

Published in final edited form as:

*Fungal Genet Biol.* 2011 October ; 48(10): 998–999. doi:10.1016/j.fgb.2011.07.003.

## Microtubule Dynamics in Mitosis in *Aspergillus nidulans*

Edyta Szewczyk<sup>1,2</sup> and Berl R. Oakley<sup>1,3</sup>

<sup>1</sup>Department of Molecular Genetics The Ohio State University 484 W.12th Ave. Columbus, Ohio 43210

### Introduction

Mitosis in *Aspergillus nidulans* is very rapid, requiring less than five minutes at 37°C in germlings (Bergen and Morris, 1983). In this time the cytoplasmic microtubules (MTs) must disassemble, the mitotic spindle assemble, function and disassemble, and cytoplasmic MTs reassemble. It follows that MTs must be extremely dynamic in this period and we were interested, in particular, in examining the processes of cytoplasmic MT disassembly in prophase and reassembly in anaphase and telophase. We observed a diploid strain that expressed GFP- $\alpha$ -tubulin. We used a spinning disk confocal microscope that allowed rapid image capture, which proved necessary because microtubule dynamics were extremely rapid. We found, for the first time, that microtubule severing occurs in prophase in a filamentous fungus and that catastrophe rather than nucleation limits astral microtubule growth.

### Microtubule severing and astral microtubule catastrophe in mitosis in *A. nidulans*

We created strain LO1052, a diploid homozygous for the GFP-TUBA ( $\alpha$ -tubulin) fusion previously constructed by Han et al. (2001). Diploid strains are larger than haploid strains and have larger mitotic apparatuses, which facilitated our observations. It grew at rates comparable to *tubA*<sup>+</sup> control diploids, indicating that the GFP-TUBA fusion supported normal growth. We were able to capture four-dimensional (Z-series stacks collected at time intervals) images of hyphae passing through the entire mitotic process. The data set shown in Video 1 and Fig. 1 illustrates our major findings. In prophase, MTs disassembled from their ends and also severed into fragments that subsequently disassembled (Fig. 1b,c). Disassembly of cytoplasmic MTs was rapid (Fig. 1a-e) and the mitotic spindle began to assemble well before the completion of the disassembly of the cytoplasmic MTs (Fig. 1c,d). Microtubule fragmentation and severing has been studied in animal cells and extracts. The key enzymes are a group of related AAA ATPase proteins that also play a role in the proliferation of MTs in plant cells and in flagellar excision (Vale, 1991; McNally and Vale, 1993; McNally, 1998, additional data reviewed in Roll-Mecak and McNally, 2010). Genes encoding AAA ATPase proteins are present in *A. nidulans* and it would be interesting to determine if any of them is involved in microtubule severing.

© 2011 Elsevier Inc. All rights reserved.

Corresponding author: Berl R. Oakley (boakley@ku.edu).

<sup>2</sup>Current address: Department of Biological and Agricultural Engineering University of California One Shields Avenue Davis, CA 95616, USA

<sup>3</sup>Current address: Department of Molecular Biosciences University of Kansas 1200 Sunnyside Ave. Lawrence, KS 66045

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The mitotic spindle initially appeared to be lobed (Fig. 1e), probably reflecting the fact that two half spindles were in the process of fusing into a bipolar spindle. It became a short rod (Fig. 1f) and then began to elongate (Fig. 1g). As it elongated, very short astral MTs assembled (Fig. 1g,h), but they disassembled immediately (Video 1), obviously undergoing catastrophe. As the spindle lengthened, the catastrophe frequency appeared to decrease resulting in longer astral MTs (Fig. 1i). These data indicate that astral microtubule growth is limited by catastrophe frequencies rather than microtubule nucleation. As mitosis proceeded, the mitotic spindle disassembled and astral MTs grew and appeared to interact with MTs growing in from adjacent nuclei (Fig. 1j). In early G<sub>1</sub> nuclei, the former poles (i.e. spindle pole bodies) nucleated a robust array of cytoplasmic MTs (Fig. 1k,l) with MTs from the two spindle pole bodies apparently interacting.

We also observed robust microtubule dynamics at the tip of a hypha next to the mitotic hypha. MTs did not come to a particular focus but probed the tip area, continually growing and shrinking.

Finally, it is worth noting that there is considerable variation in mitosis and microtubule dynamics in filamentous fungi. Unlike *A. nidulans*, which has a “semi-open” mitosis (De Souza et al., 2004) *Neurospora crassa*, for example, has a “closed mitosis and cytoplasmic MTs do not disassemble when nuclei undergo mitosis (Roca et al., 2010).

## Methods

Image acquisition with a spinning disk confocal microscope was as previously described (Horio and Oakley, 2005) except that a 60X 1.42 N.A. Planapochromatic objective was used. LO1052 is a vegetative diploid. The genotype of one parent was GFP-*tubA*, *sC12*, *pabaA1*, *cnxE16*, *wA2* and the other was GFP-*tubA*, *pyrG89*, *pabaA1*, *choA1*, *fwA1*.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We thank Dr. Stephen Osmani for the use of his confocal system. Supported by grant GM031837 from the NIH.

## Further Reading

- Bergen LG, Morris NR. Kinetics of the nuclear division cycle of *Aspergillus nidulans*. *J. Bacteriol.* 1983; 156:155–160. [PubMed: 6352675]
- De Souza CP, Osmani AH, Hashmi SB, Osmani SA. Partial nuclear pore complex disassembly during closed mitosis in *Aspergillus nidulans*. *Curr. Biol.* 2004; 14:1973–1984. [PubMed: 15556859]
- Han G, Liu B, Zhang J, Zuo W, Morris NR, Xiang X. The *Aspergillus* cytoplasmic dynein heavy chain and NUDF localize to microtubule ends and affect microtubule dynamics. *Curr. Biol.* 2001; 11:719–724. [PubMed: 11369237]
- Horio T, Oakley BR. The role of microtubules in rapid hyphal tip growth of *Aspergillus nidulans*. *Mol. Biol. Cell.* 2005; 16:918–926. [PubMed: 15548594]
- McNally FJ, Vale RD. Identification of katanin, an ATPase that severs and disassembles stable microtubules. *Cell.* 1993; 75:419–429. [PubMed: 8221885]
- McNally F. Purification and assay of the microtubule-severing protein katanin. *Meth. Enzym.* 1998; 298:206–218. [PubMed: 9751883]
- Roca MG, Kuo HC, Lichius A, Freitag M, Read ND. Nuclear dynamics, mitosis, and the cytoskeleton during the early stages of colony initiation in *Neurospora crassa*. *Eukaryot. Cell.* 2010; 9:1171–1183. [PubMed: 20207852]

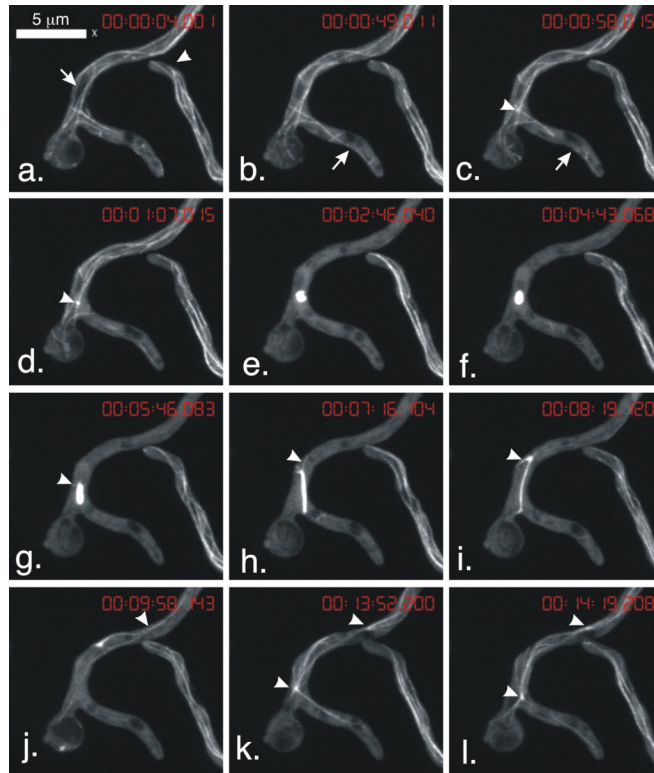
- Roll-Mecak A, McNally FJ. Microtubule-severing enzymes. *Curr. Opin. Cell Biol.* 2010; 22:96–103. [PubMed: 19963362]
- Vale RD. Severing of stable microtubules by a mitotically activated protein in *Xenopus* egg extracts. *Cell.* 1991; 64:827–839. [PubMed: 1671762]

Highlights

Microtubule severing and fragmentation occur in early mitosis in *A. nidulans*.

Microtubule catastrophe limits astral microtubule growth in mitosis.

Reduced catastrophe in telophase and G1 allows astral microtubule growth.



**Fig. 1.**

Microtubule dynamics in mitosis. Images are from Video 1 and are of strain LO1052. Each image is a maximum intensity projection of a Z-series stack. The time of completion of acquisition of each Z-series stack is shown at the top of each panel (hr:min:sec:msec). Note that internuclear distances are greater in diploid hyphae than haploid hyphae. In a haploid hypha there would be more than one mitotic nucleus in the field of view. a. Arrow shows a space of reduced tubulin fluorescence that is the nucleus that will go into mitosis. Arrowhead shows an interphase hypha. Note microtubule dynamics at tip. b,c. Arrow shows a microtubule that severs and disassembles leaving a gap in the formerly continuous microtubule. c,d. Arrowhead shows the mitotic spindle as it initially begins to form. e-i. The mitotic spindle is initially lobed but becomes a short rod that later extends into a long, thinner and somewhat flexible rod. g,h. Arrowheads show short astral MTs. i. In late anaphase the astral MTs become longer. j. An astral microtubule is adjacent to a microtubule from the adjacent spindle (out of field of view). Arrowhead shows point of intersection. k,l. Astral MTs extend from former spindle poles and apparently interact with each other.