Supplementary Materials

Tables

Table S1. *A. gossypii* strains used in this study

Strain	Genotype	Reference
ΔlΔt (referred to as wild-type)	*Agleu2*Δ *Agthr4*Δ	(1)
AgH4-GFP	*Agade2*::*AgADE2-AgHHF1-GFP Agleu2*Δ *Agthr4*Δ	(2)
*Agade2*Δ*1*	*Ag*ade2*(310-566)*Δ*::GEN3, Agleu2*Δ*, Agthr4*Δ	(3)
AgTub4-YFP	*AgTUB4-YFP-LEU2 Agleu2*Δ *Agthr4*Δ	this study
AgH4-GFP Tub4-RFP	*Agade2*::*AgADE2-AgHHF1-GFP Agleu2*Δ *Agthr4*Δ *p(AgTUB4-RedStar2-GEN3)*	this study
GFP-AgTub1	*Agade2::AgADE2-GFP-AgTUB1 Agleu2*Δ *Agthr4*Δ	this study

(1) (Altmann-Johl and Philippsen, 1996)

(2) (Helfer and Gladfelter, 2006)

(3) (Knechtle, 2002)

Table S2. Olignucleotide primers used in this study

Name	Sequence 5' – 3'
AgTUB4_F1	TGGGTGGAGATGCTGAGATGATCGATATTGAAAGTA ACGACGACATCATAAaaacgacggccagtgaattcg
AgTUB4_F2	AGGTCCCGTTTCTAGTATCTACTAATGATGAAGCCAA TGGTTACTAGCCTATTTACGCGAaccatgattacgccaagctt gc
L3	aactggtgatttaggtggttcc
Green2.2	tgtagttcccgtcatctttg
AgTUB4_A3	cgttatattcaaacgcggtgcc
AgTUB4_A4	gtccatttgcatctccaccccc
Agade2verfor_CB	gcgggttgctgactcaatcc
Agade2_verup_CB	atgcccatcctcttccaaac
Agade2verrev_CB	ctacgtggtgccacagtatgc
Agade2_verds_1	gggcagggatttattcggcg

Supplemental Figures

Figure S1. Movements of nuclei and SPBs in *A. gossypii* hyphae expressing AgH4-GFP and AgTub4-RedStar2. Images were taken for 10 min with 30 sec intervals and are projections of 3 Z planes (0.75 µm Z interval). GFP and RFP signals were imaged for 500 msec and 1500 msec, respectively. The left hypha shows two typical examples of nuclear oscillation with SPB reorientations concomitant with switch of migration direction (nuclei 1 and 2) and one fast bypassing event (nuclei 3 and 5). The right hypha shows low amplitude oscillations of nuclei 1 and 2 with one fast movement of nucleus 1 at 8 min and a slow nuclear bypassing of nuclei 4 and 5. Nucleus 5 inverts its movement at 4 min. Parameters of nuclear movements in these two hyphae are summarized in Table 1, columns 2 and 4, respectively. Bars, 5 µm.

Figure S2. Merged, deconvolved image and its six single live images from a hypha expressing GFP-AgTub1. The single images are frames 6 to 12 of the Z-stack compiled as Movie S5 (Supplementary Material). The upper SPB is in focus in frame 6, the lower SPB in frame 8 (asterisks). They both nucleate several microtubules, one of each pointing toward but never touching each other (red and green arrows). In planes 10 to 12 one long microtubule can be followed that is located on top of the two SPBs (grey arrows). The merged image gives the impression that the two SPBs are directly connected although our microtubule tracking data shows this is not the case. Bar, 5 µm.

Movies

Movie S1. Overlay of maximum projections of AgH4-GFP and AgTub4-RFP signals. Images were captured every minute. Z-distance 0.75 µm.

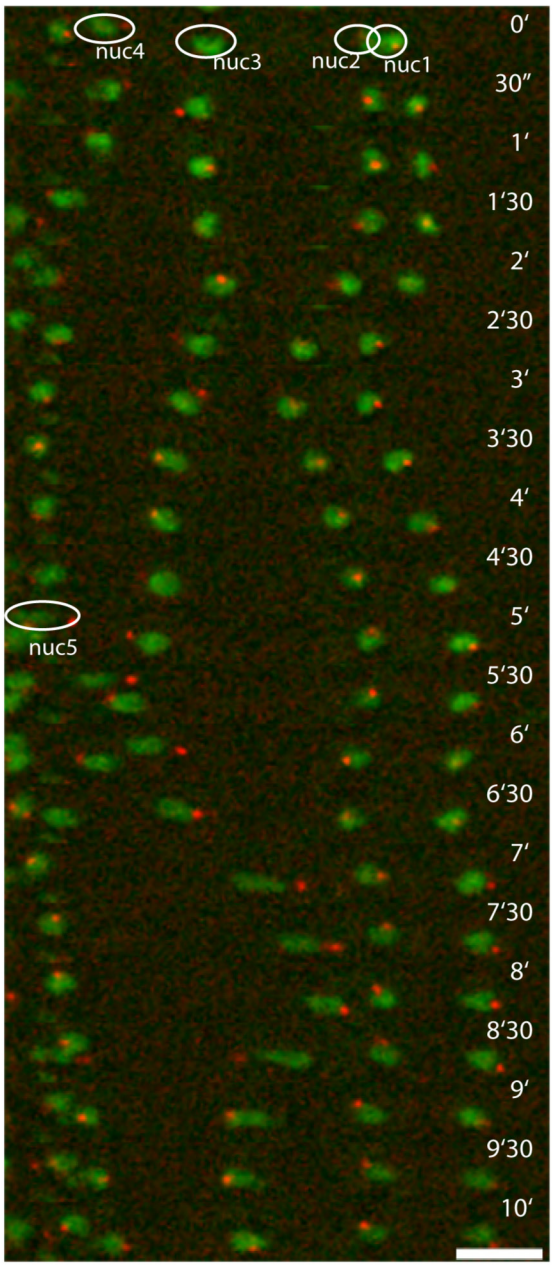
Movie S2. Overlay of maximum projections of AgH4-GFP and AgTub4-RFP signals of a hypha treated with 15 µg/ml nocodazole. Images were captured every 30 sec. Z-distance 0.75 µm.

Movie S3. Time lapse analysis of GFP-AgTub1. Pictures were taken every 6 sec. Exposure time 1500 msec. Analysis of this movie revealed that the first SPB (most proximal to the hyphal tip) was in focus during 30 time frames and moving in different directions by 0.2 µm to 0.7 µm. The second SPB was visible for all 70 frames. During 14 time intervals (20%), it did not observably change its location, but migrated, mainly in different directions, in 56 (80%) time intervals by maximal 0.9 µm within 6 sec. It kept the same direction only between frames 2 and 5 (1.8 µm in 18 sec) and frames 12 to 16 (2 µm in 24 sec). The third SPB moved in 48 (80%) of the observable 60 time intervals with maximal 1.1 µm per 6 sec, again often changing the direction of movement. It kept the same direction only between frames 5 and 9 (1.8 µm in 24 sec), frames 36 and 39 (1.3 µm in 18 sec) and between frames 44 and 46 (1.2 µm in 12 sec). In summary, about 80% of the SPBs changed their locations within 6 seconds by an average of 0.32 µm.

Movie S4. Z-stack of a GFP-AgTub1 labeled hypha. Z-distance 0.3 µm, exposure time 1500 msec.

Movie S5. Z-stack of a GFP-AgTub1 labeled hypha. Z-distance 0.3 µm, exposure time 1500 msec.

Figure S1, Lang et al. 2009a



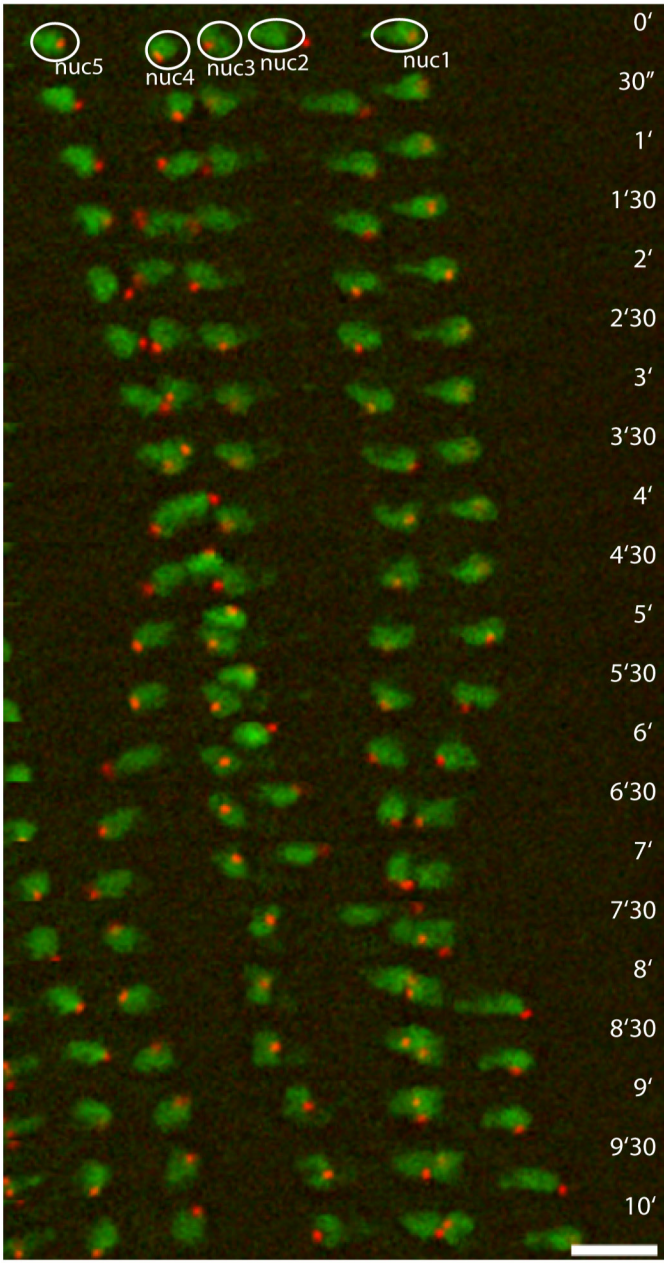


Figure S2, Lang et al. 2009a