Clustering of nuclei in multinucleated hyphae is prevented by dynein-driven bidirectional nuclear movements and microtubule growth control in Ashbya gossypii

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Running title: Role of dynein in A. gossypii

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Abbreviations used: cMT, cytoplasmic microtubule; SPB, spindle pole body
Abstract

During filamentous fungi development, multinucleated hyphae employ a system for long-range nuclear migration to maintain an equal nuclear density. A decade ago the microtubule motor dynein was shown to play a central role in this process. Previous studies with *A. gossypii* revealed extensive bidirectional movements and by-passings of nuclei, an autonomous cytoplasmic microtubule (cMT) cytoskeleton emanating from each nucleus, and pulling of nuclei by sliding of cMTs along the cortex. Here, we show that dynein is the sole motor for bidirectional movements and by-passing because these movements are concomitantly decreased in mutants carrying truncations of the dynein heavy chain *DYN1* promoter. The dynactin component Jnm1, the accessory proteins Dyn2 and Ndl1, and the potential dynein cortical anchor Num1 are also involved in the dynamic distribution of nuclei. In their absence, nuclei aggregate to different degrees, whereby the mutants with dense nuclear clusters grow extremely long cMTs. As in budding yeast, we found that dynein is delivered to cMT +ends, and its activity or processivity is probably controlled by dynactin and Num1. Together with its role in powering nuclear movements, we propose that dynein also plays (directly or indirectly) a role in the control of cMT length. Those combined dynein actions prevent nuclear clustering in *A. gossypii* and thus reveal a novel cellular role for dynein.
Migration of nuclei in multinucleated hyphae of filamentous fungi is a fascinating but also a very complex dynamic process which is far from being understood. The efficient polar growth of hyphae continuously generates new cytoplasmic space. Hyphae maintain a relatively even distribution of nuclei by adjusting the migration and the division of nuclei to the hyphal growth speed. Important components of this control system were first identified as mutants exhibiting nuclear distribution defects, and were termed nud in Aspergillus nidulans and ropy in Neurospora crassa. Most of the affected genes coded for components of the microtubule motor dynein and its activating complex dynactin (27, 30, 42, 43). Dynein and dynactin components were found to form comet-like structures at the +end of cytoplasmic microtubules (cMT) in N. crassa and A. nidulans thereby indicating that cMTs and very likely the dynamics of cMTs are important for nuclear migration (10, 27, 45). Dynein was also shown to be essential for functional distribution of nuclei in Nectria haematococca, Ashbya gossypii and the dimorphic fungus Ustilago maydis (1, 13, 37). However, surprising phenotypic differences were observed questioning the existence of a single mechanism controlling nuclear distribution in fungi. In the absence of dynein, nuclei remain in the germ bubble of A. nidulans where they form clusters whereas in A. gossypii all nuclei move out of the germ bubble and form clusters at hyphal tips (1, 42). Hyphae lacking dynein generate more stable or longer cMTs in A. nidulans and A. gossypii, whereas fewer and shorter cMTs form in N. haematococca (1, 10, 13). An additional complication is the double role of dynein in
filamentous fungi because this motor mediates nuclear migration as well as organelle transport (34, 35, 40). The only exception seems to be *A. gossypii* because in this fungus, cMTs, as in budding yeast, are only involved in nuclear migration and not in transport of other organelles or vesicles (7, 17).

Our initial *in vivo* studies on long range nuclear migration in *A. gossypii* had shown that nuclei exert autonomous forward and backward movements during all nuclear cycle stages including mitosis and that nuclei frequently bypass adjacent nuclei (1, 7). Thus, forces acting in opposite directions on nuclei result in a dynamic distribution with an average distance between nuclei of 5 to 6 µm. Bi-directional nuclear migration was also demonstrated in *A. nidulans*, and it probably also exist in young hyphae of *N. crassa* as inferred from the observed shorter and longer distances between nuclei, whereas nuclei in the much wider and very fast growing mature hyphae of *N. crassa* migrate in one direction, i.e. with the cytoplasmic flow (27, 31, 32).

Further studies with *A. gossypii* revealed that the spindle pole body (SPB) of each nucleus initiates a microtubule cytoskeleton consisting of 4 to 6 short and long cMTs. The long cMTs emanate in opposite directions with respect to the growth axis and often extend beyond adjacent nuclei. Those studies also showed that mutants growing only short cMTs still perform bi-directional movements but lack nuclear bypassing events, that mutants with no or very short cMTs lack both, bi-directional movements and by-passing of nuclei, and that mutants with slightly increased length of cMTs show higher frequencies of bi-directional movements and by-passing of nuclei (8, 18, 19). Knowing that 4
to 6 cMTs emanate from each nucleus in wild type hyphae, it is difficult to understand how nuclei attached to short and long cMTs pointing to different directions can by-pass each other without getting entangled in the narrow hyphal tube (the diameter of which is only twice the diameter of nuclei). It is possible that they sometime do and that a rescue system operates to untangle those nuclei to prevent larger nuclear aggregates to form. We hypothesize that a combination of bi-directional forces alternately acting on nuclei together with a length control for cMTs dissolve entangled nuclei thus preventing the formation of nuclear aggregates in *A. gossypii*.

Since loss of dynein activity leads to formation of nuclear clusters in filamentous fungi including *A. gossypii*, the dynein/dynactin complex is most likely an important component of such rescue system. This huge multi-subunit complex acts in all eukaryotes as a minus-end directed MT motor. It consists of two heavy chains with motor activity and MT-binding capacity, several intermediate and light chains, and accessory proteins for its activation (14). In the yeast *Saccharomyces cerevisiae* the dynein pathway has a very specific cellular function which unlikely is conserved in multinucleated hyphae. It acts together with the Kar9 pathway to position the nucleus at the bud neck and direct the pulling of one daughter nucleus into the bud (24, 29). The pulling function of dynein is restricted to anaphase in *S. cerevisiae*. According to the current model, which may also be valid for filamentous fungi, these pulling forces depend on lateral contacts of cMTs with the cortex mediated by their interactions with the minus-end motor dynein which is attached to the cell cortex via the anchor protein Num1 (6, 20, 29, 38).
In this paper we first aimed at identifying the origin of the forces responsible for bi-directional movement and by-passing of nuclei in *A. gossypii*. Then, we tested whether hyphae lacking dynactin components or other dynein accessory proteins show the same or less severe degree of nuclear aggregation originally observed in the dynein deletion mutant. Next, we monitored the localization of fluorescently labeled dynein expressed from its endogenous promoter to see potential alterations between wild type and different mutants including a mutant of the presumptive cortical anchor Num1. Finally, we compared the sizes of cMTs in mutants with decreased dynein expression and hyphae lacking dynein activity to try to differentiate between effects of overly long cMTs and lowered level of dynein in formation of nuclear aggregates. The individual results will mainly be compared with the dynein/dynactin system of budding yeast because *A. gossypii* carries a budding yeast-like genome (4) and because we want to demonstrate the evolution of the dynein pathway, as it is known from studies in *S. cerevisiae*, to an essential cellular function in multinucleated hyphae.

**MATERIALS AND METHODS**

*A. gossypii* media and growth conditions

*A. gossypii* media and culturing protocols are described in (2, 41). Strains were grown in Ashbya Full Medium (AFM medium: 1% bactopeptone, 1% yeast extract, 2% glucose and 0.1% myo-inositol) at 30°C. Transformants derived from *A. gossypii* reference strains were selected on AFM plates containing 200 mg/ml of G418/geneticin (ForMedium Ltd, England) or 50
µg/ml ClonNAT (Werner BioAgents, Germany). To test the effect of Benomyl on growth, AFM plates were prepared by adding 33 µM Benomyl (Sigma-Aldrich; dissolved in DMSO) directly into the medium before pouring the plates. 15µg/ml of Nocodazole (Sigma-Aldrich; dissolved in DMSO) was directly added in AFM liquid cultures and cells were monitored on microscopy slide 15 to 30 min after treatment.

**Strain construction**

*A. gossypii* transformation protocols are described in (2, 41). All strains constructed in this study were derived from reference strains expressing either a Histone H4-GFP fusion (ASG46 strain: ADE2-HHF1-yeGFP; (7), an exogenous GFP-Tubulin1 fusion (ADE2-GFP-AgTUB1; (19) or the BIK1-Cherry fusion (8). *A. gossypii* deletion mutants were made by a PCR-based one-step gene targeting approach (41). PCR were performed using standard methods with Taq polymerase from Roche, oligonucleotides were synthesized at Microsynth (Balgach, Switzerland). Oligonucleotide primers are listed in Table S1.

For gene deletions, *A. gossypii* cells were transformed with PCR products amplified with the pAG140 (GEN3) or pAG100 (NAT) templates and the "gene name"-del5/del3 oligonucleotide pairs, which contained 45-bp homology upstream and downstream of the ORFs. The primary transformation produces heterokaryon cells, which have a mixture of "wild-type" and transformed nuclei. Transformed heterokaryons were verified with oligonucleotide pairs "gene name"-VER5/G2.2 and "gene name"-VER3/G3.3 for GEN3 cassettes ("gene name"-VER5/V2*NAT1 and "gene name"-VER3/V3*NAT1 for NAT...
cassettes; see supplementary Table 1). For the DYN1 promoter truncations, cells were transformed with PCR products obtained with the pAG140 plasmid and the oligonucleotide pairs DYN1-180-NS1/DYN1PR-DELF2 (only the 180 bp upstream of the ATG were retained; the sequence from -673 to -181 bp was replaced by the GEN3 cassette) and DYN1-130-NS1/DYN1PR-DELF2 (only the 130 bp upstream of the ATG were retained; the sequence from -673 to -131 bp was replaced by GEN3). The correct integration of the two cassettes was then checked by PCR with the same primer pairs: DYN1-PROM-I2/DYN1-PROM-G4 and DYN1-PROM-I1/DYN1-PROM-G1 (for the absence of a WT promoter) and G2.1/DYN1-PROM-G4 and G3.3/DYN1-PROM-G1 (for the presence of the deletion cassette). Three homokaryons (obtained after sporulation of three independent verified transformants) were characterized for each mutant.

For the Dyn1-tdTomato fusion, a tdTomato-NAT-tagging cassette was produced by PCR using as a template the pAGT213 plasmid (15) and the primers DYN1-TAG3 and DYN1-TAG5 (homologous to the 45 bp upstream and downstream of the AgDyn1 stop codon respectively). The resulting PCR product was co-transformed into yeast with the pAG1011 plasmid (encoding the C-terminal part of Dyn1). After verification of the fusion by sequencing and digestion with Clal/BglIII, the fragment of DNA showing long regions of homology with the 3’ end of Dyn1 was transformed into GFP-Tub1 A. gossypii cells. Verifications of the heterokaryons and homokaryons were performed as described above with the primer pairs DYN1-VER3/V3*NAT1.
Immunofluorescence and time-lapse microscopy

We used an Axioplan 2 imaging microscope equipped with the objectives Plan-Apochromat 100x 1.40-numerical-aperture oil differential interference contrast (DIC) and Plan-Apochromat 63x 1.40-numerical-aperture oil DIC (Carl Zeiss AG, Feldbach, Switzerland) and appropriate filters (Zeiss and Chroma Technology, Brattleboro, VT). The light source for fluorescence microscopy was a Polychrome V monochromator (TILL Photonics GmbH, Gräfelfing, Germany). Images were acquired at room temperature using a CoolSNAP HQ cooled charge-coupled device camera (Photometrics, Tucson, AZ) with MetaMorph 6.2r6 software (Molecular Devices Corp., Downingtown, PA). The distance between two planes in stack acquisitions was set to 1 µm for H4-GFP movies, 0.3 µm for anti-tubulin and actin stainings. Brightness and contrast were adjusted using MetaMorph's “scale image” command. Stacks were deconvolved with MetaMorph's “2-D deconvolution” module and flattened by maximum projection with the “stack arithmetic” function. The Bik1-Cherry GFP-Tub1 and Dyn1-TdTomato GFP-Tub1 movies were one Z-plane movies treated with Flatten Background/Kernel/Equalize light functions. Images were colored and overlaid using MetaMorph's “overlay images” command. Time-lapse picture series were processed as described above and converted into QuickTime MPEG-4 movies (QuickTime Player Pro, Apple Inc.). Immunofluorescence stainings were performed as described previously (2, 7). Rat anti-alpha-tubulin (YOL1/34; Serotec, Oxford, United Kingdom) was used at a 1:50 dilution and Alexa Fluor 568 goat anti-rat IgG (Invitrogen, Carlsbad, CA) at a 1:200 dilution. For time-lapse acquisition, small pieces of...
2-days old mycelium were cultured on agarose slides as previously described (19).

RESULTS

Hyphae lacking Kar9 and Bim1 show even nuclear distributions

A strong nuclear clustering phenotype was observed in hyphae lacking the dynein heavy chain Dyn1. Since all nuclei in germlings of this mutant clustered in the tip of the first emerging hypha it was anticipated that dynein is the motor responsible for retrograde movements while another motor was responsible for the tip-directed movements (1). In an attempt to identify this motor, all six kinesin genes of A. gossypii were deleted but none of the deletions showed nuclear clustering (C. Alberti-Segui unpublished).

We then asked whether the AgKar9 protein could be responsible for the tip directed nuclear movements because of the function of its S. cerevisiae homolog. Indeed, in budding yeast, Kar9 specifically accumulates at the bud-proximal SPB and at cMTs emerging from it to ensure that only this SPB will move into the bud later, during anaphase. This bud tip-directed guidance of cMTs also depends on Bim1, the myosin motor Myo2 and actin cables. In the current model, Kar9 binds to cMT +ends via Bim1. The Kar9-loaded MTs are then pulled towards the bud tip along actin cables due to the interaction of Kar9 with Myo2 (12, 24). In A. gossypii, actin cables emerge at hyphal tips and are oriented along the polarity axis to allow directed transport of secretory vesicles (16). We hypothesized that Kar9 and Bim1 may be involved in tip-
directed (forward) nuclear movements via a mechanism similar to budding yeast. AgKar9 is 31.6% identical to ScKar9 and contains an additional 158 aa C-terminal extension. AgBim1 shares 55.4% identity with its budding yeast orthologue. We constructed Agkar9∆ and Agbim1∆ deletions in a histone H4-GFP background and studied the effect of the deletions on nuclear dynamics and distribution.

As shown in Figure 1A, the deletions of KAR9 and BIM1 only slightly reduced the radial growth rate (97% and 90% of WT (H4-GFP), respectively). We performed 1 min interval time-lapse movies with WT and mutant hyphae to quantify the ability of nuclei to oscillate and by-pass each other. These cMT-dependent movements are superimposed on the overall nuclear migration with the cytoplasmic stream, which, for nuclei close to the hyphal tip, is similar to the growth speed of the hyphae (19). For our measurements we defined “forward events” (faster than the cytoplasmic stream) and “backward events” (against the cytoplasmic stream) as follows. Forward movements during 1 min with at least twice the speed of the hyphal tip were counted as forward events. Backward movements during 1 min with at least half the speed of the hyphal tip were counted as backward events. This strict definition was also previously employed (8). The 5 nuclei closest to the hyphal tips were tracked for 30 min in 7 different WT and mutant hyphae (Figure 1B and Table 1). We also measured the distances between neighboring nuclei in WT, kar9∆ and bim1∆ strains which are presented as histograms of four size ranges (Figure 1C).

As evident from Figure 1B, nuclei in kar9∆ and bim1∆ hyphae did not form clusters and were still able to move back and forth and by-pass each other.
There was no significant difference in the number of forward events between WT and the mutant hyphae (Table 1). Interestingly, we noticed significantly more backward and double as many by-passing events in the kar9Δ strain indicating an overall increase in nuclear dynamics compared to WT (Table 1).

Hyphae lacking Bim1 reveal slightly more backward and a strong decrease in by-passing events (3 in bim1Δ, 9 in WT, and 19 in kar9Δ). This low number is probably related to the overall increase in nuclear distances in bim1Δ hyphae (Figure 1C; N-N distances > 6 µm: 27.5% in WT; 76.3% in bim1Δ). Budding yeast Bim1 is known to control the dynamics of kinetochore-MTs during anaphase (46). The A. gossypii homolog most likely plays a similar role, and it is therefore conceivable that the increased N-N distances in hyphae lacking Bim1 result from a lower mitotic index. Since budding yeast Kar9 is important to direct the nucleus towards the bud tip, we tested whether hyphae lacking Kar9 would show an increased distance between the first nucleus and the hyphal tip. No such increase was found (Figure 1D).

All together, our results strongly suggest that forward nuclear movements do not depend on the potential ability of Kar9/Bim1 to guide the cMTs/SPB along polarized actin cables and to pull nuclei (especially the nucleus closest to the tip) in the direction of the hyphal tips. The overall higher nuclear mobility in the kar9Δ mutant is partly reminiscent of the kar9Δ phenotype in S. cerevisiae with its increased dynein-dependent oscillations of the nucleus through the bud neck (44). The increased frequency in backward events in this mutant suggests a role of Kar9, even if minor, in transiently anchoring cMTs +ends on
actin cables to counter-balance dynein-dependent backward events, or in controlling the number of cMTs directed toward the hyphal tips.

**Dynein drives backward and forward nuclear movements**

Since the Kar9 homolog of *A. gossypii* does not play a role in forward movements we hypothesized that dynein, in addition to its obvious role in backward movements, could also be responsible for forward movements. It was already recently suggested that dynein could be the motor which induces cMT sliding along the cell cortex and actively pulls on nuclei, not only in the opposite direction of the hyphal tip but also toward it (8). It is not possible to test this hypothesis by directly measuring nuclear movements in a dyn1Δ strain because, already in germlings, nuclei move to the tip of emerging hyphae where they form clusters (1). Nuclei in these clusters are so close to each other that oscillatory movements cannot be analyzed by 1 min interval movies (Figure 2B). To overcome this difficulty we decided to observe the effects of reduced expression of the motor. Several attempts to exchange the strong promoter of the dynein gene with a weak *A. gossypii* promoter failed. We then decided to construct and test truncations of the DYN1 promoter. Two shortened DYN1 promoter mutants were created: the prom180-DYN1 strain carries only 180bp and the prom130-DYN1 strain only 130 bp original sequences upstream of the start codon. The prom180-DYN1 and prom130-DYN1 strains respectively grew with close to 100% and 80% of WT radial growth rate (Figure 2A). They showed only a weak tendency of nuclear clustering compared with the dynein deletion mutant which allowed us to monitor the mobility of individual nuclei with 1 min interval movies (Figure 2B).
In both strains, the ability of nuclei to oscillate and by-pass each other is impaired (Table 1). Compared to WT, the number of forward and backward events was both decreased by approx. 50% in the prom180-DYN1 mutant. The prom130-DYN1 strain displayed even stronger decrease in oscillation events (27% of WT forward events, and 7% of WT backward events). In order to compare different degrees of clustering we counted the number of nuclei within 15 µm long hyphal segments containing nuclear clusters and defined the average number as clustering index. The highest density was measured in dyn1Δ hyphae with an average of 17.5 nuclei (max. 25 nuclei) compared to an average of 4.5 nuclei in 15 µm long WT hyphal segments. The loose nuclear clusters visible in the prom 130-DYN1 mutant have a clustering index of 6 whereas the prom 180-DYN1 mutant, which retains 50% of the bidirectional nuclear movements, has a clustering index like wild type.

All together, these results strongly indicate that dynein is the key motor for both forward and backward nuclear movements in A. gossypii and that its decreased expression in the prom130-DYN1 strain leads to pronounced reduction of bidirectional mobility concomitant with the formation of loose nuclear aggregates.

Dynactin plays a key role in preventing aggregation of nuclei

Because dynein is essential for the dynamic homogeneous distribution of nuclei and the prevention of nuclear cluster formation in A. gossypii hyphae, we asked whether dynactin, the complex necessary for activating dynein, also plays a similar role. Hyphae lacking dynactin components may show different
degrees of nuclear distribution defects depending on their importance for
dynein activation. A complete loss of dynein activation could cause dense
clusters of nuclei and an almost complete absence of single nuclei as
observed in hyphae lacking the dynein heavy chain (1).

We first confirmed the dynein deletion phenotypes by deleting the ORF for the
dynein heavy chain in a strain expressing a Histone H4-GFP fusion. As
expected, this dyn1Δ strain showed a strong growth defect and dense nuclear
clustering mainly at tips of young hyphae and later also at branching sites of
mature hyphae (Figure 2B, 3A and 3B). We also noticed that the size of the
dyn1Δ nuclei was reduced in the clusters. We then deleted the complete open
reading frame of JNM1, a gene encoding the homolog of a subunit of the
dynactin complex, in the H4-GFP strain. As mentioned above, the dynactin
complex is an essential dynein adaptor, found to be involved in most of dynein
functions and its inhibition is phenotypically similar to a complete loss of
dynein function (14). Even though AgJnm1 is only weakly related to ScJnm1
(28.2% identity), its deletion induced phenotypes also observed in dyn1Δ
hyphae, i.e. a strong growth defect, severe nuclear clustering and an almost
complete absence of single nuclei (Figure 3A and 3B). The only apparent
difference between both mutants is seen during germination. All nuclei in
germinating spores carrying the dyn1Δ allele move to the tip of the first
emerging hyphae which leads to tip-located nuclear clusters, and only later,
clusters also form throughout the hyphae ((1) and Figure 2B and 3B). Nuclei
in germinating spores carrying the jnm1Δ allele distribute similarly to wild type,
most likely because these spores still carry some Jnm1 protein. Clusters form
only later throughout the branching hyphae and less frequently in tip regions (Figure 3B). In jnm1Δ hyphae, nuclear clusters contained on average 14.9 nuclei (max. 19 nuclei) which is almost as dense as nuclear clusters in dyn1Δ hyphae (Figure 3D).

Together, these results show that the dynactin component Jnm1 is also essential for nuclear distribution and for preventing formation of nuclear aggregates.

**Dyn2 and Ndl1 contribute to the dynamic distribution of nuclei**

The dynein heavy chain Dyn1 interacts with several light and intermediate chains within the dynein complex. In addition, several proteins that do not belong to the dynein complex itself are important for adapting the motor to its cellular function. We tested the deletion phenotypes of two of these factors, Dyn2 and Ndl1. In budding yeast, the role of the dynein light chain Dyn2 for dynein activity is not critical since a dyn2Δ mutant exhibits only partial defects in certain spindle position assays (29). The *A. gossypii* Dyn2 shows 71% identity with ScDyn2. In contrast to dyn1Δ, the dyn2Δ mutant only has a slightly decreased radial growth rate (85% of WT; Figure 3C). However, nuclear distribution is significantly impaired in dyn2Δ: nuclear clusters form with an average of 9.3 nuclei in 15 μm long segments (4.5 in WT), which is a lower nuclear density than in clusters of dyn1Δ and jnm1Δ strains (Figure 3D).

We also deleted the gene encoding AgNdl1, the homolog of which is involved in the accumulation of dynein at cMT +ends in budding yeast but its role in
dynein function is not clearly understood (20, 23). The radial growth rate of
the ndl1Δ mutant is only slightly decreased (94% of WT) and nuclei only rarely
form loose clusters with an average density of 7.7 nuclei in 15 µm long
segments (Figure 3C and 3D). Radial growth in these two mutants is much
less affected by nuclear aggregates compared to the dyn1Δ and jnm1Δ
mutants. Possible reasons for these growth differences may be the insufficient
distribution of mRNAs and vesicles in the tightly clustered mutants (the
fraction of non-aggregated nuclei is less than 10% in dyn1Δ and jnm1Δ, 29% in
dyn2Δ and 51% in ndl1Δ mycelia) or a severe impairment of organelle and
secretory vesicle transport by dense nuclear aggregates.

All together, the dynein adaptors AgDyn2 and AgNdl1 support nuclear
distribution and play a role in preventing nuclear cluster formation in A.
gossypii.

Dynein localizes to +ends of cMTs emanating from SPBs in both
directions of the growth axis, to cMT −ends and along cMTs
To begin to understand the underlying mechanisms of nuclear movements in
A. gossypii, we looked at dynein localization. We fused the TandemTomato
tdTomato) fluorescent protein to the C-terminal end of dynein in hyphae
expressing the GFP-Tubulin1 fusion. The Dyn1-tdTomato fusion was
expressed at endogenous level and was fully functional since the strain
expressing this fusion was growing with wild type rate (data not shown). Using
time-lapse video microscopy, we first tested whether the dynein motor, in
accordance with its role in forward and backward movements of nuclei, could
be observed at the +ends of cMTs growing in the direction of the hyphal tip and opposite to it. One example with eleven consecutive images taken in 6 sec intervals is shown in Figure 4A. Dynein (red) is seen at the +end of a cMT emanating from a SPB (green) and growing 2.7 µm towards the hyphal tip during the first four images. Starting in the sixth image, dynein is seen at the +end of a second cMT emanating from the same SPB but in the opposite direction and growing 4.5 µm during the next five images. Dynein can also clearly be observed at the SPB in all images and, during six images, along a third cMT which may or may not emanate from the same SPB. Visual inspection of many images revealed that dynein localizes mainly at SPBs (cMT –ends) during all nuclear cycle stages and, as less intense foci, along cMTs and at cMT +ends (data not shown and Figure 5A). Rarely, dynein foci were detectable at hyphal tips, probably when +ends of growing cMTs reached the tip cortex (see also below). With our label, we did not detect dynein foci along the hyphal cortex. Cortically-located dynein could be seen in budding yeast but only using Dyn1 fused to 3 GFPs, and dynein localization at cMT +ends in budding yeast is more predominant compared to A. gossypii (20, 21).

Another very important difference between budding yeast and A. gossypii is the functional distinction between the two SPBs in metaphase. In yeast, dynein loading is restricted in metaphase to the SPB/cMT which enters the bud in order to help the spindle to align along the polarity axis and to control the pulling of only one SPB through the bud neck (9). In A. gossypii, nuclear division and cytokinesis are not strictly coordinated, and nuclei are in
continuous motion. Thus, a requirement for an asymmetric distribution of
dynein at metaphase SPBs is very unlikely. Indeed, metaphase spindles often
changed orientations with respect to the growth axis prior to anaphase and a
dynein signal was always visible on both SPBs (Figure 4B).

In conclusion, dynein is seen at all SPBs, independently of the nuclear cycle
stage. It is transiently visible at +ends of cMTs emanating in both directions
from SPBs with respect to the growth axis, which is consistent with the role of
this motor in bidirectional nuclear movements.

Jnm1 is important for transient localization of dynein at cMT + ends
Since *A. gossypii* dynein accumulation at cMT +ends is transient, it is possible
that dynein gets regularly “off-loaded” from the cMT +ends to the cell cortex
as it has been proposed in budding yeast (20). The “dynein off-loading model”
requires that first an inactive form of dynein is transported to cMT +ends. The
dynein complex detaches then from the +end when it reaches the cell cortex.
This leads to a stable cortex association of dynein. At the same time, the
dynactin complex and some cortical factors (Num1 for example) may
stimulate the -end motor activity of the anchored dynein (see review by (28)).

To test whether this model is relevant in *A. gossypii*, we determined the time
dynein was visible at +ends of cMTs in WT and the *jnm1Δ* dynactin mutant,
and measured in addition the intensity of the dynein signals at cMT +ends.
Our time-lapse video microscopy (1 Z planes, 6 sec intervals; Figure 4A and
5A) revealed that the motile dynein dots (most probably associated with cMT
+ends) are only visible for short periods in WT (17.5 sec on average; Figure 5C). For comparison, the MT +end tracking protein Bik1 can be visible for more than 3 min at cMT +ends in A. gossypii (8). Compared to WT, dynein localization in jnm1Δ significantly increased at cMT +ends, and concomitantly decreased at SPBs indicating that less dynein migrates to the cMT –ends (Figure 5A). Moreover, the average time dynein spent at the cMT +ends in jnm1Δ significantly increased compared to WT (17.5 sec for WT and 47.7 sec for jnm1Δ; n=35; Figure 5C). When WT and jnm1Δ hyphae were treated with the MT-destabilizing drug Nocodazole (NZ), dynein localization at SPBs was completely lost (Figure 5B).

Together these results indicate that dynein is most likely not directly loaded on SPBs but travels along cMTs toward the –ends/SPBs. Our data also strongly suggest that dynein accumulation at SPBs is dynactin-dependent, and that dynactin is necessary to keep the accumulation of dynein at cMT +ends very transient, most probably because dynactin activates the –end motor activity of dynein. The off-loading model presented in S. cerevisiae may therefore also be valid in A. gossypii.

AgNum1 is involved in nuclear distribution and in the control of dynein accumulation at cMT +ends

Num1 has been shown in budding yeast to be necessary for the cortical attachment of dynein. Num1 associates with dynein and with the plasma membrane as discrete non-motile patches (5, 6, 11). Recent work has shown that ScNum1 is targeted to the plasma membrane via its C-terminal Pleckstrin...
homology (PH) domain (aa 2563-2692) (29, 38). The A. gossypii Num1 homolog (3645 aa) shares 29.4% identity with ScNum1 (2748 aa). Interestingly, a stretch of 178 aa in the C-terminus of AgNum1 shares 67% identity with the C-terminus of the budding yeast ortholog and also contains a PH domain. We therefore supposed that AgNum1 is also targeted to the plasma membrane, and tested whether its role in nuclear migration is conserved in A. gossypii. So far, our attempts to delete the complete 10938 bp NUM1 ORF or its 3’ half have not been successful. However, we were able to delete the last 515 bp of the ORF (encoding the PH domain) in the H4-GFP background. This num1ΔCt mutant showed a mild growth defect (91% of WT growth rate) but frequently formed loose nuclear clusters with on average 8.4 nuclei in 15 μm segments (Figure 6A, B and C). These clusters showed similar densities as in dyn2Δ and ndl1Δ strains (average 9.3 and 7.7 nuclei, respectively) and were less dense than clusters in dyn1Δ (average 17.5 nuclei). We also looked at the ability for individual nuclei to oscillate and by-pass each other. The results compiled in Table 1 reveals that the number of bypassing, forward and backward events are overall reduced in the num1ΔCt mutant compared to the WT strain but to a lesser extent than in the prom130-DYN1 mutant. Interestingly, this indicates that dynein can still exert pulling forces, although reduced, on nuclei in hyphae expressing the carboxy-terminal truncation of Num1. This remaining dynein activity exceeds the dynein activity in the low level expressing prom130-DYN1 mutant.
We also wanted to check dynein localization in a NUM1 deletion mutant. However, as mentioned above, our attempts for deletion of this long gene failed. We only could delete the first 1500 bp of the NUM1 ORF in the Dyn1-TdTomato GFP-Tub1 background. Since the GEN3 deletion cassette replaced the coding sequence of the first 500 amino acids of Num1, we assumed that the protein was not expressed in the mutant strain (we named num1ΔN1). Hyphae of this strain also exhibit nuclear clustering, visualized by regions with densely populated GFP-labeled SPBs, and grow with a similar rate than num1ΔCt (data not shown). In this mutant we also clearly observed very long cMTs. Some long growing cMTs were curving while reaching hyphal tips (see * in Figure 6D). These curvatures were probably induced by physical constrictions and not lateral cortex interactions characteristic of a sliding event. In contrast to WT cells but similar to jnm1Δ, the concentration of dynein at SPBs in the num1ΔN1 mutant was weak and significantly increased at cMT +ends (compare Figure 5A and 6D).

Together, these results indicate that Num1, like Jnm1/dynactin, plays an important role in the dynamic localization of dynein at cMT +ends in A. gossypii, and that Num1 very likely has to bind to the plasma membrane to fulfill its function. Thus, major control components for dynein localization and activity are conserved in S. cerevisiae and A. gossypii, even though the specific cellular roles of dynein in both organisms have changed during evolution.
Hyphae lacking Jnm1 exhibit very long cMTs which maintain nuclei in clusters.

Previous work has shown that the lack of Dyn1 is associated with the formation of unduly long cMTs in *A. gossypii* (1). MT immunostainings of jnm1Δ hyphae also shows that very long cMTs were spreading in the anucleated regions and that very short cMTs are only visible in nuclear clusters (Figure 7A). On average, cMT length was 9.6 µm in jnm1Δ (n=147, max length: 40 µm) and 5.6 µm in WT (n=165, max length: 26 µm) (Figure 7B). Only 9% of the WT cMTs are longer than 12 µm. This category reaches 34% in jnm1Δ cells. The Figure 7B also shows a WT-like cMT lengths distribution in hyphae expressing less dynein (prom180-DYN1 and prom130-DYN1 strains).

We then looked at cMTs behavior and polymerization rate in jnm1Δ hyphae *in vivo* (in a Bik1-Cherry GFP-Tubulin1 background). Bik1 fused to the fluorescent protein Cherry is a good marker to follow growth at cMTs +ends (8). Using this marker, we have previously shown that in *A. gossypii* WT hyphae, cMTs do not undergo dynamic instability (consecutive phases of growth and shrinkage), but spend most of their time growing. As shown in Figure 7C and 7D, cMTs in jnm1Δ cells are also mainly in growing phase. The average MT polymerization rates in the jnm1Δ mutant and in WT were very similar: 0.098 ± 0.015 µm/sec for jnm1Δ and 0.108 ± 0.035 µm/sec for WT (see (8)). Therefore, cMTs in jnm1Δ strain are much longer than cMTs in WT hyphae because of a prolonged growing phase but not because of a faster MT polymerization rate.
We hypothesized that by being more stable and/or constantly growing, long jnm1Δ cMTs (with an approx. polymerization rate of 6 µm/min) prevent nuclear progression towards the growing hyphal tips (maximal growth speeds of jnm1Δ and dyn1Δ hyphae 0.4 µm/min; WT hyphae 3.0 µm/min) and thus maintain nuclei in clusters. As shown in Figure 7C, cMTs can also enter into nuclei-free lateral branches. Since sliding of those cMTs along the cell cortex does not occur, nuclei are not pulled into the lateral branches and their progression is blocked within the main hyphae. The ability of jnm1Δ long cMTs to enter lateral branches and stably interact with them can also, at least partially, explain how nuclear clusters form in mutants. The conclusion that nuclei are maintained in dense cluster by the presence of extra-long cMTs is confirmed by the following experiment: Treatment of jnm1Δ cells with the MT-destabilizing drug Nocodazole induces dissolution of nuclear clusters. After 25-30 min incubation, jnm1Δ nuclei redistributed and migrated into nuclei-free spaces and lateral branches (Figure 7E). In addition, as it had been already demonstrated for dyn1Δ mutant, the severe growth defect of jnm1Δ mutant can be rescued by the addition of the MT-destabilizing drug Benomyl (Figure 7F) (1). This suggests that dense nuclear clusters caused by unrestricted growth of cMTs and the absence of oscillatory nuclear movements, act as a physical barrier for normal distribution of organelles and sufficient tip-directed transport of secretory vesicles thereby causing the severe growth defect.
The goals in this work were, first to identify the motor(s) responsible for the bidirectional movements of nuclei in the filamentous fungus *A. gossypii*, second, to find the underlying cellular function for this continuous nuclear motion, and third, to gain insights into the mechanism directing nuclear movements. As a result, we could demonstrate that the dynein network, from its rather restricted and non-essential role during anaphase in budding yeast, has evolved in *A. gossypii* to an essential system for maintaining a dynamic distribution of nuclei and preventing deleterious formations of nuclear clusters.

When we realized that dynein is the motor exerting bidirectional forces on nuclei, we wanted to know to which degree the partner proteins of dynein, known from work in yeast and other organisms, contribute to dynein activity and localization and bidirectional movements.

**Nuclear oscillations prevent the formation of nuclear clusters in multinucleated hyphae**

Ten years ago it was noted that nuclei in *A. gossypii* hyphae are in continuous motion and that the distribution of nuclei is dynamic resulting in an average distance between adjacent nuclei of 5 to 6 µm (1). We tried several approaches to identify a cellular function of oscillatory nuclear movements. So far, it was not possible only to eliminate or decrease these movements without affecting at the same time the microtubule cytoskeleton. First, it was shown that mutants (bik1Δ and kip2Δ) with shortened cMTs due to decreased cMT +end stability have decreased back and forth movements and do not show
any nuclear bypassing events (8). Those mutants do not show any clustering phenotype, grow almost like WT and do not reveal clues about a potential cellular role of nuclear oscillations. Second, when cMTs are slightly longer than in WT (kip3Δ mutant), the frequency of nuclear oscillation increases but no nuclear clusters or cell growth defects are observed (8). Third, loss of dynein/dynactin causes very long cMTs (much longer than in the kip3Δ mutant) concomitant with the formation of tight nuclear clusters and a strong growth defect. WT-like oscillations events and bypassing are also abolished in this mutant (Figure 8). Here, we analyzed a dynein promoter truncation (prom130-DYN1) which showed decreased oscillations and bypassing events, and triggered formation of loose nuclear aggregates without observably altered cMT growth control. We propose that nuclei, each nucleating up to six cMTs in different directions, can entangle in the narrow hyphal tube, e.g. during bypassing attempts, and that dynein driven oscillatory movements in WT-frequencies can dissolve blocked pairs of nuclei (e.g. first two nuclei of the “WT” panel in Figure 1B). Nuclei in mutants with short cMTs do not entangle since they do not bypass each other. However, in mutants with WT-like cMTs (such as the prom130-DYN1 mutant), the absence of sufficient oscillatory movements does not dissolve loose nuclear clusters. Nuclei in dynein and dynactin mutants form tight clusters and do not oscillate (Figure 8). Those clusters dissolve in the presence of MT-destabilizing drugs such as Nocodazole (NZ) suggesting that the increased stability and length of cMTs lead to tight nuclear clustering. We showed here that the dissolution of nuclear clusters by NZ takes about 30 min, a relatively long period of time.
This is probably dependent on the cytoplasmic stream since nuclear
oscillations are abolished in NZ-treated cells (19).

Dyn1, Dynactin and Num1 are involved in the control of cMT dynamics

In budding yeast, mutants lacking Num1 or dynein/dynactin display longer
MTs than WT cells (3, 5, 26). We show here that this “longer MT” phenotype
is also visible in A. gossypii dynactin and num1 mutants.

In previous work, we have shown that cMTs in A. gossypii do not exhibit the
dynamic instability characteristic of cMTs in other organisms. Therefore, even
if A. gossypii cMTs do not go through consecutive phases of growth and
shrinkage, it seems that their overall maximal length is controlled in WT cells.

This control seems to be particularly affected in the dynein/dynactin/num1
mutants. The fact that a complete loss of dynein or an abnormal accumulation
of dynein at cMT +ends (in jnm1Δ or num1Δ hyphae) induces the same “long
cMT” phenotype is still not understood. One hypothesis could be that the
absence of dynein activity directly or indirectly induces cMTs bundling. This
bundling could affect cMT dynamics by increasing cMTs stability. Our time-
lapse microscopy data on jnm1Δ Bik1-Cherry GFP-Tub1 cells go in favor of
this hypothesis since only a few very long cMTs were visible in the nuclei-free
regions (compared to the overall number of WT cMTs) and those very long
cMTs had an increased GFP fluorescence intensity (see an example in Figure
4E). The establishment of the whole set of interactions at cMT +ends in A.
gossypii will definitely help to understand the contribution of dynein and
dynein accessory proteins to MT dynamics in filamentous fungi but also in
other organisms.
Control of dynein loading on cMT +ends

In filamentous fungi like *A. nidulans* and *U. maydis*, dynein accumulation at cMT +ends is essential for the retrograde movement of early endosomes. Recent publications have shown that in *U. maydis*, approx. 55 dynein motors concentrate at cMTs +ends. The mechanism by which dynein concentrates there is a combination of stochastic accumulation and active retention by the dynactin/EB1 complex (22, 33, 34). In *A. gossypii*, dynein is not involved in cell growth and endosome movements but also accumulates at cMT +ends. However, the control of dynein loading on cMT +ends seems to be different from *U. maydis*. Indeed, in contrast to *U. maydis*, the dynactin complex prevents dynein accumulation at cMT +ends.

In budding yeast and *A. gossypii*, dynein is mainly involved in nuclear movements. As already mentioned previously, yeast dynein is first maintained in an inactive form at cMT +ends (with a maximal retention at anaphase). It gets only activated (and released from the +end) after its interaction with the cell cortex and especially Num1 (20, 25, 36). Since dynein is a highly processive –end MT motor, it must be in an inactive form to be able to stably interact with cMT +ends. This high dynein concentration then induces a strong pulling of the nucleus through the bud neck during anaphase.

Our results show that *A. gossypii* dynein requires similar mechanisms (and especially Num1 and dynactin) to get activated, and therefore released from the cMT +ends. However, our time-lapse microscopy of GFP-Tub1 Dyn1-TdTomato hyphae clearly showed that dynein accumulation at cMT +ends is weaker and more transient than in *S. cerevisae*. This suggests that dynein loading and/or retention at +ends is regulated slightly differently in the
filamentous fungus. In budding yeast, it seems that dynein +end accumulation and activation is cell-cycle regulated (36). In the multinucleated A. gossypii hyphae, mitoses are asynchronous and little is known about the mode of actions of cell cycle regulators (7). However, the fact that nuclei constantly oscillate throughout A. gossypii hyphae and that dynein localizes to SPBs during the entire nuclear cycle, suggests that dynein activation is not dependent on cell cycle regulators.

In the future, it will be necessary to test whether kinesins (such as Kip2 for example) are involved in dynein loading on cMT +ends. It will also be interesting to test whether AgNum1 spreads all over the hyphal cortex like its budding yeast orthologue (25, 29, 38).

Another important question to solve in the future is: how do nuclei coordinate the (opposite) pulling forces applied on their SPBs? One attractive hypothesis, in agreement with our dynein localization data, is that the loading of dynein on cMTs only occurs on specific cMTs. It has been shown in the fission yeast S. pombe that dynein can quickly relocated from one MT to another during meiotic nuclear oscillations. In their model, Vogel et al. proposed that in response to load forces, dynein motors detaches from the trailing cMTs (39). Dyneins first detach from the cell cortex, and then from the cMTs. After redistributing via the cytoplasm, they attach along the leading/sliding MTs, thereby producing asymmetric forces necessary for oscillations. We hope to be able to generate in the future new variants of fluorescent Tubulin allowing a better visualization of cMTs dynamics and sliding and to create strong fluorescent variants of dynein. These tools will be...
necessary to better characterize the relationship between the dynamic
distribution of dynein and cortical pulling forces.

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FIGURE LEGEND

Figure 1:
The absence of Kar9 and Bim1 does not significantly affect maximal hyphal growth and nuclear movements. (A) Radial growth of wild type, kar9Δ and bim1Δ strains after 5 days at 30°C on full medium (AFM). (B) Nuclear dynamics in wild type, kar9Δ and bim1Δ hyphae with H4-GFP labeled nuclei. Images of selected time-lapse movies are presented (green: H4-GFP, red: DIC). Yellow circles show examples of backward nuclear movements, whereas white circles represent forward events. Time is indicated in min. Bars: 5 µm. (C) Dynamic distributions of nuclei. Distances between neighboring nuclei were measured in wild type and the promoter mutants, and plotted in four different size ranges. (D) Distances between the hyphal tip and the first nucleus were measured in wild type and the kar9Δ strain.

Figure 2:
Dynein is the motor for forward and backward nuclear movements. (A) Radial growth of wild type and strains with shortened DYN1 promoters on full medium (AFM); 4 days at 30°C. prom180 and prom130 indicate the length (in base pairs) of the remaining DNA sequence upstream of the start codon. (B) Nuclear distribution in the dyn1Δ, prom180-DYN1 and prom130-DYN1 mutants. Images of time-lapse movies are shown (green: H4-GFP, red: DIC). Time is indicated in min. Note the dense nuclear cluster in the tip region of the dyn1Δ hyphae and the loose nuclear aggregate in the tip region of the prom130-DYN1 hyphae. Bars: 5 µm. (C) Nuclear clustering index of the
dyn1Δ and the DYN1 promoter mutants. The average number of nuclei within fifteen hyphal segments, each 15 µm long, showing a higher nuclear content was determined in the different mutants and compared with the average density of nuclei in randomly picked 15 µm long regions in wild type hyphae. Analyses were performed with images as in (B). Bars indicate standard deviations.

Figure 3:
Radial growth and nuclear distribution in dyn1Δ, jnm1Δ, dyn2Δ, and ndl1Δ mutants. (A) Size of wild type, dyn1Δ and jnm1Δ mycelium after 5 days at 30°C on full medium (AFM). (B) Nuclear distribution in branched mycelium of wild type, dyn1Δ and jnm1Δ strains. Hyphae were grown at 30°C in AFM for 18h (green: H4-GFP, grey: DIC). Nuclei strongly cluster in dyn1Δ and jnm1Δ hyphae. (C) Radial growth and nuclear distribution in wild type, dyn2Δ and ndl1Δ strains. Left panels: Size of mycelia after 5 days at 30°C on full medium (AFM). Right panels: Nuclear distribution in 17 h-old mycelia (green: H4-GFP, red: DIC). Note the loose nuclear aggregates in the mutant hyphae. (D) Nuclear clustering index of indicated mutants. The average number of nuclei within fifteen micrometers long regions with a higher nuclear content was determined in mutant hyphae and compared with the average nuclear density in wild type. Bars indicate standard deviations. Scale bars: 5 µm.

Figure 4:
A. gossypii dynein localizes at cMT +ends and -ends, and along cMTs. Hyphae expressing Dyn1-tdTomato (red) at endogenous levels and GPF-
Tub1 (green) were monitored by time-lapse microscopy. (A) Dynein interacts with cMTs directed toward the hyphal tip (arrowheads) or backward (*). Numbers indicate time in sec. Pictures represent 1Z plane. (B) Pictures are stacks of 5 Z planes with 0.75 µm interval. Dynein localizes symmetrically on both SPBs during metaphase and anaphase. Numbers indicate time in min. Bars: 5 µm.

**Figure 5:**
Dynein strongly accumulates at cMT +tips in the absence of Jnm1. (A) 1Z plane movies of Dynein-TdTomato GFP-Tub1 wild type and jnm1Δ cells. Time is in sec. Arrowheads indicate +tips of cMTs. (B) Dynein-TdTomato GFP-Tub1 wild type and jnm1Δ cells treated with 15 µg/ml Nocodazole for 15 min (5Z plane pictures). (C) Quantification of the lifetime and fluorescence intensity (in arbitrary units) of dynein-TdTomato dots in wild type (dyn1-tdTomato GFP-Tub1) and jnm1Δ cells. Quantifications were made with movies as shown in (A). Bars: 5 µm.

**Figure 6:**
Num1 is involved in the control of nuclear distribution and dynein localization. (A) Radial growth of wild type and num1ΔCt strains after 5 days at 30°C on full medium (AFM). (B) Average numbers of nuclei within 15 clusters. 15 µm long regions with a higher nuclear content were analysed for the different strains (see example in C). (C) Nuclear dynamics in H4-GFP num1ΔCt cells (green: H4-GFP, red: DIC). Some nuclei move individually towards the hyphal tip whereas others form clusters. Time is indicated in min. (D) Dynein (in red)
only weakly localizes at SPBs (green dots) but mainly concentrates at cMT +ends in the num1ΔN1 mutant. The arrowheads focus on the +end of a growing cMT. The * symbols show the curvatures of the cMT after its +end has reached the hyphal tip. Time is indicated in sec. Bars: 5 µm.

**Figure 7:**

The very long cMTs observed in dynein/dynactin mutants are responsible for nuclear clustering.

(A) Anti-alpha-tubulin immunostaining of jnm1Δ, prom130-DYN1, prom180-DYN1, and wild type strains. Pictures correspond to stacks of 5Z planes with 0.75 µm distance. (B) Quantification of cMT length in WT and mutant cells using anti-Tub1 immunostaining pictures as shown in (A). (C) Localization of Bik1-Cherry (red) at cMTs +ends (GFP-Tub1 in green) in jnm1Δ cell. Nuclei/SPBs cluster at some places (*) while long cMTs investigate the nuclei-free lateral branch. The arrow points to one growing cMT +end. The dashed line represents the position of this +end at time point 0. Time is indicated in sec. (D) Quantification of cMT polymerization rate using 1Z plane movies as shown in (C). cMTs were measured from their -end (SPB observed with GFP-Tub1) until their +tip (Bik1-Cherry signal). Due to increased cMTs length in the jnm1Δ mutant, SPBs and +tips were often in different focal planes (*) corresponds to the real length of the cMTs (SPB to +tip) whereas the other cMTs have an underestimated length (visible part of the cMT to the +tip). 1 frame corresponds to 6 sec. The average cMT polymerization rate in wild type is shown in black as a reference (see Grava and Philippsen, 2010). (E) Nuclear clusters disappear and nuclei redistribute after treatment of jnm1Δ.
H4-GFP cells with Nocodazole 15 µg/ml (red: DIC; green: H4-GFP). Time indicates minutes after addition of Nocodazole. (F) Growth defects of jnm1Δ and dyn1Δ cells are partially rescued by the MT-stabilizing drug Benomyl. Bars: 5 µm.

Figure 8:
The absence of oscillation and the presence of very long cMTs observed in dynein/dynactin mutants are responsible for nuclear clustering. In kip3Δ mutant, cMTs are slightly longer than in WT and induce more frequent nuclear oscillations and higher oscillation amplitudes (see grey double arrows). In bik1Δ, cMTs are much shorter than in wild type. Nuclear dynamics is significantly decreased in this mutant (Grava and Philippsen, 2010). In jnm1Δ, num1Δ, or dyn1Δ mutants, MTs are also longer than in WT but since dynein activity is abolished, no oscillations are observed. MTs push nuclei apart and prevent their progression within the hyphae. The black arrows illustrate the direction of cell growth and of the cytoplasmic stream responsible for the inactive forward nuclear movements. A reduced numbers of cMTs emerging from each SPB is shown in this figure for reasons of clarity.
Figure 2
Figure 3
Figure 5
Table 1  Quantification of numbers and amplitude of oscillation events in WT and mutant A. gossypii strains

<table>
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<th>Strain</th>
<th>Forward Events</th>
<th>Fwd events P-values</th>
<th>Backward Events</th>
<th>Bwd events P-values</th>
<th>By-passing Events</th>
<th>Maximal Forward Amplitude</th>
<th>Maximal Backward Amplitude</th>
<th>Average Growth Speed</th>
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(a) Total number of events observed during 30 min for 35 nuclei. The 5 front nuclei of 7 different hyphae were followed for 30 min. (see examples of movies in Figure 1B). Time interval was 1 min. Definitions for Forward and Backward is given in the Results part.
(b) P-values derived from two-tailed Fisher’s exact test (R software).
(c) Amplitudes (in µm/min) indicate the maximal distance covered by a nucleus within a 1 min time interval.
(d) This speed is an average of the growth speed of the 7 hyphae used for the quantification.