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Analysis of the role of the Spitzenkörper in fungal morphogenesis by computer simulation of apical branching in *Aspergillus niger*

(hyphoid model/image analysis/growth patterns)

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ABSTRACT **High-resolution video microscopy, image analysis, and computer simulation were used to study the role of the Spitzenkörper (Spk) in apical branching of *ramosa-1*, a temperature-sensitive mutant of *Aspergillus niger*. A shift to the restrictive temperature led to a cytoplasmic contraction that destabilized the Spk, causing its disappearance. After a short transition period, new Spk appeared where the two incipient apical branches emerged. Changes in cell shape, growth rate, and Spk position were recorded and transferred to the FUNGUS SIMULATOR program to test the hypothesis that the Spk functions as a vesicle supply center (VSC). The simulation faithfully duplicated the elongation of the main hypha and the two apical branches. Elongating hyphae exhibited the growth pattern described by the hyphoid equation. During the transition phase, when no Spk was visible, the growth pattern was nonhyphoid, with consecutive periods of isometric and asymmetric expansion; the apex became enlarged and blunt before the apical branches emerged. Video microscopy images suggested that the branch Spk were formed anew by gradual condensation of vesicle clouds. Simulation exercises where the VSC was split into two new VSCs failed to produce realistic shapes, thus supporting the notion that the branch Spk did not originate by division of the original Spk. The best computer simulation of apical branching morphogenesis included simulations of the ontogeny of branch Spk via condensation of vesicle clouds. This study supports the hypothesis that the Spk plays a major role in hyphal morphogenesis by operating as a VSC—i.e., by regulating the traffic of wall-building vesicles in the manner predicted by the hyphoid model.**

The Spitzenkörper (Spk) is a characteristic complex structure found in growing hyphal tips of higher fungi (1–8). Spk morphology differs considerably among fungi (7). In general, the Spk consists of three main parts: a central core of variable composition, a cluster of vesicles surrounding the core, and an outer cloud of vesicles with imprecise boundaries. The core may not be readily apparent by light microscopy, but it is unmistakably evident with transmission electron microscopy of thin-sections of chemically fixed (5) or freeze-substituted hyphal tips (9). Examined by phase-contrast microscopy, the dark cluster of vesicles is usually the most salient structure. Most of our observations on the Spk of *Aspergillus niger* pertain to the vesicle cluster—i.e., the phase-dark spheroidal body in close proximity to the apical pole.

Since the discovery of the Spk in 1924 (1), Brunswik suggested that it plays a role in apical growth, but its exact function still is not well understood. Girbardt's studies (2, 6) on living hyphae associated the presence of a Spk with hyphal elongation and correlated Spk position with growth direction. Other studies on living hyphae have confirmed the presence of a Spk in growing hyphal tips of diverse higher fungi (3, 5, 7, 8, 10).

Our working hypothesis for the operation of the Spk was formulated from an exercise in computer simulation of fungal morphogenesis (11). Accordingly, the Spk is believed to function as a vesicle supply center (VSC) regulating the traffic of wall-building vesicles. Vesicles are released at random in all directions from the VSC. In this model, the movement of the VSC generates shape. Specifically, a hyphal tube is generated when the VSC advances in a straight line, a process described mathematically by the hyphoid equation $y = x \cot (x V/N)$. This equation embodies two quantitative parameters that are key to morphogenesis: N, the amount of vesicles released, and V, the rate of advance of the VSC. A plot of this equation produces tubular shapes with tips of similar if not identical morphology to those seen in hyphae of Spk-bearing fungi.

Previously, the hyphoid model was used to explain the role of the Spk in a simple hyphal deformation (12). Recently, we isolated a temperature sensitive mutant of *A. niger, ramosa-1*, capable of undergoing apical branching at the restrictive temperature of 34°C (13). This mutant allowed us to induce apical branching *in situ* and to make a detailed video microscopic study of the behavior of the Spk during branch formation. With this information, we tested the premise that the Spk functions as VSC in a more complex morphogenetic sequence where, basically, a single center of polarized growth was transformed into two. Our results corroborate the usefulness of the hyphoid model and the FUNGUS SIMULATOR program in the analysis of fungal morphogenesis.

MATERIALS AND METHODS

Organism. The test fungus was *ramosa-1*, an apical branching, temperature-sensitive mutant of *A. niger* (13). The mutant and its parent wild-type strains were grown in a sterile slide-culture chamber (14) on Vogel's complete medium solidified with 17% gelatin and 0.36% phytagel (VCMPG) (13). Apical branching was induced in the temperature sensitive mutant *in situ* by increasing the temperature of the slide chamber directly on the microscope stage with a household electric hair dryer (13). Slide chamber temperature was determined with an ultra-thin thermocouple model CO1-T (Omega Engineering, Stamford, CT) placed inside the chamber over a layer of VCMPG.

Video Microscopy. Microscopic observations were made with an Olympus Vanox microscope fitted with a phase contrast ×100 oil-immersion-objective lens (n.a. 1.25). Video images were obtained with a Hamamatsu C2400–07 camera and enhanced with an Argus-10 real-time image processor (Hamamatsu Photonic Systems, Bridgewater, NJ). Images were recorded on S-VHS videotapes.

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Abbreviations: Spk, Spitzenkörper; VSC, vesicle supply center.

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FUNGUS SIMULATOR. To test the applicability of the VSC model to actual morphogenetic examples, the original computer program used to simulate fungal morphogenesis (11) was thoroughly revamped and converted into a Windows program named the FUNGUS SIMULATOR (15) (this program is available through the internet at http://www.cs.ucr.edu/~davidb/model/fs.html). This improved program facilitated comparisons between the real shapes of fungi with those generated by the computer.

Data Collection and Processing. Individual images from the videotaped sequences were captured in 8-bit gray scale with an Imagraph Imascan/Chroma frame grabber. Image Pro Plus for Windows (Media Cybernetics, Silver Spring, MD) was used for image analysis. Spk position and cell outlines were manually traced on captured images as sets of *X Y* coordinates. Spk coordinates were measured at the center of the phase-dark vesicle cluster and then shifted by 0.36–0.66 μm (depending on Spk size) to place the VSC at the mid-back point of the Spk (see *Discussion*). Without such correction the computer-generated shapes ran slightly ahead of the actual cell profiles.

Correction factors were calculated to compensate for the slight but persistent tendency of the microscope stage to drift during the course of the observations (16). Growth, measured as area increase, was calculated from the captured cell profiles. All data required to drive the simulation were imported into an EXCEL spreadsheet. Areas were converted to simulator units (15) and used to calculate a vesicle release rate for the VSC(s). Spk trajectory was used to calculate speed and direction of the VSC.

RESULTS

Video Microscopy of Apical Branching in *ramosa-1*. Apical branching was induced in hyphae of *ramosa-1* by a shift to the restrictive temperature (13). Fig. 1 *A–H* shows eight frames from a videotaped sequence selected to illustrate the key morphological changes in apical branching. These plus other intermediate stages (not shown) were used to generate the computer simulation in Fig. 1 *a–h*. During apical branching, major changes in Spk behavior took place. Initially, as the temperature shifted gradually from permissive (23°C) to restrictive (34°C), hyphae of *ramosa-1* continued to elongate normally for 20–60 min (Fig. 1*A*). At the permissive temperature, there was no detectable difference in hyphal tip morphology between *ramosa-1* and its wild-type parent; the hyphal apex of *ramosa-1* (Fig. 2*A*) exhibited a distinct morphology that approximated the perfect shape defined by the hyphoid equation (11). During normal hyphal elongation, the Spk maintained an essentially constant distance from the apical pole. Following the shift to a higher temperature, the first noticeable event in the apical branching sequence was a cytoplasmic contraction (time zero). This contraction, lasting about 1 s, consisted of a sudden synchronous movement of organelles toward the apex. Shortly thereafter (5–13 s), the Spk migrated away from its usual position next to the apical pole (Fig. 1*B*), reversed its direction briefly (15–20 s), and disappeared gradually (30–58 s). After a period (60–260 s) when no Spk was visible (Fig. 1*C*), two new Spk began to form on opposite sides of the slightly enlarged apex, the first one became visible at 263 s (see Fig. 1*D*), the second one at 360 s (Fig. 1*E*). Concomitant with the appearance of each Spk, incipient branches began to emerge. They continued to elongate (Fig. 1 *F–H*), giving rise to two well-developed branches that grew in opposite directions (at about 50°) from the parent hypha until the end of the observation (570 s). Each branch displayed a typical Spk and hyphoid morphology (Fig. 2*C*).

Growth Rates. Whenever a forward-moving Spk was clearly visible—i.e., prior to branching (Fig. 1*A*) or after the branches had emerged (Fig. 1 *E–H*)—the rate of growth (measured as area increase) was fast and the shape of the growing apex tended to be distinctly hyphoid. After the cytoplasmic contraction, growth rate decreased rapidly (Fig. 3). The decrease coincided with the retraction of the Spk from its position next to the apical pole (Fig. 1*B*); by the time the Spk of the parent hypha had disappeared (60 s), growth was reduced to about 20–25% of the original value. Growth rate continued to decrease reaching a minimum of about 5% of the original value between 120 s and 150 s and then began to increase. By the time the first branch Spk appeared (263 s), the growth rate had recovered to about 25%; it continued to increase reaching a maximum value at about 360 s and remained so for the duration of the observation (570 s). This maximum rate, measured from the sum of the two branches, was only 72% of the initial growth rate in the parent hypha.

Growth Patterns. In fast-growing hyphae, the growth increments (shaded areas in Fig. 4 *A* and *F*) were hyphoid increments—i.e., growth zones delimited by two consecutive (nested) hyphoid curves (17). During the disappearance and reappearance of the Spk, and coincident with the changes in growth rate, there were marked changes in growth patterns. As the Spk began to migrate and then disintegrate, the hyphoid pattern was replaced with a more isometric expansion that caused a slight enlargement of the apex (Figs. 4 *B* and *C* and 2*B*). The brief phase of isometric expansion (60–90 s) was followed by an asymmetric apical expansion (Fig. 4*D*) that began on one side of the apex (90–180 s) and extended to its front (180–246 s). This gave the tip an unusually blunt shape. From this blunt apex, coincident with the appearance of new Spk, two branches began to form on opposite sides and both acquired a distinct hyphoid shape (Figs. 4*E* and 2*C*).

FUNGUS SIMULATOR and Hyphoid Model. The FUNGUS SIMULATOR generates shapes by a process that mimics a vesicle-based mechanism for cell wall growth. Vesicles released from a VSC move to the periphery where they contribute to the enlargement of the cell perimeter. Vesicles are programmed to move radially from the VSC in all directions at random (360° arc) (Fig. 5). The program allows adjustment of the arc of vesicle release; for reasons described below, an arc of 320°, oriented with the 40° opening pointing away from the apical pole (Fig. 5), was used throughout the simulation. Also the VSC is shown as a circle (representing the surface of a round Spk) rather than a point source as in the original model (11). [VSC diameter is not critical because exactly the same shapes are produced from either a point source VSC or a circular VSC (unpublished work).] In this computer model, cell size is determined by the number of vesicles released whereas morphology depends on the movement of the VSC as it releases vesicles. To operate the FUNGUS SIMULATOR, two sets of data were collected from digitized images of apical branching: the amount of new surface generated at each interval (Fig. 3) and the trajectory of the Spk (Fig. 6).

Simulation of Apical Branching. Fig. 1 *a–h* shows a computer simulation of the actual apical branching sequence in Fig. 1 *A–H*. The beginning and the end of the simulation posed no new challenge because Spk were distinctly evident (Fig. 6). Accordingly, a single VSC was programmed to follow the Spk trajectory of the parent branch (Fig. 6, points a–b), whereas two separate VSCs were programmed to follow the Spk trajectories of the two branches (Fig. 6, points c–d and e–f). The new challenge was how to run the simulation when no Spk was visible (from 60 to 260 s). Different simulations were run to account for the morphological changes during this transition period. In all, the same growth rate was used as dictated by the values in Fig. 3, only the trajectory and number of VSCs was varied.

Spk division. Although the highly visible vesicle cluster of the original Spk disappeared from view at 60 s (point a in Fig. 7 *A* and *B*), we assumed that its invisible core remained active, continued to function as a VSC, and subsequently divided. The amount of growth for each daughter VSC was apportioned to

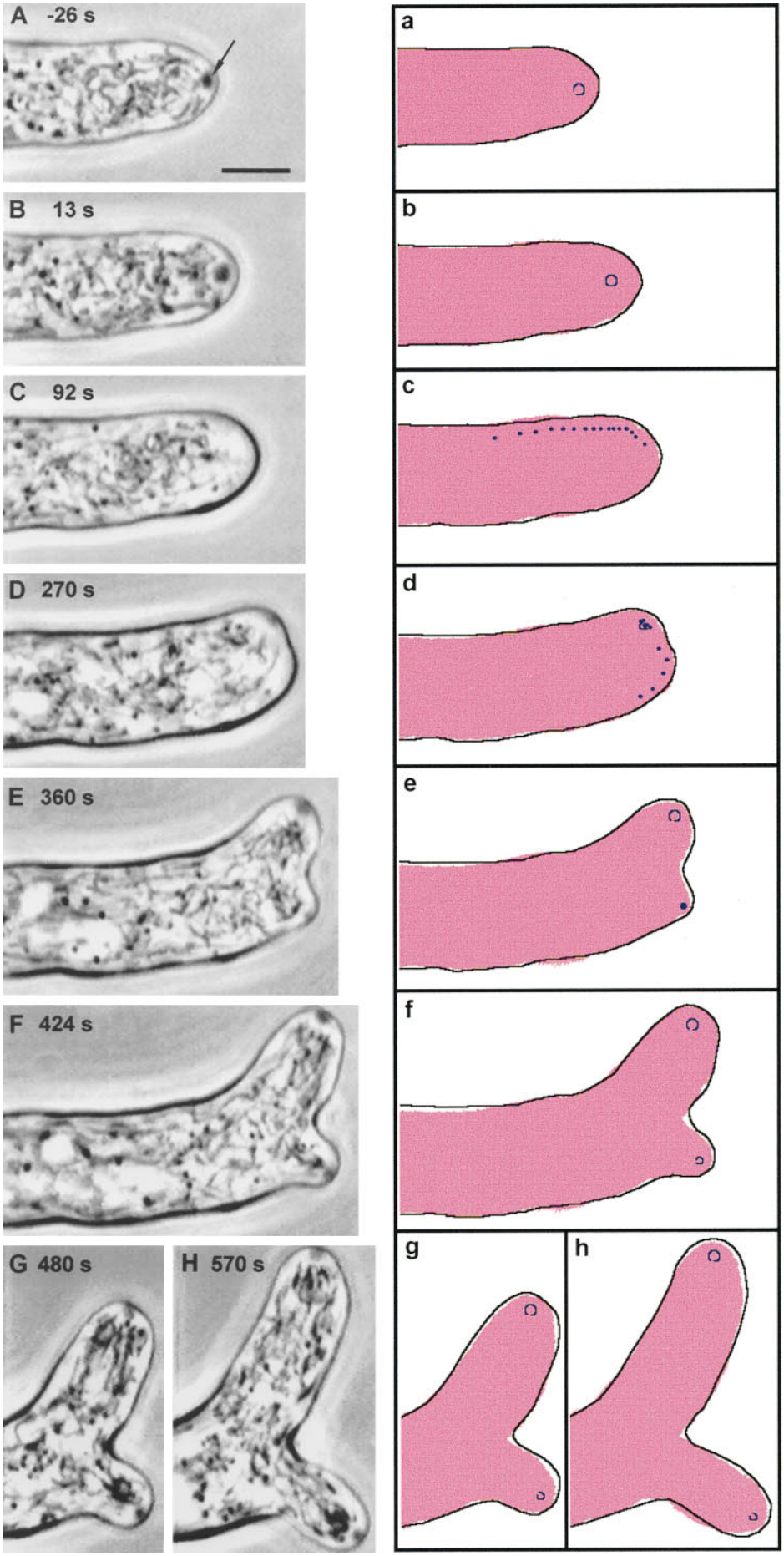

FIG. 1. Computer simulation of apical branching in the *ramosa-1* mutant of *A. niger*. (*A–H*) Phase contrast micrographs of the actual branching sequence. (*a–h*) Corresponding computer simulation of apical branching morphogenesis. Times shown (seconds) are relative to the occurrence of the cytoplamic contraction (zero time). (*A*) Normal apical growth (arrow points to Spk). (*B*) Spk retraction. (*C*) Spk disappearance. (*D*) Appearance of the first branch Spk. (*E*) Appearance of the second branch Spk. (*F–H*) Progressive stages in branch development. In the simulation sequence (*a–h*), the black outlines are the actual cell profiles captured from the corresponding micrographs (*A–H*); the surface generated by the simulator is shown in pink. The simulation is a continuous process. The program starts by filling the first available cell outline (*a*); subsequently, the expanding cell surface is generated by the VSC mechanism (see text). The elongation of the parent hypha (*b*) and the two branches (*e–h*) was simulated by VSCs (shown by black circles) programmed to follow the Spk trajectories illustrated in Fig. 6. The transition phase (*c* and *d*), when no Spk was visible, was simulated by the coalescence of mini-VSCs (shown as black dots) imitating the condensation of a vesicle cloud into a Spk (see text and Fig. 8). (Bar = 5 μm.)

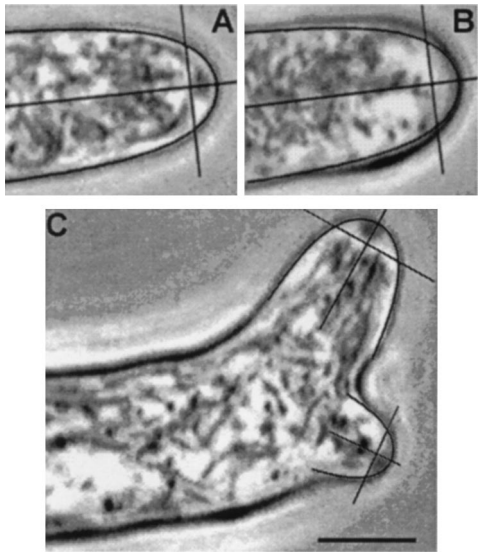

FIG. 2. Hyphal tip morphology and Spk location during apical branching in the *ramosa-1* mutant of *A. niger*. (*A*) Normal hyphal apex appearance 1 s before the cytoplasmic contraction. (*B*) Enlarged apex 90 s later. (*C*) Apical branches 424 s after the cytoplasmic contraction. Hyphoid plots were superimposed to show correspondence (*A* and *C*) or lack of it (*B*) with the hyphoid equation. The VSC position of the plotted hyphoid is at the intersection of the axes; note that this position corresponds to the back of the Spk.

match the size ratio between incipient branches. Two main possibilities were tested. (*i*) The VSC was programmed to divide into two at 90 s and each VSC started moving toward the site where each branch Spk first became visible (points b and c in Fig. 7*A*). (*ii*) The VSC was moved to the geometric center of the apex (point b in Fig. 7*B*) and remained stationary between 60 and 150 s to account for the isometric expansion observed in this period. At 150 s, the single VSC began to move toward the site where the first branch Spk became visible. At point d (Fig. 7*B*), the VSC divided. One continued on the same trajectory, the other moved toward the site where the second branch Spk was first seen (point c, Fig. 7*B*). This and other similar simulations varying the position/time of VSC division all produced shapes that deviated significantly from the actual cell profiles. The area mismatch between simulation and cell profile was calculated to be 12.5% and 6.8% for the simulations shown in Figs. 7 *A* and *B*, respectively.

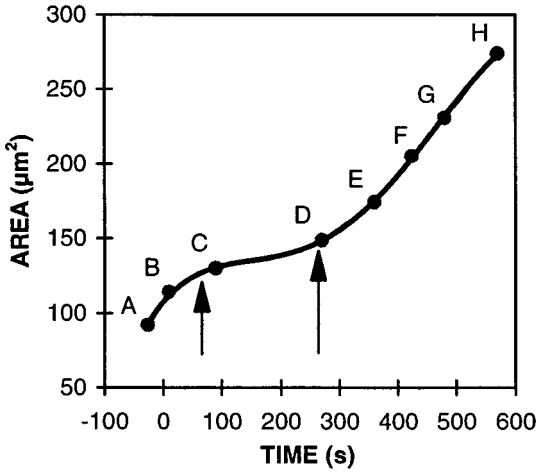

FIG. 3. Growth kinetics during apical branching. Growth was estimated as two-dimensional area increase. Letters correspond to the same developmental stages shown in Fig. 1 *A–H*. Arrows depict beginning and end of the period when no Spk was visible

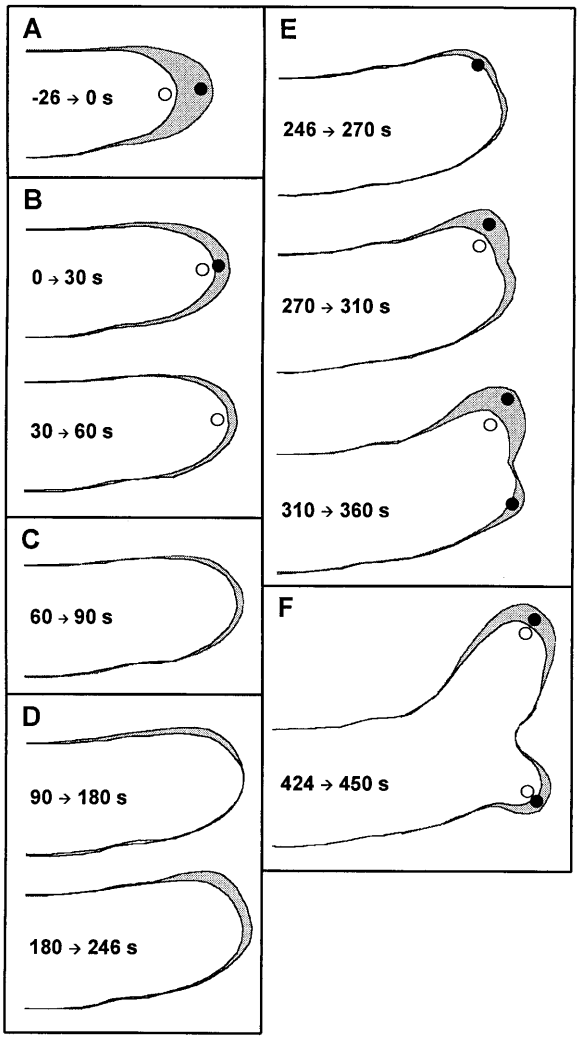

FIG. 4. Growth patterns during apical branching. Cell profiles were traced from the developmental sequence in Fig. 1 *A–H* plus additional intermediate stages not shown there. Consecutive pairs of profiles are shown to illustrate the growth patterns during the listed time intervals (shaded areas). Spk position is shown: ○, first cell profile; ●, second profile. (*A*) Hyphoid growth during normal hyphal elongation. (*B*) End of polarized growth during retraction of the Spk and its gradual disappearance. (*C*) Isometric expansion after the Spk disappears. (*D*) Asymmetric expansion prior to branch emergence. (*E*) Reestablishment of hyphoid growth in incipient branches. (*F*) Hyphoid growth in well-developed branches.

De novo origin of branch Spk. This simulation assumed that the original Spk ceased to exist after a brief phase of isometric expansion (60–90 s). The latter was programmed by displacing the VSC to the center of the apex (point b, Fig. 7*B*). To simulate the next phase of growth (90–246 s), we took into account the observed changes in growth pattern (Fig. 4*D*) and previous observations showing the presence of diffuse vesicle clouds next to the expanding wall areas and the apparent condensation of these clouds into distinct Spk (13). Because the FUNGUS SIMULATOR does not currently have a feature to simulate a diffuse VSC condensing into a discrete VSC, we modeled this option by placing a multitude (sixteen) of mini-VSCs next to the area where growth occurred. Each mini-VSC was programmed to generate a proportional fraction (1/16th) of the required growth increment. The mini-VSCs were spread under the projected area of growth (Fig. 8), and the distance between adjacent VSCs was calculated to match the growth increment expected in the vicinity of each mini-VSC. The mini-VSCs were programmed to move progressively and co-

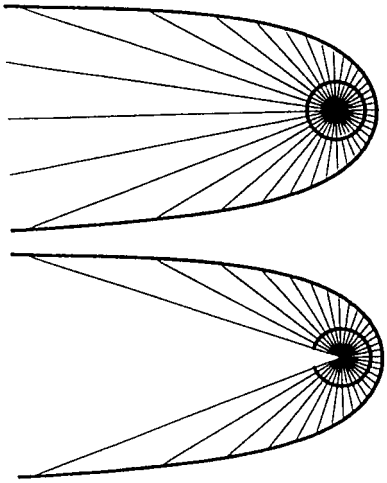

FIG. 5. Relationship between arc and hyphoid expansion. (*Upper*) VSC releasing vesicles in all directions (360°). (*Lower*) The 320° restriction in the arc of vesicle release.

alesce into a single VSC at the position where the Spk of the branch was first detected. The mini-VSCs responsible for the first branch coalesced at 270 s, whereas the VSCs associated with the formation of the second branch coalesced at 360 s. The arrangement and movement of the mini-VSCs for both branches is shown in Fig. 8. This simulation exercise duplicated the morphology of apical branching more faithfully than the others (Fig. 7*C*; area mismatch = 2.3%) and was therefore used for the final simulation in Fig. 1 *a–h*.

DISCUSSION

This study extends previous observations on the physiology of apical branching in fungi (18, 19). The availability of a temperature-sensitive apically branching mutant of *A. niger* (*ramosa-1*) made it practical to induce and observe branching in a hypha growing under an oil immersion lens (13). The use of video microscopy, with electronically enhanced contrast, allowed us to record, at high magnification, the behavior of the Spk. The videotaped images could be played back, frame by frame, to scrutinize cytological events that occurred in a fraction of a second. Digitization of selected images greatly

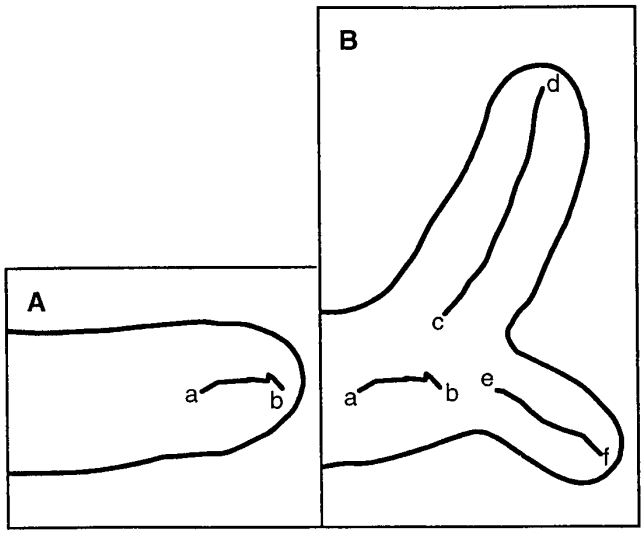

FIG. 6. Spk trajectories during apical branching. (*A*) Cell profile at 60 s. (*B*) Cell profile at 570 s. The Spk positions were mapped during elongation of the parent hypha (points a–b) and the first (points c–d) and second branch (points e–f).

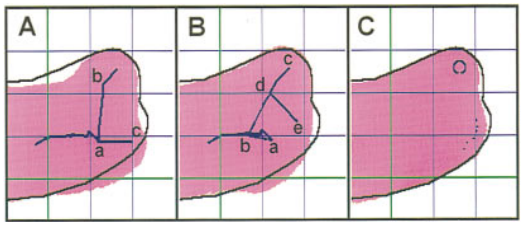

FIG. 7. Simulations of Spk division. The FUNGUS SIMULATOR was programmed to test three different options: (*A*) Spk divides at point a and the two daughter Spk move to points b and c. (*B*) Spk first moves to point b, and then while advancing toward point c it divides at point d. (*C*) No division. Spk disappears at point a; vesicle cloud condenses into Spk (see Fig. 8).

facilitated their morphometric analysis. Finally, the FUNGUS SIMULATOR program permitted testing different options to explain the genesis of form in apical branching.

Elongation of the parent hypha and its branches could be explained by the VSC concept as originally formulated (11)—namely, the Spk functions as a site for the collection of vesicles from their subapical sources and their subsequent distribution to the wall. Vesicles are not targeted to a specific site on the cell surface. It is the forward movement of the Spk, as it releases vesicles, that is the critical factor. Such a process generates tubular cells with a characteristic hyphoid shape.

Our present findings give us some insight into one of the most basic questions in fungal biology: the origin of apical growth. Because a single center of polar growth is converted into two centers, within a small space and in a short time, apical branching is particularly amenable to exploring the basis of Spk ontogeny and the origin of polarized growth. We found no microscopic evidence in favor of a division mechanism where the existing Spk would have simply split into two to give rise to the branch Spk. Had this occurred, the growth rate might have been maintained with only a minor dislocation. Instead, the growth rate came to a near halt as the original Spk disintegrated and the pattern of cell expansion ceased to be hyphoid.

Other observations argue against a simple division mechanism. (*i*) The short delay before the appearance of the second Spk in *ramosa-1* and the uneven size of the branches argue against a simple split as the origin of the branch Spk. (*ii*) Previous extensive studies on Spk behavior have not reported instances of Spk division (2, 5, 7, 10). (*iii*) Our attempts to simulate apical branching by assuming that the original VSC split into two VSCs produced simulated shapes that deviated significantly from actual cell morphology (Figs. 7 *A* and *B*). Although a simple Spk division mechanism seems unlikely, we

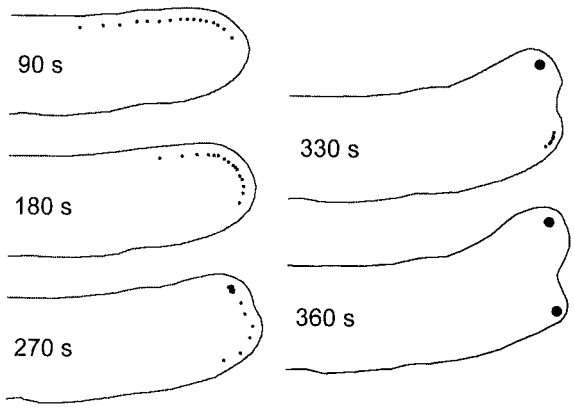

FIG. 8. Position of the mini-VSCs in an improvised simulation of condensation of a vesicle cloud into a Spk.

cannot rule out the possibility that fragments from the original Spk may have served to nucleate the new Spk.

Instead of Spk division, the evidence suggests a *de novo* origin by a gradual condensation of the vesicle cloud detected in the vicinity of the site where the branch Spk eventually formed (13). This cloud was an active, wall-building entity responsible for the observed one-sided enlargement of the apex that preceded the appearance of the Spk. Whether an invisible core structure served as the nucleus for this condensation remains to be determined. Our interpretation is in line with observations made by López-Franco *et al.* (10) on the so-called satellite Spk. These small Spk usually end up merging with the main Spk, but if they remain stationary, they are capable of producing localized zones of wall expansion with consequent deformations in the profile of the hypha.

Previously, the hyphoid model was successfully applied, without modifications, to the simulation of a simple hyphal deformation in *Rhizoctonia solani* caused by the momentary dislocation of its Spk (12). However, the more elaborate example of morphogenesis in apical branching required adjustments in mapping the position of VSC and reduction of the vesicle release arc. Neither change affected the main premise of the hyphoid model.

When hyphoid plots are superimposed on electron micrographs of thin sections of hyphal tips (11, 20) there is a good positional coincidence between the center of the Spk and the VSC. However, in highly magnified, phase-contrast micrographs of fungal apexes, the center of the Spk tends to lie between the VSC and the apical pole. The reason for this discrepancy is not entirely clear. Perhaps the Spk image is displaced forward by the highly curved and refractive cell wall of the apical dome. In *R. solani,* the VSC position was measured from the position of the Spk core, which in this fungus is a distinct light-phase structure located toward the back of the Spk (12). In *A. niger*, no such core was evident, and it became necessary to shift backwards the centrally mapped Spk.

The restriction in the arc of vesicle release of the VSC, from the original 360° to 320° (Fig. 5), was critical during branch elongation but not during the elongation of the parent hypha. The reason is that in a straight-growing hyphoid, vesicles programmed to travel directly backwards (in an arc of $\pm 20°$ relative to the longitudinal axis) would impact the cell surface at a great distance from the growing tip and would have no perceptible effect on cell expansion. However, when there is an abrupt turn in growth direction—e.g., during the development of the two apical branches—vesicles released backwards within this rear arc of 40° would impact the wall of the parent hypha causing its expansion. Because no such expansion occurred, we must conclude that the straight backward flow of vesicles in real cells is restricted. For all practical purposes, hyphal diameter does not increase significantly beyond a relatively short distance from the tip, and this is compatible with the 40° restriction. [Theoretically, the 320° arc would abolish growth beyond $9\,d$ (d = distance between apical pole and VSC; see ref. 11). In the hyphae of *ramosa-1* $9\,d$ was about 12–13 μm.] Mathematically, the same result ensues if instead of a restriction in the arc of vesicle release from the VSC, one restricts the maximum distance that a vesicle can travel.

During the course of the final simulation shown in Fig. 1 *a–h*, the percent mismatch between simulated shape and real shape varied from 1.2 to 4.2% (average = 2.3%). This discrepancy seems quite reasonable given the inevitable imprecision in determining cell profiles and Spk position in the highly magnified images. A similar discrepancy was obtained in a previous test (12). The examples we selected were those where the parent and branch hyphae remained on the same focal plane, but inevitable small displacements of the hyphae or the Spk in the Z axis may have contributed to the discrepancy.

Although the molecular events expressed when *ramosa-1* is shifted to the restrictive temperature are yet to be elucidated, it is clear they unleashed cytological/physiological disturbances that, in turn, brought about profound morphogenetic changes. In the absence of experimental evidence, we can only speculate that the *ramosa-1* mutation affected two critical features related to the operation of the Spk: (*i*) regulation of vesicle traffic in the hyphal apex and (*ii*) mechanism that advances the Spk. Because the visible part of the Spk is not a static structure but an ephemeral body created by the dynamic accumulation of vesicles, its disappearance prior to apical branching could be due either to a net reduction in the flow of vesicles to the tip or perhaps to a loss of the presumed guidance activity of the Spk core. Whereas the overall reduction in growth rate suggests that the flow of vesicles to the tip was disrupted, the retraction of the Spk prior to its disappearance suggests a disturbance in the mechanism responsible for the forward motion of the Spk.

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1. Brunswik, H. (1924) in *Botanische Abhandlung*, ed. Goebel, K. (Fischer, Jena, Germany), pp. 1–152.
2. Girbardt, M. (1957) *Planta* **50,** 47–59.
3. McClure, W. K., Park, D. & Robinson, P. M. (1968) *J. Gen. Microbiol.* **50,** 177–182.
4. Girbardt, M. (1969) *Protoplasma* **67,** 413–441.
5. Grove, S. & Bracker, C. E. (1970) *J. Bacteriol.* **104,** 989–1009.
6. Girbardt, M. (1973) in *Grundlagen der Cytologie,* eds. Hirsch, G. C., Ruska, H. & Sitte, P. (Fischer, Jena, Germany), pp. 441–460.
7. López-Franco, R. & Bracker, C. E. (1996) *Protoplasma* **195,** 90–111.
8. Howard, R. J. & Aist, J. R. (1977) *Protoplasma* **92,** 195–210.
9. Bourett, T. M. & Howard, R. J. (1991) *Protoplasma* **163,** 199–202.
10. López-Franco, R., Howard, R. J. & Bracker, C. E. (1995) *Protoplasma* **188,** 85–103.
11. Bartnicki-Garcia, S., Hergert, F. & Gierz, G. (1989) *Protoplasma* **153,** 46–57.
12. Bartnicki-Garcia, S., Bartnicki, D. D., Gierz, G., López-Franco, R. & Bracker, C. E. (1995) *Exp. Mycol.* **19,** 153–159.
13. Reynaga-Peña, C. G. & Bartnicki-Garcia, S. (1997) *Fungal Genet. Biol.,* in press.
14. López-Franco, R. (1992) Ph.D. dissertation (Purdue Univ., West Lafayette, IN).
15. Bartnicki, D. D. (1994) M.Sc. dissertation (Univ. of California, Riverside).
16. Bracker, C. E. (1995) *Zool. Studies* **34** (Suppl. 1), 154–156.
17. Bartnicki-Garcia, S. (1995) *Can. J. Bot.* **73s1,** 372–378.
18. Trinci, A. P. J. (1970) *Trans. Br. Mycol. Soc.* **55,** 17–28.
19. Trinci, A. P. J. (1978) *Trans. Br. Mycol. Soc.* **71,** 107–120.
20. Bartnicki-Garcia, S. (1990) in *Tip Growth in Plant and Fungal Cells*, ed. Heath, I. B. (Academic, San Diego), pp. 211–232.