The spindle pole body of *Aspergillus nidulans* is asymmetrically composed with changing numbers of gamma-tubulin complexes

Xiaolei Gao[1], Marjorie Schmid[1], Ying Zhang[1], Sayumi Fukuda[2]
Norio Takeshita[2] and Reinhard Fischer*[1]

running head: MTOCs in *A. nidulans*

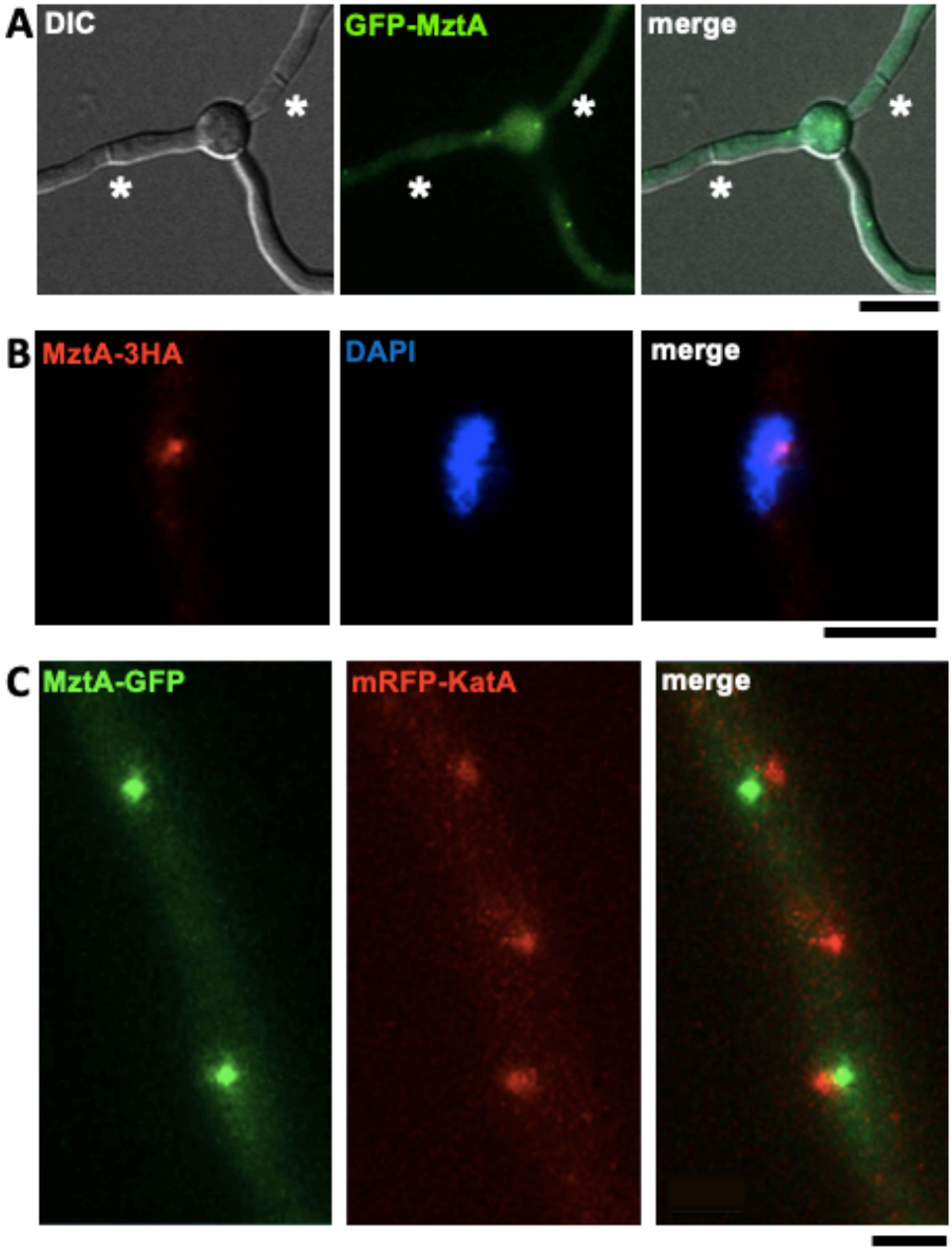
Address: [1]Karlsruhe Institute of Technology (KIT) - South Campus
Institute for Applied Biosciences
Dept. of Microbiology
Fritz-Haber-Weg 4
D-76131 Karlsruhe
Phone: +49-721-6084-4630
Fax: +49-721-6084-4509
E-mail: reinhard.fischer@KIT.edu
Homepage: www.iab.kit.edu

[2]Tsukuba University
Faculty of Life and Environmental Sciences
Tsukuba, 305-8572
Japan

*corresponding author

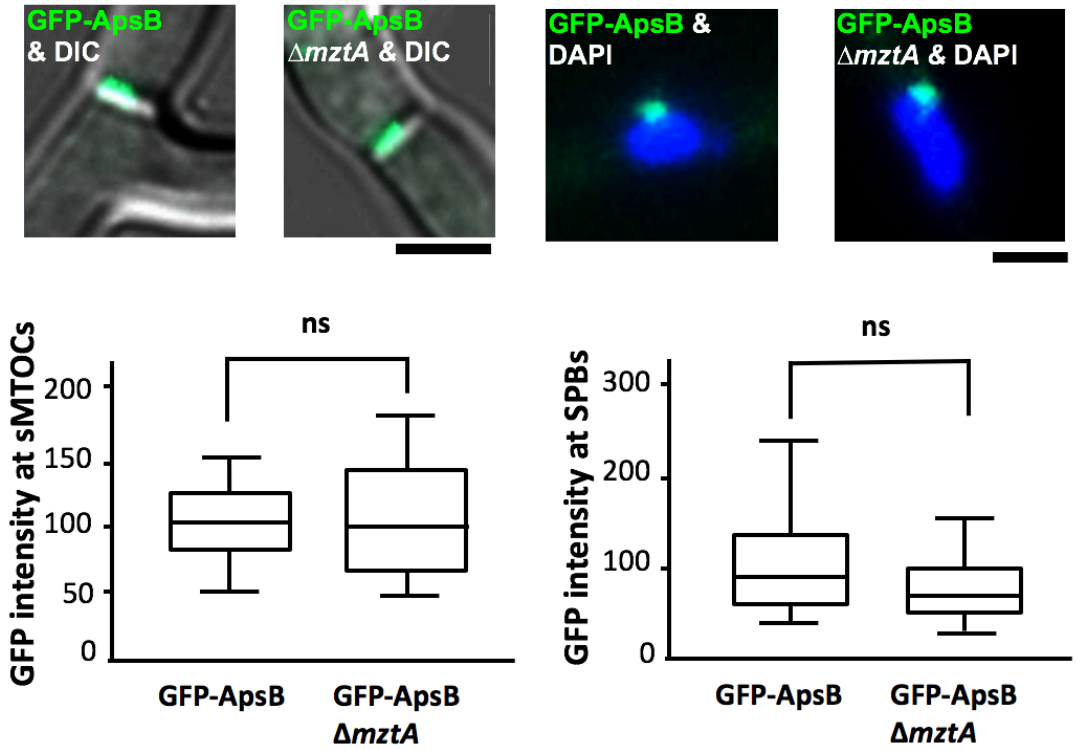
Key words: Aspergillus, microtubules, gamma-tubulin, MTOC, SPB, Gcp, MOZART

Suppl. Figure S1



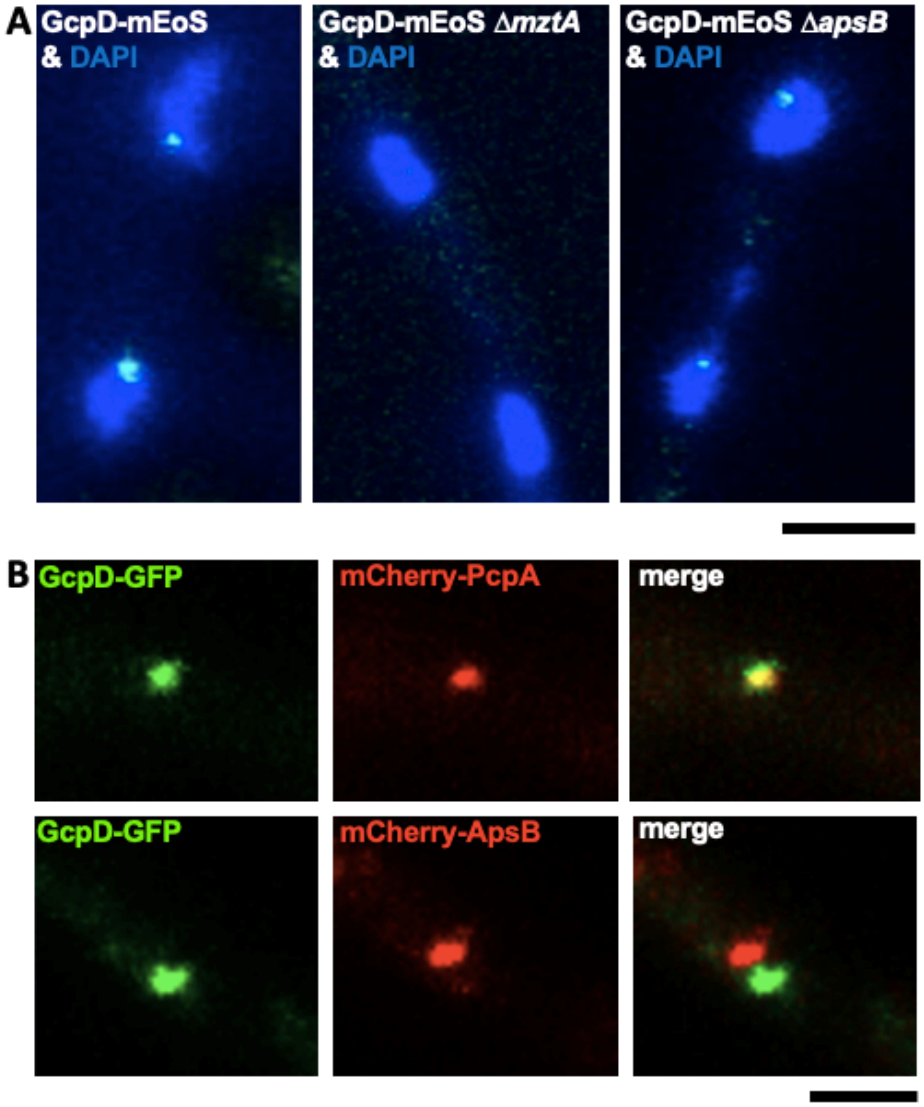
Suppl. Fig. S1: Analysis of MztA localization using different tagging strategy. (A) GFP fusion at the N-terminus of MztA interrupted the septal localization. Strain SXL142 (*alcA(p)::GFP::mztA*) was incubated in MM (2% glycerol) with supplements at 28°C overnight. Asterisks indicate the septa positions. Scale bar, 2 µm. **(B)** Immunofluorescence images of MztA tagged with 3HA at its C-terminus. Strain SXL143 (*mztA::3HA*) was performed with anti-HA mouse 1st antibody and cys3 2nd antibody against mouse. Nuclei was stained with DAPI. **(C)** Localization of MztA compared to the kinetochore marker. Strain SXL68 (*alcA(p)::mRFP::katA*; *mztA::GFP*) was incubated in MM (2% glycerol) at 28°C overnight and imaged. Scale bar, 2 µm.

Suppl. Figure S2



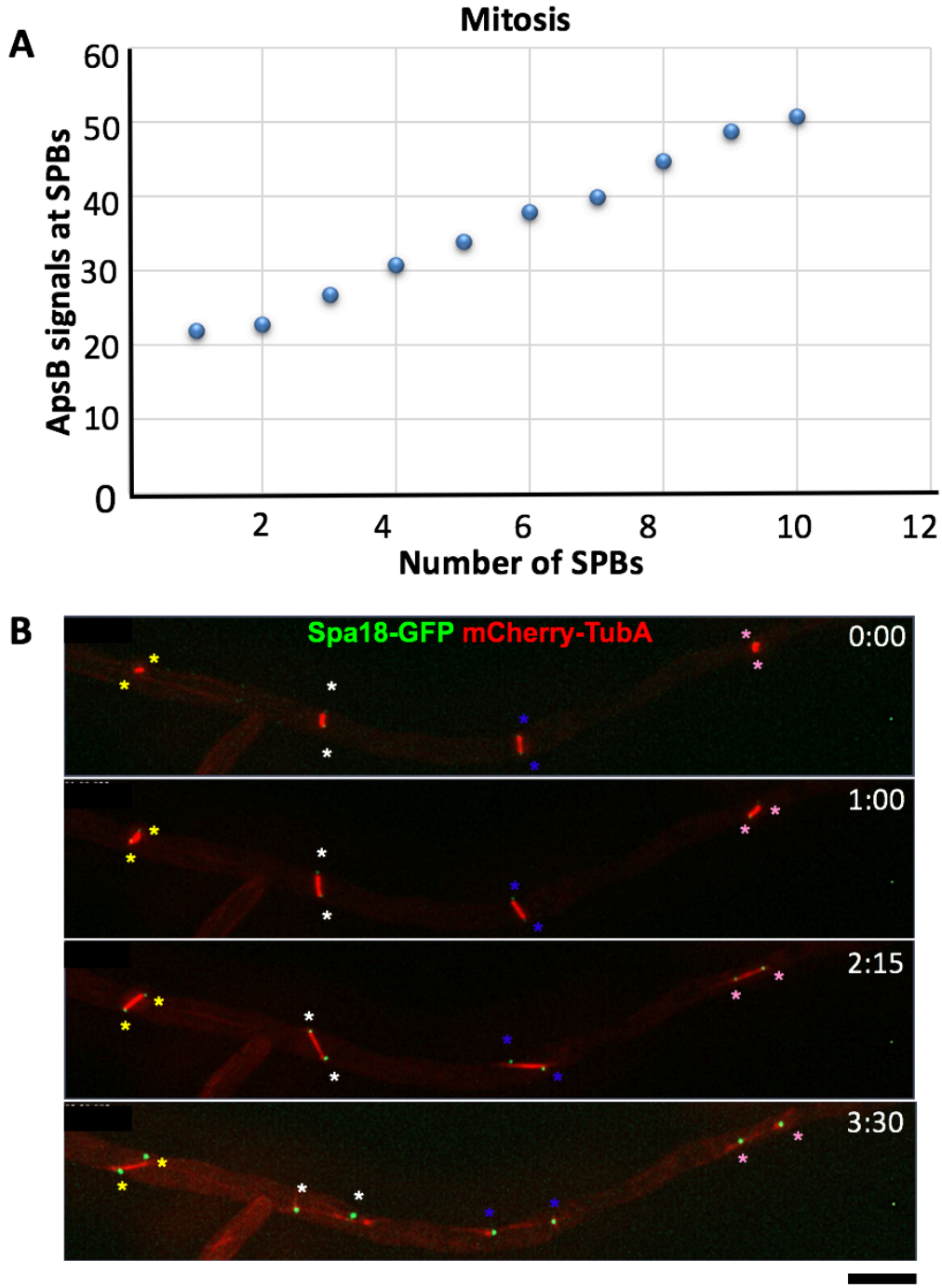
Suppl. Fig. S2: ApsB localization at sMTOCs and SPBs was not affected in a *ΔmztA* strain. Strains SYZ2 (*alcA(p)::GFP::apsB*) and SXL63 (*alcA(p)::GFP::apsB*; *ΔmztA*) were incubated in MM (2% glycerol) at 28°C overnight with supplements and imaged. Nuclei were stained with DAPI. In total 30 SPBs or 30 septa were analyzed for the quantification. Images of 10-15 sections were taken along the Z-axis at 0.27-µm increments. Maximum projection images were obtained and maximum fluorescence intensities over the background intensity were used for statistical analysis. The exposure time and shutter level were set to be identical for each strain. Mann-Whitney U test was performed with GraphPad Prism 7. The boxes mark the region of the SD. The vertical lines indicate the range of all data. The ns above the graph indicate no significant differences compared to wild type ($p > 0.05$). Scale bar, 2 µm.

Suppl. Figure S3



Suppl. Fig. S4: Role of MztA in recruitment of the γ-TuRC-specific component GcpD and co-localization analysis of GcpD with PcpA or ApsB. (A) GcpD localization on SPBs depends on MztA but not on ApsB. Strain SXL99 (*gcpD::mEosFP*), SXL101 (*gcpD::mEosFP; ΔapsB::pyroA*) and SXL111 (*gcpD::mEosFP; ΔmztA(L)::pyroA*) were incubated in MM (2% glycerol) at 28°C with supplements overnight and observed in the GFP channel. Nuclei were stained with DAPI. Scale bar, 2 µm. **(B)** Localization of GcpD with PcpA or ApsB. Strains SXL147 (*gcpD::GFP; alcA(p)::mCherry::apsB*) and SXL148 (*gcpD::GFP; alcA(p)::mCherry::pcpA*) were incubated in MM (2% glycerol) at 28°C overnight and imaged. Scale bar, 2 µm.

Suppl. Figure S4



Suppl. Fig. S4: ApsB and Spa18 concentrations at SPBs are increasing during mitosis. (A) PALM data showed increasing numbers of ApsB during mitosis. 10 SPBs were counted. Strain SXL100 (*apsB::mEoS*) was analyzed with PALM. **(B)** Spa18 started to be assembled into short spindles and gradually increasing intensities as mitosic spindles elongated. Time lapse images were taken every 1 min. Strain SXL21 was incubated in 8 well u-slides at 28°C overnight. Scale bar, 5 µm.

suppl. Table S1: List of *A. nidulans* strains used in this study.

suppl. Table S2: List of plasmids used in this study.

suppl. Table S3. Primers used in this work.

Suppl. Table S4: Molecular numbers of MTOC components at SPBs. Four MTOC components ApsB, GcpC, GcpD and MztA were quantified at SPBs and sMTOCs with super-resolution microscopy (PALM). In interphase, 32 SPBs for ApsB, 66 SPBs for GcpC, 20 SPBs for GcpD and 34 SPBs for MztA were counted. 20 septa for GcpC, 10 sepat for GcpD and 10 septa for MztA were counted at interphase. In mitosis, each protein was counted at 10 SPBs. Strains SXL114 (*mztA::mEoS*; *alcA::GFP::tubA*), SXL115 (*gcpC::mEoS*;*alcA::GFP::tubA*), SXL116 (*apsB::mEoS*; *alcA::GFP::tubA*) and SXL117 (*gcpD::mEoS*; *alcA::GFP::tubA*) were used where spindles were observed with the GFP channel. The average numbers of each protein are shown in the Table with SD. SD of interphase SPB is much higher than that of mitosis, indicating the high dynamics in interphase.

	ApsB	**GcpC**	**GcpD**	**MztA**
Interphase	20±8.8	31.9±22.7	10.6±4.8	30.7±12.6
Mitosis	41.6±6.6	46.7±4.2	15.6±2.6	58.6±6.0

Suppl. Movie 1 & 2: GFP-KipA in the *mztA* mutant and in WT. SXL87 (Δ*mztA, alcA(p)::GFP::kipA*) and SSH27 (*alcA(p)::GFP::kipA*) were cultured in 8 well u-slides at 28°C overnight. 20 frames were captured every 5s and movie speed is 5fps. Arrows indicated the position of the septum. Scale bar, 2 μm.