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On the role of microtubules, cell end markers, and septal microtubule organizing centres on site selection for polar growth in *Aspergillus nidulans*

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ABSTRACT

Tip growth of filamentous fungi depends on continuous polarized growth and requires the actin and microtubule (MT) cytoskeleton. Cortical proteins at polarity sites, also known as cell end markers, play important roles in polarity maintenance. Deletion of the cell end marker *teaA* results in zigzag hyphal morphologies, which is contrary to the normal rectilinear growth pattern. Here we studied the role of TeaA and MTs in the establishment of polarity during tip growth of *Aspergillus nidulans*, including conidia germination, second germtube formation, hyphal branching and conidiophore development. TeaA is delivered to the cortex by growing MTs. In conidia TeaA appeared at the germination site prior to germtube formation, and deletion of *teaA* resulted in germination at multiple sites, increased branching and abnormal conidiophores. The formation of a second germtube opposite the first conidial germtube depended on the presence of a septum at the base of the first germtube. An MT-organizing centre, associated to the septum, produced microtubules, which delivered TeaA towards the opposite side of the conidium. These results suggest a new function for TeaA in polarity establishment. It can be a positive function, but TeaA could also suppress polarity sites in the vicinity of the first germtube.

Introduction

Filamentous fungi have highly polarized cells that form hyphae and mycelium *via* tip growth and branching. A continuous flow of secretion vesicles, from the hyphal cell body to the growing tip, is essential for cell wall and cell membrane extension (Harris *et al.* 2005; Taheri-Talesh *et al.* 2008). Microtubules (MTs) and actin comprise the hyphal cytoskeleton and, in concert with their corresponding motor proteins, play important roles in the secretion process (Steinberg 2007a; Fischer *et al.* 2008). This indicates that cytoskeleton arrangement and organization is crucial to the establishment and maintenance of polarity. Cortical proteins, also known as cell end markers, have been shown to be associated with MT and actin cytoskeletons, as well as play important roles in the maintenance of cell polarity (Fischer *et al.* 2008).

Investigations of cell end marker function in fission yeast *Schizosaccharomyces pombe* have found that Tea1 reaches the MT plus end *via* kinesin-7 Tea2, and is then delivered *via* growing MTs to the cortex of cell ends (Mata & Nurse 1997; Browning *et al.* 2000; Browning *et al.* 2003). Tea1 is anchored to the membrane through interaction with the prenylated protein Mod5 (Snaith & Sawin 2003). At the cell end, Tea1 interacts with additional cellular components, resulting in

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recruitment of formin For3 and Cdc42 GTPase-activating protein (GAP) (Martin & Chang 2003; Feierbach *et al.* 2004; Tatebe *et al.* 2008). For3 then forms the actin cables that are required for secretion vesicle transport and polarized growth (Kovar 2006). The cell end marker Tea1 is thought to transmit MT-dependent positional information to the actin cytoskeleton, thereby contributing to polarized growth (Martin & Chang 2005; Martin 2009).

Recent studies have revealed that cell end marker proteins also contribute to polarity maintenance in the filamentous fungus *Aspergillus nidulans* and probably other filamentous fungi (Fischer *et al.* 2008). The cell end marker TeaA, the Tea1 orthologue, localizes at hyphal tips depending on MTs and anchors to the cortex tip *via* interaction with the membrane-associated protein TeaR (Takeshita *et al.* 2008). TeaA also interacts with TeaC, which possibly interacts with formin SepA (Higashitsuji *et al.* 2009). SepA, the only formin found in *A. nidulans*, localizes at hyphal tips and septation sites, and plays an essential role in tip growth and septation (Harris *et al.* 1994; Sharpless & Harris 2002). The fact that the deletion of *teaA* or *teaR* exhibits zigzag or curved growing hyphae rather than the normal rectilinear growth suggests that the polarity site is not stable at hyphal tips in the mutants. The cell end markers are thought to function in the stabilization and maintenance of polarity growth sites at tips (Fischer *et al.* 2008).

The establishment of polarity is an important process for hyphal growth. First, spores start to swell and then the polarized initial emerges. This germination process is comparable to the budding process of *Saccharomyces cerevisiae*, in which the bud site selection mechanism is well understood (Casamayor & Snyder 2002). However, since *S. cerevisiae* bud site markers are poorly conserved in filamentous fungi (Harris & Momany 2004), the mechanism of germination site selection in filamentous fungi has not yet been elucidated in these organisms. Fungal hyphae are composed of multiple compartments separated by septa. While tip compartment growth is ongoing, sub-apical compartments generate new polarized hyphae by way of lateral branching, which results in complex mycelia. The mechanism of branch site selection also remains largely unknown (Harris 2008). Polarity establishment and its dynamic regulation are also required during development. *A. nidulans* reproduces by means of asexual conidiospores (Adams *et al.* 1998). These conidiophore structures consist of a conidiophore stalk with a terminal vesicle, from which up to 70 metulae emerge. Each metula produces 2–3 phialides, which in turn continuously generate conidiospores. As a difference to hyphae, the cell cycle is strictly linked to cytokinesis during conidiation, and the formation of metulae, phialides and conidia resembles bud formation in *S. cerevisiae* (Dutton et al. 1997). Besides asexual development, *A. nidulans* is able to reproduce sexually. A complex morphological structure, the cleistothecium is produced in which meiotically formed ascospores are generated (Busch & Braus 2007).

Here, we demonstrate that the cell end marker protein TeaA localizes at the plus ends of MTs and is transported to hyphal tips *via* MT growth. We present evidence for a novel role of TeaA and MTs in polarity establishment at distinct stages and show the importance of hyphal morphology to polarization site selection.

Materials and methods

Strains, plasmids and culture conditions

Supplemented minimal medium for *Aspergillus nidulans* was prepared as described and standard strain construction procedures were described by Hill & Käfer (2001). A list of *A. nidulans* strains used in this study is provided in Table 1.

Molecular techniques

Standard DNA transformation procedures were used for *Aspergillus nidulans* (Yelton *et al.* 1984) and *Escherichia coli* (Sambrook & Russel 1999). For PCR experiments, standard protocols were applied using a personal Cycler (Biometra) for the reaction cycles.

Light/fluorescence microscopy

For live-cell imaging of germlings and young hyphae, cells were grown on cover slips in 0.5 ml MM + 2 % glucose or 2 % glycerol (derepression of the *alcA* promoter, low expression level). Cells were incubated at room temperature or 27 °C either overnight or 24 h. For short time imaging, cells were grown on cover slips in 0.5 ml medium. For long time imaging, cells were grown in FluoroDish, cover-glass bottom culture

Table 1 – *A. nidulans* strains used in this study. All strains harbor in addition the *veA1* mutation.

Strain	Genotype	Source
TN02A3	*pyrG89; argB2, ΔnkuA::argB; pyroA4*	(Nayak *et al.* 2006)
GR5	*pyrG89; pyroA4*	(Waring *et al.* 1989)
SJW02	*pyroA4; alcA$_{(p)}$-GFP-tubA; ΔargB::trpCΔB*	J. Warmbold, Marburg, Germany
SNT49	*teaA$_{(p)}$–mRFP1-teaA; pyroA4*	(Takeshita *et al.* 2008)
SNT56	*alcA$_{(p)}$–GFP-teaR; teaA$_{(p)}$–mRFP1-teaA*	(Takeshita *et al.* 2008)
SSK91	*ΔteaA::argB; pyrG89; ΔargB::trpCΔB; pyroA4* (Δ*teaA*)	(Takeshita *et al.* 2008)
SNT21	*pyroA4; alcA$_{(p)}$-GFP-tubA; ΔargB::trpCΔB;* pDC1(plasmid including *argB*)	(Takeshita *et al.* 2008)
SNT52	*pabaA1; teaA$_{(p)}$–mRFP1-teaA; ΔargB::trpCΔB*	(Takeshita *et al.* 2008)
SDV25	*alcA$_{(p)}$–GFP-kipA; apsB6*	(Veith *et al.* 2005)
SYH03	*alcA$_{(p)}$–GFP-teaC; pyroA4*	(Higashitsuji *et al.* 2009)
SNT65	SNT21 crossed to SNT52, (*alcA$_{(p)}$-GFP-tubA*, *teaA$_{(p)}$–mRFP1-teaA*)	This study

dishes, FD35-100 (WPI). Benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate (Aldrich Chemical, Milwaukee, WI), was used at 0.05–1 μg/ml in medium from a stock solution of 50 mg/ml in dimethyl sulfoxide (DMSO). Images were captured at room temperature using an Axiophot microscope (Zeiss, Jena, Germany) using a Planapochromatic 63× oil immersion objective lens, the Zeiss AxioCam MRM camera (Zeiss, Jena, Germany), and the HBO103 mercury arc lamp (Osram). Images were collected and analyzed with the AxioVision system (Zeiss). A Lambda DG4 system (Sutter Instrument) including 175 W light source and high-speed wavelength switching system, has been used. Images were collected and analyzed with the AxioVision system (Zeiss).

Results

TeaA is transported to hyphal tips via microtubule growth

In fission yeast, it has been shown that Tea1 is transported by means of kinesin-7 Tea2 towards microtubule (MT) plus ends and travels to the cell ends *via* MT growth (Browning *et al.* 2003). In *Aspergillus nidulans*, an analysis using the MT-destabilizing drug benomyl showed that TeaA localization at hyphal tips also depended on MTs (Takeshita *et al.* 2008), and that low benomyl concentrations (0.3 μg/ml) impaired directional growth to a similar extent as deletion of cell end markers

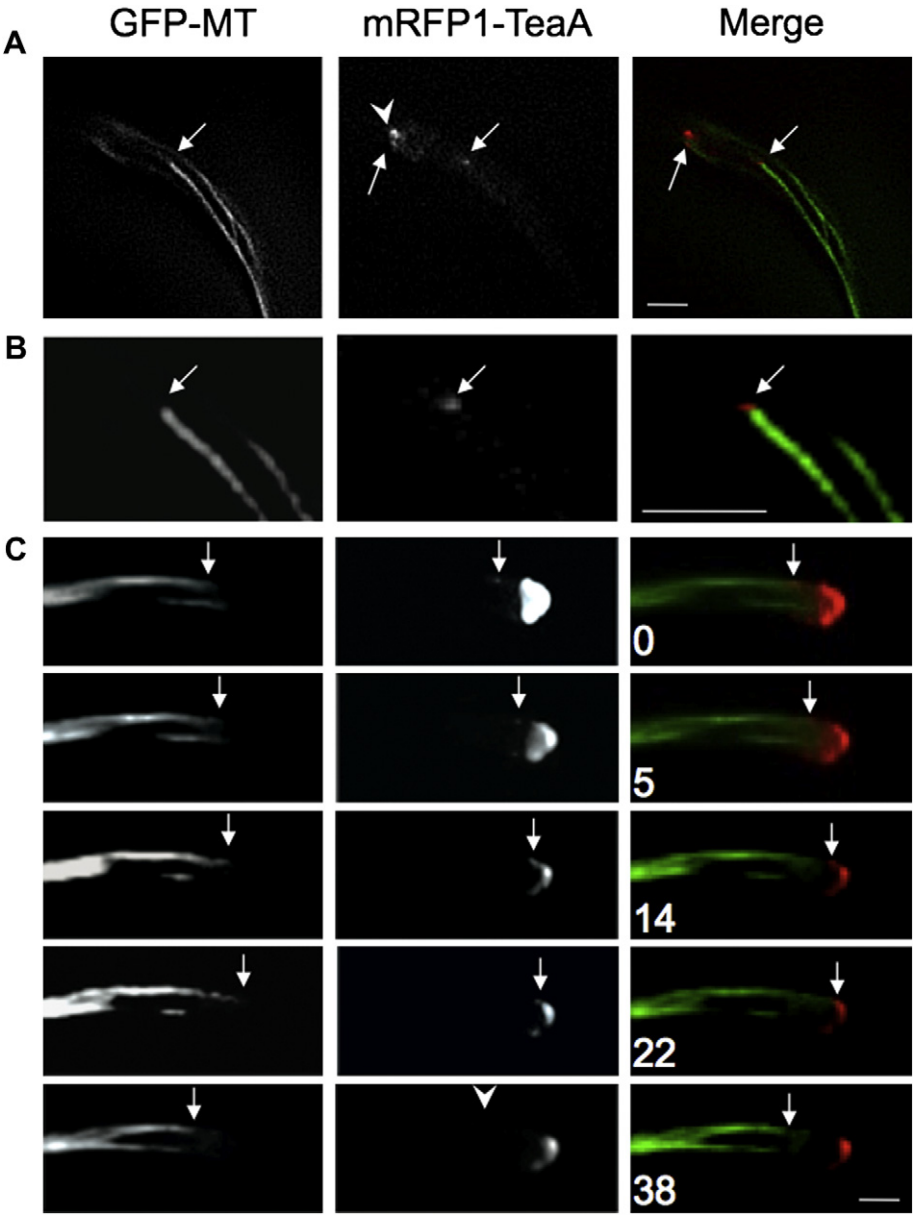

Fig 1 – mRFP1-TeaA localization at growing MT plus ends. (A) mRFP1-TeaA localized at hyphal tips (arrowhead) and small TeaA dots in the cytoplasm co-localized with MT plus ends (arrows). (B) TeaA enlargement at a MT plus end (arrow). (C) The TeaA dots were observed at growing MT plus ends (arrows), but not during shrinkage of corresponding MTs upon reaching the tip (arrowhead). Elapsed time is reported in seconds. SNT65 (GFP-MTs, mRFP1-TeaA) was grown on minimal medium with glycerol as carbon source. Scale bars are 2 μm.

(Supplementary Fig 1A) and remarkably decreased the signal intensity of TeaA at tips (Supplementary Fig 1B, C). These data suggest a conserved transport mechanism. In order to obtain direct evidence for TeaA localization at the MT plus end, we fused TeaA to mRFP1 and alpha-tubulin to GFP. TeaA was present at hyphal tips (Fig 1A, arrowhead) and very weak mRFP1-TeaA signals were indeed visible at the MT plus ends (Fig 1A,B, arrows). The mRFP1-TeaA dots moved to hyphal tips as MTs elongated (Fig 1C arrows), but mRFP1-TeaA dots were not observed at shrinking MTs (Fig 1C arrowhead).

Role of TeaA and microtubules in germination

While the role of TeaA in polarity maintenance of mature hyphae has been well studied, we analyzed the importance of this protein to initial germtube formation. Time-lapse imaging revealed that mRFP1-TeaA appeared at growth sites during conidia germination (Fig 2A and Movie 1). GFP-labelled MTs displayed dynamic elongation and shrinkage prior to germination (Fig 2B). As soon as a small germination bud appeared, some MTs contacted the cortex of the tip (Fig 2C), while the polarized MTs likely deliver

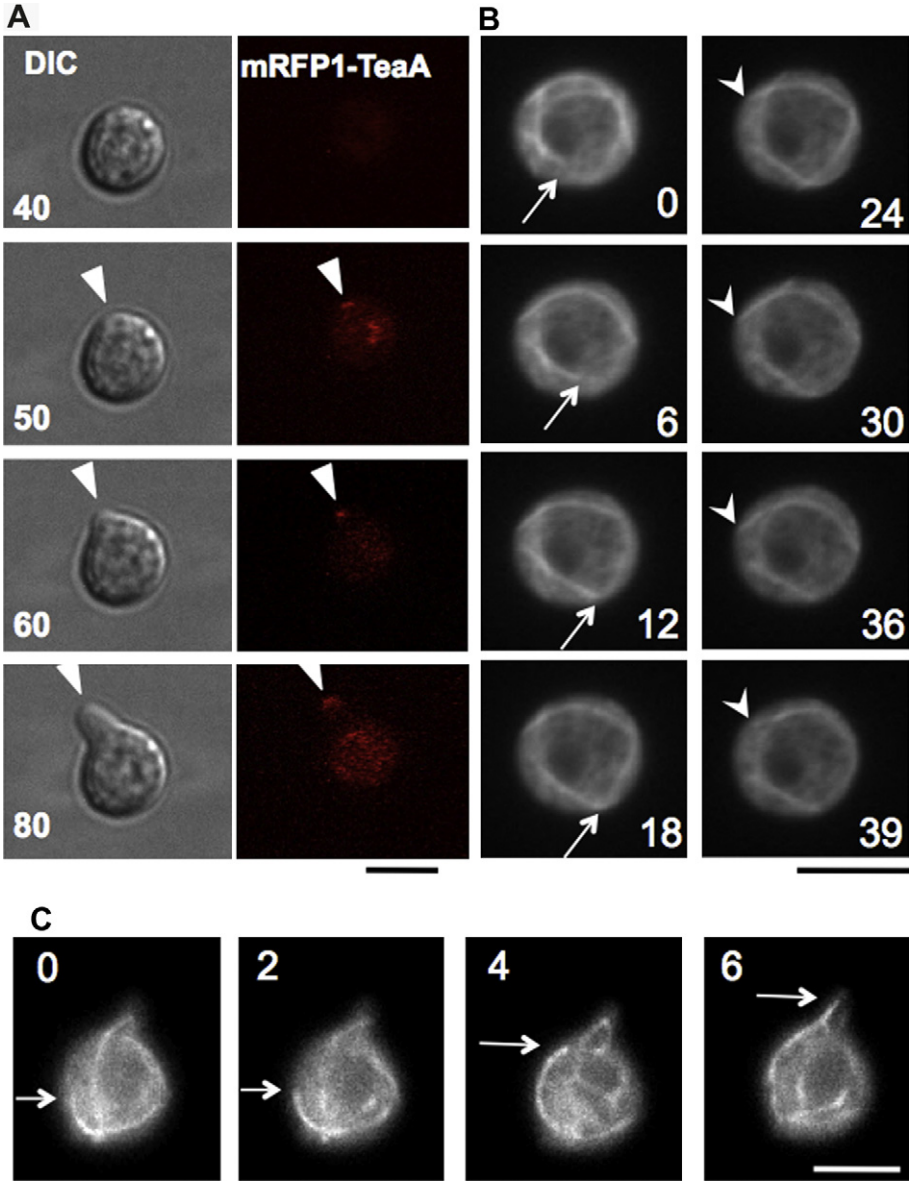

Fig 2 – Localization of TeaA and MT organization during germination. (A) mRFP1-TeaA localized at the germination site. Spores of SNT49 strain were incubated in minimal medium with glucose at room temperature. Elapsed time is given in 10 min increments. (B) GFP-MTs displayed dynamic elongation and shrinkage in spores prior to germination. Dynamic MT plus ends were marked by arrows and arrowheads. (C) After a small germination bud had appeared on the spore, some MTs elongated into the emerging germtube and attached to the hyphal tips. (B, C) Spores of SJW02 were incubated in minimal medium with glycerol at room temperature. Elapsed time is given in second increments. The scale bars are 5 μm.

more TeaA and other proteins to the bud site, enforcing polar growth.

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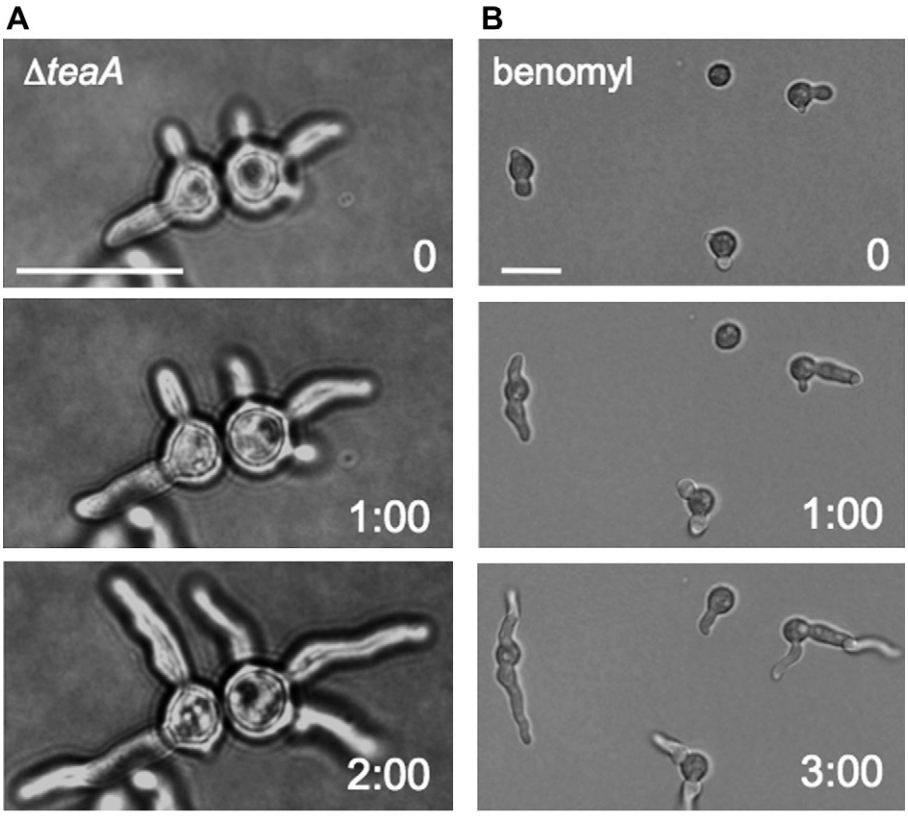
In order to further investigate the function of TeaA on germination, a *teaA*-deletion strain was analyzed. In contrast to wild-type spores, which always germinate at only one site to form one hypha, spores of the *teaA*-deletion strain often germinated at two (40 %) or three sites (3 %) simultaneously (Fig 3A,C). We restricted our investigations here to spores with germtubes shorter than 10 μm, in order to avoid counting spores that produced additional germtubes after the first had grown beyond a certain length (see below). A similar phenotype of multi-germtube formation was observed using the MT-destabilizing drug benomyl. It had been shown already that MTs are not essential for the germination process itself (Oakley & Morris 1980). Our results indicate that TeaA and MTs are not necessary for the emergence of the



C

WT	100	0	0
Δ*teaA*	57	40	3
benomyl	54	42	4

(%, N=100)

Fig 3 – Deletion of *teaA* and a MT inhibitor result in germination at multiple sites. (A, B) Spores frequently germinated at multiple sites simultaneously. (A) Spores of SSK91 strain (Δ*teaA*) were grown in minimal medium with glucose and (B) spores of TN02A3 strain (wild type) were grown in the same medium supplemented with 1.0 μg/ml benomyl at room temperature. Elapsed time is reported in hours. The scale bars are 10 μm. (C) Quantification of the number of germlings with the indicated germination pattern. For each strain, 100 germlings were counted.

germtube, but probably rather for restricting germination to a specific place.

Formation of the second germtube depends on septation

Once the first hypha reaches a determinate length, a second germtube appears on the spore. This second germination site normally lies opposite of the first hypha (Fig 4A arrowheads, Movie 2, and Fig 6B) (Harris *et al.* 1999). Using time-lapse experiments, we observed that the second germtube appeared after the first septum at the base of the first hypha was formed (Fig 4A arrows, and Movie 2). Septa were normally located close to the spore within a distance of less than 10 μm (88 % of the spores) (Fig 4C). In order to confirm that formation of a second germtube is dependent on septation, the percentage of germlings with/without a second germtube, and with/without a septum was calculated. Our results showed that germlings possessing a septum could be lacking a second germtube, but germlings lacking a septum never had a second hypha (Fig 4B), suggesting that the formation of a second germtube opposite the first conidial germtube depends on the presence of a septum at the base of the first germtube.

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TeaA localizes at the second germination site

TeaA localization was analyzed using time-lapse imaging during the formation of the second germtube (Fig 5A). mRFP1-TeaA appeared at the second germination site, opposite the first hypha, after septation in the first hypha. Given that the polarization of TeaA depends on MT structure (see Fig 1), the next question was how the MT cytoskeleton is organized in germlings, and how MTs become oriented towards the germination site opposite the first germtube. In *Aspergillus nidulans*, MTs are formed from the spindle pole body (SPB) and from septum-associated MT-organizing centres (septal MTOCs) (Konzack *et al.* 2005; Veith *et al.* 2005; Xiong & Oakley 2009). MTs emanating from the septum of the first hypha grew towards the first germtube as well as in the direction of the spore and reached the second germination tip where TeaA localized (Fig 5B). These findings suggest that MTs originating from the septal MTOCs are important for TeaA delivery and site selection for the second germtube. In comparison, SPBs moved randomly in the spore (data not shown), suggesting no correlation between the SPB location and the second germination site selection.

We analyzed a *teaA*-deletion strain, to further investigate the role of TeaA on second germination site selection. The second germtube often appeared at random positions on the

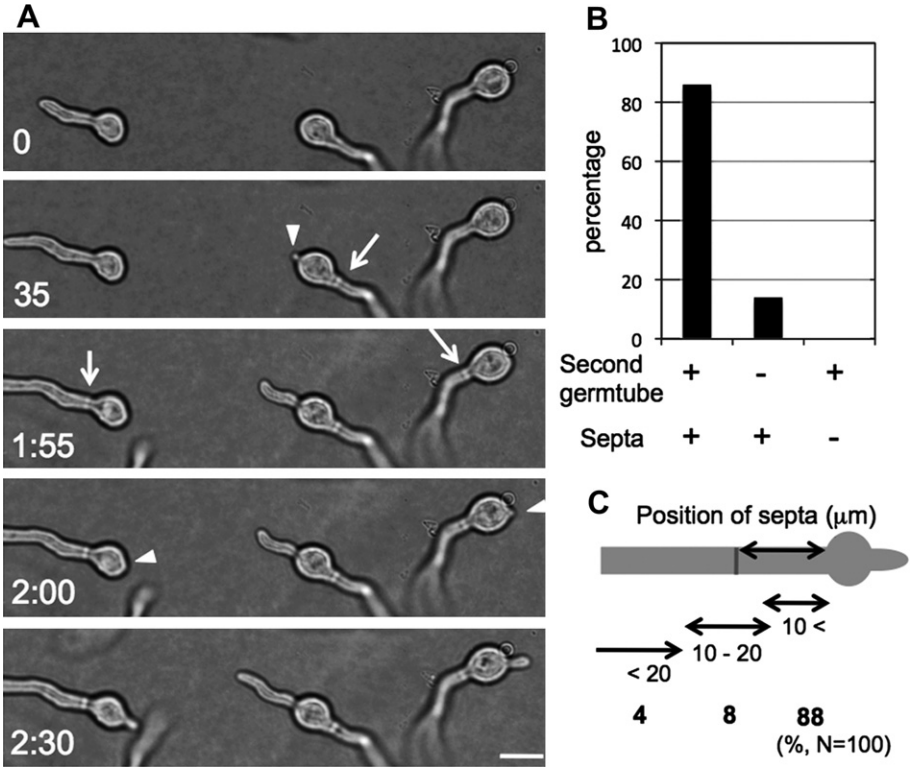

Fig 4 – The formation of a second germtube is septation-dependent. (A) A second germtube normally appeared at a location on the spore opposite the germination site of the first hypha (arrowheads), but only once the first hypha had septated (arrows). Spores of a wild-type strain (TN02A3) were grown in minimal medium with glucose at room temperature. Elapsed time is reported as hours:minutes. The scale bar is 5 μm. (B) Germling quantification with/without a second germtube and with/without septa. (C) Quantification of the septal position in the first germtube. 100 germlings with second germtubes were counted.

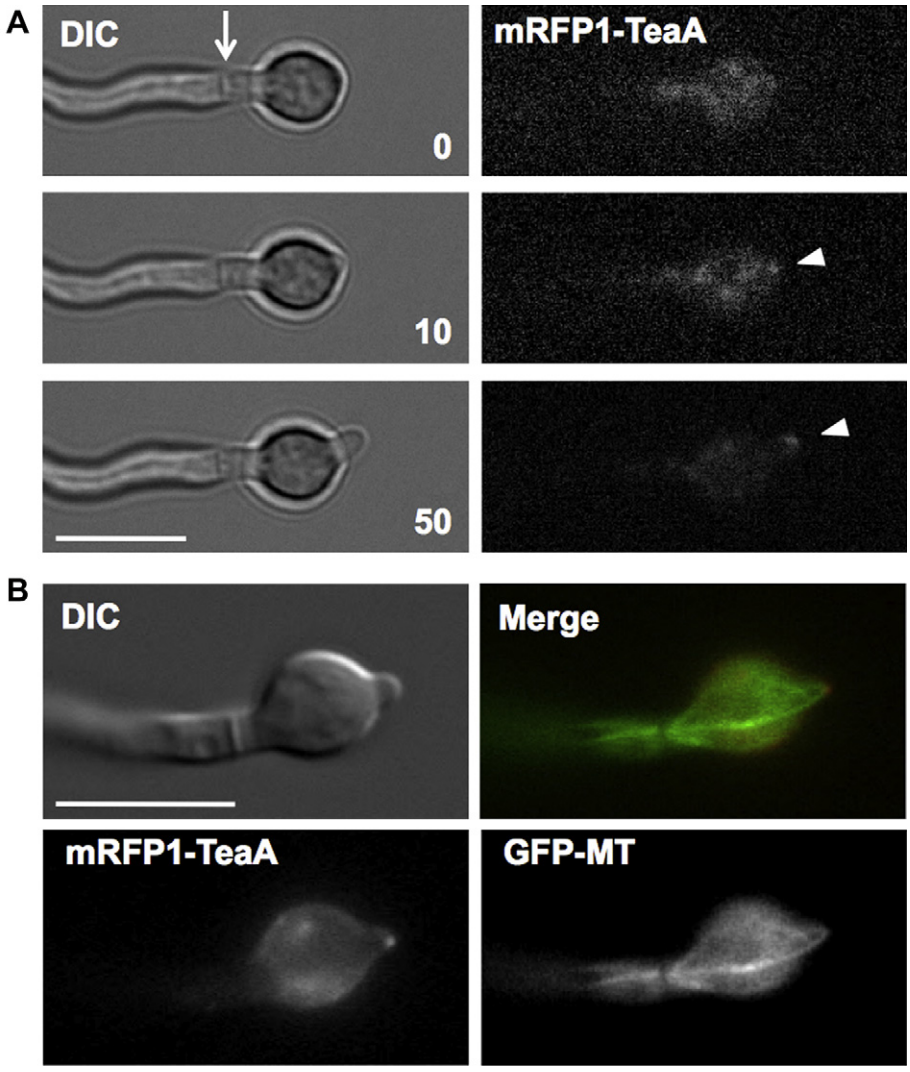

Fig 5 – TeaA localization at the second germination site. (A) Time course of the second germination. mRFP1-TeaA localized at the second germination site. Spores of SNT49 (mRFP1-TeaA) were incubated in minimal medium with glucose at room temperature. Elapsed time is reported in minutes. The scale bar is 5 μm. (B) Localization of TeaA and MTs during the second germination. mRFP1-TeaA localized at the second germtube tip and GFP-MTs elongated from septal MTOC to the second germtube tip. SNT65 (GFP-MTs, mRFP1-TeaA) was grown in minimal medium with glycerol. The scale bar is 5 μm.

conidium and, sometimes (9 %), at two sites simultaneously (Fig 6A,B). These results show that TeaA is required for specific site selection of the second germtube. To distinguish germlings possessing multiple germtubes (see Fig 3), we selected germlings with one long hypha and one or two short second germtubes for counting. Such germlings had a septum at the base of the first hypha.

An *apsB6* mutant was analyzed in order to further investigate the function of MTs, derived from septal MTOC, in the selection of a site for second germination. ApsB is a MTOC component that interacts with gamma-tubulin (Zekert *et al.* 2010). The *apsB6* mutant was shown to form fewer MTs out of SPBs, compared to the wild type (less than 70 %), and substantially fewer MTs from septa (less than 40 % compared to wild type) (Veith *et al.* 2005). The *apsB6* mutant also showed defects in selection of the second germination site. Interestingly, 35 % of the spores did not produce a second germtube from the spore but produced a second hypha by branching from the first hypha between septum and spore (Fig 6B, C), although the timing of septation in the first hypha and branching was not clear in the analysis. These results suggest that MTs originating from the septum in the first hypha are important for second germtube formation from the spore. Other means of determining the importance of septal MTOCs involved in addition the use of a *teaC* overexpressing strain. TeaC links TeaA and formin, and septation is inhibited upon overexpression of the gene (Higashitsuji *et al.* 2009). More than 80 % of the spores of the strain did not form a second hypha from the spore under conditions, where no septum is formed (Fig 6C), while almost all the wild-type spores formed the second hypha under the same conditions.

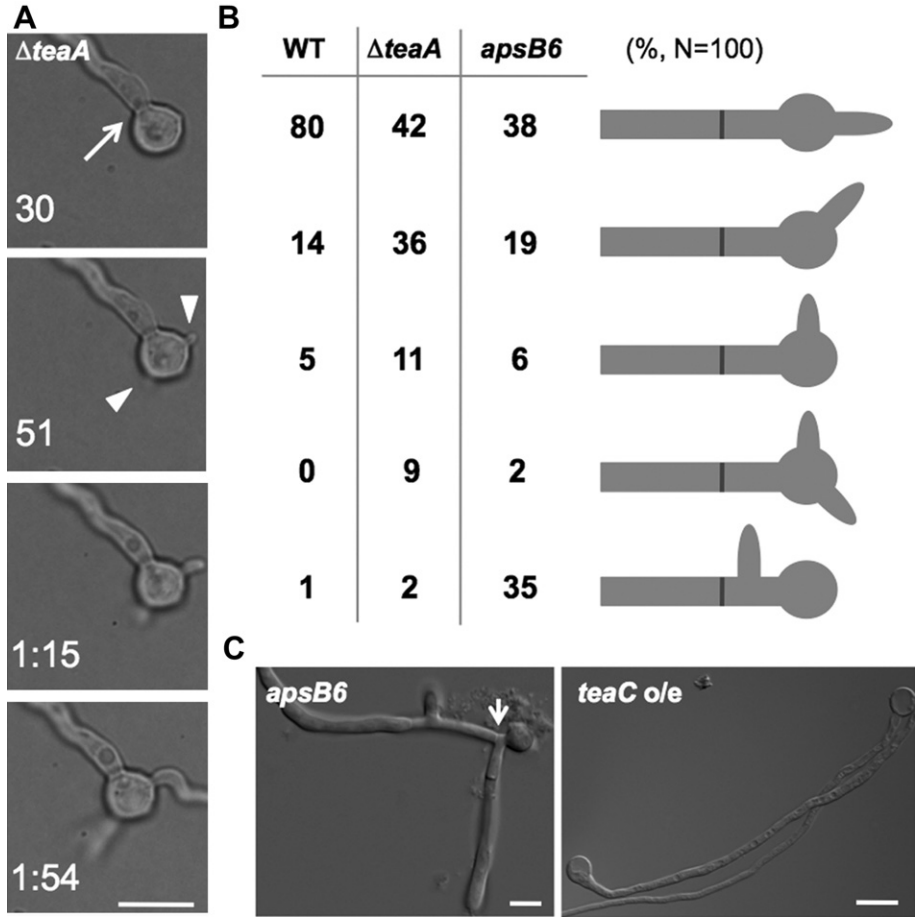

Fig 6 – Formation of a second germtube depends on septation and septal MTOC function. (A) Time course of the second germination in the Δ*teaA* strain (SSK91). Second germtubes appeared at two sites after septation of the first hypha. The second germination site is marked by arrowheads and the septum is marked by an arrow. Elapsed time is reported as hours:minutes. The scale bar is 5 μm. (B) Quantification of the number of germlings with a second germtube in the wild-type strain (TN02A3), the Δ*teaA* strain (SSK91), and the *apsB6* mutant (SDV25). Spores were incubated in minimal medium with glucose at room temperature. For each, 100 germlings were counted and grouped into five categories according to the position of the second germtube or branch. (C) Germlings without second germtube from the spores in an *apsB6* mutant (SDV25) and a *teaC* overexpression strain (SYH03 grown in minimum medium with 2 % threonine) despite the extremely long first germtube. Septum is marked by an arrow. The scale bars are 5 μm.

TeaA localizes at branch sites

Deletion of *teaA* increased hyphal branching, particularly in mature hyphae (Takeshita *et al.* 2008). Time-lapse imaging showed mRFP1-TeaA dot at branch site and was also localized at branch tips (Fig 7). These results suggest that TeaA is involved in branch formation, although an increased branching frequency may be an indirect effect due to the reduced growth rate of *teaA*-deletion strains.

TeaA localization during conidiophore development

In order to investigate the function of TeaA in polarity establishment during conidiophore development, the localization of mRFP1-TeaA was analyzed at different stages of conidiophore development. There are three stages, which require polarity establishment, (1) formation of metulae emerging from the vesicle of the conidiophore, and (2) formation of phialides from the tips of metulae and (3) production of conidiospores at the tips of phialides. However, one important difference is that in the case of metula formation up to 70 polarity sites need to be selected simultaneously, whereas for phialide and conidia production only one site at a time is required. Interestingly, no mRFP1-TeaA signals were observed at the cortex of the conidiophore vesicle prior to metulae formation, but appeared at the tip of elongated metulae prior to phialide formation, and at the tip of phialides (Fig 8A–C). There were no signals in the chain of conidia between the conidiospores (Fig 8D). This localization pattern might suggest that TeaA is not involved in budding but bud elongation. On the other hand, it could be that TeaA has a repressing effect on polarity establishment in its vicinity and therefore is not used for the budding process during metulae formation. Deletion of *teaA* also sometimes caused abnormal conidiophore development

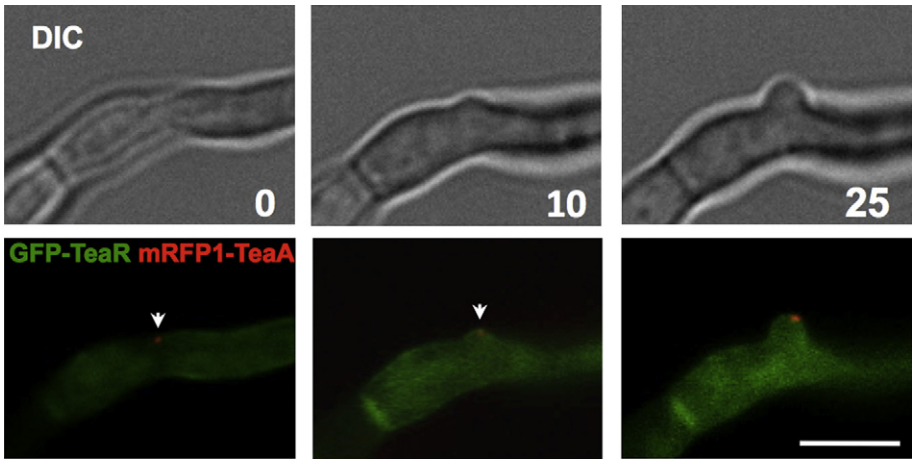

Fig 7 – Localization of TeaA during branching. TeaA localized at the branch site. The SNT56 strain (mRFP1-TeaA, GFP-TeaR) was incubated in minimal medium with glycerol at room temperature. Elapsed time is reported in minutes. The scale bar is 5 μm.

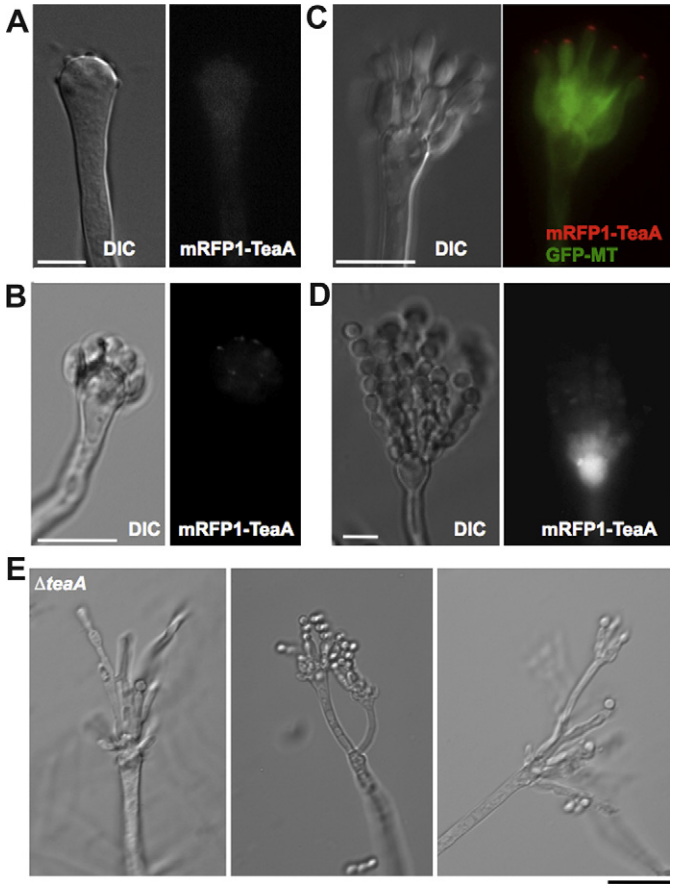

Fig 8 – Role of TeaA in conidiophore development. (A–D) Localization of mRFP1-TeaA during conidiophore development in a SNT65 strain (GFP-MTs, mRFP1-TeaA). TeaA did not localize in young metulae (A), but at the tips of mature metulae (B) and phialides (C). TeaA did not localize at conidia, but at septa between conidia and phialides (D). The scale bars are 10 μm. (E) Abnormal morphology of conidiophores in a Δ*teaA* strain (SSK91). Long metulae (left), branched conidiophore (middle) and secondary conidiophores (right) were visible. The scale bar is 20 μm.

(<10 %), such as elongated metulae, branched conidiophores and secondary conidiophores (Fig 8E).

Discussion

In this paper, we investigated the roles of the cell end marker TeaA and MTs on polarity establishment. TeaA localized at first and second germination sites, branching sites and the tips of metulae and phialides in conidiophores. Deletion of *teaA* or the application of MT inhibitors caused multiple germtube formation. After septation of the first hypha, the second germtube emerged at a location opposite to where the first hypha had developed. Our results suggest that TeaA and MTs from septal MTOC in the first hypha are involved in site selection of the second germination sites.

The role of interphase MTs in polarized growth has been studied in different fungi and as a main function motor-driven vesicle transportation along the cytoskeleton has been shown (Seiler *et al.* 1997; Requena *et al.* 2001; Horio & Oakley 2005; Steinberg 2007b; Zekert & Fischer 2009). However, the result that MT inhibitors impaired the growth direction had suggested another role of MTs on polarity maintenance (Riquelme *et al.* 1998). The result that TeaA localizes at plus ends of MTs and is transported by growing MTs to the tip of hyphae, with consideration of previous work, suggests that interphase MTs transmit positional information to hyphal tips (Fischer *et al.* 2008; Takeshita *et al.* 2008; Higashitsuji *et al.* 2009). Here, we present evidence that TeaA is also involved in the establishment of polarity in germinating conidia. MTs are not essential for germination itself (Oakley & Morris 1980), but without MTs and TeaA, germination often occurred at multiple sites. TeaA appeared to localize at the germination site just prior to germination (Figs 2, 5 and 7) suggesting a very early role of TeaA in the process. However, it remains to be solved if and which other factors exist prior to TeaA localization. Several cortical landmarks determine axial budding or bipolar budding in budding yeast (Casamayor & Snyder 2002). Given that conidia in *Aspergillus nidulans* are formed in a budding-like process at the tip of phialides, the bud scar in each spore has the potential to mark the future germination site. Despite the similarities in the morphology of the two processes, *Saccharomyces cerevisiae* bud site markers are poorly conserved in *A. nidulans* (Harris & Momany 2004). Clearly more work is required to fully understand the sequence of germination site selection, MT polarization, deposition of TeaA and germtube formation.

The proposed positive function of TeaA in the germination site selection process is mainly based on the proposed function in the maintenance of polarized growth through the polarization of the actin cytoskeleton. However, TeaA could also serve a negative function to suppress polarity establishment at multiple sites. Such a suppressor function could perhaps explain the observation that the deletion of *teaA* caused germination at multiple sites and that the simultaneous formation of up to 70 metulae from the vesicle dome of the conidiophore stalk appeared to be independent of TeaA. Such an explanation is also supported by evidence in *Saccharomyces pombe*, where it was shown that the deletion of cell end marker proteins exhibited T-shaped branched cells (Mata & Nurse 1997). Moreover, although in different processes, cell end marker proteins have a suppressor function to inhibit the division-septum assembly at cell ends (Huang *et al.* 2007).

Another surprising phenomenon concerns formation of the second germtube in *A. nidulans*. The second germtube normally emerges at a site opposite where the first hypha developed, depending on the integrity of the actin and MT cytoskeletons, and results in a bipolar germination pattern (Harris *et al.* 1999). Here, we discovered that MTs emanating from septal MTOC appear to be particularly important for this bipolar germination pattern. Septa are formed perpendicular to hyphae probably because the shrinkage of the actomyosin ring prior to septum formation ensures the shortest distance within the hypha. Septa are formed at the base of the first germtube, then septal MTOCs polymerize MTs towards the growing tip of the first germtube, as well as in the direction of the spore. Due to the stiffness of the MTs and the geometry of the hyphae, the main growth direction of the MTs is perpendicular to the septum and along the longitudinal axis of the germling (Fig 9, left). This is an example of how hyphal morphology and cytoskeletal arrangement may determine new sites for polarized growth. This transmission of positional information by MTs from septal MTOC might be applied to tip growth of hyphae, too (Fig 9, right). The possibility that other proteins also play a role, such as the proteins involved in the bipolar budding of *S. cerevisiae*, cannot be excluded at the moment (Harris & Momany 2004). The model presented here does not explain yet how branch sites are selected. Although a random pattern of branching is observed in *A. nidulans*, the mechanism of branch site selection remains largely unknown (Harris 2008).

The establishment of polarity during germination is comparable to the bipolar growth model in fission yeast. In *S. pombe* MTs originating from the nuclear envelope are

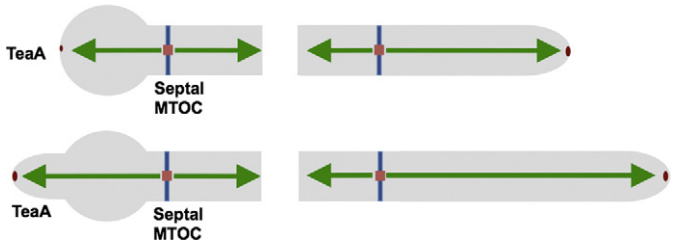

Fig 9 – Model of second germination site selection. Septa are formed perpendicular to the hypha tube. After septation at the base of the first hypha, MTs emanating from the septal MTOC grow bi-directionally along the hyphal axis. The MTs growing into the spore reach the spore cortex at the opposite site of first hypha development and deliver TeaA there.

organized in anti-parallel bundles (Sawin & Tran 2006), and after cellular fission, MTs transmit positional information from the old end to the new end *via* transport of cell end markers (Martin & Chang 2005; Martin 2009). In *A. nidulans*, MTs from septal MTOCs are organized in opposite directions and play a role in the transmission of positional information.

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Supplementary data

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