Available online at www.sciencedirect.com
SCIENCE DIRECT®
Fungal Genetics and Biology 41 (2004) 842–851
FUNGAL GENETICS AND BIOLOGY
www.elsevier.com/locate/yfgbi

Key differences between lateral and apical branching in hyphae of *Neurospora crassa*☆

Meritxell Riquelme and Salomon Bartnicki-Garcia*

Department of Plant Pathology, University of California, Riverside, CA 92521-0122, USA
Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), 22860 Ensenada, Baja California, Mexico

Received 1 March 2004; accepted 21 April 2004
Available online 2 June 2004

Abstract

We examined in fine detail growth kinetics and intracellular events during lateral and apical branching in hyphae of *Neurospora crassa*. By high-resolution video-enhanced light microscopy, we found remarkable differences in the events preceding lateral vs apical branching. While apical branching involved a significant disturbance in the apical growth of the parental hypha, lateral branching occurred without any detectable alterations in the growth of the parental hypha. Prior to the emergence of a lateral branch, an incipient Spitzenkörper was formed about 12–29 μm behind the apex. Lateral branch formation did not interfere with the elongation rate of the primary hypha, the shape of its apex or the behavior of its Spitzenkörper. In sharp contrast, apical branching was preceded by marked changes in physiology and morphology of the parental hypha and by a sharp drop in elongation rate. The sequence involved a cytoplasmic contraction, followed by a retraction, dislocation, and disappearance of the Spitzenkörper; hyphal elongation decreased sharply and a transient phase of isotropic growth caused the hyphal apex to round up. Growth resumed with the formation of two or more apical branches, each one with a Spitzenkörper formed by gradual condensation of phase-dark material (vesicles) around an invisible nucleation site. The observed dissimilarities between lateral and apical branching suggest that these morphogenetic pathways are triggered differently. Whereas apical branching may be traced to a sudden discrete disruption in cytoplasmic organization (cytoplasmic contraction), the trigger of lateral branching probably stems from the subapical accumulation of wall precursors (presumably vesicles) reaching a critical concentration.
© 2004 Elsevier Inc. All rights reserved.

Index Descriptors: Apical branching; Lateral branching; Hyphae; *Neurospora crassa*; Spitzenkörper; Video microscopy

1. Introduction

The present study was undertaken to investigate in fine detail the intracellular events that lead to branching in living hyphae of *Neurospora crassa*. Hyphal branching is an essential feature in the development of a fungal colony. Through branching, fungi maintain their intrinsic tendency to increase their biomass exponentially (Trinci, 1984). In a colony of *N. crassa*, lateral branching is the most frequent type of branching though occasionally, branching occurs at the apex. Most studies on colony development have been done by low-resolution microscopy (Buller, 1931; Prosser, 1990; Robertson, 1959; Smith, 1924; Trinci, 1969, 1984). The main objective of this study was to examine cytological events at the highest optical magnification and measure kinetics of branching with greater precision.

We were particularly interested in examining the role of the Spitzenkörper (Spk) during branch formation. It is well known that hyphae increase in length by apical growth, a polarized process that involves the coordinated transport of vesicles. At the hyphal apex, wall-building vesicles congregate temporarily in the Spk (Brunswik, 1924; Girbardt, 1957; Grove and Bracker, 1970; McClure et al., 1968). According to the hyphoid model for fungal morphogenesis (Bartnicki-Garcia et al., 1989), the Spk behaves as a vesicle supply center

☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fgb.2004.04.006.

* Corresponding author. Fax: +52-646-174-4729.
E-mail address: bartnick@cicese.mx (S. Bartnicki-Garcia).

1087-1845/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.fgb.2004.04.006

(VSC) directing the traffic of vesicles to the plasma membrane. It has been previously shown that the position and behavior of the Spk or VSC determine the growth, morphology, and direction of growth in fungal hyphae (Bartnicki-Garcia et al., 1995; Girbardt, 1957; López-Franco and Bracker, 1996; Reynaga-Peña and Bartnicki-Garcia, 1997; Riquelme et al., 1998). Branching is the initiation of a new center of polarized growth and therefore it brings forth the question of how the new Spk is generated.

A previous video-enhanced light-microscopy study on apical branching revealed an intriguing pattern of Spk behavior in a temperature-sensitive mutant of *Aspergillus niger* (Reynaga-Peña and Bartnicki-Garcia, 1997), namely the disappearance of the original Spk with a major disruption in elongation rate followed by resumption of growth with the appearance of two new Spk. The question remained as to whether Spk behavior and ontogeny would be significantly different during lateral branching. To address this question, we conducted a high-resolution comparative morphometric analysis of apical and lateral branching in a wild-type strain of *N. crassa*, as part of an ongoing study on the cell biology of this fungus.

2. Materials and methods

2.1. Fungal strains

Neurospora crassa wild type (FGSC 988) was obtained from the Fungal Genetics Stock Center. The strain was grown and maintained in 8.5 cm plastic Petri dishes containing 20 ml of Vogel's Complete Medium agar (Vogel, 1956) with 1.5% (w/v) sucrose as the carbon source. Cultures were grown at 28 °C.

2.2. Video microscopy

The fungus was grown on a modified slide culture chamber as previously described (López-Franco, 1992; Riquelme, 2000). Growing hyphae were observed with an Olympus Vanox-S microscope fitted with a phase-contrast 100× oil-immersion objective (n.a. 1.25) and a 10× WF eyepiece (American Optical). Video images were produced with a Hamamatsu C2400-07 high-resolution camera (Hamamatsu Photonic Systems), enhanced with an Argus-10 image processor (a real-time digital contrast and low light enhancement system), and displayed on a black and white, 12-in., high-resolution monitor (Sony Corporation of America; Model PVM-122). Sequences were videotaped in real time with a S-VHS recorder (JVC Model BR-S822U). Videotaped sequences were played on a variable-tracking player (JVC Model BR-S525U) and observed on a Sony Trinitron model monitor. Individual hyphal images were captured from the videotaped sequences in 8-bit gray scale with an Imascan/Chroma frame grabber (Imagraph).

2.3. Growth kinetics

Hyphal elongation rates were calculated from videotaped sequences by following the advance of the apical pole of individual hyphae. The *XY* coordinates of the apical pole were mapped at 1 s intervals. Running averages of five consecutive values were calculated for each point to minimize reading errors. The distances traveled were computed on Excel spreadsheets.

To measure growth rate in sequences involving shape changes, we calculated area increase rather than elongation rat. Cell profiles were traced with Image Pro Plus Software for Windows (Media Cybernetics, Silver Spring, MD) as a set of *XY* coordinate values. These values were automatically collected into a text file with a Windows application program interfaced with the Argus-10 analyzer (Bartnicki et al., 1994) and the text files were then imported into Microsoft Excel spreadsheets for further processing. The area delimited by the cell profiles was computed by using Green's formula (Marsden et al., 1993). Hyphal diameter and Spk diameter were measured on the monitor screen with the line command of the measure option in the Argus-10 menu. Hyphal diameter was routinely measured at a distance 2d from the apical pole (d is defined in the hyphoid equation as distance between the VSC and the apical pole) (Bartnicki-Garcia et al., 1989).

3. Results

Leading hyphae from the actively growing edge of colonies of *N. crassa* were examined. A conspicuous Spk was always present in the apical dome (Fig. 1 and Table 1). In the confinement of the microscope growth chamber, the increase in hyphal length was linear for short periods but the overall rate decreased over time (Fig. 2). A linear elongation rate was maintained for the first 4 min ($R^2 = 0.99$), which dropped by 7 and 14% on prolonged incubation (Fig. 2).

3.1. Lateral branching

Contrary to the impression obtained by examining the morphology of a fungal colony, where lateral branches appear to arise at a considerable distance from the tip of the parental hyphae, in *N. crassa* lateral branches were recorded emerging from primary hyphae at a surprisingly short distance from the pole of the growing tip (Fig. 3). Lateral branches were formed at a distance ranging from 12 to 29 μm (average 17 μm) behind the tips of leading hyphae. This is similar to the values found for *Aspergillus nidulans*, in which

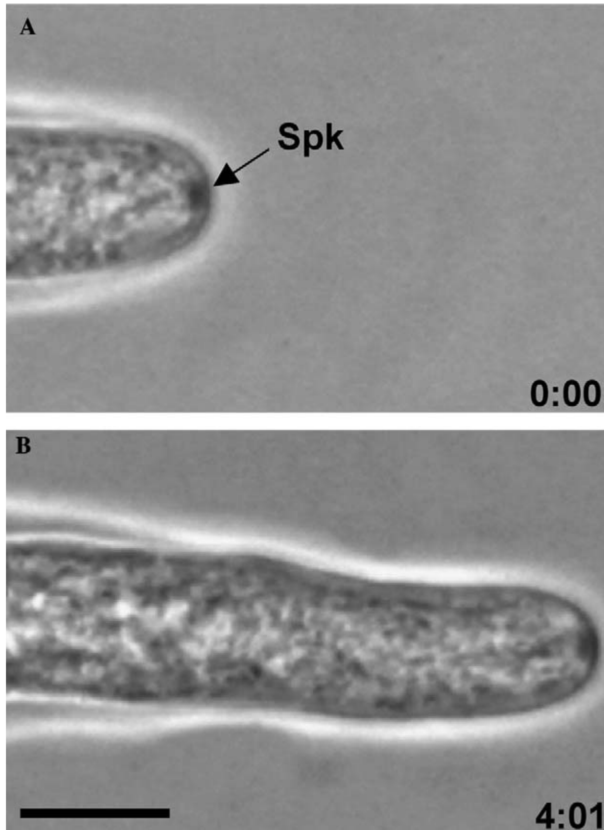

Fig. 1. Phase-contrast video-microscopy of a growing hypha of *N. crassa* showing a typical Spk. Time in min:s. Scale bar, 10 μm.

lateral branches form at distances of 7–70 μm (mean 30 μm) behind the tips of the parental hyphae (Trinci, 1970).

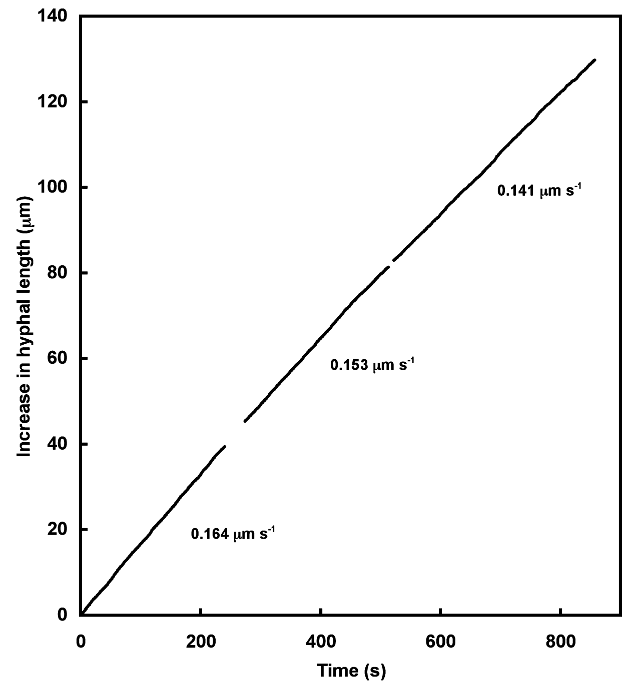

Fig. 2. Elongation rate of the hypha shown in Fig. 1. Data were collected every second. Rates were calculated by linear regression for each set of values. The gaps correspond to the times when the microscope stage was repositioned.

3.1.1. Growth kinetics and Spitzenkörper ontogeny

By examining the videotaped record of laterally branching hyphae, the first indication of branching was an outward deformation of the lateral wall of the parental hypha (Fig. 3b) that grew into a conic protrusion (Fig. 3c) to later evolve into a characteristic hyphal

Table 1
Parameters of branching hyphae of *N. crassa*

Cell types	Sequence total time (min:s)	Average hyphal diameter (μm)	Average Spk diameter (μm)	Elongation rate ($\mu m\ s^{-1}$; mean ± SE)[a]	Increase in area ($\mu m^2\ s^{-1}$)[b]	Increase in volume ($\mu m^3\ s^{-1}$)[c]
Unbranched (Fig. 1)	4:01	9.8	1.9	0.164 ± 0.025	1.8	13.85
Lateral branching (Fig. 3)	2:29					
Parental hypha		9.3	2.1	0.215 ± 0.025	2.2	16.07
Lateral branch		5.4	0.9	0.076 ± 0.012	0.5	2.12
Apical branching (Fig. 5)	20:21					
Parental hypha[d]		10.4	1.8	0.27 ± 0.016	2.94	23.98
Parental hypha[e]		11.7	—	0.12 ± 0.06	1.21	11.14
Subapical branch		5.9	0.8	0.095 ± 0.025	0.77	3.54
Parental hypha[f]		9.3	1.6	0.22 ± 0.038	6.99	51.08
Parental hypha[g]		11.4	—	0.084 ± 0.06	1.06	9.49
Upper apical branch		8.8	1.5	0.16 ± 0.023	1.80	12.44
Lower apical branch		9.6	1.6	0.17 ± 0.022	2.35	17.69

[a] Elongation rate calculated by linear regression.
[b] Calculated by Green's formula.
[c] Calculated as cylinder volume: area increase × diameter × ($\pi/4$).
[d] Before contraction.
[e] During isotropic growth.
[f] Before Spk retraction.
[g] During isotropic growth.

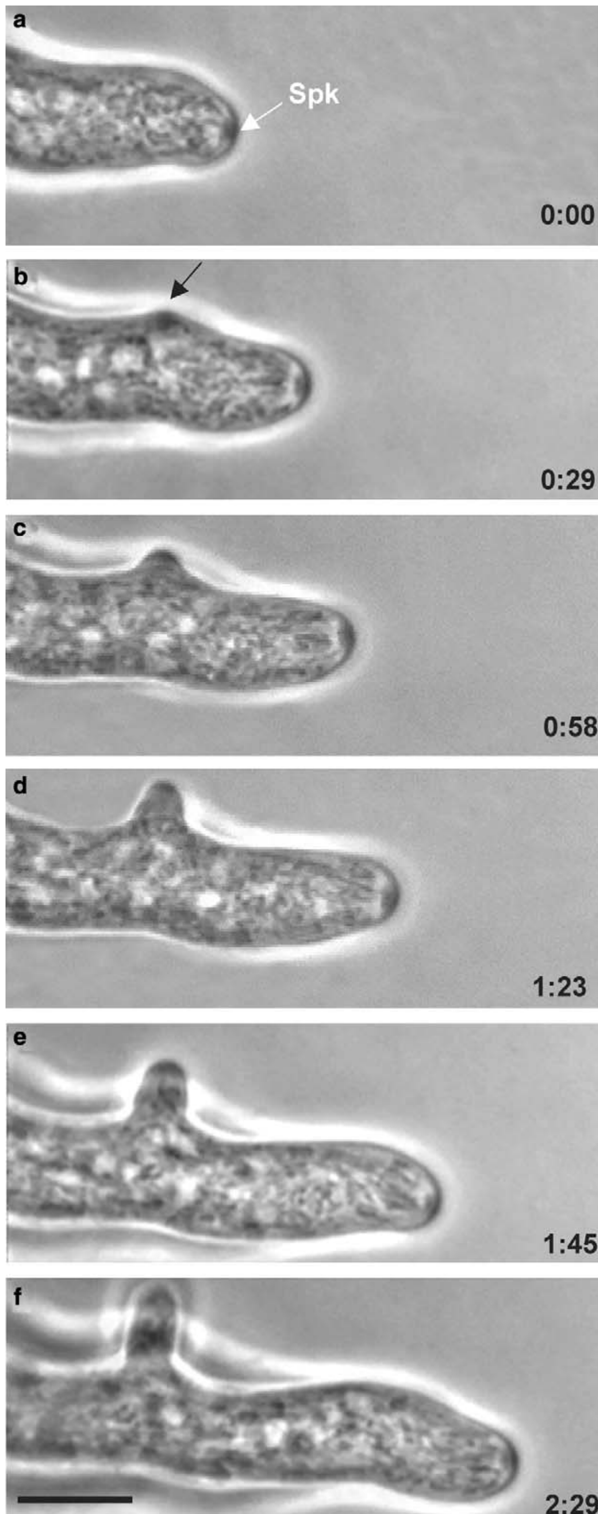

Fig. 3. Phase-contrast video-microscopy showing Spk ontogeny during lateral branching (a). Arrow in (b) points at the Spk inside the incipient emerging lateral branch. Note the falcate shape of the Spk in (c). In (d) a spheroid Spk is already visible in the lateral branch. In (e,f) the branch develops a characteristic tubular shape. Time in min:s. Scale bar, 10 μm.

shape (Figs. 3d–f) (real-time videotaped sequence available in http://boyce3427.ucr.edu/Riquelme/riquelme.htm). Cell measurements, in both length and area, indicated no changes in the growth rate of the parental hypha during the formation of a lateral branch (Fig. 4; Table 1). The first stage in the emergence of the lateral branch was followed frame by frame on the recorded sequence. During the initial phase, lasting 20 s, the elongation rate was 0.045 $\mu m\, s^{-1}$. As the branch continued to emerge, its elongation rate increased to 0.076 $\mu m\, s^{-1}$. Overall, both parental hypha and branch maintained an essentially linear rate of elongation during the course of the observation. Both the elongation rate and diameter of the lateral branch were considerably smaller than those in the parental hyphae (35 and 58%, respectively).

By the time an incipient branch was visible, a Spk was also seen as a dense accumulation of phase-dark material noticeable inside the protrusion (Fig. 3b, arrow). As the lateral branch continued to elongate, the phase-dark material changed shape reversibly from a falcate-shaped structure (Fig. 3c) into a more typical spheroid Spk (Fig. 3d). The changes in Spk shape were variable (Figs. 3e and f). During emergence of the lateral branch, there were no perceptible changes in apical morphology of the parental hypha. No unusual changes in movement or appearance of organelles in the parental apical region were detected. Mitochondria could be seen entering the developing branch. The Spk of the primary hypha behaved in a normal fashion with the subtle changes in mobility and growth direction noted earlier for *N. crassa* (Riquelme et al., 1998).

3.2. Apical branching

In the course of studying lateral branch formation, we encountered occasionally instances of apical branching. The developmental sequence in Fig. 5 is a good example of subapical and apical branches emerging consecutively from the same hypha of *N. crassa.* To record the whole sequence, lasting about 20 min, several displacements of the microscope stage were needed to keep the hyphal apices in view. To illustrate the totality of these branching events, 15 individual profiles were assembled into an overall profile (Fig. 6).

3.2.1. Spitzenkörper ontogeny and growth kinetics

In contrast to lateral branching, there were major internal changes in the parental apex prior to the emergence of apical branches (Figs. 5a–d). These changes entailed: (1) *cytoplasmic contraction*: the organelles suffered a momentary spasmic movement forward (real-time sequences in http://boyce3427.ucr.edu/Riquelme/riquelme.htm); (2) *Spk retraction*: 12 s after the cytoplasmic contraction, the Spk moved 0.8–0.9 μm away from its normal position in the apical dome. This displacement was accompanied by a change in the shape of the Spk, from a spheroid shape to a falcate shape (convex site facing the apex) (Figs. 5b and c); (3) *Spk*

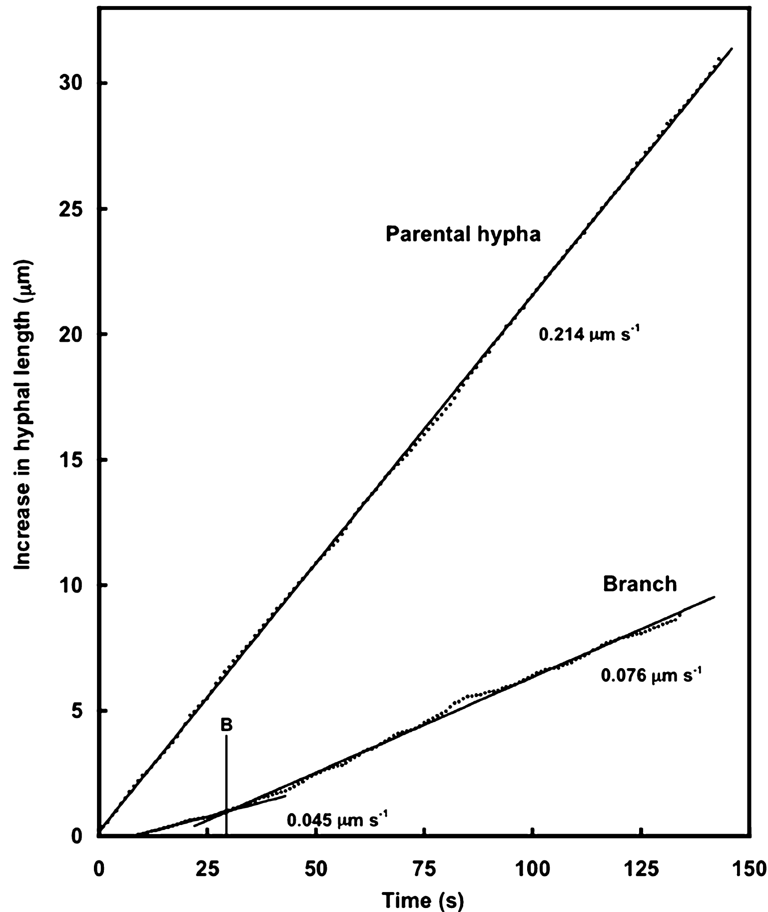

Fig. 4. Elongation rates of the parental hypha and its lateral branch shown in Fig. 3. Data were collected every second. Elongation rates were calculated by linear regression (solid straight lines). Separate regression lines were plotted for values before and after B; Time B corresponds to the image in Fig. 3b.

disappearance: 47 s after it retracted, the Spk disappeared completely (Fig. 5d); and (4) *isotropic growth*: during the 10 s when the Spk retracted and disappeared, the hyphal apex lost its hyphoid profile, and became more rounded (Figs. 5c and d). During the period of isotropic growth elongation rate decreased 56%; if measured by area increase the drop was 59% (Table 1; Fig. 6).

The apical-branching sequence began with the reappearance of a new Spk (Fig. 5e) and the parental hypha resuming elongation but with a smaller diameter and a slower elongation rate (Table 1; Figs. 5f and 6). Shortly thereafter, a new Spk appeared at 14 μm from the apical pole, where a subapical branch emerged subsequently (Figs. 5g and h). Both parental hypha and subapical branch continued elongating for about 11 min at which time the Spk of the parental hypha disappeared (Fig. 5m), and another period of isotropic growth begun with a sharp decrease in growth rate measured by elongation rate (62%) or area increase (85%) (Table 1; Fig. 6). About 45 s after the start of isotropic growth, a cloud of dense phase-dark material started accumulating on the lower side of the enlarged hyphal apex (Fig. 5n). An additional cloud started developing on the upper side 7 s later (Fig. 5o). Both clouds became more distinct and developed into two Spk, one for each of the newly formed apical branches (Fig. 5p). In both upper and lower apical branches the diameter and size of the Spk was similar as were also their elongation rates (Figs. 5q–s, Table 1).

4. Discussion

4.1. Branch ontogeny: lateral vs apical branching

Because of the rapid rate of elongation of primary hyphae it is difficult to establish the precise moment of emergence of a lateral branch and its distance from the primary tips by conventional microscopy. One of the virtues of video microscopy is that it allows a retrospective examination of the phenomenology of any specific event. Accordingly, we could focus attention on the cytoplasmic region where we knew a branch would later emerge. In this manner, remarkable differences were found between lateral and apical branching in hyphae of *N. crassa*.

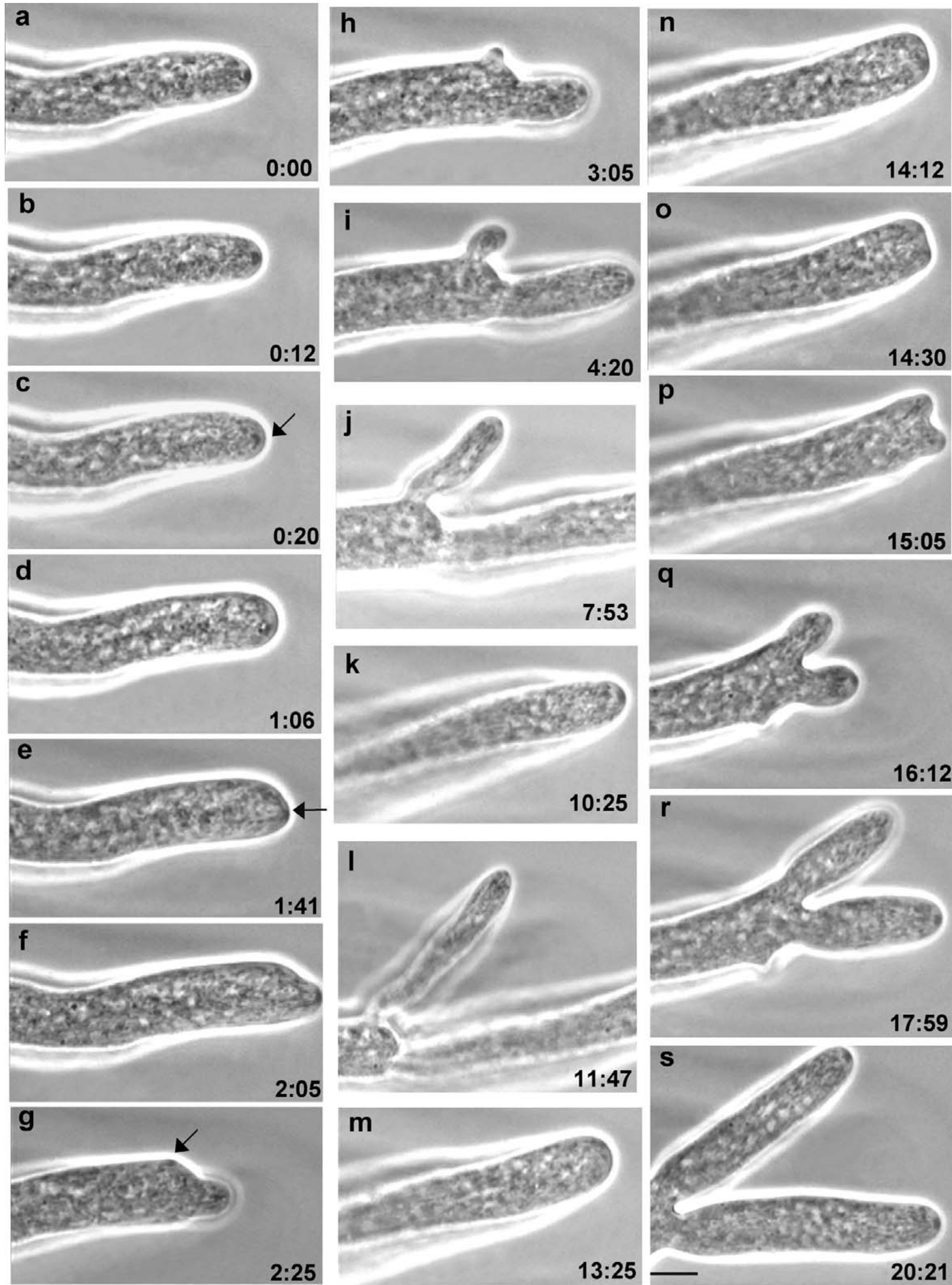

Fig. 5. Phase-contrast video-micrographs of *N. crassa* showing the ontogeny of apical branching. (a,b) Growth before branching; (c) retracted falcate-shaped Spk; (d) the Spk has disappeared and the hyphal apex has started swelling and has lost its hyphoid shape; (e) a new Spk appears in the lower half of the hyphal apex; (f) the parental hypha resumes growth; (g) dense phase-dark material accumulates in the upper side of the hypha (arrow) about 14 μm behind the pole of the emerging branch; (h) a small subapical branch emerges subapically with a small falcate Spk; (i,j,l) the subapical branch continues to grow and its Spk becomes more apparent; (k) the lower parental hypha continues to grow; (m) the Spk of the parental hypha disappeared and its apex lost its hyphoid profile; (n,o) two new Spk appear at opposite sides of the apex; (p–s) Spk become more apparent as two correspondent apical branches develop. Time in min:s. Zero time corresponds to the time of the cytoplasmic contraction. Scale bar, 10 μm.

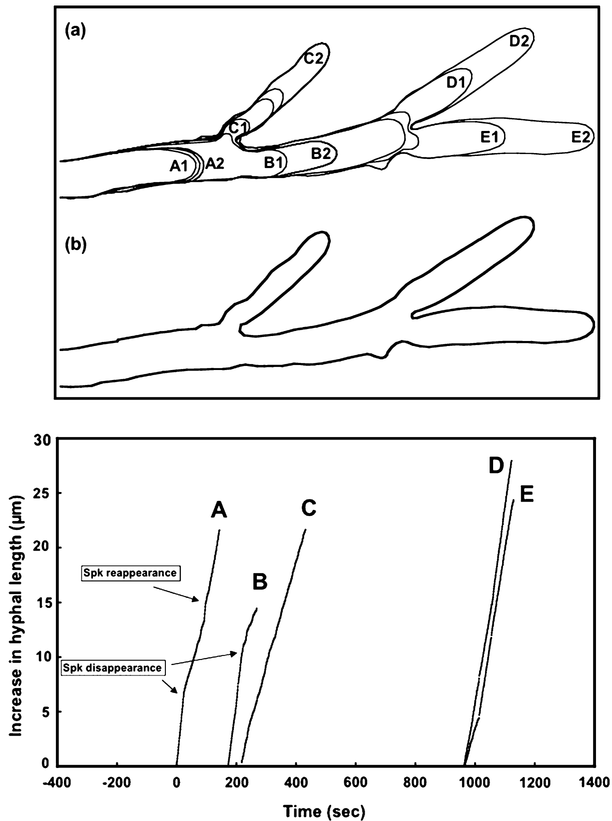

Fig. 6. Reconstruction and analysis of apical branching in the morphogenetic sequence illustrated in Fig. 5. (Top panel) Reconstruction of the entire sequence. Intermediate profiles (a) used for assembling the overall hyphal profile (b); (Bottom panel) growth kinetics. A, parental hypha; B, parental hypha after subapical branching; C, subapical branch; D, upper apical branch; E, lower apical branch. In each case elongation was measured for the periods corresponding to the profiles shown in the top panel, e.g., A1 to A2, B1 to B2, etc.

In lateral branching, the elongation rate of the parental hypha continued undisturbed while the branch emerged and developed. There were no ostensible changes in the appearance or behavior of the Spk of the parental hypha either prior to or during the emergence of the lateral branch. Clearly, the new Spk was formed without affecting the primary Spk. In contrast, apical branching followed a massive disruption of apical growth of the parental hypha. There was a retraction and disappearance of the main Spk, during which time elongation rate dropped sharply. When growth resumed, two new Spk had formed in each of the newly formed apical branches. In all instances, new Spk formed by gradual condensation of phase-dark material (vesicles) around an invisible nucleation site. The ontogeny of individual Spk during apical branching observed in *N. crassa* resembles the ontogeny reported for apical branching in *A. niger ramosa-1* mutant strain (Reynaga-Peña and Bartnicki-Garcia, 1997).

4.2. Kinetic aspects

In *A. nidulans*, Trinci (1970) found that the growth of a lateral branch increases exponentially for about 10 min before attaining a constant rate of elongation. This behavior is substantially different from the one we describe here for *N. crassa* where the lateral branch achieves a constant linear rate within seconds.

The fact that an emerging lateral branch of *N. crassa* attains a steady linear growth rate almost immediately without disturbing the growth of its parent hypha is an indication that both parent and branch derive their precursors from a sufficiently large pool of cytoplasmic reserves. The different behavior shown by *A. nidulans* may be a reflection of the smaller size of their hyphae and a correspondingly smaller reservoir of cytoplasmic precursors.

4.3. Distinction between apical, subapical or lateral branching

Since the demarcation between the apical and subapical zones of a hypha is largely arbitrary, a positional distinction between apical, subapical, and lateral branching becomes a problem. Our findings indicate that Spk behavior may be a better criterion to distinguish branching types. Accordingly, both apical and subapical branching, the latter defined by Trinci (1970) as occurring within about 4 µm from the tip, belong to the same category (i.e., apical branching) and involve the disappearance of the parental Spk and the appearance of new Spk. In contrast, lateral branching occurs independently of the parental Spk which remains unaltered.

4.4. The causes of branching

It is commonly believed that new apices arise from a weakening of the cell wall (Gooday and Gow, 1994; Gooday and Schofield, 1995; Wessels, 1984). It is, however, somewhat misleading to focus on wall weakening as the starting point of branching (Bartnicki-Garcia, 1999). The emergence of a germ tube implies new wall growth and thus it involves both synthetic as well as lytic processes. The same enzymes and precursors that are secreted to support apical growth participate in creating a new growth point. The deformation of the wall, the first visible indication of branch emergence, is not a mere deformation of a weakened wall under high turgor but the protrusion of a growing cell wall. Other authors have attributed branching to restricted areas of expandable cell wall (Virag and Griffiths, 2004). However, the same reasoning discussed above applies and therefore all areas of the cell wall are potentially expandable. What makes an area expand is the presence of an organized and active Spk that directs the exocytotic vesicles and their lytic and synthetic components to the cell membrane.

What causes branching is one the most intriguing and prevalent questions in fungal biology. Most studies have concentrated on lateral branching, which is the most common type of branching in fungal hyphae. The dissimilarities we found between lateral and apical branching suggest that these morphogenetic pathways are triggered differently (see below). But in either case, the processes unleash the formation of new Spk by a mechanism which is largely unknown.

4.5. Triggers of lateral branching

Contemporary thinking on the causes of hyphal lateral branching date back to the pivotal physiologic studies of Robertson (1959), Trinci (1969, 1970), and Katz et al. (1972) who traced the origin of branching to the interplay between the intrinsic tendency of the mycelium to grow exponentially and the inability of individual hyphal tips to exceed a certain maximum rate of elongation. Katz et al. (1972) concluded "a new branch is formed when the capacity of the hypha to elongate exceeds that of the existing tips." Or as currently phrased by Watters and Griffiths (2001): "the formation of a lateral branch is determined by the accumulation of tip-growth vesicles caused by the excess of the rate of supply over the rate of deposition at the apex."

Trinci (1970) concluded that the formation of a lateral branch does not significantly affect the extension of the primary leading hypha. Our observations at high magnification strengthen this conclusion: the formation of lateral branches caused no interference at all in the growth rate of the primary hyphal apex. This finding demonstrates that lateral branches do not drain the resources that support the growth of the primary apex and supports the idea that lateral branching is triggered by an excess of wall-building precursors (presumably vesicles) which cannot be efficiently transported to the increasingly more distant primary apex and accumulate in the hyphal tube. Accordingly, lateral branching may be viewed as the generation of an alternative route for the increasing number of secretory vesicles produced in the continuously enlarging parental hypha.

By comparing the relative size of the parent hypha and its branch, we estimated that the lateral branches of *N. crassa* described here require a 12% increase in wall-building precursors over that needed to maintain the steady growth of the parent hypha; hence, we suggest that this relatively small increase in precursor availability would be sufficient to trigger the ontogeny of a new Spk and the development of the lateral branch.

4.6. Triggers of apical branching

Our results suggest the trigger of apical branching may be traced to a sudden discrete disruption in cytoplasmic organization (cytoplasmic contraction). The localized cytoplasmic contractions reported here for *N. crassa* are similar although more subtle than the contractions reported previously for an apical branching temperature-sensitive mutant strain of *A. niger* (Reynaga-Peña and Bartnicki-Garcia, 1997). Momentary cytoplasmic contractions have also been detected in wild-type hyphae of *Rhizoctonia solani* (Bartnicki-Garcia et al., 1995; López-Franco and Bracker, 1996), *A. niger*, *N. crassa*, and *Trichoderma atroviride* (Reynaga-Peña et al., 1995). A major consequence of these contractions is usually the dislocation of the Spk with ensuing morphological changes. As the dislocated Spk stops functioning, the polarized distribution of vesicles is interrupted, and elongation ceases until new Spk are generated. Although the cause of cytoplasmic contractions remains undiscovered, they have been attributed to the disruption of a contractile cytoskeletal network (actin–myosin) linking the Spk to other cell organelles. Since Ca^{2+} has been long identified as a signal for apical branching in *N. crassa* (Regalado, 1998; Reissig and Kinney, 1983), it is conceivable that internal changes in Ca^{2+}, mediated by SPRAY and calcineurin (Bok et al., 2001), are involved in triggering the contraction. The observations that (1) hyphae exposed to environmental stresses (Reissig and Kinney, 1983; Riquelme et al., 1998; Robertson, 1959) commonly respond by apical branching and (2) internal physiologic stresses induced by mutations often result in apical-branching morphotypes (Dicker and Turian, 1990; Reynaga-Peña and Bartnicki-Garcia, 1997; Virag and Griffiths, 2004) suggests that the Spk-cytoskeleton link is highly susceptible to disruption by either internal or external disturbances. The temporary dislocation/disappearance of the primary Spk results in an accumulation of wall-building vesicles sufficiently large to support the genesis of two new Spk.

4.7. Spk ontogeny

Prior to branch initiation, whether laterally or apical, phase-dark material accumulates at the subapical or apical regions, respectively. By a process that remains the central mystery of polarity initiation, the phase-dark material congregates around a nucleation center to give rise to a new Spk and the resulting branch. As reviewed earlier (Bartnicki-Garcia, 2002), these phase-dark concentrations of vesicles coalesce around a Spk core to form a functional Spk. The nature of the Spk core is still unknown (López-Franco and Bracker, 1996), but recent findings have revealed intriguing possibilities: e.g., γ-tubulin, a component of microtubule organizing centers was found in the Spk of *Allomyces macrogynus* (MacDaniel and Roberson, 1998). Knechtle et al. (2003) reported the presence of GFP-labeled AgSpa2p, a component of the so-called polarisome, at the growing apices of parental hyphae and branches of *Ashbya gossypii*, and suggested that AgSpa2p functions in the

organization of secretory vesicles at the tip. The location of polarisome components appears to coincide with the core of the Spk. In *Saccharomyces cerevisiae*, ScSpa2p interacts with the formin ScBni1p (Evangelista et al., 2002). Formins are a family of multidomain scaffold proteins involved in actin-dependent morphogenetic events (Sharpless and Harris, 2002). Sharpless and Harris (2002) have shown that the formin SepA controls the assembly of actin cables at hyphal tips and septation sites.

The relationship between lateral branching and other cellular events is not fully understood. Lateral branching in *A. nidulans* has been associated with septation and mitosis (Dynesen and Nielsen, 2003; Fiddy and Trinci, 1976; Harris and Momany, 2004). Septins are proteins that act as organizational scaffolds in areas of cell division and new growth. Septin AspB is the only marker found at sites of newly emerging lateral branches but not at the hyphal tip of *A. nidulans* (Westfall and Momany, 2002). However, Dynesen and Nielsen (2003) found that two septum-lacking mutants of *A. nidulans*, *sepG*, and *sepH*, have normal nuclear distribution and lateral branching pattern identical to the wild-type strain. They concluded that "branch initiation is driven by the existence of a minimum required cytoplasmic volume per nucleus."

Clearly, there is a need to integrate physiologic data with the growing number of molecular and cytologic findings associated with the onset of hyphal morphogenesis and branching (Bartnicki-Garcia, 2002; Harris and Momany, 2004; Harris et al., 1999; Seiler and Plamann, 2003). A major challenge is to discern cause–effect hierarchy in this diverse and complex phenomenology to elucidate the origin and the sequence of events triggering branching.

Acknowledgments

M.R. was supported by a Research Assistantship from the Microbiology Graduate Program of the University of California, Riverside. The experimental portion of this work was supported in part by grants from the National Institutes of Health (GM-48257), the National Science Foundation (IBN-9204541), and a special grant from Consejo Nacional de Ciencia y Tecnología, Mexico (DG/2003-1158).

References

Bartnicki, D.D., Gierz, G., Bartnicki-Garcia, S., 1994. "Fungus Simulator": a Windows application to model fungal morphogenesis. In: Abstracts for the 5th International Mycological Congress, Vancouver, Canada, p. 12.

Bartnicki-Garcia, S., 1999. Glucans, walls, and morphogenesis: on the contributions of J. G. H. Wessels to the golden decades of fungal physiology and beyond. Fungal Genet. Biol. 27, 119–127.

Bartnicki-Garcia, S., 2002. Hyphal tip growth: outstanding questions. In: Osiewacz, H.D. (Ed.), Molecular Biology of Fungal Development. Marcel Dekker, New York, pp. 29–58.

Bartnicki-Garcia, S., Bartnicki, D.D., Gierz, G., López-Franco, R., Bracker, C.E., 1995. Evidence that the Spitzenkörper behavior determines the shape of a fungal hypha: a test of the hyphoid model. Exp. Mycol. 19, 153–159.

Bartnicki-Garcia, S., Hergert, F., Gierz, G., 1989. Computer simulation of fungal morphogenesis and the mathematical basis for hyphal (tip) growth. Protoplasma 153, 46–57.

Bok, J., Sone, T., Silverman-Gavrila, L.B., Lew, R.R., Bowring, F.J., Catcheside, D.E.A., Griffiths, A.J.F., 2001. Structure and function analysis of the calcium-related gene *spray* in *Neurospora crassa*. Fungal Genet. Biol. 32, 145–158.

Brunswik, H., 1924. Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyzetengattung *Coprinus*. Botanische Abhandlungen 5, Gustav Fisher, Jena, pp. 1–152.

Buller, A.H.R., 1931. Further observations on the Coprini together with some investigations on social organisation and sex in the Hymenomycetes. In: Researches on Fungi, vol. IV. Longmans, Green and Co, London.

Dicker, J.W., Turian, G., 1990. Calcium deficiencies and apical hyperbranching in wild-type and the "frost" and "spray" morphological mutants of *Neurospora crassa*. J. Gen. Microbiol. 136, 1413–1420.

Dynesen, J., Nielsen, J., 2003. Branching is coordinated with mitosis in growing hyphae of *Aspergillus nidulans*. Fungal Genet. Biol. 40, 15–24.

Evangelista, M., Pruyne, D., Amberg, D.C., Boone, C., Bretscher, A., 2002. Formins direct Arp2/3-independent actin filament assembly to polarize cell growth in yeast. Nat. Cell Biol. 4, 32–41.

Fiddy, C., Trinci, A.P.J., 1976. Mitosis, septation, branching and the duplication cycle in *Aspergillus nidulans*. J. Gen. Microbiol. 97, 169–184.

Girbardt, M., 1957. Der Spitzenkörper von *Polystictus versicolor* (L.). Planta 50, 47–59.

Gooday, G.W., Gow, N.A.R., 1994. Shape determination and polarity in fungal cells. In: Ingram, D.S., Hudson, A. (Eds.), Shape and Form in Plants and Fungi. Academic Press, London, pp. 329–344.

Gooday, G.W., Schofield, D.A., 1995. Regulation of chitin synthesis during growth of fungal hyphae: the possible participation of membrane stress. Can. J. Bot. 73, S114–S121.

Grove, S.N., Bracker, C.E., 1970. Protoplasmic organization of hyphal tips among fungi: Vesicles and Spitzenkörper. J. Bacteriol. 104, 989–1009.

Harris, S.D., Momany, M., 2004. Polarity in filamentous fungi: moving beyond the yeast paradigm. Fungal Genet. Biol. 41, 391–400.

Harris, S.D., Hofmann, A.F., Tedford, H.W., Lee, M.P., 1999. Identification and characterization of genes required for hyphal morphogenesis in the filamentous fungus *Aspergillus nidulans*. Genetics 151, 1015–1025.

Katz, D., Goldstein, D., Rosenberger, R.F., 1972. Model for branch initiation in *Aspergillus nidulans* based on measurements of growth parameters. J. Bacteriol. 109, 1097–1100.

Knechtle, P., Dietrich, F., Philippsen, P., 2003. Maximal polar growth potential depends on the polarisome component AgSpa2 in the filamentous fungus *Ashbya gossypii*. Mol. Biol. Cell 14, 4140–4154.

López-Franco, R., 1992. Organization and dynamics of the Spitzenkörper in growing hyphal tips. Ph.D. dissertation. Purdue University, West Lafayette, IN.

López-Franco, R., Bracker, C.E., 1996. Diversity and dynamics of the Spitzenkörper in growing hyphal tips of higher fungi. Protoplasma 195, 90–111.

MacDaniel, D.P., Roberson, R.W., 1998. γ-Tubulin is a component of the Spitzenkörper and centrosomes in hyphal tip cells of *Allomyces macrogynus*. Protoplasma 203, 118–123.

Marsden, J.E., Tromba, A.J., Weinstein, A., 1993. Basic Multivariable Calculus. Springer-Verlag, New York.

McClure, W.K., Park, D., Robinson, P.M., 1968. Apical organization in the somatic hyphae of fungi. J. Gen. Microbiol. 50, 177–182.

Prosser, J.I., 1990. Growth of fungal branching systems. The Mycologist 4, 60–65.

Regalado, C.M., 1998. Roles of calcium gradients in hyphal tip growth: a mathematical model. Microbiology 144, 2771–2782.

Reissig, J.L., Kinney, S.G., 1983. Calcium as a branching signal in *Neurospora crassa*. J. Bacteriol. 154, 1397–1402.

Reynaga-Peña, C.G., Bartnicki-Garcia, S., 1997. Apical branching in a temperature sensitive mutant of *Aspergillus niger*. Fungal Genet. Biol. 22, 153–167.

Reynaga-Peña, C.G., Bracker, C.E., Bartnicki-García, S., 1995. Cytoplasmic contractions, Spitzenkörper behavior and apical branching in *Aspergillus niger*. In: Abs. Annu. Meet. Mycol. Soc. America, San Diego, CA. *Inoculum* 46, p. 34.

Riquelme, M., 2000. The role of the Spitzenkörper in hyphal growth and morphogenesis. Ph.D. dissertation. University of California, Riverside, CA.

Riquelme, M., Reynaga-Peña, C.G., Gierz, G., Bartnicki-García, S., 1998. What determines growth direction in fungal hyphae? Fungal Genet. Biol. 24, 101–109.

Robertson, N.F., 1959. Experimental control of hyphal branching and branch form in hyphomycetous fungi. J. Linnean Soc. London Bot. 56, 207–211.

Seiler, S., Plamann, M., 2003. The genetic basis of cellular morphogenesis in the filamentous fungus *Neurospora crassa*. Mol. Biol. Cell 14, 4352–4364.

Sharpless, K.E., Harris, S.D., 2002. Functional characterization and localization of the *Aspergillus nidulans* formin SepA. Mol. Biol. Cell 13, 469–479.

Smith, J.H., 1924. On the early growth rate of the individual fungus hypha. New Phytol. 23, 65–78.

Trinci, A.P.J., 1969. A kinetic study of growth of *Aspergillus nidulans* and other fungi. J. Gen. Microbiol. 57, 11–24.

Trinci, A.P.J., 1970. Kinetics of apical and lateral branching in *Aspergillus nidulans* and *Geotrichum lactis*. Trans. Br. Mycol. Soc. 55, 17–28.

Trinci, A.P.J., 1984. Regulation of hyphal branching and hyphal orientation. In: Jennings, H.H., Rayner, A.D.M. (Eds.), The Ecology and Physiology of the Fungal Mycelium. Symposium of British Mycological Society, pp. 24–52.

Virag, A., Griffiths, J.F., 2004. A mutation in the *Neurospora crassa* actin gene results in multiple defects in tip growth and branching. Fungal Genet. Biol. 41, 213–225.

Vogel, H.J., 1956. A convenient growth medium for *Neurospora* (Medium N). Microbial Genet. Bull. 13, 42–43.

Watters, M.K., Griffiths, J.F., 2001. Tests of a cellular model for constant branch distribution in the filamentous fungus *Neurospora crassa*. Appl. Environ. Microbiol. 67, 1788–1792.

Wessels, J.G.H., 1984. Apical hyphal wall extension. Do lytic enzymes play a role? In: Nombela, C. (Ed.), Microbial Cell Wall Synthesis and Autolysis. Elsevier Science Publishers, Amsterdam, pp. 31–42.

Westfall, P.J., Momany, M., 2002. *Aspergillus nidulans* septin AspB plays pre- and postmitotic roles in septum, branch, and conidiophore development. Mol. Biol. Cell 13, 110–118.