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Computer simulation of fungal morphogenesis and the mathematical basis for hyphal (tip) growth

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Summary. A novel mathematical model is proposed to explain how a tubular shape (e.g., a fungal hypha) is generated by a tip-growing cell. The model derived from a computer simulation of morphogenesis assumes that: i) the cell surface expands from materials discharged by wall-destined vesicles, ii) vesicles are released from a postulated vesicle supply center (VSC), iii) vesicles move from the VSC to the surface in any random direction. The position and movement of the VSC become the critical determinant of morphogenesis: a stationary VSC releases vesicles that reach the cell surface in about equal numbers in all directions, and the cell grows as a sphere. Any displacement of the VSC from its original central position distorts the spherical shape. A sustained linear displacement of the VSC generates the typical cylindroid shape of fungal hyphae. The model yields the equation

$$y = x \cot \frac{V \cdot x}{N}$$

which defines both the shape and size (diameter) of a hypha by two parameters, to which physiological significance can be ascribed: N, the amount of wall-destined vesicles released from the VSC per unit time; V, the rate of linear displacement of the VSC. There is a remarkable coincidence between the position of the VSC in the model and the position of the Spitzenkörper in real hyphae. The model affords a simple mechanism to generate a tubular shape from a tip-growing cell; it obviates the need to postulate specific targets for vesicles on the apical cell surface or to invoke gradients in the properties of the apical wall. Other common morphogenetic transitions of fungi and other organisms can be simulated with the same basic model.

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Abbrevation: VSC vesicle supply center.

Introduction

The manner in which hyphae, the tip-growing tubular cells typical of fungi, attain their characteristic morphology has long been an appealing subject for experimental and theoretical studies. From earlier findings with surface markers (Castle 1958) or radioisotopes (Bartnicki-Garcia and Lippman 1969, 1977; Gooday 1971) it became clear that apical growth entailed a gradient of surface expansion that was maximum at the tip and declined towards the base of the extension zone (Green 1969).

Various attempts have been made to describe in mathematical terms the pattern of surface growth that can generate a nearly cylindrical[1] shape from a tip-growing cell (Reinhardt 1892, da Riva Ricci and Kendrick 1972, Trinci and Saunders 1977, Prosser and Trinci 1979, Koch 1982; for a review of mathematical models, see Prosser 1979). Although most of these models afford equations that approximate the shape of fungal hyphae, they appear to have a serious shortcoming. They are basically geometric exercises that formulate equations from artificial coordinates and reference points for

[1] The shapes are not truly cylindrical since the tubes have a slight taper.

which there are no corresponding subcellular structures underlying these markers in a hypha (e.g., apical pole, base of the apical dome, and related angular coordinates). Any dimensions given to a hyphal apex are somewhat arbitrary since the base of the apical dome cannot be defined precisely because of the lack of a sharp demarcation between the acutely tapered portion of the apex and the quasi-cylindrical portion of the hypha.

The present study[2] was an attempt to develop a more realistic mathematical model of hyphal growth based on what is clearly the main subcellular structure involved in cell wall growth in fungi: secretory vesicles. This model quantitates an earlier scheme of fungal wall growth based on vesicles (Bartnicki-Garcia 1973). Although our initial goal was to understand the genesis of fungal shapes, the present model can be extended to other organisms with comparable morphogenesis.

Methods

The computer simulation was carried out on a Sun 4/260S-16 computer running Sun Unix. Graphics were displayed on Tektronics terminals and printed with a color dot-matrix printer. The computer program was written in the language C. Plots of the hyphal equation (hyphoid) were made with Lotus 123 software in an IBM-AT computer and printed with a Hewlett-Packard plotter model 7475.

Computer simulation of morphogenesis

The model was built by imagining that a cell is a container under pressure bombarded continuously from *within* by a myriad of tiny vesicles. Upon impact, each vesicle becomes inserted into the wall of the container; since wall thickness remains constant, vesicle insertion increases the surface area of the cell by one unit. Although there is evidence for more than one kind of vesicle being involved in fungal wall growth (Bartnicki-Garcia 1987), the model considers that one type of vesicle delivers all necessary materials to produce a unit of growth, including ingredients needed to give the wall a localized measure of transient plasticity. Also, no distinction between wall and plasma membrane is made. The entire mass of the vesicle is assumed to be incorporated to the cell surface. The model does not take into account that much of the wall is made in situ. However, any mass produced in situ is likely to be proportional to the amount of enzyme delivered by the vesicles. The model was formulated in two-dimensions but the conclusions can be extrapolated to the corresponding three-dimensional solid of revolution. The resulting shapes represent median sections of three-dimensional cells. The simulation is done on a grid with squares representing a unit of area (Bartnicki-Garcia et al. 1989) each filled square is derived from a "vesicle" (Fig. 1). A square can be either full or empty. The union of full squares constitutes the fungal cell, the empty squares are the surrounding medium. The vesicles are released at random, in all directions from an idealized point source inside the cell: the vesicle supply center (VSC). The vesicles travel in the direction they were initially emitted until they reach the cell boundary (wall or "shell"). The program chooses an angle at random. This angle defines a ray emanating from the VSC and represents the direction in which the vesicle will travel. The probability distribution is uniform, i.e., all angles have the same likelihood. The program searches for the first empty square along the ray and changes its status from empty to full. Cell area is thus increased by one unit. To facilitate observation, the simulation of growth processes was recorded stepwise, in alternating colors, with the same number of vesicles released per step.

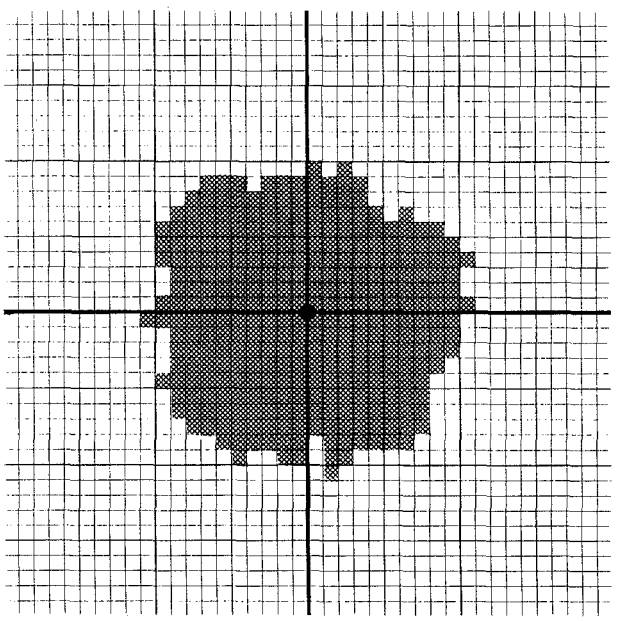


Fig. 1. A magnified schematic representation of the programming grid. Individual growth events are shown as filled squares. Each event is triggered by a "vesicle" emanating from the center (VSC) and traveling in a randomly chosen direction

Simulation of spherical growth: stationary VSC

This is the simplest case of growth simulation. The VSC is held stationary while vesicles are released ran-

[2] A preliminary description of the model was first presented at a Harden Workshop of The Biochemical Society in Oxford, U.K., April 24–27, 1988 (see Bartnicki-Garcia et al. 1989).

domly in all directions. The resulting shape is a growing circle, i.e., a two-dimensional simulation of spherical growth (Fig. 2a–g; Fig. 2a–o, see p. 52).

Simulation of cylindrical (hyphal) growth: moving VSC

If after a period of spherical growth generated by vesicles released from a stationary VSC, the VSC is programmed to move in a fixed direction while releasing vesicles at the same rate, the shape gradually changes from spherical to tubular (Fig. 2h–o). The circle will continue to enlarge but with increasing asymmetry. Although vesicles are continually released by the VSC, uniformly in all directions, the number of vesicles reaching different points on the cell surface becomes increasingly disproportionate; the frequency of vesicle impacts per unit of surface will be increasingly greater on the advancing side of the VSC than on the rest of the cell surface. As the VSC approaches the cell surface, the deformation becomes more and more apparent and a conspicuous protrusion begins to emerge from the circular shape (Fig. 2h–j). The protrusion grows longer until a well defined tube is generated (Fig. 2k–o). As long as the simulation continues with the same parameters, namely the same frequency of vesicle release and the same rate of displacement of the VSC, the tube will elongate and produce the two-dimensional equivalent of a long cylinder with a slight taper. The entire sequence in Fig. 2 is a two-dimensional simulation of spore germination and hyphal morphogenesis in fungi (Bartnicki-Garcia 1981) or equivalent morphogenetic processes in other organisms, e.g., zygote germination in algae (Quatrano et al. 1985); or pollen tube germination in higher plants (Steer and Steer 1989).

Mathematical basis for hyphal growth

The mathematical function that describes the growth of a hyphal tube shown in Fig. 2 was derived under the following assumptions:

(1) The VSC moves along the y-axis with a velocity equal to the constant V.

(2) The shape of the fungal tube is symmetric with respect to the growth axis; since the y-axis is the growth axis, rotation of a certain curve $y = f(x)$ about the y-axis will generate the three-dimensional boundary of the cell.

(3) In an equilibrium state, the shape of the hypha would remain constant while it elongates at a speed V.

Let $y = f(x, t)$ denote the equation of the curve defining the two-dimensional boundary of the cell at time t (Fig. 3). We would expect that

$$f(x, t + \Delta t) = f(x, t) + V \cdot \Delta t$$

or, equivalently

$$f(x, t) = f(x, 0) + V \cdot t .$$

In order to define $f(x, t)$ for any value of t, the coordinate system is adjusted so that at time $t = 0$ the VSC is positioned at the origin, and a version of polar coordinates is used in which β is the angle to the y-axis instead of the usual x-axis. Hence we have the transformations:

$$x = r \sin\beta$$
$$y = r \cos\beta .$$

For the differentials this means

$$dx = \sin\beta\, dr + r \cos\beta\, d\beta$$
$$dy = \cos\beta\, dr - r \sin\beta\, d\beta$$

and we obtain

$$y' = \frac{dy}{dx} = \frac{\cos\beta \dfrac{dr}{d\beta} - r \sin\beta}{\sin\beta \dfrac{dr}{d\beta} + r \cos\beta} .$$

Let $r = \rho(\beta, t)$ be the equation for the curve $y = f(x, t)$ in our polar coordinate system. Then,

$$\rho(\beta, t) \cos\beta = f(x, t)$$
$$\rho(\beta, t) \sin\beta = x .$$

Hence

$$\rho(\beta, t) \cos\beta = f(\rho(\beta, t) \sin\beta, t)$$
$$= f(\rho(\beta, t) \sin\beta, 0) + V \cdot t$$

and therefore

$$\rho_t(\beta, t) \cos\beta = f_x(\rho(\beta, t) \sin\beta, 0) \cdot \rho_t(\beta, t) \sin\beta + V .$$

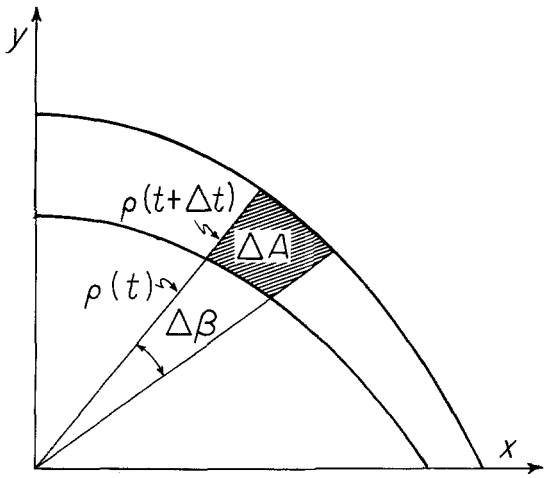


Fig. 3

For $t = 0$ we obtain

$$\rho_t(\beta, 0)\cos\beta = f_x(\rho(\beta, 0)\sin\beta, 0)\cdot\rho_t(\beta, 0)\sin\beta + V.$$

Solving the equation for ρ_t yields

$$\rho_t(\beta, 0) = \frac{V}{\cos\beta - f_x(\rho(\beta, 0)\sin\beta, 0)\cdot\sin\beta}.$$

Using the above expression for $dy/dx = f_x$ in this equation, we obtain

$$\begin{aligned}\rho_t &= \frac{V}{\cos\beta - \frac{dy}{dx}\sin\beta} \\ &= \frac{V}{\cos\beta - \sin\beta\frac{\rho_\beta\cos\beta - \rho\sin\beta}{\rho_\beta\sin\beta + \rho\cos\beta}} \\ &= \frac{V}{\rho}(\rho_\beta\sin\beta + \rho\cos\beta).\end{aligned}$$

Since the curve is symmetric to the y-axis, the tangent line at $x = 0$ would be parallel to the x-axis. Therefore

$$y'(0) = 0.$$

If $x = 0$, then $\beta = 0$. Hence $y'(0) = 0$ is equivalent to

$$\frac{dr}{d\beta}(0) = 0.$$

The area A added to the fungus *surface* in time Δt in an angular region $\Delta\beta$ (see Fig. 3) is equal to

$$\Delta A = (\rho(t + \Delta t) - \rho(t))\rho(t)\Delta\beta$$

Since vesicles are emitted at random and uniformly in all directions, the increase of area per angle per time (N) is constant. Hence sending Δt and $\Delta\beta$ to 0 in the above equation, we obtain

$$\rho_t\rho = N.$$

Using our expression for ρ_t, we get

$$\rho\cos\beta + \rho_\beta\sin\beta = \frac{N}{V}$$

or

$$\frac{d}{d\beta}(\rho\sin\beta) = \frac{N}{V}$$

which yields

$$\rho = \frac{N}{V}\frac{\beta}{\sin\beta}.$$

In Cartesian coordinates the equation of this curve is

$$y = x\cot\frac{V\cdot x}{N}. \qquad \text{(I)}$$

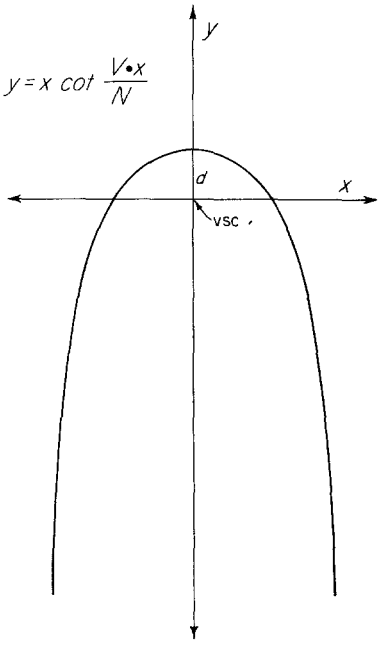


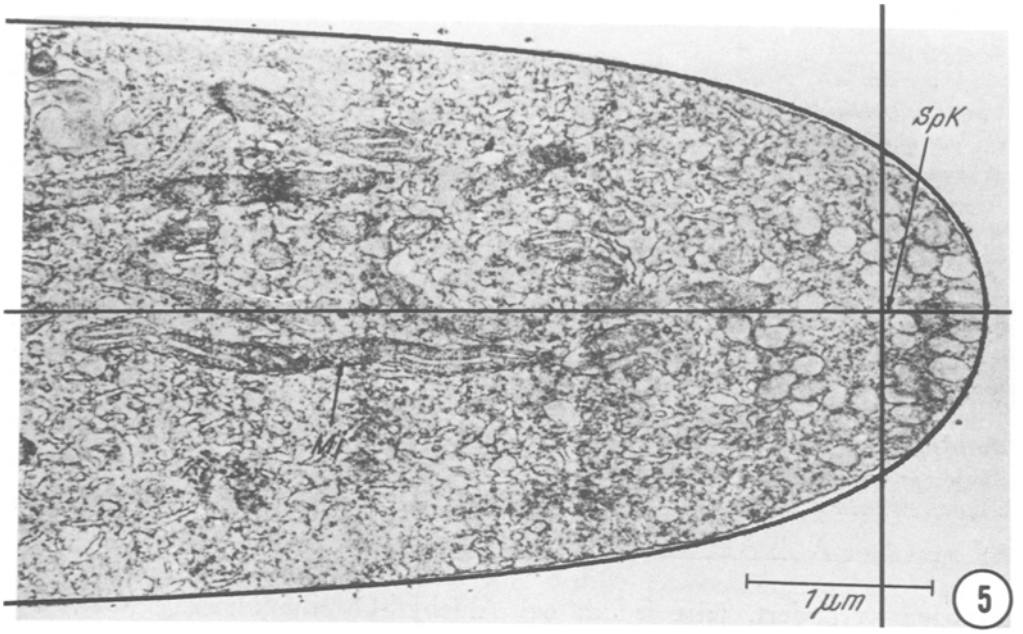
Fig. 4. A hyphoid curve plotted on an arbitrary scale from Eq. (I). The distance between the origin (VSC) and the tip is $d = \frac{N}{V}$

Equation (I) defines the overall shape of a hypha in longitudinal, median cross-section (Fig. 4). Accordingly, the dimensions of the hypha are governed by two parameters which have *physiological* significance: N, rate of increase in area = number of vesicles released by the VSC per unit time; V, rate of linear displacement of the VSC.

The ratio $\frac{N}{V}$ defines a key parameter in a hypha: the distance (d) between the VSC and the apical wall (Fig. 4). This single value determines the size of a hypha:

$$y = x\cot\frac{x}{d}. \qquad \text{(II)}$$

Values of d can be readily calculated for actual hyphae (e.g., from published micrographs showing hyphal tips in median longitudinal section). With two simple measurements: (1) diameter of the hyphae at any given point and (2) distance of this point from the hyphal tip, we calculated d using a numerical solution for Eq. (II). These d values, entered in Eq. (II), were used to plot curves which matched accurately the corresponding profiles of hyphae from a wide variety of fungi. Figures 5–7 show the close correspondence between plotted curves and actual profiles of *Polystictus versicolor, Phytium aphanidermatum,* and *Armillaria mellea*. Similarly, the profiles of hyphae of *Gilbertella per-*



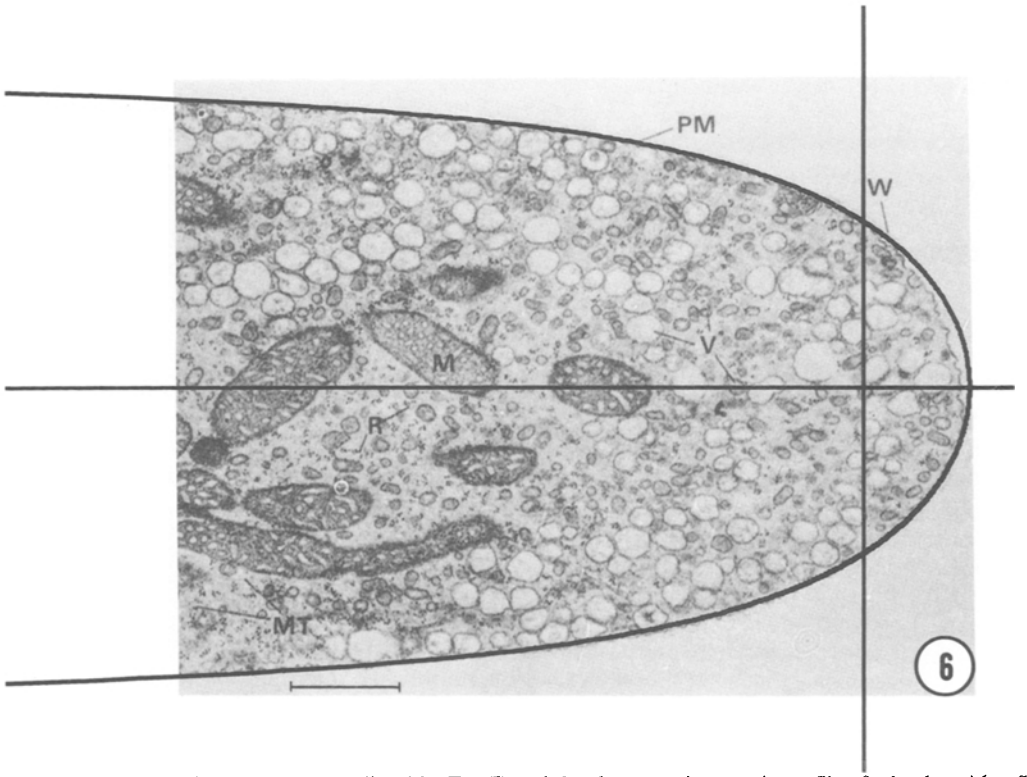


Fig. 5. Correspondence between curve predicted by Eq. (I) and the electron microscopic profile of a hypha with a Spitzenkörper (*Polystictus versicolor*). The curve plotted from the calculated value of *d* was superimposed on a photographic copy of Fig. 1 from Girbardt (1969). Note that the originally marked position of the Spitzenkörper (*Spk*) coincides with the position of the VSC in the model curve. Micrograph reproduced with publisher's permission

Fig. 6. Correspondence between shape predicted by Eq. (I) and the profile of a hypha from a fungus devoid of Spitzenkörper, *Pythium aphanidermatum*. Montage prepared as in previous figure for micrograph in Fig. 1 from Grove and Bracker (1970). Micrograph reproduced with author's permission. Bar = 1 µm

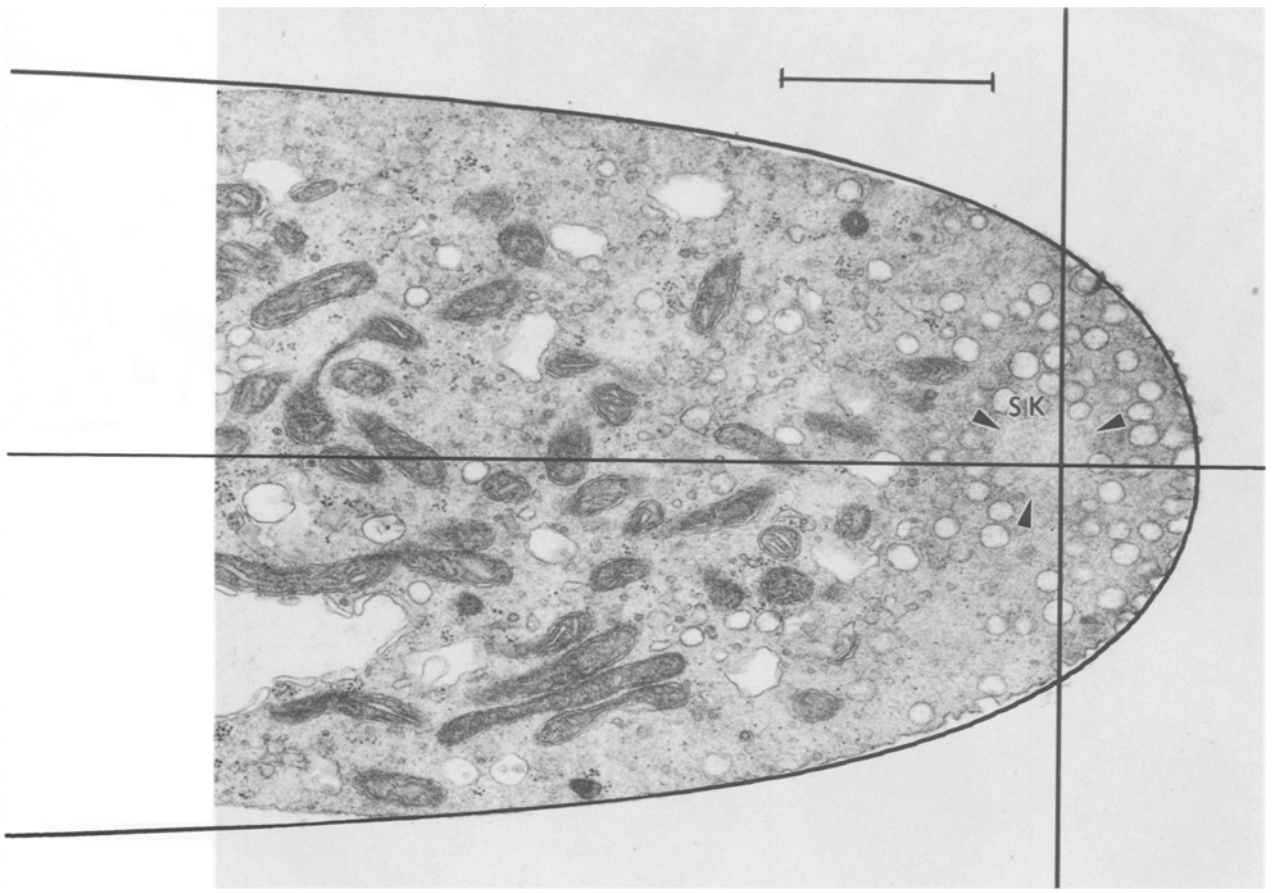


Fig. 7. Correspondence between curve predicted by Eq. (I) and the electron microscopic profile of a hypha of *Armillaria mellea* published by Grove and Bracker (1970). The arrows delineate a vesicle-free center core of this Spitzenkörper (*SK*). Note the coincidence of the VSC with Spitzenkörper. Micrograph courtesy of Charles E. Bracker. Bar = 1 μm

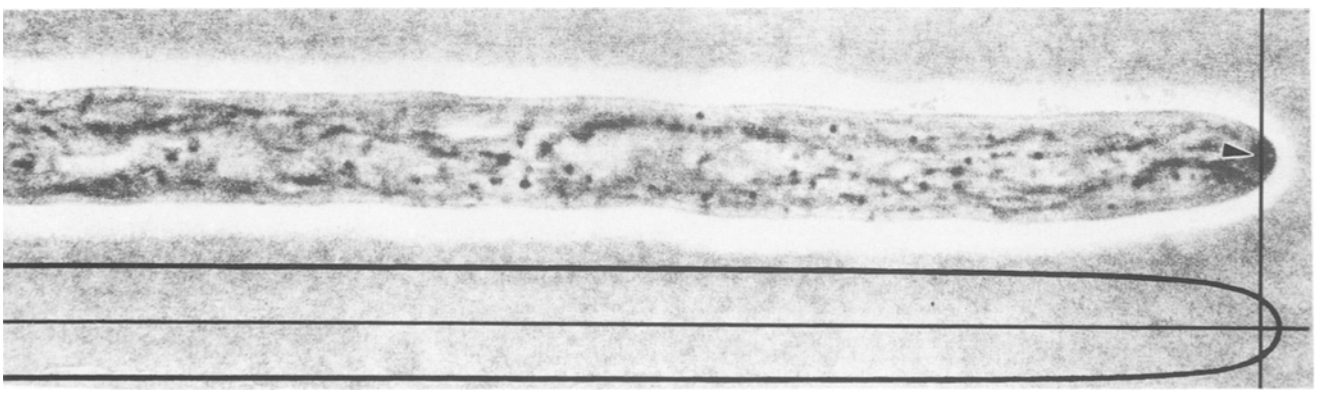


Fig. 8. Correspondence between shape predicted by Eq. (I) and the profile of a long living hypha of *Fusarium acuminatum* observed under a phase-contrast microscope by Howard and Aist (1977). Note the correspondence between the VSC and the Spitzenkörper (▶). Micrograph courtesy of Richard J. Howard

sicaria, Aspergillus niger, Neurospora crassa, Fusarium oxysporum, and *Ascodesmis nigricans* [Figs. 7, 16, 22, 30, and 33 in the classic paper on hyphal tips of fungi by Grove and Bracker (1970)] were found to fit the equation (not shown). Needless to say, a good match requires a true median longitudinal section of a hyphal tip and little or no ostensible shape distortion or specimen damage during processing for electron microscopy.

The equation describes hyphal shape not only in the

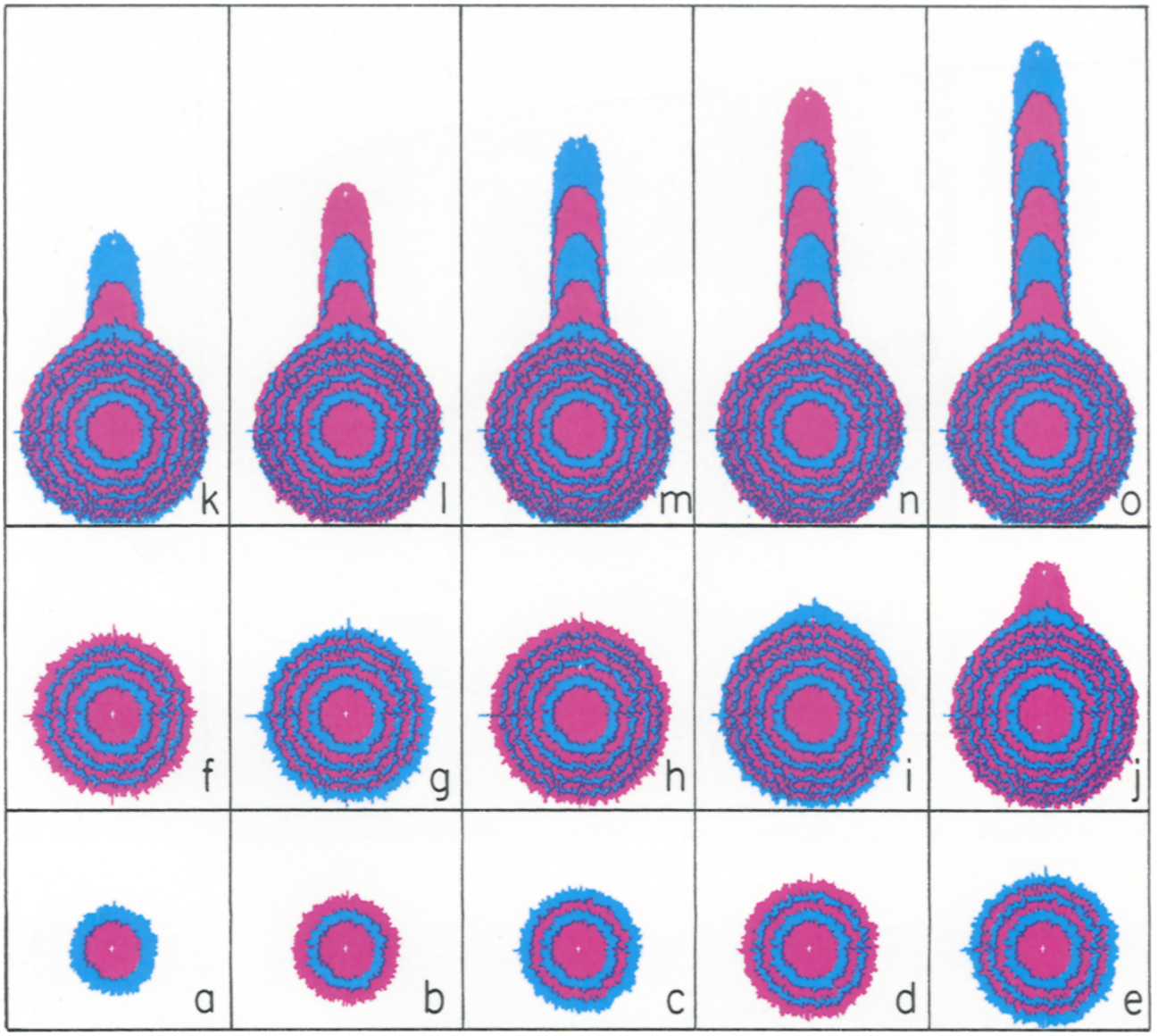


Fig. 2. Computer simulation of spherical growth and hyphal morphogensis. This exercise emulates the two-stage sequence of morphological development commonly observed during fungal spore germination (Bartnicki-Garcia 1981). First, the *spherical growth* or "swelling" of the spore is simulated by the random discharge of vesicles from a stationary VSC (white + cursor). Second, the emergence of a germ tube, i.e., the initiation of hyphal morphogenesis is achieved by displacing the VSC toward the periphery while maintaining the same rate of vesicle discharge. In each frame, 8,000 "vesicles" were released from the VSC (+). Alternating color bands show the amount of growth in each frame. **a–g** A gradually expanding circle results from the vesicles released by a fixed VSC. **h–o** The VSC was made to advance continuously while releasing the same number of vesicles: as the VSC approaches the cell surface a protrusion (**i**) emerges from the circle, which acquires a characteristic hyphal tube shape as it continues to elongate (**j–o**)

apical region but over the entire length of the hyphal tube. Thus, the plotted curve for a hypha of *Fusarium acuminatum* can closely trace its long profile observed by light microscopy (Fig. 8).

From Eq. (I) the diameter of a hypha can also be derived. Thus, since the first poles of Eq. (I) occur at

$$\frac{V \cdot x}{N} = \pm \pi$$

it follows that the maximum diameter (D) of the hyphae would be

$$D = 2\pi \frac{N}{V} . \qquad \text{(III)}$$

Also since $d = \frac{N}{V}$, the position of the VSC in the apical dome is directly proportional to hyphal diameter

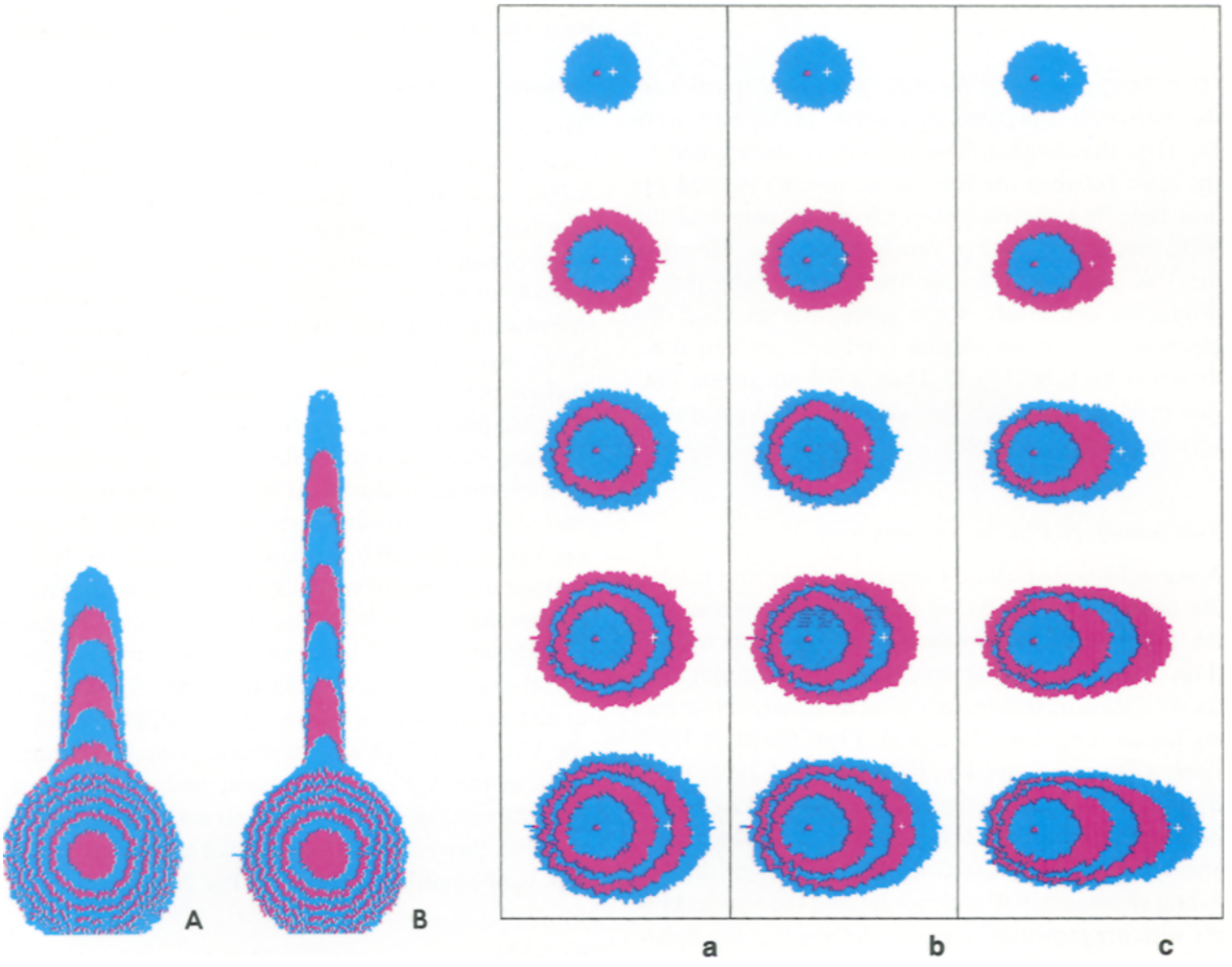


Fig. 9. Generation of hyphae of different diameter. Simulations were performed as in Fig. 2. in **A, B** The total number of "vesicles" was the same, as was the quantity of vesicles released (N) per color band, the only difference was that in **B** the VSC (V) was programmed to advance twice as fast as in **A**

Fig. 10a–c. Computer simulation of yeast cell morphogenesis. The production of ovoidal/ellipsoidal shapes typical of ordinary yeast cells can be simulated by invoking a limited displacement of the VSC and a high rate of vesicle generation. In each frame, 8,000 "vesicles" were released from the VSC (+). In each vertical sequence, three different yeast shapes of increasing cylindricity were generated by varying the rate of advance of the VSC (V) in a 1 : 1.5 : 2.0 ratio (**a–c**). The initial position of the VSC is marked by the red dot

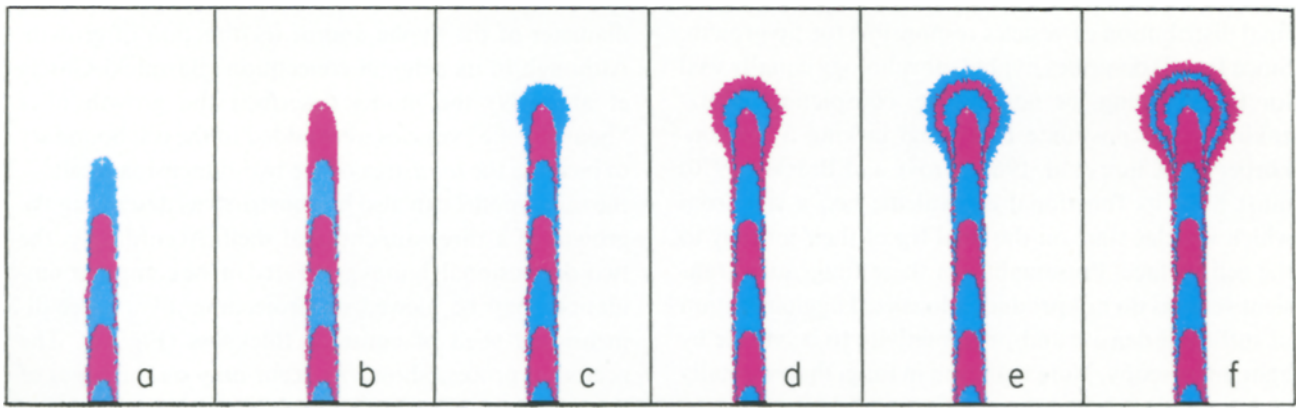


Fig. 11a–f. Computer simulation of sporangium development. A common morphology seen in reproductive structures of fungi consists of a long tube (sporangiophore) with a large spheroidal reservoir at one end (sporangium, gametangium). The process can be simulated by generating a long narrow hypha (**a, b**) as was done in Fig. 9B, and then stopping the linear displacement of the VSC ($V = 0$) without interrupting vesicle release (**c–f**). In each frame, 8,000 "vesicles" were released from the VSC (+)

($D = 2\pi d$): the closer the VSC lies to the apical wall, the narrower the tube produced. It follows from Eq. (III) that hyphal diameter will be determined by the ratio between the number of vesicles emitted per unit time and the rate of linear displacement of the VSC. For a given rate of vesicle generation, the faster the VSC advances, the narrower is the tube that is produced; conversely, for a given rate of VSC displacement, the more vesicles produced per unit time, the wider the tube (Fig. 9). Thus, a fast advancing VSC that produces relatively few vesicles would generate a very narrow hyphal tube.

Relationship of VSC to Spitzenkörper

A remarkable correlation emanates from the model: the position of the VSC in the model corresponds to the position of the Spitzenkörper in living hyphae (Figs. 5 and 7). The Spitzenkörper is a conspicuous accumulation of vesicles in hyphal tips of *actively* growing higher fungi (McClure et al. 1968, Girbardt 1957, 1969; Grove and Bracker 1970, Howard 1981, Roberson and Fuller 1988). Spitzenkörper were discovered long ago (Brunswik 1924) but their significance in hyphal growth has remained unclear. Although there is strong circumstantial evidence linking the Spitzenkörper with the growth of a hypha, the absence of a Spitzenkörper in the hyphae of lower fungi (McClure et al. 1968, Grove and Bracker 1970) has caused some to question whether the Spitzenkörper played an essential role in hyphal growth. The close correlation we find between the position of the VSC in the model hyphae ($= d$) and the position of the Spitzenkörper in real hyphae supports the notion that the Spitzenkörper is a manifestation of an essential feature of hyphal morphogenesis, namely the existence of a center for the final distribution of vesicles responsible for tip growth. Since Eq. (I) describes hyphal morphology equally well for fungi having, or not having, conspicuous Spitzenkörper, we postulate that fungi lacking a Spitzenkörper (McClure et al. 1968, Grove and Bracker 1970) must have its functional equivalent; i.e., a site from which vesicles start on the final leg of their journey to the cell surface. Presumably, in these fungi such transient vesicles do not produce a localized agglomeration of sufficient density and/or refractivity to be visible by light microscopy. Note that even in fungi that normally have a visible Spitzenkörper, this may disappear during partial growth inhibition with anti-tubulin agents yet the hypha continues to elongate (Howard and Aist 1977).

Simulations of morphogenetic processes

One virtue of our model is that one can simulate actual morphogenetic transitions simply by adjusting the ratio of the two vesicle parameters N and V. When V is zero, a spherical shape would result, when V has a finite value, an elongated shape would be produced that may vary from a slightly prolate spheroid to an extremely narrow and long tube. With these two parameters the basic shapes of fungal cells may be constructed. These include the processes of spore germination depicted in Fig. 2 (a phase of stationary VSC followed by a phase of linear displacement of the VSC); the development of a sporangium illustrated in Fig. 11 (the reverse situation where a growth phase with a relatively fast moving VSC is followed by a phase with a stationary VSC); and various types of yeast-like shapes shown in Fig. 10 (generated by the VSC advancing over a short distance at different rates of movement). These ovoidal/ellipsoidal shapes typical of ordinary yeast cells were simulated by invoking a relatively slow displacement of the VSC and a high rate of vesicle generation. Variations in the N/V ratio produced buds of increasing cylindricity (Fig. 10). If there is no net displacement of the VSC, then spherical buds typical of yeasts of *Mucor* spp. would result (simulated in Fig. 2a–g).

Discussion

As presently constructed, the model generates the shape of a straight hypha with a regular shape and size. Although living hyphae deviate to different extents from this highly idealized morphology, the present model also provides a basis for explaining the more convoluted or irregular shapes seen in actual hyphae. Oscillations in the V/N ratio and/or drift in the path followed by the VSC could be the cause of fluctuations in the diameter of the hypha and/or its direction of growth. Although in its original conception (Bartnicki-Garcia et al. 1989) the model described the growth of a "board"—i.e., vesicles were added to the cell boundary to increase the *total* area of the two-dimensional cell[3]—the same model can also be construed as describing the growth of a three-dimensional shell. Accordingly, the two-dimensional figures generated in the computer simulation may be viewed as projections of a three-dimensional shell of constant thickness (Fig. 12). The accretion process shown to occur only on the edges of the "shadow" (Fig. 3), should be regarded as insertion

[3] We are indebted to Paul B. Green for drawing our attention to this conceptual limitation of our original model.

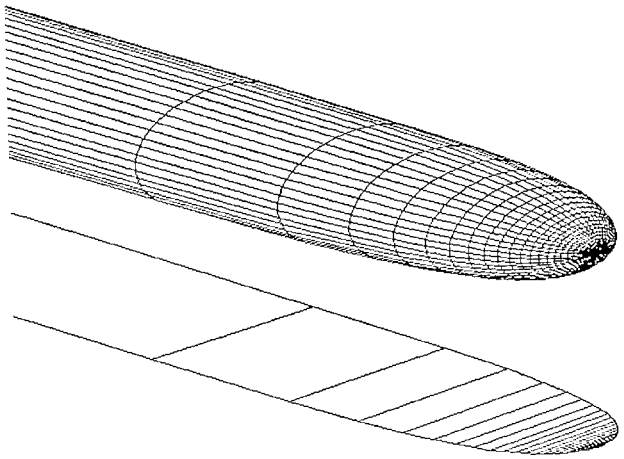


Fig. 12. Two-dimensional projection (shadow) of a three dimensional tubular shell. For clarity, meridional lines were omitted from shadow

of material over the entire circumference of the shell. Interpreted in this fashion, the model embodies a more realistic simulation of the growth of a "shell", namely the cell envelope (wall and plasma membrane).

The present model assumes that growth is isotropic (allometric coefficient of 1) (Prosser 1979, Green 1965), i.e., materials accrued from vesicle discharge expand the surface equally in transverse and longitudinal directions and should therefore be applicable to the generation of tubular cell walls in other tip-growing systems, e.g., root hairs, pollen tubes. The present model does not explain intercalary tubular growth (e.g., the subapical growth in elongating sporangiophores of *Phycomyces* during stage III) (Castle 1953) or the diffuse growth of elongating plant cells (Green and King 1966). For such growth, additional parameters need to be considered that would restrict wall expansion in girth while allowing longitudinal extension, namely, anisotropy of wall expansion (Green 1969, Green and King 1966).

The VSC concept and mechanisms for its displacement

The main premise of the present model for hyphal growth, that vesicles are released randomly from a linearly advancing source (VSC), may be a coarse simplification but one that need not be in serious conflict with the obviously more complex situation of a living hypha. The model shows that, in theory, a single source of vesicles, i.e., a single Golgi apparatus, could generate a hypha provided that during the course of cell growth, it advances linearly as it releases its vesicles. A single source of vesicles could not possibly account for the prodigious rate of hyphal extension in many fungi [in a rapidly growing fungus, tens of thousands of vesicles are discharged per minute per growing hyphal tip (Grove and Bracker 1970, Gooday and Trinci 1980)]. Hence, it is reasonable to propose that fungi have evolved an efficient mechanism to collect vesicles over a large portion of the hyphal tube (Grove and Bracker 1970, Barstow and Lovett 1974, Collinge and Trinci 1974, Howard and Aist 1979, Heath et al. 1971) and translocate them to the tip. The model predicts that the collected vesicles would be first delivered to a supply center (VSC) from which they are then free to migrate in any random direction towards the cell surface. Presumably, the VSC is a terminal collection point for vesicles that may be traveling along cytoskeletal tracks to the tip. The observation that the apex is the preferred nucleation site for microtubules in fungal hyphae (Hoch and Staples 1985) gives us reason to speculate that the VSC of a hypha might be a microtubule organizing center, or a structure intimately associated with it.

In spheroidal cells the VSC may be regarded as the geometric center for multiple sources of vesicles dispersed throughout the cytoplasm.

For the purpose of correlating parameters of the model with actual features of living cells, we regard any displacement of the VSC as being a displacement of an *entire* vesicle-generating unit. There are two different ways to generate the linear displacement of the VSC: pulling or pushing mechanisms. The model would work the same with either one, and both can be supported in principle by current cytological evidence. The cytoskeleton may either anchor the VSC to the apical pole (pulling mechanism) or may provide a scaffolding for the continuous advance of the VSC (pushing mechanism). A structural linkage between the VSC and the fungal cell surface is not far-fetched; this might be established through microfilaments anchoring the VSC plus its supporting structures to the apical plasma membrane. Extensive arrays of actin microfilaments have been seen in hyphal tips and other wall growing regions of fungal cells (Adams and Pringle 1984, Anderson and Soll 1986, Hoch and Staples 1985, Runeberg and Raudaskoski 1986, Heath 1987) and it has been proposed that they *pull* the cytoplasm in a tipward direction (McKerracher and Heath 1987).

As this study has provided new insights into the mechanism of morphogenesis in a vesicle-mediated growth process, it allowed us to predict the critical factors in such processes. Foremost was the realization that a simpler mechanism than was previously anticipated can explain the apical growth of fungal hyphae: by simply

advancing an existing vesicle-generating apparatus in a continuous, linear fashion, the cell would automatically establish the polarized pattern of surface expansion that is so typical of fungal hyphae. The present model obviates the need for mechanisms to move vesicles to specific or preferential targets on the cell surface (apical pole). Vesicles need only be endowed with the ability to move towards the cell surface in any random direction. (The massive polarized transport of vesicles from the subapical to the apical region of a hypha should be viewed as an additional feature required not for morphogenesis *per se* but to provide an ample supply of vesicles to support the fast growth of fungal hyphae). Our model is in accord with newer evidence (McGillviray and Gow 1987, Schreurs and Harold 1988) questioning whether the electric currents which flow through the tip of tubular cells (Jaffe 1968) play a determining role in morphogenesis.

The observed or predicted gradients of wall properties in the hyphal apex, e.g., elasticity/rigidification (Robertson 1965, Saunders and Trinci 1979), plasticity (Bartnicki-Garcia 1973), or polymer cross-linking (Wessels and Sietsma 1981, Wessels 1986) are probably not the cause of hyphal morphogenesis but, rather, a reflection of the pattern of vesicle discharge which generates a graded distribution of biochemical/biophysical activities on the cell surface. Accordingly, the key to hyphal morphogenesis probably does not lie in the apex *per se*, as believed previously (Robertson 1965, Bartnicki-Garcia 1973, Gooday and Trinci 1980) but in the mechanisms that cause the linear displacement of the VSC. Conceivably, primary control of hyphal morphogenesis resides in the machinery that assembles elements of the cytoskeleton—a suggestion supported circumstantially by experimental observations on the distortion of hyphal growth caused by inhibitors of microtubules (Howard and Aist 1977) or microfilaments (Betina et al. 1972, Grove and Sweigard 1980, Tucker et al. 1986).

The proposed Eq. (I) for a hypha goes beyond earlier models which were mainly approximations of the shape of the apical dome. This function termed *hyphoid* describes the profile of a hypha in its entirety. Accordingly, there is no sharp boundary between the dome portion at the tip and the rest of the quasi-cylindrical shape. Hence, the definition and demarcation of what has been variously called the "extension zone", "tapered zone", "apical dome" of a hypha is an artificial distinction with no mathematical foundation. It should be noted that the cotangent function in Eq. (I) bears no conceptual relationship to the cotangent function derived by Saunders and Trinci (1979) in their model of the tapered apical dome of fungal hyphae.

The model can simulate a wide spectrum of cell morphogenesis (hyphal development, spore germination, sporangium formation, yeast cell growth) by the simple device of modifying the two parameters of the equation: the number of wall-destined vesicles emitted per unit time and the rate of linear displacement of the vesicle supply center.

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