

Apical growth and mitosis are independent processes in *Aspergillus nidulans*

Short communication

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Summary. It is well established that cytoplasmic microtubules are depolymerized during nuclear division and reassembled as mitotic microtubules. Mounting evidence showing that cytoplasmic microtubules were also involved in apical growth of fungal hyphae posed the question of whether apical growth became disrupted during nuclear division. We conducted simultaneous observations of mitosis (fluorescence microscopy) and apical growth (phase-contrast microscopy) in single hyphae of *Aspergillus nidulans* to determine if the key parameters of apical growth (elongation rate and Spitzenkörper behavior) were affected during mitosis. To visualize nuclei during mitosis, we used a strain of *A. nidulans*, SRS27, in which nuclei are labeled with the green-fluorescent protein. To reveal the Spitzenkörper and measure growth with utmost precision, we used computer-enhanced videomicroscopy. Our analysis showed that there is no disruption of apical growth during mitosis. There was no decrease in the rate of hyphal elongation or any alteration in Spitzenkörper presence before, during, or after mitosis. Our findings suggest that apical growth and mitosis do not compete for internal cellular resources. Presumably, the population of cytoplasmic microtubules involved in apical growth operates independently of that involved in mitosis.

Keywords: Spitzenkörper; Tip growth; Mitosis; Cytoplasmic microtubule; Green-fluorescent protein; *Aspergillus nidulans*.

Abbreviations: MTs microtubules; GFP green-fluorescent protein.

Introduction

Cytoplasmic microtubules (MTs) play a key role in nuclear positioning and migration (Girbardt 1968, Oakley and Morris 1980, McKerracher and Heath 1987, Aist 1995).

More recently, it has become clear that cytoplasmic MTs also play a major role in apical growth of fungal hyphae. Cytoplasmic MTs are not only the tracks for the transport of vesicles from Golgi equivalents to the hyphal apex (Howard and Aist 1977, 1980; Howard 1981; Gooday 1983; Gow 1989; Hasek and Bartnicki-García 1994; Heath 1995; Seiler et al. 1999) but also for providing directionality and/or stability to the Spitzenkörper and therefore maintaining normal hyphal growth and morphology as was described for *Neurospora crassa* (Riquelme et al. 1998, 2000, 2002).

The present work was designed to test whether apical growth and mitosis competed for MTs during hyphal elongation. A number of assorted reports suggested that this could be the case. Various studies on the role of cytoplasmic MTs in nuclear division indicated that most cytoplasmic MTs disassembled during mitosis (prometaphase) and their tubulin subunits were repolymerized to make mitotic MTs and their associated astral MTs (Gambino et al. 1984, Osmani et al. 1988, Salo et al. 1989, Oakley et al. 1990, Doonan 1992, Pitt and Doonan 1999). Robinow (1963) reported that hyphal extension fell by 30–50% during mitosis in *Basidiobolus ranarum*. Other circumstantial evidence suggested the possibility that mitosis might interfere with apical growth. For instance, in seemingly undisturbed colonies of *Achlya bisexualis* and *Saprolegnia monoica*, elongation of individual hyphae was temporarily interrupted, the tips bulged, and elongation resumed a few minutes later (Bartnicki-García and Lippman 1997).

On the basis of the notion that cytoplasmic MTs provide the tracks for long-distance travel of secretory vesicles to

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the hyphal apex, it was reasonable to hypothesize that the previously reported depolymerization of cytoplasmic MTs during mitosis could cause a significant decrease in the flow of vesicles migrating to the tip. The resulting decrease in new cell wall construction would produce a drop in growth rate. To test this working hypothesis, we have conducted a simultaneous analysis of nuclear division by fluorescence microscopy and apical growth by phase-contrast microscopy to determine if growth rate and Spitzenkörper behavior are affected during mitosis in mature individual hyphae of *Aspergillus nidulans*. They were not.

Material and methods

Strains, media, and growth conditions

We used a strain of *Aspergillus nidulans* (SRS27) whose nuclei are labeled with green-fluorescent protein (GFP) (Suelmann et al. 1997). The fungus was grown and maintained on agar plates of *Aspergillus* Minimal Medium (AMM) (Käfer 1977) containing 1% D-glucose as the carbon

source, sodium nitrate as the nitrogen source, and supplemented with uridine (1.2 g/l), uracil (1 g/l), and pyridoxine (0.001 g/l) at 37 °C.

Video microscopy

For low-magnification images of hyphal growth, the fungus was grown in 8 cm diameter plastic petri dishes containing supplemented AMM agar at 37 °C and observed with bright-field optics (10× or 20× objectives). For high-resolution work, cultures were grown on coverslips coated with a thin layer of AMM solidified with 16% gelatin. Conidia were inoculated in the center of the layer. The inoculated coverslips were placed on two glass rods inside a petri dish humid chamber and incubated at 25 °C for 24–48 h. After the colonies had reached 1 cm in diameter, advancing hyphal tips from the edge of the colony were observed and recorded with an inverted Zeiss Axiovert microscope fitted with a phase-contrast 100× oil immersion objective lens, a 0.5× WF eyepiece, and a 3 charge-coupled-device Intas Seescan video camera system (Intas, Göttingen, Federal Republic of Germany). For fluorescence microscopy we used the Zeiss filter settings for GFP fluorescence (filter combination nr. 17: excitation, 485 nm; beam splitter, 510 nm; emission, 515–565 nm wavelength). Individual images were captured from the videotaped sequences in 8-bit gray scale with an Imascan/Chroma frame grabber (Imagraph) and processed with Adobe Photoshop version 5.5.

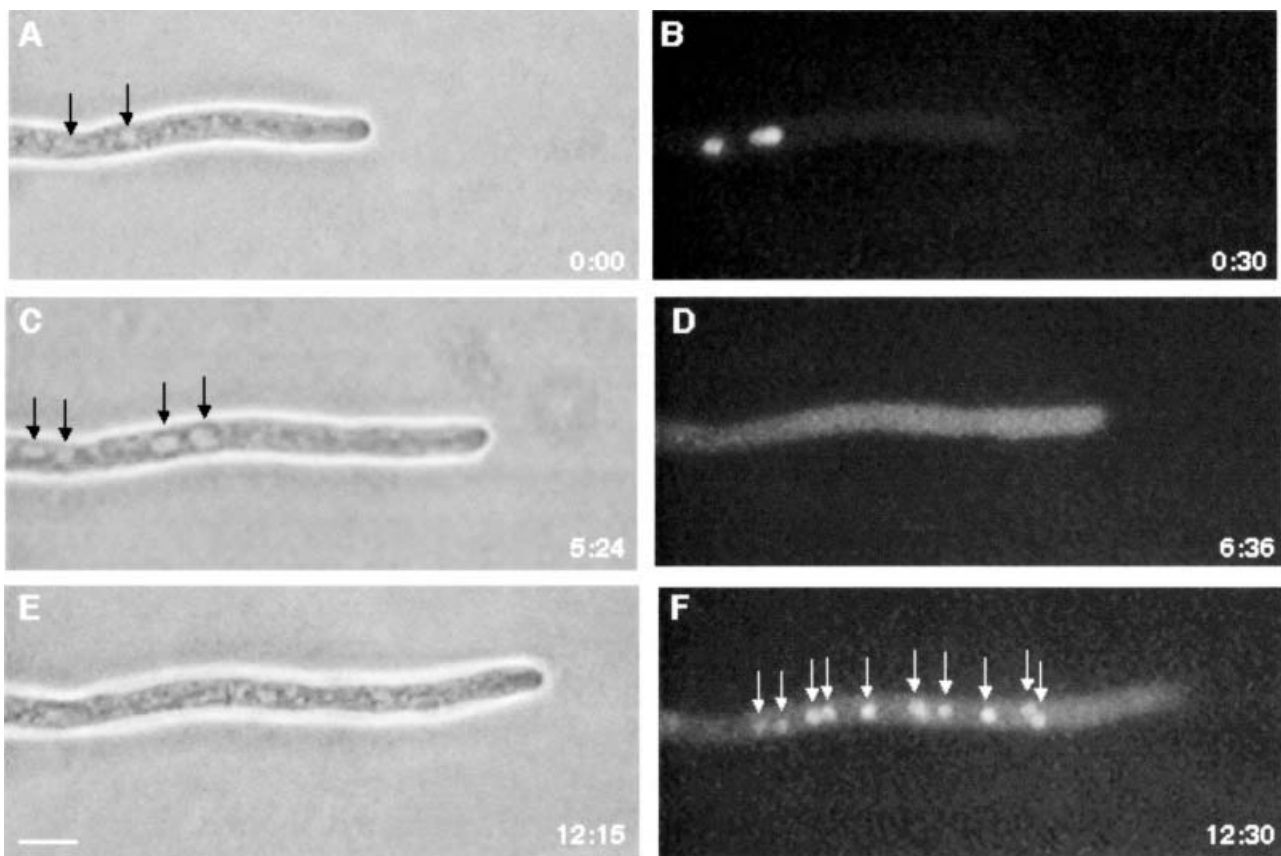


Fig. 1. Alternating observations of mitosis and apical growth in a hypha of *A. nidulans* strain SRS27 by phase-contrast (A, C, and E) and epifluorescence microscopy (B, D, and F). Each set of left and right images is the closest pair of phase-contrast and fluorescent images. Because of time needed to change optics, there is a variable delay of 15–68 s between phase-contrast and fluorescent images. A and B Cell in interphase. Two nuclei are vaguely visible by phase-contrast (A, arrows) but can be clearly distinguished by GFP fluorescence (B). C Nuclei about to enter mitosis; by this time two more nuclei had migrated into view and all four nuclei appeared enlarged (arrows). D During mitosis, nuclei were no longer distinguished by epifluorescence microscopy. E and F Mitosis was completed 6 min after it was first detected and the newly divided nuclei were faintly visible by phase-contrast but clearly visible by fluorescence microscopy (F, arrows). Time in minutes and seconds. Bar: 8 μ m

Effect of cytoskeleton inhibitors

Filter paper disks (7 mm in diameter) containing either 10 μ l of benomyl (0.1 mg/ml stock solution) in 0.1% dimethyl sulfoxide or cytochalasin A (0.1 mg/ml stock solution) in 0.1% dimethyl sulfoxide were placed at a distance of 1.5 cm from the margin of 3–4 cm diameter colonies of *A. nidulans* wild type growing on supplemented AMM agar plates at 37 °C.

Results and discussion

In growing hyphae of *A. nidulans*, interphase nuclei can be discerned with variable difficulty as phase-light ellipsoidal organelles by phase-contrast microscopy (Fig. 1A). Before entering mitosis, nuclei became more conspicuous (Fig. 1C), but during mitosis they were no longer visible by phase-contrast microscopy. Nuclear visibility was greatly improved by using a strain whose nuclei were tagged with GFP and observed by fluorescence microscopy (Fig. 1B, F). Under fluorescence microscopy, nuclei were also invisible during mitosis as the GFP-labeled fusion protein contained in the nuclei was released and the entire hypha became diffusely fluorescent (Fig. 1D). The nucleus closest to the apex entered mitosis first and a wave of mitosis moved quickly along the hypha as previously reported (Doonan 1992). After 5–10 min, mitoses within the apical compartment were completed and the divided nuclei were again clearly visible by fluorescence microscopy (Fig. 1F). In phase-contrast microscopy, not all of the newly divided nuclei were readily visible (Fig. 1E).

Concurrent with nuclear observations, growth measurements made at 15 s intervals showed no change in elongation rate of the hyphae of *A. nidulans* before, during, or after mitosis (Fig. 2). It had been previously reported that hyphae of *Schizophyllum commune* continued to extend at a linear rate throughout the duplication cycle (Volz and Niederpruem 1968). However, measurements of hyphal extension were done at hourly intervals, and considering that the mitotic process lasts 5 to 10 min, any concomitant disturbance in growth rate would have been easily overlooked. Our precise measurements of growth rate showed conclusively that the apical growth of *A. nidulans* was not interrupted during mitosis – a clear indication that the cellular mechanism responsible for apical growth operates independently of that involved in nuclear events.

The sequences of hyphal growth we analyzed showed no disruption in the overall appearance of the Spitzenkörper before, during, or after mitosis (Fig. 1). At the medium magnification used to follow both apical growth and nuclear division, it was not possible to observe fine details in the appearance of the Spitzenkörper as has been done in other studies (López-Franco and Bracker 1996; Reynaga-Peña and Bartnicki-García 1997; Riquelme et al. 1998,

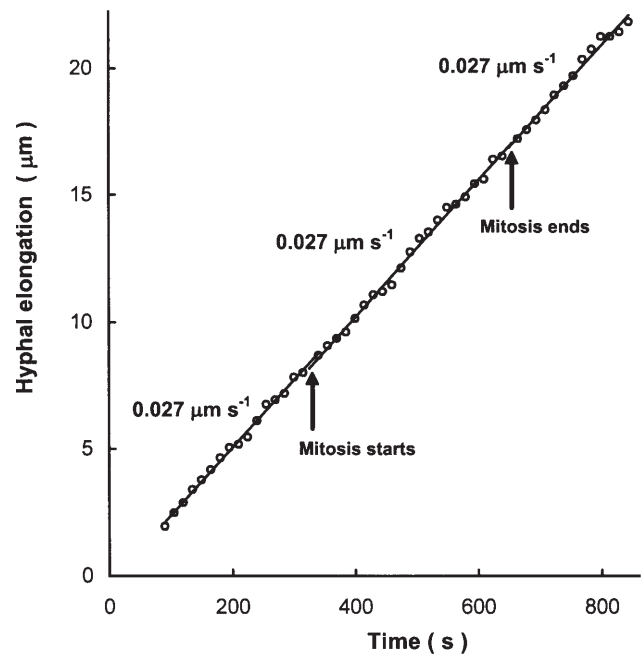


Fig. 2. Elongation rate of the hypha shown in Fig. 1. The XY coordinates of the apical pole were mapped at 15 s intervals on the monitor screen and the distances traveled were computed on an Excel spreadsheet. Three sets of data were considered: before, during, and after mitosis. Circles indicate data points. The lines are regression lines for each set of data. Rates shown are the slope of each regression line

2000). There was however no indication of any significant disturbance in the behavior of the Spitzenkörper.

There is good evidence that both the actin and the microtubular cytoskeletons are involved in apical growth of fungal hyphae albeit with different roles (Bartnicki-Garcia 2002, Heath 1994). There is no evidence that the actin located at the hyphal apex plays any role in mitosis; but reports indicating a disappearance of cytoplasmic microtubules during mitosis (Gambino et al. 1984, Osmani et al. 1988, Salo et al. 1989, Oakley et al. 1990, Doonan 1992, Pitt and Doonan 1999) have prompted us to investigate whether such a transient loss of cytoplasmic MTs would hinder apical growth. Since we found no evidence that the growth of *A. nidulans* was diminished while nuclei were undergoing synchronized mitosis, we must consider two explanations: MTs are not essential for apical growth, or not all cytoplasmic MTs are recycled during mitosis and those that persist suffice to supply the needs of the apex. We favor the second explanation because it has been shown that MTs do play a role in hyphal growth: interference with MT structure by inhibitory drugs (Howard and Aist 1980, Riquelme 1998), or by mutational disruption of MT motors has serious effects on the growth and morphogenesis of hyphae of *N. crassa* (Seiler et al. 1997, Minke et al. 1999, Riquelme et al. 2000), *Nectria*

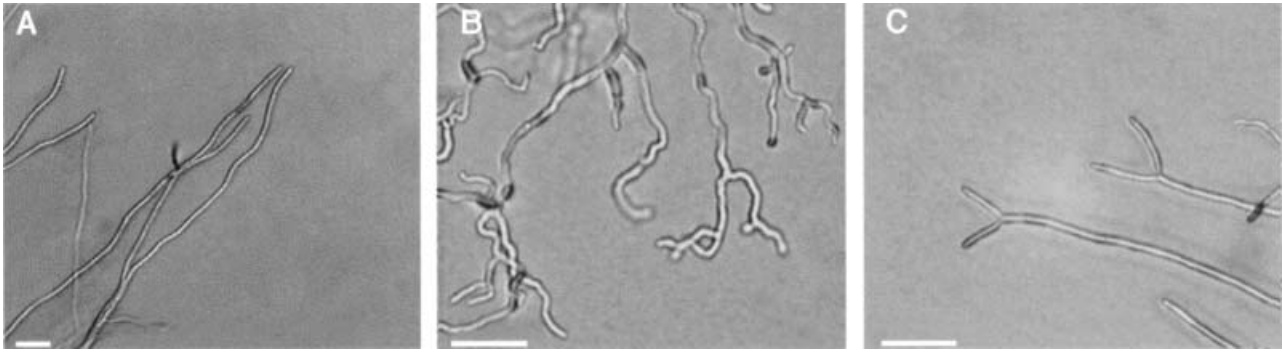


Fig. 3 A–C. Effect of cytoskeleton inhibitors on hyphal morphogenesis of *A. nidulans*. **a** Control hyphae. **b** Benomyl elicits branching and a marked disturbance of hyphal directionality. **c** Cytochalasin A elicits dichotomous branching but does not have a marked effect on the directionality of individual hyphae. Bar: 50 μm

haematococca (Inoue et al. 1998), and *Ustilago maydis* (Steinberg et al. 2001); our own findings (Fig. 3) demonstrate that the microtubule inhibitor benomyl causes a severe inhibition and distortion of hyphal growth of *A. nidulans*, similar to that shown earlier for *N. crassa* (Riquelme et al. 1998).

We must keep in mind that the claims of a complete disappearance of MTs during mitosis based on immunocytochemical techniques may be inconclusive. These procedures are prone to show losses due to the inherent difficulties in stabilizing microtubules during fixation procedures. This caveat is particularly important for germlings of *A. nidulans* which contain few MTs. Gambino et al. (1984) noted that cytoplasmic MTs sometimes persisted during mitosis. Our own preliminary studies on immunolocalization and in vivo visualization of GFP-labeled microtubules in *A. nidulans* showed that some cytoplasmic MTs were still present during mitosis (unpubl. results). The persistence of cytoplasmic MTs in or near the apical region of hyphae while some nuclei were in mitosis has been reported for other fungi (Salo et al. 1989, Minke et al. 1999). In the light of these observations and our own findings, we favor the view that a portion of the population of cytoplasmic MTs is preserved during mitosis and this satisfies the needs of apical growth.

Minke et al. (1999) suggested that the persistence of cytoplasmic MTs during mitosis in *N. crassa*, but not in *A. nidulans*, was related to the lack of synchronized nuclear division and the much higher growth rate of *N. crassa*. However, we believe there is no qualitative difference in microtubular needs between these two fungi. Despite widely different growth rates, both slowly and fast-growing fungi depend on cytoplasmic MTs for orderly hyphal growth and morphogenesis.

Although we endorse the view that MTs are principally responsible for the orderly movement of secretory vesicles to the tips of well developed hyphae, we cannot rule

out the possibility that an alternate transport mechanism (actin-based) may compensate for any loss of cytoplasmic MTs during mitosis. To resolve these issues unequivocally, the dynamics and fate of cytoplasmic MTs need to be clearly defined in living cells.

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