kanR tea1(Δ100):kanR leu1-32 ura4-D18 ade6-M216 h-*	This study
RB130	*tea2GFP:kanR tea1(Δ200):kanR leu1-32 ura4-D18 ade6-M216 h-*	This study
RB2500	*tea2GFP:kanR tea1Δ::ura4+ ura4-D18 h-*	Browning et al., 2000
RB3643	*tip1GFP:kanR ura4-D18 leu1-32 ade6-M216 h+*	D. Brunner[a]
RB131	*tip1GFP:kanR tea1(Δ100):kanR leu1-32 ura4-D18 ade6-M210 h-*	This study

[a]EMBL, Heidelberg, Germany.

Time-lapse live imaging

Live images were taken at room temperature with a Zeiss Axioplan microscope equipped with a chilled video CCD camera (C4742-95; Hamamatsu) and controlled by Kinetic Imaging AQM software (Kinetic Imaging Ltd). All strains were grown to mid-log phase in EMM2 at 25°C and imaged at room temperature (23–25°C). In mixing experiments strains RB112 and RB10546 were grown separately, then aliquots were mixed followed by imaging. For time-lapse microscopy, fresh cells were taken for each film series, and cells were imaged in a single optical plane. Movies typically consisted of 50 frames (totaling ~4 min), with images acquired at 5-s intervals at varying exposure times (0.5–1 s). For microtubule live imaging, strain RB22 was transformed with plasmid pDQ105 (*nmt-GFP-atb2*) (Ding et al., 1998), cells were grown in the presence of 15 μM thiamine to repress the *nmt* promoter to a low level, and imaged for <15 min (<200 frames). For two-color YFP/CFP imaging, plasmid pRL72 (*nmt-CFP-atb2*) (Glynn et al., 2001) was transformed into strains RB134, RB135, and RB136, respectively, and transformants were imaged in the presence of 0.2 μM thiamine.

Quantification of morphology defects and microtubule curling

The level of bending/branching was determined by scoring 200 interphase cells for each strain in three independent experiments. For quantification of microtubule curling around cell ends, methanol-fixed cells were stained for tubulin and analyzed by fluorescence microscopy. For each strain, microtubules were examined in 300 interphase cells and the analysis was repeated in three independent experiments. All strains were grown in log phase in YE5S at 32°C.

Quantification of image data

The speed of movement of tea1GFP dots was determined using tracking software (Motion Analysis, Kinetic Imaging Ltd). Dots were tracked in >15 cells of each strain and movement rates were calculated by linear regression analysis over 5–20 displacements for dots moving toward cell ends, and over 4–8 displacements for dots moving toward the nucleus. Correspondingly, microtubular dynamics were determined using GFP-tubulin movies. Distances were measured using Lucida software (Kinetic Imaging Ltd). The number of dots per cell was determined by analyzing randomly selected images obtained using the same conditions. The rates of dots reaching and leaving the cell ends were determined by analyzing >25 cells for each strain for a total time of >3,000 s. Only the first 25 frames of each movie (~2 min) were examined to avoid loss of information by bleaching. Correspondingly, GFP-tubulin movies were used to determine the frequency of microtubules reaching and leaving the cell ends; 15 cells over ~9,200 s of total movie time were analyzed. The frequency of changes in the direction of dot movement outside the region of the nucleus (2.3 ± 0.3 μm, $n = 13$ cells) was determined by tracking a total of 92 dots in 18 cells.

Prediction of coiled-coil motifs

Predictions for coiled-coil domains in tea1p were obtained using the program MULTICOIL (cut off $P = 0.01$, window = 21) available at http://nightingale.lcs.mit.edu/cgi-bin/multicoil (Berger et al., 1995).

Online supplemental material

Time-lapse experiments shown in Figs. 1 B, 2 B, and 4 A are also viewable as QuickTime videos (available at http//:www.jcb.org/cgi/content/full/jcb.200112027/DC1). Video 1 shows movement of tea1GFP from the nucleus to the cell ends (Fig. 1 B); Video 2 shows movement of tea1GFP in a *tea2Δ* cell (Fig. 2 B); and Video 3 shows movement of tea1(Δ200)GFP from the nucleus to the cell ends (Fig. 4 A).

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