Genetic evidence for a microtubule-capture mechanism during polar growth of

Aspergillus nidulans

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Abstract

The cellular switch from symmetry to polarity in eukaryotes depends on the microtubule and actin cytoskeletons. In fungi such as *Schizosaccharomyces pombe* or *Aspergillus nidulans*, the microtubule cytoskeleton determines the sites of actin polymerization through cortical cell-end marker proteins. Here we describe *A. nidulans* MigA (*microtubule guidance*) as the first orthologue of the karyogamy protein Kar9 from *Saccharomyces cerevisiae* in filamentous fungi. *A. nidulans* MigA interacts with the cortical ApsA protein and is involved in spindle positioning during mitosis. MigA is also associated with septal and nuclear microtubule organizing centers (MTOCs). PALM super-resolution microscopy revealed that MigA is recruited to assembling and retracting microtubule plus-ends in an EbA-dependent manner. MigA is required for microtubule convergence in hyphal tips and plays a role in correct localization of the cell-end markers TeaA and TeaR. In addition, MigA interacts with a class V myosin, suggesting an active microtubule capture and pulling mechanism of microtubule ends along actin filaments. Hence, the organization of microtubules and actin depend on each other, and positive feedback loops ensure robust polar growth.
Introduction

Polarity establishment and maintenance is an essential mechanism conserved from simple unicellular organisms to higher eukaryotes. It plays an important role in various biological processes such as embryogenesis, organogenesis, cell morphogenesis or asymmetric cell division. Neurons are among the most polarized cells and the actin and microtubule (MT) cytoskeletons play essential roles for correct guidance of axons (Dent et al., 2011).

Simple models for polarized growth are the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe but also filamentous fungi such as Aspergillus nidulans or Neurospora crassa (Arkowitz, 2011; Casamayor and Snyder, 2002; Peñalva, 2010; Riquelme, 2013; Takeshita et al., 2014). In filamentous fungi polarized growth is the dominant growth form and requires continuous extension of the hyphal tip with massive transport of enzymes, and cell wall and plasma membrane components. The actin and MT cytoskeletons along with their respective motor and other associated proteins play crucial roles in these transport processes and are also required for establishing and maintaining the polarity axis (Fischer et al., 2008; Takeshita et al., 2014). Whereas MTs emerge from spindle pole bodies and septal MTOCs and span the entire hyphae, the actin cytoskeleton is organized very differently (Konzack et al., 2005). Actin patches are found along the hyphae at the cortex and actin filaments emerge mainly from the hyphal tip and are restricted to a short area behind the tip (Upadhyay et al., 2008). The two cytoskeletons are linked through a class of cortical proteins, restricted to the apex. They are called cell-end marker proteins and were discovered in S. pombe (Snell and Nurse, 1994). Here, one key protein is Mod5, which is prenylated and serves as an anchor for further proteins in the apical membrane (Snaith et al., 2003). It recruits other cell-end marker proteins like Tea1 and ultimately the formin For3, which polymerizes actin cables (Feierbach and Chang, 2001). Tea1 is associated with MT plus ends and is delivered by growing MTs (Mata and Nurse, 1997). Hence the MT cytoskeleton organizes the actin cytoskeleton. In A. nidulans cell-end markers are essentially conserved, although sequence similarities are in general very low (Higashitsuji et al. 2009; Takeshita et al., 2008). In contrast to S. pombe MTs converge at one prominent spot at the hyphal tip in A. nidulans. This convergence depends on TeaA (Tea1) and TeaR (Mod5) (Takeshita et al., 2008). In addition, it was also shown that the microtubule polymerase AlpA (XMAP215) interacts with TeaA at the cortex and that polymerase activity is controlled by AlpA (Takeshita et al., 2013). However, the exact mechanism how MTs converge into a single spot remained unclear. One could hypothesize that growing MTs follow the dome-shaped hyphal apex passively, although this would not explain the observed
misguided MTs in the absence of TeaA or TeaR. An alternative mechanism would involve active MT capture and guidance. This hypothesis is based on a model in S. cerevisiae.

In S. cerevisiae polarized growth is restricted to a short time of the cell cycle (Martin et al., 2014). When the yeast cell forms a daughter bud, the nucleus divides and migrates to the budding neck. This migration depends on astral MTs, which contact the cortex and are subsequently pulled by dynein. In addition to the so-called dynein pathway, a second pathway has been described, which ensures proper spindle alignment and nuclear migration during mitosis (Liakopoulos et al., 2003; Miller and Rose, 1998). The key component of this pathway is Kar9. It localizes initially to the spindle pole body (SPB) but remains only at the SPB facing the daughter. This asymmetry involves multiple phosphorylations of Kar9 by the human Clip-170 orthologue Bik1 and the Clb4/Cdc28 complex at the SPB, which remains in the mother cell (Liakopoulos et al., 2003; Maekawa et al., 2003; Moore and Miller, 2007; Pereira et al., 2001). After loading Kar9 onto the MT, it is Bim1 dependently transported to the MT plus-end, which classifies Kar9 as a MT plus-end associated protein (+TIP) (Akhmanova and Steinmetz, 2010; Liakopoulos et al., 2003; Miller et al., 2000). Once a MT plus-end reaches the actin cables, emerging from the bud tip, Kar9 interacts with the class V myosin Myo2, which in turn pulls Kar9, the attached MT and the SPB along an actin cable into the daughter cell. (Beach et al., 2000; Hwang et al., 2003; Lee et al., 2000; Liakopoulos et al., 2003; Miller et al., 2000; Yin et al., 2000). Hence actin cables guide MTs towards the bud tip.

In this work, we describe MigA (Microtubule guiding protein A) as the first orthologue of Kar9 in filamentous fungi. A. nidulans MigA is involved in mitotic spindle positioning, and also in MT capture at the hyphal tip. Furthermore, it is required for cell-end marker positioning and, thereby, for the organization of the MT and actin cytoskeletons during polar growth.

Results
Identification of a Kar9 orthologue in A. nidulans
The A. nidulans database (www.aspgd.org) was searched for proteins with sequence similarity to S. cerevisiae Kar9 (Cerqueira et al., 2013). The best candidate was AN2101, although the similarity was restricted to a short stretch and the e-value was only 3e-06, but the two protein sequences showed a high overall identity of 22 %. Nevertheless, here we present strong evidence that the two proteins are orthologues. Since the abbreviation kar was already used in A. nidulans, we named the gene migA to refer to the proposed function of microtubule guidance (see below). The migA gene does not contain introns (RNAseq data); the derived protein product consists of 1010 amino acids, with a calculated molecular mass of 109.75 kD and an
isoelectric point of 9.01 (Fig. 1A). Pfam analysis revealed similarities of the region ranging from amino acid 300 to 1004 to the Kar9 protein family, with a bit score of 683.3 and an e-value of 2.4e-205 (Finn et al., 2014). Further analyses revealed other conserved structural features of the two proteins (Fig. 1A). Two putative dimeric coiled coil domains were identified, one between amino acids 573 and 607 and another one between amino acids 692 and 719, by using the Multicoil algorithm with a maximum search window length of 28 and a p-score of 0.97 and 0.59, respectively (Wolf et al., 1997). Within the alkaline C-Terminus of MigA, a SxIP motif was found at position 873 to 876 (STIP). Such a motif is also present in Kar9, APC and other proteins known to bind EB1 and, hence, is a +TIP localization signal (as reviewed by Honnappa et al., 2009). Phosphorylation sites essential for asymmetric loading onto SPB’s in S. cerevisiae, as described by Liakopoulos et al. 2003, were not found in MigA, although it possesses numerous other predicted phosphorylation sites (data not shown). The MigA protein is well conserved in other filamentous ascomycetes. For instance, A. nidulans MigA shares 59 % sequence identity with its orthologue in Penicillium chrysogenum, and 43 % with the one in Neurospora crassa (Fig. 1B and Supplemental Fig. 1A).

**Deletion of migA partially phenocopies mutations in cell-end marker genes**

To characterize the function of MigA in vivo, a migA-null mutant was created (Fig. 2A, Supplemental Figs. 2A, B, C). The migA knock-out cassette was obtained from the Fungal Genetics Stock Center (Kansas State University, Manhattan, USA) and transformed into the nku-deletion strain TN02A3. To ensure that the phenotypes are not caused by the nkuA deletion, we back-crossed a ∆migA strain to an A. nidulans wild type (SRF201) and selected a ∆migA, nku+ strain. Colonies of all three strains grew as fast as wild type colonies (Fig. 2A). However, hyphal morphology was affected and resembled the phenotype of mutants lacking the kinesin-VII KipA or cell-end markers such as TeaA or TeaR (Higashitsuji et al., 2009; Konzack et al., 2005; Takeshita et al., 2008). Deletion mutants lacking TeaA, TeaR or KipA failed to maintain the internal polarity axis, resulting in curved or zig-zag growth patterns, which is most apparent in medium with 2% glucose as carbon source. Furthermore, tip splitting could be observed (Fig. 2A, B, D). In addition, polarity establishment as required during germination of conidiospores was affected. The angle of emerging secondary hyphae was significantly different from wild type, and a third germ tube occurred more frequently. This resembled the effects of a lacking cell-end marker TeaA (Figure 2 B, C).
MigA localizes to mitotic spindles and facilitates contact between astral MTs and cortical ApsA
To determine the localization of MigA, eGFP was fused to the C-terminus of MigA and expressed under the control of the endogenous promoter. MigA:eGFP localized along the mitotic spindle including SPBs (Fig. 3A). In addition, a small cluster was found at septa (data not shown). This suggests that MigA is present at septal and nuclear MTOCs. The localization of the protein appeared very dynamic, and the signal intensities at the SPBs changed over time before it appeared at astral MT (Fig. 3A, Supplemental M1). When cells were treated with benomyl, MigA localized in clusters at the plasma membrane (Supplemental Fig. 3A). To test whether MigA interacts with the cortical protein ApsA (Fig. 3D, Supplemental Fig. 3B) (Fischer and Timberlake, 1995) in the same fashion as S. cerevisiae Kar9 interacts with Num1 (Farkasovsky and Kuntzel, 2001), bimolecular fluorescence complementation (BiFC) and yeast two-hybrid analyses were performed. BiFC analysis showed an interaction of the two proteins at the plasma membrane throughout the fungal hyphae and also occasionally at septa (Fig. 3B). Since false-positive results can be obtained in a BiFC analysis (Kerppola, 2008), we performed additional BiFC experiments with MT associated proteins such as KipA and AlpA and the cell-end markers TeaR and TeaC. No signals were obtained in any of the combinations with MigA (data not shown). The yeast-two hybrid assay indicates that MigA interacts with the N-terminal part of ApsA under medium stringency conditions (Fig. 3C). As a negative control MigA/TeaR was included (Fig. 3C).

Since spindle motility in apsA-deletion strains is nearly abolished, we analyzed this phenotype in a ∆migA strain and compared it to the ∆apsA strain. In both cases, spindle motility was significantly reduced in comparison to the wild type, although the effect was stronger in the absence of ApsA (Fig. 3E). In the migA-deletion strain, astral microtubules failed to make contact to the cortex in early stages of mitosis (Supplemental M2) and thus we reasoned that MigA like Kar9 in S. cerevisiae has a role in nuclear positioning during early stages of mitosis. This is consistent with the fact, that ∆migA strains do not show a nuclear misdistribution phenotype like ∆apsA strains do (Fig. 3F).

MigA associates with growing and retracting MT plus-ends in an EbA-dependent manner
Time-lapse analyses of MigA:eGFP revealed its transport to the hyphal tip in interphase cells (Fig. 4A, B, C). This behavior resembles the behavior of the MT plus-end associated motor protein KipA. The velocity of KipA was determined to be $9.5 \pm 1.8 \mu m/min$ (Schunck et al., 2011) whereas the growth rate of MT was shown to be $13.7 \pm 3.1 \mu m/min$ (Han et al., 2001). MigA
comets were imaged in vivo and velocities of 11.9 ± 9.5 μm/min were calculated and thus resembles the velocities of KipA and growing MTs (Fig. 4C). Dual labeling of TubA and MigA revealed that MigA is loaded onto the SPBs and, from there, actively transported towards the MT plus-end (Fig. 4B, Supplemental M3). Overexpression of eGFP::MigA led to complete decoration of cytoplasmic MT (Supplemental Fig. 4A, Supplemental M4). In addition to eGFP fusions, we generated a fusion with photoconvertible mEosFPthermo (Wiedenmann et al., 2004; Wiedenmann et al., 2011), which allows super-resolution microscopy such as Photoactivated Localization Microscopy (PALM) (for a review about super-resolution microscopy see Patterson et al., 2010). Super-resolution single particle tracking analysis of MigA::mEosFPthermo (MigA C-terminally tagged) clusters provided essentially background free images and showed localization of single MigA clusters at growing and retracting MTs (Fig. 4D, Supplemental M5).

Furthermore, we investigated the potential roles of the Kar9 domain and the conserved N-terminal stretch of MigA. Whereas deletion of the N-terminal stretch did not alter the localization and dynamics of the protein, deletion of the Kar9 domain affected both. The corresponding protein was observed mainly in the cytoplasm and as accumulations in a subapical region resembling the endocytic collar (Fig. 4E, F). Thus the Kar9 domain is required for MT association.

In order to test if MT plus-end association of MigA depends on the A. nidulans EB1 orthologue EbA (Zeng et al., 2014), BiFC assays were performed to demonstrate their interaction. A strong signal along short and long filamentous structures was observed in hyphae. The features observed in the image resembled MT, which suggests that MigA and EbA interact at the MT lattice (Fig. 5A). Other MT-associated proteins such as KipA and AlpA did not interact with MigA (Enke et al., 2007; Zekert and Fischer, 2009)(data not shown). The EbA-MigA interaction was confirmed in a Y2H assay (Fig. 5B). The predicted SxIP motif at position 873-876 in MigA was not essential for the MigA/EbA interaction (Fig. 5B). Surprisingly, the SxIP domain was crucial for the transport of MigA in vivo and accumulated in non-motile clusters in the hyphae (Fig. 5C).

In order to address the question whether MigA is loaded onto MT in an EbA-dependent manner, we analyzed MigA::eGFP in a ∆ebA-deletion strain. In contrast to, e.g., KipA (Zeng et al., 2014), MigA still localized to MTs. However, MTs were more uniformly decorated and MT plus-end accumulation was abolished although this did not completely phenocopy a deletion of the SxIP motif (Fig. 5C, D, E). We also observed MigA at septa and uniformly decorated mitotic spindles (data not shown), suggesting EbA-independent binding of MigA to septa, mitotic spindles, SPBs and MTs. Direct interaction of MigA and TubA was further proven by BiFC and
in a Y2H assay (Fig. 5B, F). In this series of experiments, strong self-interaction of MigA was observed in the Y2H assay (Fig. 5B).

**MigA plays a role in cell-end marker positioning and MT convergence**

Because the phenotype of ΔmigA strains resembled that of null mutations of cell-end marker mutants, we anticipated MigA being involved in cell-end marker positioning. To test this, tagged cell-end markers eGFP::TeaR (N-terminally tagged) and mRFP1.2::TeaA, expressed from their natural promoters, were analyzed in ΔmigA and wild type strains (Fig. 6A). Indeed the number of hyphae with mis-positioned TeaA or TeaR was higher than in wild type. Next we analyzed direct interaction of MigA with cell-end marker proteins using the BiFC and Y2H assays. Whereas TeaA did interact, TeaC and TeaR did not (Fig. 6B, C and Fig. 3C, BiFC assay MigA/TeaC and MigA/TeaR not shown). The interaction of MigA and TeaA was restricted to the hyphal tip, and occasionally to septa. We did not observe transport of any assembled BiFC complexes, which suggests that the interaction only takes place at the tip. Furthermore, we observed a strong dominant-negative phenotype on polar growth in these strains, where hyphae displayed meandering growth similar to a migA/teaA double-deletion strain (Fig. 2B). The observed phenotypes were not due to the tagging of MigA or TeaA with the split YFP halves (Supplemental Fig. 4B). As suggested by the interaction of MigA with TeaA, MT convergence in the hyphal tip was similarly affected in ΔmigA as in ΔteaA strains (Fig. 6D, Supplemental M6).

**MigA-MyoE interaction allows an active guidance mechanism for MTs along actin filaments**

A possible mechanism for MT convergence in the hyphal tip is an active pulling of the MT plus-ends along actin cables originating from the cell-end marker complex. To test this hypothesis, we examined an interaction of MigA with the class V myosin MyoE (MyoV), which localizes in vivo to the hyphal tip and associates with secretory vesicles (Taheri-Talesh et al., 2012; Zhang et al., 2011). Bimolecular fluorescence complementation revealed a strong fluorescence signal at the hyphal tip and along some filamentous structures originating from the cortex (Fig. 7A). Strains overexpressing migA display a slightly curvy phenotype and no phenotype on the colony level, whereas hyphae in myoE-overexpressing strains are considerably thicker and show a growth defect on solid media (Supplementary Figs. 4A, C, E). The corresponding BiFC strain also shows strong growth defects with smaller colonies, a defect in spore formation, and the diameter of hyphae is gradually increasing from the spore to the tip (Supplementary Fig. 4D,
The phenotype of the BiFC strain resembles also a myoE-deletion phenotype, which suggests that MyoE is not functional probably due to the irreversible interaction of the two split YFP halves. Thus in vivo the interaction can only be transient. The interaction between MigA and MyoE was further confirmed in a Y2H assay (Fig. 7B).

Colocalization studies with GFP tagged MigA and mCherry tagged MyoE should show a cotransport of both proteins. However, the MyoE concentration - even after expression under its native promoter – is too high to resolve such a cotransport (Taheri-Talesh et al., 2012). In order to lower the concentration of the tagged MyoE protein, we constructed a strain with mCherry tagged MyoE, which has a modified stop codon (TGACTA) between the coding sequence of myoE and mCherry. This stop codon was shown to frequently trigger translational readthrough (Freitag et al., 2012; Stiebler et al., 2014). In this strain only a small fraction of MyoE is labeled with mCherry and this allowed tracking of smaller clusters of MyoE at the tip. Using this construct we observed partial co-localization of MigA and MyoE in the tip (Fig. 7C). Although being almost below the detection limit and thus time resolution was challenging we were able to detect co-transport of both proteins at the hyphal tip (Supplemental M7). In the corresponding time-lapse series signals of MyoE moved away from the tip and returned again together with MigA comets (Supplemental M7).

**Discussion**

The interaction and attachment of MTs to chromosomal kinetochores and the temporal interaction of MTs to defined cortical regions during polar cellular extension are two prominent examples for the necessity of MT capture in eukaryotic cells (Carminati and Stearns, 1997; Fodde et al., 2001; Reilein et al., 2005; Lu et al., 2001). The mechanisms require the spatial and temporal interaction between MT plus-end associated proteins and target protein complexes, which transmit information to downstream processes. If only a small number of MT plus-end associated proteins (or only one) were required for different MT interactions, one would assume that the specificity also relies on different interacting proteins. Here we found that the +TIP protein MigA is able to interact with two cortical proteins, ApsA and TeaA. The downstream processes are very different, however, for the two cases. Whereas the interaction with ApsA promotes spindle oscillations and most likely involves activation of the dynein pathway as in S. cerevisiae, dynein is not activated upon interaction with the cell-end marker protein TeaA. The two processes are well separated because ApsA does not reach the hyphal tip, whereas TeaA is restricted to the hyphal tip (Fig. 3D, Supplementary Fig. 4F).
The interaction of MigA with ApsA is conserved in relation to *S. cerevisiae*. However, nuclear division in yeast is correlated with nuclear migration and asymmetric movement of the dividing nucleus into the bud neck. This asymmetry is generated in the first place by asymmetric loading of Kar9 onto the two SPBs. Such asymmetry is not required in vegetative hyphae of filamentous fungi because interphase nuclei migrate within the hyphae (Suelmann *et al.*, 1998). Nevertheless, the dynamic behavior of Kar9 appears to be conserved in MigA. When the MigA concentration increased at one SPB, it decreased at the other one. This oscillation was repeated several times during mitosis. Such fluctuations of MigA came as a surprise because, in *S. cerevisiae*, asymmetric loading of Kar9 was shown to result from phosphorylation of a number of serine residues (Liakopoulos *et al.*, 2003). However, because these serines are not conserved in MigA, a different mechanism is likely to play a role. In any case, the fluctuations itself reveal the potential for stable asymmetric loading of MigA onto the SPBs upon regulation. This may be of importance during mitotic events during conidiophore development. The formation of primary and secondary sterigmata indeed closely resembles the budding process in *S. cerevisiae*. Without MigA, astral MTs fail to establish contact with the plasma membrane/ApsA and retract. As in *S. cerevisiae*, where the Kar9 pathway is predominantly active during pre-anaphase, MigA is important in nuclear positioning during early stages of mitosis, because in later stages of mitosis astral MT are able to establish contact with the cortex (Supplementary M2). Following the yeast model (Miller *et al.*, 1998; Liakopoulos *et al.*, 2003), the MigA and dynein pathway are partially redundant and therefore dynein can fulfill functions of MigA. This is consistent with our observation that nuclear distribution is not significantly altered in a \( \Delta \text{migA} \) strain (Fig. 3F). This leads to the suggestion that MigA is not essential for astral MT binding to cortex patterns such as ApsA but rather is a promoting factor and facilitates contact between ApsA and astral MT (Fig. 8A, Supplementary M2).

In interphase cells MigA is actively transported to the hyphal tip. This transport is dependent on the Eb1 orthologue EbA (Fig. 5A, B, C, D, E), although MigA is able to bind TubA (\( \alpha \)-Tubulin) autonomously (Fig. 5B, F). In a Y2H screen the *in silico* identified SxIP motif in the C-Terminus of MigA turned out to be not essential for the interaction of the two proteins. It is not unusual for Eb1 interaction partners to harbor more than one and/or degenerated SxIP motifs or alternatively MT plus-end and/or Eb1 binding sites which do not match the SxIP consensus sequence (van der Vaart *et al.*, 2011). However, the SxIP motif was crucial for MigA motility (Fig. 5C). Surprisingly, deletion of the SxIP did not completely phenocopy an ebA deletion.

The novel key finding in this work is that MigA is able to transiently interact with the cell-end marker protein TeaA. Apparently, MigA plays a role in correct positioning of TeaA. This may
be explained by a MT capture mechanism in the hyphal tip (Fig. 7C, 8B and Supplementary M7). The interaction of MigA with TeaA would ensure docking of the MT plus-end to the TeaA protein complex. The establishment of such a complex involves a positive feed-back loop. Initially, only a few molecules of TeaA are delivered to one position at the cortex. From there, some actin cables are launched, which in turn guide more MT plus-ends (through the action of MigA and MyoE) to this spot and thus again increase the TeaA concentration (Ishitsuka et al., 2015, submitted). Another possible explanation for the guidance mechanism of the MT plus ends along actin cables could be a bridging of the two cytoskeletons by secretion vesicles, which are associated to both, kinesin and MyoE (Pantazopoulou et al., 2014). However, this mechanism would not explain why deletion of migA would affect MT convergence in the hyphal tip.

Another explanation for the interaction between TeaA and MigA could be regulation of TeaA. TeaA interacts with the MT polymerase AlpA and controls its activity (Takeshita et al., 2013). However, both, TeaA and AlpA are transported to the MT plus-end and we have no evidence that they interact there. It would actually be very disadvantageous if TeaA already were to interact with AlpA at the MT plus-end because this could lead to inactivation of the AlpA activity, which was proposed to happen only at the cortex. MigA also appears to interact only at the hyphal tip with TeaA and this interaction could change the activity of AlpA. TeaA thus appears to be a scaffold protein engaged in stable interactions with proteins including TeaR or TeaC, and also transient interactions with proteins such as MigA or AlpA. In S. cerevisiae, it was not yet reported that Kar9 interacts with the TeaA orthologue Kel1. However, cell-end marker proteins (landmark proteins) in S. cerevisiae do not play a direct role in polarized growth. Cells lacking kel1 are defective in cell fusion during mating due to failure of membrane fusion and cytoplasmic mixing. In contrast, cells lacking the kel1 paralog kel2 do not show any phenotype during cell fusion (Philips et al., 1998). Given the high conservation of MigA and its long N-terminal extension in all analyzed filamentous ascomycetes, the proposed mechanism of MT capture in the hyphal tip may be a novel evolutionary function and adds another piece towards understanding the mechanism of polar growth in filamentous fungi.

The orthologue of MigA, Kar9 is frequently referred to as the functional orthologue of the human adenomatous-polyposis-poli (APC) protein in S. cerevisiae (Liakopoulos et al., 2003; Miller and Rose, 1998). Although Kar9 possesses only a short amino acid sequence similar to APC, it might share some functions with APC (Bloom, 2000). APC is an extensively studied tumor suppressor with a well-known role in the (canonical) Wnt signaling pathway, where APC is part of a protein complex that triggers degradation of β-catenin (Behrens et al., 1998; Groden
et al., 1991). In neuronal tissue, however, APC plays another important role and the MT and actin cytoskeletons are highly disturbed if APC is missing (Chen et al., 2011). It has been shown that APC contains a functional MT binding site at the C-terminal part, which can stimulate MT assembly as well as bundling in vitro, and stabilizes MTs in vitro and in vivo (Munemitsu et al., 1994; Zumbrunn et al., 2001).

EB1 (end binding protein 1) is an important interaction partner of APC that was discovered in a yeast-two hybrid screen with APC as bait (Su et al., 1995; for a review about EB1 proteins see Tirnauer et al., 2000). The APC-EB1 interaction was proposed to play a critical role in chromosomal stability because it is necessary for physical interaction between MT plus-ends and chromosomal kinetochores during mitosis (Fodde et al., 2001).

Since MigA is more closely related to APC than Kar9 to APC (Fig. 1B), it is possible that this potentially evolutionarily developed mechanism and the influence on cell-end markers is also conserved in human cells. Indeed MigA and APC share several MT-associated functions. In the absence of the MT cytoskeleton, MigA localizes in cortical clusters. A similar localization is known for APC, which accumulates at the cortex at the very periphery of actively extending membranes (Barth et al., 2002; Barth et al., 1997b; Näthke et al., 1996). APC deficient neuronal cells have a highly disturbed cytoskeleton (Chen et al., 2011), which, with a high number of non-converging MTs, is also true for A. nidulans migA-deletion strains. Furthermore, APC and MigA are transported to the MT plus-end in an Eb1-dependent manner, although they both bind tubulin autonomously as well (Deka et al., 1998). It was also reported that APC partially localizes at the basal cortex, and passing MT plus-ends pause at the APC puncta. Therefore, APC was proposed as a template that guides MT network formation (Reilein et al., 2005). This behavior resembles the mechanism described here, where MigA interacts with the cell-end marker TeaA to ensure docking of MTs to the cell cortex.

The interplay between the actin and the MT cytoskeletons is a key step in many cellular processes. Although many open questions remain, the comparative analysis of key components in different organisms helps to develop a general picture.

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Materials and methods
Strains, Plasmids and Culture Conditions
Supplemented minimal medium for *A. nidulans* was prepared as described, and standard strain construction procedures were used (Takeshita et al., 2008). *A. nidulans* strains used in this study are listed in Table 1. The *S. cerevisiae* strains AH109 and Y187 (Clontech) were used for yeast two-hybrid interaction studies. *S. cerevisiae* cells were grown in YPDA complete medium, or on minimal medium (SD) supplemented with the dropout-mix needed for selection, as described in the Clontech Matchmaker™ GAL4 Two-Hybrid System 3 Manual (http://www.clontech.com). *S. cerevisiae* strains used in this study are listed in Table 2. Standard laboratory *Escherichia coli* strains (Top 10 F') were used. Oligonucleotides are listed in Table 3 and plasmids in Table 4.

Molecular Techniques
Standard DNA transformation procedures were used for *A. nidulans*, *S. cerevisiae* and *E. coli*. For PCR experiments, standard protocols were applied using a personal Cycler (Biometra, Göttingen, Germany) for the reaction cycles. DNA sequencing was performed commercially (MWG Biotech, Ebersberg, Germany). DNA analyses and Southern hybridizations were performed as described by Sambrook and Russel, 1999.

Yeast-Two-Hybrid analysis
Screening for an interaction of MigA with other proteins was performed according to the Matchmaker™ GAL4 Two-Hybrid System 3 Manual (Clontech). Plasmids harboring the *migA* open reading Frame (ORF) were generated by PCR amplification from genomic DNA (strain TN02A3), introducing *Sfi*/EcoRI restriction sites (Primer: KarAFull_Y2HSfiI / KarAFull_Y2HEcoRI) for subsequent ligation into pGBK7 (Clontech) and *EcoR*I/XhoI sites (Primer: FullKarA_EcoRIF / FullKarA_XhoIR) for ligation into pGADT7-Rec (Clontech) yielding pRM32 or pRM36, respectively. The C-terminal region of *migA* was amplified by PCR from cDNA (strain TN02A3) and subsequently ligated into pGADT7-Rec using *Nde*I and *EcoR*I restriction sites (Primer: KarACT_Y2HNdIF / KarAFull_Y2HEcoRI) for ligation into pGBK7 (Clontech) and pGADT7-Rec with the N-terminal part of *apsA* were generated by PCR amplification (Primer: ApsA_Y2HNdF / ApsA_Y2HBamHI) from cDNA (strain TN02A3) and subsequent ligation into the respective vectors via *Nde*I/BamHI sites. The same approach was applied for *tubA* (Primer: TubA_Y2HNdFw / TubA_Y2HBamHIr), *eba* (Primer: EBA_Y2HNdelfor / EBA_Y2HEcoRrev) and *myoE* (Primer: MyoV_Ndel / MyoV_EcoRI).
In order to generate a plasmid with the mutated SxIP motif (MigA$_{CT}^{Δ873-876}$) pRM27 was mutagenized. In a PCR with Pfu Polymerase and 5’ phosphorylated oligonucleotides flanking the coding region (Primer: MigACT_Eb1Mut_fw / MigACT_Eb1Mut_rv) a linear fragment was amplified. The complete reaction was digested with DpnI to cut all methylated original vector molecules and ligated. The final plasmid (pRM104) was partially sequenced to confirm the deletion.

Strains AH109 and Y187 were transformed using the lithium chloride method and transformants were selected on selective synthetic dropout media as described in the Matchmaker™ GAL4 Two-Hybrid System 3 Manual. Expression of all constructs was verified by Western Blot (except for AD MigA$_{CT}^{Δ873-876}$) and appropriate tests for self-activation have been performed (Supplementary Fig. 3C).

**Tagging with eGFP and gene deletion**

MigA was tagged at the C-terminal end with eGFP. The 1 kb C-terminal region of migA was PCR amplified with genomic DNA (strain SO451) with the primer pair KarA_P4 and KarA_P6, and the 1 kb terminator region of the gene with primer pair KarA_P5 and KarA_P8. A fragment of the eGFP::pyrG cassette was amplified from pFNO3 using primer pair GA_linker and pyrG_cas_rev. The three fragments were fused together in a subsequent Fusion PCR (Nayak et al., 2006) with primer pair KarA_P4 and KarA_P7. In order to introduce a C-terminal mEosFPthermo tag we amplified mEosFPthermo with primer pair Linker_mIRIS_fwd and IRIS_Linker_rev, the pyrG fragment from pFNO3 with primer pair pyrG_cas_for and pyrG_cas_rev and fused together in a Fusion PCR with primer pair GA_linker and pyrG_cas_rev. The mEosFPthermo::pyrG fragment was also fused to the C-Terminal and right border of migA as described before. The resulting migA::mEosFPthermo::pyrG cassette was subcloned into cloning vector pJet1.2 (Fermentas) resulting in pRM35. In order to generate a construct of MigA with a mutated SxIP (MigA$_{^{Δ873-876}}$) pRM35 was mutagenized in the same way as pRM104 was generated resulting in pRM105.

The migA$_{^{ΔNT}}$::eGFP::pyrG construct was generated by amplifying the promoter region with KarA_P3 / MigA_P12, the Kar9 domain with primer pair MigA_P11 / MigA_P10. In a subsequent Fusion PCR with primer pair KarA_P2 / KarA_P7 the obtained fragments were fused together with the before described eGFP::pyrG cassette and right border. Similarly, the migA$_{^{Δkar9}}$::eGFP::pyrG was generated by amplifying the promoter and N-terminal region of migA.
with primer pair KarA_P3 / MigA_P9. In the subsequent Fusion PCR with primer pair KarA_P2 and KarA_P7 the fragment was fused together with the eGFP::pyrG cassette and right border.

In order to tag MyoE at the C-terminus with mCherry and a modified stop codon between the coding sequence of myoE and mCherry again Fusion PCR was used. The 1 kb C-terminal region of myoE was PCR amplified with genomic DNA (strain SO451) with the primer pair MyoV_P1 and MyoV_P2_TGACTA, and the 1 kb terminator region of the gene with primer pair MyoV_P3 and MyoV_RB_rev. A fragment of the mCherry-pyrG cassette was also amplified using primer pair GA_linker and pyrG_cas_rev. The three fragments were fused together in a subsequent Fusion PCR (Nayak et al., 2006) with primer pair MyoV_nested_for and MyoV_nested_rev. The resulting myoE::TGACTA::mCherry::pyrG cassette was subcloned into pJet1.2 (Fermentas). Insertion of the modified stop codon was confirmed by sequencing (MWG Biotech, Ebersberg, Germany).

PCR products were transformed into uridine/uracil-auxotrophic A. nidulans ΔnkuA strain SO451, in order to increase the frequency of homologous integration.

For tagging MigA at the N-terminus, the 1 kb N-terminal region of the gene was amplified from genomic DNA (strain TN02A3) with primer pair KarA_750bp_for and KarA_750bp_rev, digested with Ascl/PacI and ligated into pCMB17apx yielding pRM6. The same approach was applied for ApsA (Primer: ApsA_1kb_AscI / ApsA_1kb_PacI) and MyoE (Primer: AN8862_for_AscI / AN8862_rev_PacI) and ligated into pDV7, pSH44, pMCB17apx, and pJR1, respectively. The plasmids were transformed into the ΔnkuA strain TN02A3.

To delete migA, the 1-kb promoter region of the gene was amplified with primers KarA_P1 and P3. A fragment of the pyrG marker cassette was amplified with primers pyrG_cas_for and pyrG_cas_rev. PCR products of the promoter region, pyrG, and the terminator region amplified by KarA_P5 and KarA_P8 were fused together via Fusion PCR with primer pair KarA_P2 and KarA_P7. The PCR products were transformed into the ΔnkuA strain SO451.

Knockout cassettes were also obtained from the Fungal Genetic Stock Center (FGSC, http://www.fgsc.net/Aspergillus/KO_Cassettes.htm). Amplification of the FGSC migA deletion cassette via PCR was carried out with primer pair FGSC_KarA_LB_for and FGSC_KarA_RB_rev, the teaA deletion cassette with primer pair TeaA_nested_for and TeaA_nested_rev and the myoE-deletion cassette with primer pair FGSC_dMyoVnes-fw and FGSC_dMyoVnes_r. The deletion cassettes were transformed into ΔnkuA strains SO451 and TN02A3. The primary transformants were screened microscopically and by
PCR for correct integration of the eGFP tagging or deletion cassette. Integration events were confirmed by Southern blotting.

**Light/fluorescence microscopy**

Live-cell imaging of germlings and young hyphae: Up to $4 \times 10^4$ spores were grown on 170 ± 5 µm high precision microscope cover glasses (Roth, Karlsruhe, Germany) in 0.5 ml minimal medium + 2% glycerol and appropriate selection markers. Cells were incubated for 12 to 14 hours at 28°C following 2 h at room temperature. Alternatively, for *in vivo* time-lapse microscopy, cells were incubated in 35 mm Fluorodish cell culture chambers from World Precision Instruments (Sarasota, Florida, USA) in 2 ml minimal medium + 2% glycerol and appropriate selection markers and additional 7 ml medium after ON incubation. For PALM microscopy, cells were incubated in µ-Slide 8-well glass bottom chambers (ibidi, Thermo Fisher Scientific, Martinsried, Germany).

Conventional fluorescence images were captured at room temperature using a Zeiss Plan-Apochromat 63x/1.4 Oil DIC and Zeiss EC Plan-Neofluar 100x/1.3 Oil objective attached to a Zeiss AxioImager Z.1 combined with an AxioCamMR. Images were collected and analyzed using AxioVision v4.8.1, Zen 2012 Blue Edition v1.20 (Zeiss, Jena, Germany) and ImageJ 1.48p (National Institutes of Health, MD, USA). Image specifications are indicated in the respective legends.

Confocal images were captured at 21°C using a Leica HCX PL APO 63x/1.20W Corr objective attached to a Leica TCS SP5 (DM5000) and conventional PMT detectors (Leica, Wetzlar, Germany). If not otherwise stated, the pinhole size was set to 1 AU and a 458/514 nm or 488/561/633 Notch filter was used. Images were collected and analyzed using LAS AF v2.6 (Leica, Wetzlar, Germany) and ImageJ 1.48p. Acquisition specifications are indicated in the respective figure legends.

PALM imaging was performed as previously described (Ishitsuka *et al*., 2014; Ishitsuka *et al*., 2015, submitted). Briefly, images were acquired at room temperature on a modified inverted microscope (Axiovert 200, Zeiss) equipped with a high N.A. water immersion objective (C-Apochromat, 63x, N.A. 1.2, Zeiss). We employed three solid-state lasers, with wavelengths 561 nm (Cobolt Jive, Cobolt, Solna, Sweden), 473 nm (LSR473-200-T00, Laserlight, Berlin, Germany) and 405 nm (CLASII 405-50, Blue Sky Research, Milpitas, CA) for excitation and photoactivation of the fluorophores. The laser sources were combined via dichroic mirrors (AHF, Tübingen, Germany) and guided through an AOTF (AOTFnc- 400.650, A-A, Opto-Electronic, Orsay Cedex, France). Cells were incubated for 2 hours at 28°C followed by 12 to 14 hours at
room temperature in a chambered cover glass. The photoconvertible fluorescent proteins were converted from their green to their red emitting forms using high intensity 405 nm light for 10 s to preconvert sufficient fluorescent protein molecules, followed by simultaneous illumination with low intensity (0 – 50 W/cm²) 405 nm and 561 nm excitation illumination (20 – 40 W/cm²). After passing through the excitation dichroic (z 405/473/561/635, AHF, Tübingen, Germany), fluorescence emission was filtered by a 607/50 band-pass filter (AHF, Tübingen, Germany) and recorded with a back-illuminated EMCCD camera (Ixon Ultra 897, Andor, Belfast, Northern Ireland). Recorded images with MigA clusters were localized in each image frame and single particle tracking analysis have been further applied by using our custom written PALM analysis software, a-livePALM (Li et al, 2013). For single particle analysis, maximum displacement of 300 nm, memory of 2 frames (allowed frames to skip) and the minimum trajectory length of 5 frames were used.
References


Figures

Figure 1. Scheme of the MigA protein and relatedness analysis of MigA orthologues. (A) Comparison of the protein structure of MigA from *A. nidulans* and Kar9 from *S. cerevisiae*. MigA possesses an N-terminal stretch, which is conserved in filamentous fungi. Domains and motifs were determined with Pfam (Finn *et al.*, 2014), Protparam (Gasteiger *et al.*, 2005) and MultiCoil (Wolf *et al.*, 1997). Furthermore, domains and motifs of Kar9 are indicated as described by (Liakopoulos *et al.*, 2003, Miller and Rose, 1998). (B) MigA groups together with putative orthologues of other *Aspergilli* and filamentous fungi. Putative orthologues were identified using a blastp search with the full-length protein sequence of MigA as query sequence (Altschul *et al.*, 1990). The alignment was done with CLC Sequence Viewer 6.6.1 (Qiagen, Venlo, Netherlands) (gap open cost: 10.0; gap extension cost: 1.0) and a phylogenetic tree was created with a neighbor joining algorithm and bootstrapping analysis (replicates: 100) using MEGA5.2 (Tamura *et al.*, 2011).
Figure 2. Phenotypic analysis of a migA-deletion strain. (A) Top: Colonies of wild type (SRF201), ΔmigA (SRM11), ΔteaA (SRM127), ΔteaR (SNT34) and ΔkipA (SSK44) strains. Strains were grown on MM agar plates supplemented with appropriate vitamins and 2% glucose for 3 days at 37°C. (B) Hyphae of wild type (I) (TN02A3), ΔmigA (II, III) (SRM11), migA under alcA(p) control (SRM12) and repressed with 2% glucose (IV), derepressed with 2% glycerol (V), or induced with 2% threonine and 0.01% glucose (VI), ΔteaR (VII) (SNT34), ΔteaA (VIII) (SRM127), ΔkipA (IX) (SSK44) and ΔteaA/ΔmigA (X) (SRM117). Strains were grown as
described with 2% glucose or as indicated (Scale bar: I, III: 5 µm; II, IV – VI, X: 10 µm; VI – IX: 8 µm). (C) Quantification of the impact of a \textit{migA} deletion on second germ tube formation. Since the normal distribution of the data is not given (as determined using a Kolmogorov-Smirnov and chi-squared test), a Mann-Whitney U Test was applied. Germ tube emergence is significantly altered in $\Delta\text{migA}$ ($p=0.00298$) and $\Delta\text{teaA}$ ($p=0.00038$) strains compared to the wild type at $p \leq 0.01$. However, emergence does not differ significantly ($p=0.16152$) between $\Delta\text{migA}$ and $\Delta\text{teaA}$ strains ($n_{[\text{WT}]}=120$, Mean=153.74±25.28; $n_{[\Delta\text{migA}]}=143$, Mean=145.27±34.65; $n_{[\Delta\text{teaA}]}=84$, Mean=141.15±41.75). Conidia of wild type (SRF201), $\Delta\text{migA}$ (SRM11) and $\Delta\text{teaA}$ (SRM127) strains were grown as described and the angle of emergence of a second germ tube in relation the first one was measured. The acquired data sets were sorted in 10° groups and plotted in a radar plot. (D) Quantification of tip splitting events in wild type, $\Delta\text{migA}$, $\Delta\text{teaA}$ and $\Delta\text{teaR}$ strains. Tip splitting events in $\Delta\text{migA}$ ($p = 0$) and $\Delta\text{teaA}$ ($p = 0$) strains are significantly higher in comparison to the wild type at $p < 0.01$ whereas in $\Delta\text{teaR}$ ($p = 0.024$) strains it only differs at $p < 0.05$. In contrast, the number of split tips between $\Delta\text{migA}$ and $\Delta\text{teaA}$ ($p = 0.47$) does not differ significantly at $p < 0.1$. However, $\Delta\text{teaR}$ differ significantly from $\Delta\text{migA}$ ($p = 0.0003$) and $\Delta\text{teaA}$ ($p = 0.003$) at $p < 0.1$. In comparison to the WT, where no tip splitting was observed, the occurrence of this events in $\Delta\text{migA}$ (21.57%), $\Delta\text{teaA}$ (17.59%) and $\Delta\text{teaR}$ (4.76%) strains is significantly higher (WT: $n_{[\text{cells}]}=104$; $\Delta\text{migA}$: $n_{[\text{cells}]}=102$; $\Delta\text{teaA}$: $n_{[\text{cells}]}=108$; $\Delta\text{teaR}$: $n_{[\text{cells}]}=105$). Conidia of wild type (SRF201), $\Delta\text{migA}$ (SRM11), $\Delta\text{teaA}$ (SRM127) and $\Delta\text{teaR}$ (SNT34) strains were grown as described on 2% glucose agar plates and screened for tip splitting events at the periphery of the colony.
Figure 3. Localization of MigA and its role in mitotic spindle dynamics. (A) Dynamic localization of MigA::eGFP at both spindle poles (arrowheads), along the mitotic spindle and on astral MTs. Hyphae of SRM22 (migA::eGFP, alcA(p)::mCherry::tubA) were grown as described (exposure times 450 - 490 nm: 500 ms; 538 - 562 nm: 500 ms; Scale bar: 1 µm). (B) Confocal scanning image of the interaction of MigA and ApsA at the hyphal membrane. Hyphae of the strain SRM14 (alcA(p)::YFP::migA, alcA(p)::YFPN::apsA) were grown as described (frame
accumulation: 2; line average: 16; AOTF 514: 25%; gain: 900 V; offset: -0.2; scan speed: 1000 Hz; emission bandwidth: 522 nm - 648 nm; maximum projection of a 5.16 µm z-stack; Scale bar: 5 µm). (C) Y2H analysis of MigA and ApsA. Strains expressing different versions of MigA and TeaR serve as controls. Positive and negative controls as provided in the Matchmaker™ Gold Yeast Two-Hybrid System by Clontech Laboratories, Inc.. Dilution series of respective strains were grown on SD-LW and SD-LWH at 30°C for 3 days. (D) Confocal scanning image of the localization of ApsA in distal parts of the hyphae (a). ApsA does not localize to hyphal tips (b). Hyphae of the strain SRM176 (alcA(p)::eGFP::apsA) were grown as described (Frame Accumulation: 2; Line Average: 4; AOTF 488: 5%; Gain: 900 V; Offset: -0.1; Scan speed: 400 Hz; Emission bandwidth: 492 nm – 652 nm; maximum projection of a 6.29 µm z-stack; Scale: 10 µm). (E) Boxplot of spindle motility analysis of wild type (SRM118), ∆migA (SRM124) and ∆apsA (SRM136). The respective strains were grown as described and time-lapse images were taken from mitotic spindles every 4 s (Exposure time 450 – 490 nm: 50 ms). Distance of spindle movement was measured every frame until the end of mitosis or disappearance of fluorescence. Measured distances were grouped in 20 s intervals and plotted. Since the normal distribution of the data is not given (as determined using a Kolmogoroff-Smirnoff and chi-squared test) a Mann-Whitney U test was applied. The boxplot was created with Boxplot 1.0.0 (8). Spindle motility is significantly altered in ∆migA (p=0.00496) and ∆apsA (p=0) strains compared to the wild type at p ≤ 0.01. However, emergence does only differ significantly (p=0.0703) between ∆migA and ∆apsA strains at p ≤ 0.1. (WT: n_{[cells]}=22, n_{[spindle]}=29, n_{[data points]}=142; ∆migA: n_{[cells]}=15, n_{[spindle]}=20, n_{[data points]}=151; ∆apsA: n_{[cells]}=13, n_{[spindle]}=29, n_{[data points]}=190). (F) Boxplot of nuclear distribution analysis of wild type (SRM118), ∆migA (SRM124) and ∆apsA (SRM136). The respective strains were grown as described and nuclei stained with DAPI (Vector Laboratories, VECTASHIELD Mounting Medium with DAPI, #H-1200) and distance between neighboring nuclei was measured. Since the normal distribution of the data is not given (as determined using a Kolmogoroff-Smirnoff and chi-squared test) a Mann-Whitney U test was applied. The boxplot was created with Boxplot 1.0.0 (8). Nuclear distribution is significantly altered in ∆apsA strains compared to the wild type (p < 2e-06) and ∆migA (p < 2.2e-06) at p < 0.001. Nuclear distribution between wild type and ∆migA strains differs not significantly (p=0.089) at p ≥ 0.05. (WT: n_{[cells]}=32, n_{[nuclei]}=336; ∆migA: n_{[cells]}=31, n_{[nuclei]}=337; ∆apsA: n_{[cells]}=30, n_{[nuclei]}=396).
Figure 4. Localization of MigA at growing and retracting MT plus-ends. (A) Kymograph of MigA::GFP comets travelling towards the tip. Retrograde movement can also be observed (arrowhead). Hyphae of SRM1 (migA::eGFP) were grown as described (exposure times 450 - 490 nm: 500 ms; Scale bar x: 1 µm, y: 20 sec). (B) MigA binds to MTOCs (asterisk) at the nucleus (N) and is transported to the MT plus-ends (arrowhead). Hyphae of the strain SRM22 (migA::eGFP, alcA(p)::mCherry::tubA) were grown as described (exposure time 450 – 490 nm: 500 ms/538 - 562nm: 500 ms; N = nucleus; Scale bar: 2 µm). (C) Velocity of MigA::GFP comets in vivo. Calculated mean velocity is 11.91 ± 9.49 µm/min (n[cells]=8; n[MigA signals]=219; time-lapse sequences lasting a total of 1272 sec). 63.47% of measured velocities were between 5 and 15 µm/min. Hyphae of SRM1 (migA::eGFP) were grown as described and time-lapse images were taken (exposure times 450 - 490 nm: 500 ms). Velocities were measured using kymographs. Measured velocities were grouped and plotted. (D) Analyzed positions of mEosFPthermo
labeled MigA molecules from PALM single particle tracking analysis. Snapshots taken from a 18 s time-lapse image (total imaging time). Images show the maximum projection of 16 individual images acquired during each 3.3 s interval. Overlay shows the computed positions of all MigA::mEosFPthermo clusters detected in the time-lapse image. MigA localizes to growing and retracting MT plus-ends (arrowheads). Lines shown in the bottom image indicate trajectories of individual MigA clusters, and colors indicate different initial times of the trajectories. Hyphae of the strain SRM40 (migA::mEosFPthermo, alcA:::eGFP::tubA) were grown as described (exposure time 200 ms; Scale bar: 1 µm). (E) MigAΔNT (arrowhead) comets move towards the tip of the hyphae. Hyphae of SRM199 (migAΔNT::eGFP) were grown as described and time-lapse images were taken (exposure times 450 - 490 nm: 800 ms; Scale bar x: 2 µm, y: 15 sec). (F) MigAΔkar9 localizes to the cytoplasm and also accumulates in a subapical region (Maximum projection of a 100 sec time-lapse image). Hyphae of SRM198 (migAΔkar9::eGFP) were grown as described and time-lapse images were taken (exposure times 450 - 490 nm: 800 ms; Scale bar x: 2 µm, y: 25 sec). False color heat map (bottom) shows fluorescence intensities as color scheme.
Figure 5. Interaction of MigA with EbA and TubA. (A) Confocal scanning image of the BiFC of MigA and EbA at filamentous structures. Hyphae of the strain SRM105 (alcA\textsubscript{(p)}::YFPC::migA, alcA\textsubscript{(p)}::YFPN::ebA) were grown as described (line average: 128; AOTF 514: 10%; gain: 1000 V; offset: -0.2; emission bandwidth: 522 nm - 658 nm). (B) Y2H analysis of MigA and EbA, TubA. Positive and negative controls as provided in the Matchmaker™ Gold Yeast Two-Hybrid System by Clontech Laboratories, Inc.. Dilution series of respective strains were grown on SD-LW, SD-LWH and SD-LWHA at 30°C for 3 days. (C) MigA\textsubscript{Δ873-876} localizes to cytoplasmic clusters and also accumulates at the hyphal tip (arrowhead). Motility of these clusters is impaired in comparison to WT MigA. Hyphae of SRM201 (migA\textsubscript{Δ873-876}::mEosFPthermo) were grown as described and time-lapse images were taken (exposure times 450 - 490 nm: 500 ms). Kymograph shows motility of MigA\textsubscript{Δ873-876} (Scale bar x: 2 µm, y: 1 min). (D) MigA binds to MTs in the absence of EbA. Hyphae of SRM125 (alcA\textsubscript{(p)}::mCherry::tubA, migA::eGFP, ΔebA) strain were grown as described (Exposure time 450 – 490nm: 500 ms/538 - 562 nm: 500 ms; Maximum projection of a 1.82 µm deconvolved z-stack. Deconvolution was done with Zen 2012 Blue Edition v1.20 [Zeiss, Jena, Germany]; Scale bar: 2 µm). (E) MigA predominantly localizes to the MT plus-end in the presence of EbA (arrowheads). Hyphae of SRM22...
(alcA_p::mCherry::tubA, migA::eGFP) strain were grown as described (Exposure time 450 – 490nm: 600 ms/538 - 562 nm: 500 ms; Scale bar: 2 µm). (F) Confocal scanning image of the interaction of MigA and TubA. Hyphae of the strain SRM105 (alcA_p::YFPC::migA, alcA_p::YFPN::tubA) were grown as described (frame accumulation: 2; line average: 16; AOTF 514: 25%; gain: 900 V; offset: -0.2; scan speed: 1000 Hz; emission bandwidth: 522 nm - 648 nm; Scale bar: 5 µm).
Figure 6. Role of MigA in cell-end marker positioning. (A) MigA affects positioning of the cell-end marker proteins TeaA and TeaR. Hyphae of the wild type strain SNT173 (eGFP::teaR, mRFP1.2::teaA) and strain SRM16 (ΔmigA, eGFP::teaR, mRFP1.2::teaA) were grown as described and localization of TeaA and TeaR were determined according to the indicated pattern (n[WT]=101, n[ΔmigA]=101; data in percent; * equals p < 0.05; ** equals p < 0.01; a Z Test
was applied). **(B)** Confocal scanning image of the interaction of MigA and TeaA at a prominent point at the hyphal tip. Hyphae of the strain SRM18 \((\text{alcA}_{<p>}::\text{YFPC}::\text{migA}, \text{alcA}_{<p>}::\text{YFPN}::\text{teaA})\) were grown as described (frame accumulation: 2; line average: 8; AOTF 514: 25%; gain: 900 V; offset: -0.2; scan speed: 1000 Hz; emission bandwidth: 522 nm - 648 nm; maximum projection of a 5.22 µm z-stack). **(C)** Y2H analysis of MigA and TeaA. Positive and negative controls as provided in the Matchmaker™ Gold Yeast Two-Hybrid System by Clontech Laboratories, Inc.. Dilution series of respective strains were grown on SD-LW and SD-LWH at 30°C for 3 days. **(D)** Frequency of MT convergence in wild type (SRM164), \(\Delta\text{migA}\) (SRM166a), \(\Delta\text{teaA}\) (SRM168) and \(\Delta\text{migA}/\Delta\text{teaA}\) (SRM173) strains. eGFP labeled KipA under the control of the \(\text{alcA}\) promoter was used to visualize MT plus-ends. Respective strains were grown as described and time-lapse images were taken every 378 ms (Exposure time 450 – 490 nm: 200 ms). Trajectories of eGFP::KipA signals in growing tips of respective strains were imaged until fluorescence was depleted. The point where signals attached for the first time to the membrane was monitored and the distance from that point to the exact center of the hyphal tip was measured. Signals moving along the membrane were set to zero. The number of converging MTs in \(\Delta\text{migA}\) \((p = 0)\), \(\Delta\text{teaA}\) \((p = 0.00084)\) and \(\Delta\text{migA}/\Delta\text{teaA}\) \((p = 0.00138)\) strains is significantly lower in comparison to the wild type at \(p < 0.01\) (Z Test). In contrast, the number of converging MTs between the deletion strains does not differ significantly at \(p < 0.1\) (\(\Delta\text{migA}\) to \(\Delta\text{teaA}\): \(p = 0.33706\); \(\Delta\text{migA}\) to \(\Delta\text{migA}/\Delta\text{teaA}\): \(p = 0.41794\); \(\Delta\text{teaA}\) to \(\Delta\text{migA}/\Delta\text{teaA}\): \(p = 0.9442\)). In comparison to the WT \((78\%)\) less MT converge in one point in \(\Delta\text{migA}\) \((53.26\%)\), \(\Delta\text{teaA}\) \((59.17\%)\) and \(\Delta\text{migA}/\Delta\text{teaA}\) \((59.14\%)\) strains (WT: \(n_{\text{cells}}=27, n_{\text{MT}}=150\); \(\Delta\text{migA}\): \(n_{\text{cells}}=15, n_{\text{MT}}=184\); \(\Delta\text{teaA}\): \(n_{\text{cells}}=15, n_{\text{MT}}=120\); \(\Delta\text{migA}/\Delta\text{teaA}\): \(n_{\text{cells}}=25, n_{\text{MT}}=93\).
Figure 7. MigA interacts with the class V myosin MyoE. (A) Left: Confocal scanning image of BiFC of MigA and MyoE at the hyphal tip and along filamentous structures in distal parts of the hyphae. False color heat map (middle) shows fluorescence intensities as color scheme. Hyphae of the strain SRM17 (alcA(p)::YFPC::migA, alcA(p)::YFPN::myoE) were grown as described (frame accumulation: 2; line average: 6; AOTF 514: 20%; gain: 900 V; offset: -0.2; scan speed: 1000 Hz; emission bandwidth: 522 nm - 658 nm; maximum projection of a 1.38 µm z-stack). (B) Y2H analysis of MigA and MyoE. Positive and negative controls as provided in the Matchmaker™ Gold Yeast Two-Hybrid System by Clontech Laboratories, Inc.. Dilution series of respective strains were grown on SD-LW and SD-LWH at 30°C for 3 days. (C) Colocalization of MigA and MyoE at the hyphal tip. Hyphae of SRM192 (migA::eGFP; myoE::TGACTA::mCherry) strain were grown as described (Exposure time 450 – 490nm: 400 ms/538 - 562 nm: 500 ms; Scale bar: 2 µm).
Figure 8. Model of the MigA-pathway during (A) Mitosis: MigA localizes dynamically to both spindle poles and along the mitotic spindle. From spindle pole bodies, MigA is loaded onto astral MTs and transported towards the MT plus-ends. At the plasma membrane, MigA facilitates the interaction between astral MTs and ApsA. This mechanism is predominantly important during early stages of mitosis. (B) Interphase: MTs are growing towards the hyphal apex. MigA is able to bind TubA independently, is transported to the MT plus-end in an EbA-dependent manner and reaches the hyphal tip. In the tip region, MigA interacts with MyoE, which drags MigA and thus the bound MT along the actin filaments towards the cell-end marker complex. Once reached the cortex MigA interacts with the cell end marker TeaA and thus anchors the MT for a short time to the polarization site. The model was created with ChemBioDraw Ultra (PerkinElmer, Cambridge, USA).
Supplemental Figure 1. Alignment of MigA and putative orthologues. The N-terminal stretch of MigA, which has no similarities with Kar9, is conserved in filamentous fungi. Several domains of unknown function in this stretch are highly conserved. Sequences were aligned (gap open cost: 10.0; gap extension cost: 1.0) with CLC Sequence Viewer 6.6.1 (Qiagen, Venlo, Netherlands).
Supplemental Figure 2. Scheme of migA deletion and verification. (A) Scheme of the genomic locus of wild type and ΔmigA mutants. Primer for PCR and probes for the Southern Blot analysis as well as expected fragment sizes are indicated. (B) PCR with genomic DNA from wild type and ΔmigA strains as template according to scheme in A. Standard PCR protocols were applied and primers were used as indicated. (Marker: 1kb DNA Ladder, #N3232L, NEB). (C) Southern Blot analysis from genomic DNA of wild type (SO451) and ΔmigA (SRM2) strains according to scheme in A with both probes. 40 µg of genomic DNA was digested with 10 U SalI-HF (#R3138L, NEB) for 16 h. 100 ng of each probe was used for hybridization. Standard Southern Blot protocols were applied.
Supplemental Figure 3. MigA localizes to cortical clusters in the absence of the MT cytoskeleton and self-activation tests for Y2H constructs. (A) Maximum projection of a confocal scanning image of cortical MigA clusters upon treatment with the MT destabilizing agent benomyl. Additionally, MigA localizes to SPB’s (arrowheads). Hyphae of the strain SRM22 (migA::eGFP, alcA(p)::mCherry::tubA) were grown as described. After 12 h incubation the medium was removed and replaced with benomyl-containing medium (10 µg/ml for 1 h). Subsequently, medium was replaced with medium containing benomyl and membrane-staining dye FM 4-64 (20 µg/ml for 1 min). Hyphae were checked visually if the MT cytoskeleton was disassembled. MigA-eGFP and FM 4-64 staining shown in false color heat map. (frame accumulation: 4; line average: 3; AOTF 488: 30%; gain: 800 V; offset: -0.1; emission bandwidth: 493 nm – 556 nm; gain: 550 V; offset: -0.03; emission bandwidth: 566 nm – 706 nm; scan speed: 400 Hz; maximum projection of a 3.02 µm z-stack; Scale: 2 µm). (B) Maximum projection of a confocal scanning image of MigA and ApsA. MigA partially colocalizes with cortical ApsA. Hyphae of the strain SRM193 (alcA(p)::mCherry::migA; alcA(p)::GFP::apsA) were grown as described (frame accumulation: 4; line average: 4; AOTF 488: 5%; gain: 800 V; offset: -0.1; AOTF 561: 5%; gain: 800 V; offset: -0.03; scan speed: 1000 Hz; emission bandwidth: 493 nm – 556 nm / 566 nm – 750 nm maximum projection of a 5.54 µm z-stack; Scale: 5 µm). (C) Self-activation tests for respective Y2H constructs. Four day old single colonies of same sizes were selected, resuspended in 1 ml sterile H₂O, 5 µl spot-inoculated on appropriate medium and screened for growth after 5 days incubation at 30°C. Positive and negative controls as provided in the Matchmaker™ Gold Yeast Two-Hybrid System by Clontech Laboratories, Inc.. Protein extracts were prepared as described in the Matchmaker™ GAL4 Two-Hybrid System 3 Manual. 15 µl of crude protein extract was loaded onto appropriate SDS-Gel. Western Blot was performed with either anti HA (sigma-aldrich, #M4776) or anti c-myc antibodies (sigma-aldrich, #M5546) and anti mouse-HRP antibodies (sigma-aldrich, #M4820) according to manufacturers manual.
Supplemental Figure 4. Phenotypes of MigA BiFC and double-deletion strains (A) Upon incubation with 2% glycerol and induction of the alcA promoter, MigA uniformly decorates the MT cytoskeleton. Hyphae of the strain SRM12 (alcA(p)::eGFP::migA) were grown as described (exposure time 450 – 490nm: 500 ms; maximum projection of a 4.68 µm z-stack; scale bar: 10 µm). (B) Phenotypes of YFPC::MigA, YFPN::TeaA and the corresponding BiFC strain in repressing and derepressing medium. Strains were grown on MM media plates with appropriate supplements and 2% glucose or glycerol for 3 days at 37°C. Hyphae of (I-II) wild type (TN02A3), (III-IV) YFPC::MigA (SRM135), (V-VI) YFPN::TeaA (SRM202) and (VII-VIII) YFPC::MigA/YFPN::TeaA (SRM18) in media with 2% glucose (odd numbers) or 2% glycerol (even numbers). The strain SRM18 (alcA(p)::YFPC::migA, alcA(p)::YFPN::teaA) shows a dominant-negative phenotype in polar growth. Hyphae show an extremely curved phenotype due to irreversible interaction of MigA and TeaA (VIII: Exposure time 515 nm: 500 ms; maximum projection of a 4.16 µm z-stack; Scale bar: I, III, IV, VIII: 10 µm; II, VII: 8 µm; V, VI: 5 µm). (C) Phenotype resulting from overexpression of MyoE. MyoE localizes in a dense cluster at the hyphal tip as well as in distal parts of the hyphae. Hyphae of the strain SRM15 (alcA(p)::mCherry::myoE) were grown as described (Exposure time 538 - 562nm: 150 ms; Scale bar: 5 µm). (D) Dominant-negative phenotype in strain SRM17. Cells show an inconsistent diameter and swell at the end of the hyphae. Furthermore, they display a slightly curvy phenotype (exposure time 515 nm: 500 ms; maximum projection of a 2.6 µm z-stack; scale bar: 10 µm). (E) Colonies of (I) wild type (TN02A3), (II) ΔmigA (SRM86), (III) ΔmyoE (SRM89), (IV) alcA(p)::eGFP::migA (SRM12), (V) alcA(p)::mCherry::myoE (SRM15) and (VI) alcA(p)::YFPC::migA / alcA(p)::YFPN::myoE (SRM17) strains on glucose and glycerol as carbon source. Strains were grown on MM plates with appropriate supplements and 2% glucose or glycerol for 3 days at 37°C. (F) Localization of TeaA at the hyphal apex (false-color heat map). Hyphae of the strain SRM173 (teaA(p)::mRFP1.2::teaA::pyr4, teaR(p)::eGFP::teaR::pyr4) were grown as described. (Exposure time 538 - 562nm: 500 ms; maximum projection of a 2.18 µm z-stack; Scale bar: 2µm).
Supplemental movie 1. MigA localizes dynamically to both spindle poles. Dynamic localization of MigA::eGFP (A) at both spindle poles (colored circles in D), along the mitotic spindle and on astral MTs (B). Hyphae of SRM22 (migA::eGFP, alcA[p]::mCherry::tubA) were grown as described (exposure time 450 – 490nm: 500 ms/538 – 562nm: 500 ms; scale bar: 1 µm).
Supplemental movie 2. Comparison of spindle motility in WT (left) \( \Delta \text{migA} \) (middle) and \( \Delta \text{apsA} \) (right) strains. Confocal scanning time-lapse image of mitotic spindles. Astral MT fail to establish contact with the plasma membrane in early stages of mitosis. Spindle motility is significantly altered in \( \Delta \text{migA} \) (\( p=0.00496 \)) and \( \Delta \text{apsA} \) (\( p=0 \)) strains compared to the wild type at \( p \leq 0.01 \). However, emergence does only differ significantly (\( p=0.0703 \)) between \( \Delta \text{migA} \) and \( \Delta \text{apsA} \) strains at \( p \leq 0.1 \). (WT: \( n_{\text{cells}}=22, n_{\text{spindle}}=29, n_{\text{data points}}=142 \); \( \Delta \text{migA} \): \( n_{\text{cells}}=15, n_{\text{spindle}}=20, n_{\text{data points}}=151 \); \( \Delta \text{apsA} \): \( n_{\text{cells}}=13, n_{\text{spindle}}=29, n_{\text{data points}}=190 \)). Hyphae of the strains SRM118 (\( \text{alcA}_{(p)}::\text{eGFP}::\text{tubA} \)), SRM124 (\( \Delta \text{migA}, \text{alcA}_{(p)}::\text{eGFP}::\text{tubA} \)) and SRM136 (\( \Delta \text{apsA}, \text{alcA}_{(p)}::\text{eGFP}::\text{tubA} \)) were grown as described (frame accumulation: 2; line average: 4; AOTF 488: 5%; gain: 850 V; offset: -0.1; scan speed: 1000 Hz; maximum image acquisition speed; emission bandwidth: 493 nm – 655 nm; Scale: 1 \( \mu \text{m} \)).
Supplemental movie 3. Time-lapse image of the localization of MigA at growing MT plus-ends. MigA is moving along the MT to the plus-ends and, therefore, is transported to the hyphal tip. Hyphae of the strain SRM22 (migA::eGFP, alcA(ρ)::mCherry::tubA) were grown as described (exposure time 450 – 490nm: 500 ms/538 – 562nm: 500 ms; scale bar: 2 µm).
Supplemental movie 4. Overexpression of MigA leads to complete decoration of cytoplasmic MTs. Upon incubation with 2% glycerol and induction of the alcA promoter MigA decorates uniformly the MT cytoskeleton. Hyphae of the strain SRM129 (alcA(p)::eGFP::migA, alcA(p)::mCherry::tubA) were grown as described (frame accumulation: 3; line average: 6; AOTF 488: 15%; gain: 900 V; offset: -0.1; AOTF 561: 15%; gain: 800 V; offset: -0.03; scan speed: 400 Hz; emission bandwidth: 495 nm – 556 nm / 581 nm – 717 nm; scale: 5 µm).
Supplemental Movie 5. Localization of MigA at growing and retracting MT plus-ends.
Calculated positions of single mEosFPthermo labeled MigA molecules. MigA localizes to growing and retracting MT plus-ends. Overlay shows the mathematical computed positions of all MigA::mEosFPthermo particles detected in the time-lapse image. Hyphae of the strain SRM40 (migA::mEosFPthermo, alcA(p)::eGFP::tubA) were grown as described (exposure time 200 ms; 473 nm laser: 1%; 561 nm laser: 1%; 405 nm laser: 2%; scale bar: 1 µm).
Supplemental movie 6. Comparison of MT convergence in WT (top) ΔmigA (middle) and ΔteaA (bottom) strains. MT convergence in wild type (SRM164), ΔmigA (SRM166a) and ΔteaA (SRM168) strains. eGFP labeled KipA under the control of the alcA promoter was used to visualize MT plus-ends. Arrowheads indicate positions were MT made first contact with the plasma membrane at the hyphal tip. Respective strains were grown as described (exposure time 450 – 490nm: exposure time: 200 ms; scale bar: 2 μm).
Supplemental movie 7. Co-transport of MigA and MyoE. A fraction of MyoE is moving away from the tip and then moves back to the cortex. During anterograde movement MyoE colocalizes with MigA. Arrowheads indicate the position of MigA and MyoE, respectively. Hyphae of SRM192 (migA::eGFP; myoE::TGACTA::mCherry) strain were grown as described (Exposure time 450 – 490nm: 400 ms/538 - 562 nm: 500 ms; Scale bar: 2 µm).
Table S1

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Table S2

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Table S3

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