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Critical evaluation of the VSC model for tip growth

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The vesicle supply centre (VSC) model (Bartnicki-Garcia et al., 1989) for hyphal tip growth is powerful because it can model diverse developmental morphologies and predicts cellular organization based in current cell biology. It predicts that tip growth results from the random distribution of cell surface synthesizing vesicles from a point in the tip, the VSC, which determines their pattern of impact and fusion at the plasma membrane. We derive equations for tip-high gradients of vesicle fusions, generated by mechanisms not related to a supply centre, which create typical hyphal morphologies. These equations direct the conceptual basis for tip growth to vesicle fusion gradients, presumably mediated by a putative membrane skeleton associated with the plasma membrane. We also show that the organization and behaviour of motile organelles in growing hyphal tips of the oomycete, *Saprolegnia ferax*, argue against the presence of an apparatus capable of generating the distribution of vesicles postulated by the VSC model. We conclude that the VSC model is unlikely to describe the mechanistic basis of tip growth in *S. ferax*, and therefore, at best, it is not universally applicable.

Key Words——mathematical model; *Saprolegnia ferax*; tip growth; VSC model.

Tip growth is a very widespread process among plant and fungal cells, and is the hallmark of the fungal kingdom (Heath, 1990). It is a complex process because it involves the highly localized synthesis and expansion of cell wall and plasma membrane, diverse aspects of intracellular movements, turgor control and the extraordinarily fine regulation of the interaction of all these processes in order to generate the characteristically uniform-diameter tube-shape of most hyphae. Perhaps the most critical aspect of the system is the regulation of the extensibility of the cell surface, so that the shape of the hypha can be generated. There are two main cellular components which have been implicated in this regulation, the cell wall (e.g. Wessels, 1990; Bartnicki-Garcia, 1990; Gooday, 1995) and elements of the cytoskeleton associated with the plasma membrane (the membrane skeleton) (Heath, 1995). The relative contribution of either component is an open question which is not central to this paper, which only aims to test a model of the process centred upon vesicles which contribute to both the cell wall and the plasma membrane, and probably also the membrane skeleton.

Because the dynamic complexity of tip growth makes it difficult to formulate experiments to unambiguously determine which factors dominate the morphogenic process, there have been many attempts to develop models which will predict and explain the process. One of the most attractive of these is the vesicle supply centre (VSC) model (Bartnicki-Garcia et al., 1989), because it makes specific predictions about well known aspects of cell organization. It is based on the established premise that major components of the cell surface (both wall and membrane) are delivered by exocytosis of vesicles (wall vesicles) derived from Golgi bodies (or their functional equivalents). The contents of the vesicles are not critical to the model, only the very likely assumption that each vesicle contributes a "unit" to the synthesis and expansion of the cell surface is postulated.

The key feature of the model is that it predicts the existence of a specific region in the cell (the VSC) which acts both as the focal point for receipt of vesicles from their sub-apical source and their subsequent random radial distribution to the cell surface. Critical to the model is the implication that the site of exocytosis is determined by the site of impact of the vesicles at the end of their trajectories from the VSC. The strengths of the model are that a) computer simulations show that it can generate most of the diverse growth forms encountered in the fungi (Bartnicki-Garcia, 1990), b) it makes specific predictions about the movement of vesicles in the tip of a growing hypha, c) it can generate an equation, the "hyphoid equation," which accurately generates the outline of at least some hyphal tips and d) it can be universally applied to all tip-growing cells. Critical to the model are the demonstration of a structure with the properties of the VSC and the postulated behaviour of the vesicles in growing tips. In many fungi, the predicted position of the VSC is coincident with a structure known as the Spitzenkörper (Bartnicki-Garcia et al., 1989), but this structure has not been shown to have the postulated functions in vesicle distribution (its functions are unknown) and Spitzenkörpers are not seen in many tip-growing cells. Furthermore, there are no published descriptions of the behaviour of the wall vesicles in living, growing

hyphal tips.

Hyphal tips of *Saprolegnia ferax* (Gruith.) Thuret, a representative of the oomycetes, a group to which the VSC model is said to apply (Bartnicki-Garcia et al., 1989; Bartnicki-Garcia, 1990), provide two opportunities to test predictions of the VSC model. Firstly, many of their wall vesicles are tubular, rather than spherical, thus their direction of travel is likely to be parallel to their long axes, the direction of least resistance. Thus the orientation of wall vesicles in hyphal tips which have been freeze substituted to preserve the vesicles in the distribution patterns characteristic of living hyphae should indicate their direction of migration at the instant of freezing. Secondly, it is possible to see the organization and movements of large organelles such as mitochondria and unidentified spherical vesicles in growing tips. The organization, behaviour and effects of movements of these organelles should be compatible with the predictions of the VSC model. This report describes the results of such tests of the VSC hypothesis, and indicates that the key predictions of the model are not met. Conversely, it is mathematically shown that plausible gradients of vesicle fusions with the plasma membrane, not dependent on any postulated supply behaviour, can generate diverse tip shapes which match the diversity of known hyphal tip forms more accurately than the single "hyphoid" shape generated by the VSC model.

Materials and Methods

Hyphae of *Saprolegnia ferax* (ATCC 36051) were grown on dialysis membrane and freeze substituted, or in liquid medium for observations of growing tips, as previously described (Heath and Kaminskyj, 1989; Heath, 1987). Electron micrographs of near-median longitudinal sections of hyphal tips were reproduced at magnifications from 20,700x to 45,700x and the distributions and shapes of the characteristic wall vesicles were traced onto acetate sheets and brought to the same final magnifications by differential reduction on a photocopier. The approximate positions of the VSCs were determined by formula 4 in Bartnicki-Garcia (1990), using the largest diameter on each tracing as the maximum diameter of that hypha.

Video recordings of growing hyphae were made with Nomarski differential interference contrast (DIC) optics using a 100x, 1.32 NA objective and final magnifications of 6350x on the screen and 3000x and 4200x on a video printer. Movements of organelles were analyzed with the "trace" mode of a Hamamatsu DVS 3000 image enhancement and analysis system. In this mode, the time of analysis is set and any object which moves during that time appears as a white line (e.g. Figs. 7, 8), the shape and length of which indicate the path and velocity of the object.

Results

Mathematical modelling The basic feature of the VSC model is that vesicles arrive at the plasma membrane in a pattern determined by their export from the VSC. However, implicit in the model is that they also fuse with the plasma membrane at their points of impact. It is this gradient of vesicle fusion which uniquely determines the shape and growth rate of the hypha. The shape of the gradient of vesicle fusions predicted by the VSC model can be calculated as follows, using the equations from Bartnicki-Garcia et al. (1989). We assume, as did Bartnicki-Garcia et al. (1989), that the hypha is axially symmetric, and designate its long axis to be the y-axis of a Cartesian coordinate system. Because of the axial symmetry, rotation of a function $y=f(x)$ about the y axis can completely describe the shape of the hypha, thus we can disregard the third dimension. In the xy-plane, the membrane of the hypha will be described at time t by $y=f(x, t)$. We assume that the hypha grows with constant speed V along the y-axis and that the hypha has exactly the same shape for all times $t>0$, so that $y=f(x, t)=f(x, 0)+Vt$ describes the shape of the hypha at all $t>0$. Consider the shape of the hypha at times t and $t+\Delta t$. The increase in volume of the hypha in the slice between y and $y+\Delta y$ is proportional to $\Delta W=\Delta y\Delta x$, where x is the change in the radius of the slice in time t. Obviously $\Delta y=V\Delta t$, and if $\Delta t\rightarrow 0$, then

$$\Delta y=\frac{df(x, t)}{dx}\Delta x.$$

Thus (since $y=f(x, t)$)

$$\Delta W=V\Delta t\cdot\left(\frac{dy}{dx}\right)^{-1}\Delta y$$

Dividing by $\Delta t\Delta y$, gives the rate of change in volume per unit distance at y. If this rate of change is due to the number of vesicles absorbed between y and $y+\Delta y$, then taking the limits $\Delta t\rightarrow 0$ and $\Delta y\rightarrow 0$ gives the rate of vesicle absorption as

$$R(x, t)=V\frac{dx}{dy}$$

where $y=f(x, t)$ is the shape of the hypha at time t. Implicitly, $R(x, t)$ is a function of y, the distance from the tip of the hypha.

In developing the VSC model, Bartnicki-Garcia et al. (1989) generated a model of hyphal growth which can be used to solve for hyphal shape and thus also for the above equation. Suppose that in polar coordinates $x=\rho(\beta, t)\sin(\beta)$ and $y=\rho(\beta, t)\cos(\beta)$ defines the curve $y=f(x, t)$, where β is the angle measured from the y-axis. The assumption that $y=f(x, t)=f(x, 0)+Vt$ can be used to show that ρ must satisfy the following equation

$$\rho\rho_t=\rho\frac{\partial\rho}{\partial t}=V\frac{\partial}{\partial\beta}(\rho\sin(\beta))$$

which is a non-linear first order partial differential equation. There are infinite families of functions which satisfy this equation; to select those families which correspond to physical solutions one must make an assumption about the mechanism of hyphal growth. If ΔU is the change in volume of the hypha in an angle $\Delta\beta$ and time Δt, then Bartnicki-Garcia et al. (1989) showed that

$$\rho\rho t = \lim_{\Delta t \to 0} \lim_{\Delta\beta \to 0} \frac{\Delta U}{\Delta\beta \cdot \Delta t}.$$

In other words, $\rho\rho t$ can be interpreted as the rate of vesicle absorption per unit angle in the hyphal membrane at time t and position (in polar coordinates) $(\beta, \rho(\beta, t))$. This rate was assumed to be constant, leading to the VSC model. If the VSC moves with speed V along the y-axis, then this leads to elongation of the hypha. Assume that the VSC is at the origin, then the shape of the hyphal tip is given by $\rho = \rho(\beta, 0)$ in polar coordinates. Assume that the rate of vesicle production at the VSC is $4\pi N$, then the absorption rate per unit time per unit solid angle is N, and so $\rho\rho t = N$, and from the equation above we can solve for the shape of the hyphal tip;

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

giving

$$\rho(\beta, 0) = \frac{N}{V} \frac{\beta}{\sin(\beta)}.$$

In Cartesian coordinates this is

$$y(x, t) = x \cot\left(\frac{Vx}{N}\right) + Vt,$$

where we have added the explicit dependence on time. The ratio V/N is the distance between the VSC and the tip of the hypha at time $t=0$. We can now solve for $R(x, 0)$, obtaining

$$R(x, 0) = \frac{1}{\cot\left(\frac{Vx}{N}\right) - \left(\frac{Vx}{N}\right) \csc^2\left(\frac{Vx}{N}\right)}.$$

Note that by inverting $y = x\cot\frac{Vx}{N}$ to obtain x as a function of y we can plot $R(x, 0)$ as a function of y. This distribution of vesicle fusions along the length of the hypha, corresponding to the hypothesized moving point source of vesicles and the "hyphoid equation" for the shape of the tip (Bartnicki-Garcia et al., 1989), is illustrated in Fig. 1.

Interestingly, this pattern of vesicle fusion from a moving point source, depending as it does on the assumed random and uniform emission of vesicles in all directions, is essential to the generation of a hyphal tip, other patterns of vesicle emission will not generate a tip. For example, suppose that $\rho\rho t = Nh(\rho \sin(\beta)) = Nh(x)$, where h is some function which we shall study to leading order. In this case, the vesicles are not necessarily moving in a uniformly random direction from the VSC, for a given $h(x)$. Generally we can integrate the partial differential equation above to obtain the integral equation:

$$\int \frac{d(\beta, 0) \sin(\beta))}{h(\rho(\beta, 0) \sin(\beta))} = \frac{N}{V}\beta + C_0,$$

where C_0 is an integration constant. In all of the cases which we shall examine, $C_0 = 0$, otherwise we shall obtain unphysical singularities in our solutions. We can now examine the solutions of the differential equation for

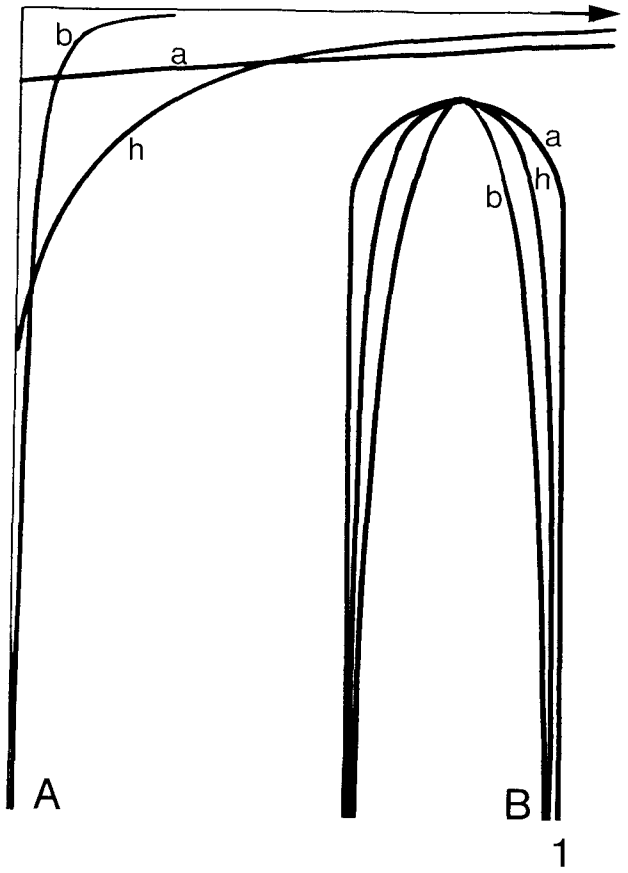


Fig. 1. Hyphal shapes (B) and vesicle fusion gradients (A) generated by the "hyphoid equation" (h) and the equations for a hemisphere atop a cylinder (a) and a more tapered tip (b). The vesicle fusion profiles are normalized such that the same number of vesicles are produced in each curve. Ordinate is distance from the tip and abscissa is the number of vesicles fusing with the wall.

various choices of $h(x)$. These correspond to different distributions of vesicles hitting the membrane after being released from the VSC (as a function of x, the distance from the axis of the hypha). In each of the following cases we assume that $h(x)$ is a function which to leading order has the assumed behaviour:

$h(x) = Nx^p$, where $p > 1$: Then direct integration gives:

$$y = x \cot\left(-\frac{V}{N} \frac{x^{1-p}}{p-1}\right)$$

which oscillates between $-\infty$ and ∞ as $x \to 0$, and so is unphysical.

$h(x) = Nx$: then

$$y = x \cot\left(\frac{V}{N} \log|x|\right)$$

which oscillates as $x \to 0$, and so is unphysical.

$h(x) = Nx^p$, where $0 < p < 1$: Then

$$y = x \cot\left(\frac{V}{N} \frac{x^{1-p}}{1-p}\right)$$

and if $p \neq 0$, then $y > 0$ if $x = \pm\varepsilon$, where ε is small, but $y = 0$ if $x = 0$. That is, the tip of the hypha has a dimple, which is not observed, and can thus be ruled out. If $p = 0$, then we obtain the solution of the VSC model above described by Bartnicki-Garcia et al. (1989).
$h(x) \approx Nx^p$, where $p < 0$: Then

$$y = x \cot\left(-\frac{V}{N}\frac{x^{1-p}}{p-1}\right)$$

which is infinite if $x = 0$, and so is unphysical.

We conclude that in the VSC model, the assumption that $\rho\rho_t = N$ to leading order in the vicinity of the hyphal tip is essential to give any physical solution: any other distribution gives an unphysical shape to the hypha.

However, if the VSC model is not operating and we look for other possible vesicle fusion profiles which can generate hyphal tip shapes which are close approximations of real tips, a variety of other candidates exist. A simple example, which appears to fit some hyphae (e.g. McKerracher and Heath, 1987), is a hemisphere atop a cylinder. Assume that the rate of vesicle absorption per unit angle per unit time is:

$$\rho\rho t = \begin{cases} N\cos(\beta), & \text{if } -\frac{\pi}{2} \leq \beta \leq \frac{\pi}{2}; \\ 0, & \text{otherwise,} \end{cases}$$

and this is leading order N as $\beta \to 0$. Then direct integration gives

$$\rho \sin(\beta) = \begin{cases} N\sin(\beta) + C_0, & \text{if } -\frac{\pi}{2} \leq \beta \leq \frac{\pi}{2}; \\ C_1, & \text{otherwise,} \end{cases}$$

where C_0 and C_1 are integration constants. Since ρ is

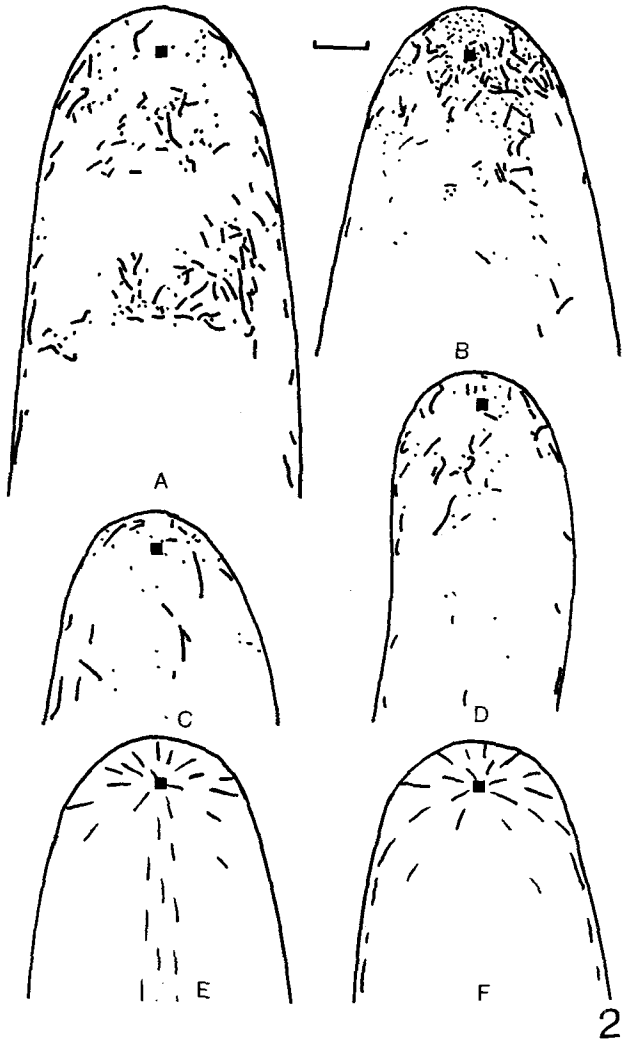


Fig. 2. Tracings of four median longitudinal sections of freeze substituted hyphal tips (A–D), showing the patterns of the wall vesicles and the predicted location of the VSCs (squares). Alternative predicted patterns of wall vesicle distributions, assuming either a single central (E) or peripheral (F) supply of elongated vesicles to the VSC are shown for comparison. Scale = 1 μm.

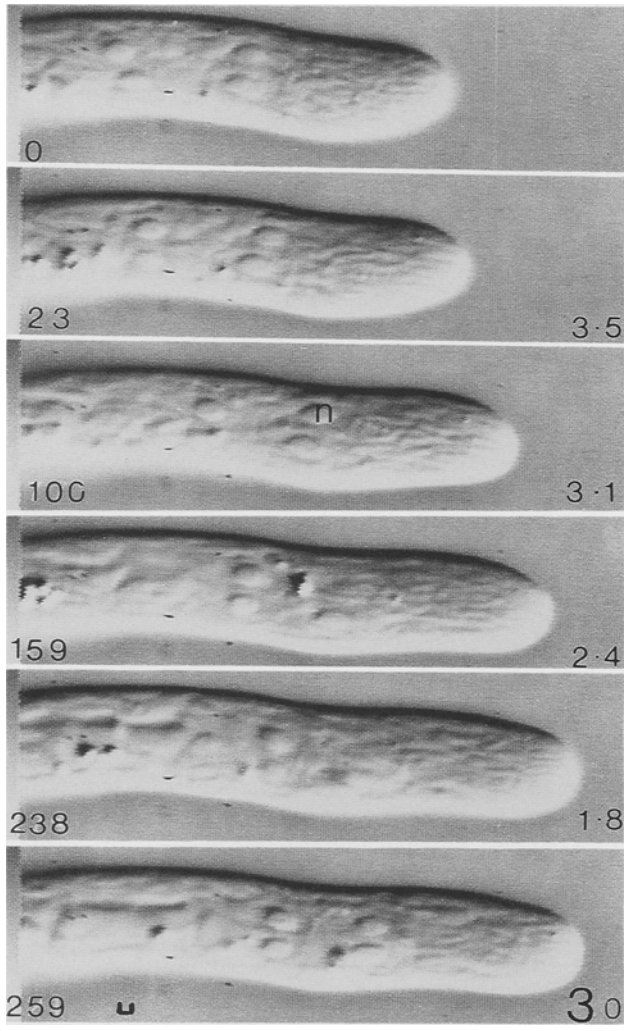


Fig. 3. Median optical sections of a growing hyphal tip showing an abundance of elongated mitochondria filling the tip, and oriented parallel to it's long axis. Nuclei (n) do not occur in the tip. Note that the organisations of the tip did not change as it slowed down and ceased to extend. Growth rates for the intervals between images (lower right, in μm/min) and total elapsed time (lower left, in sec) are indicated on each print. Scale = 1 μm.

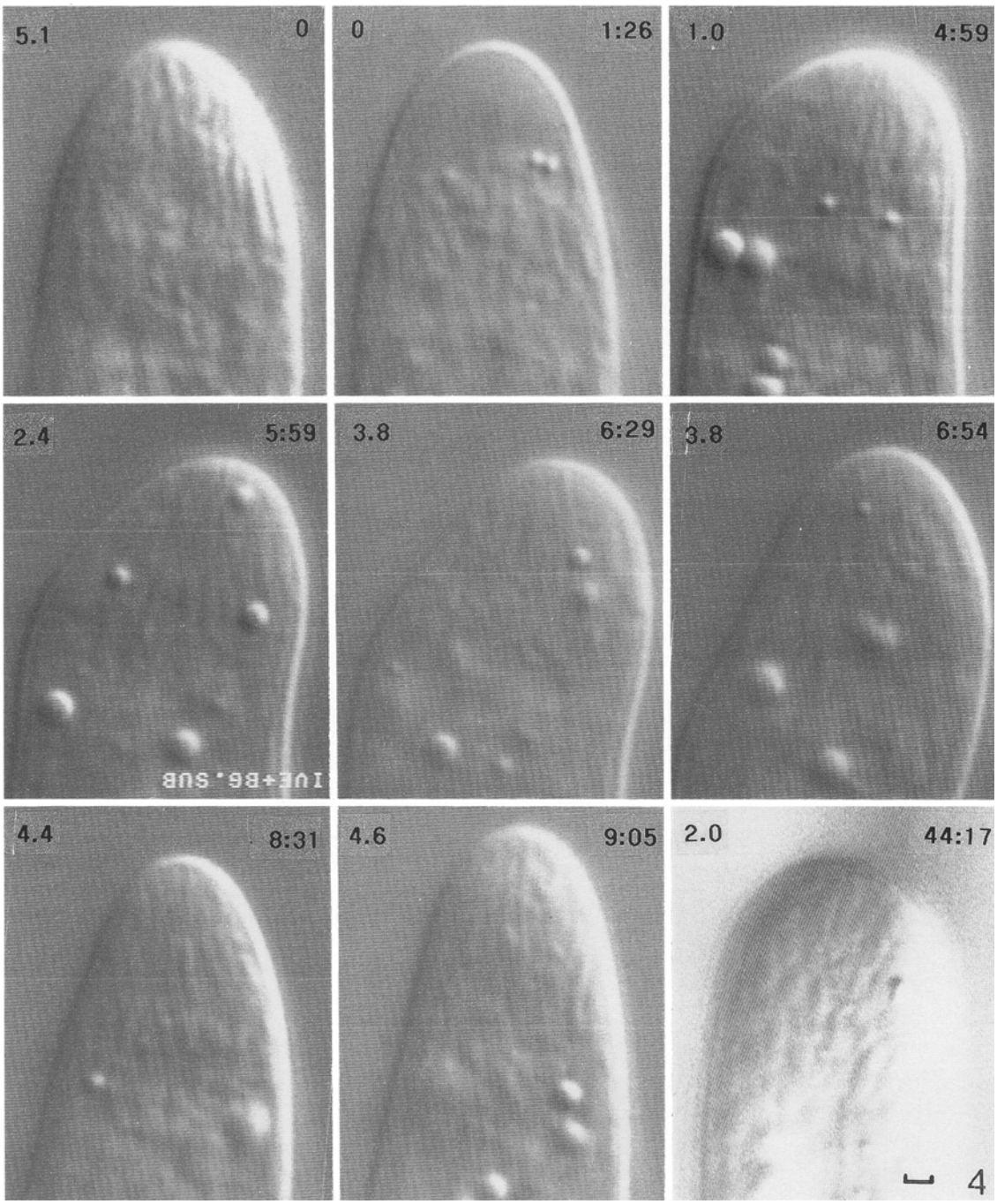


Fig. 4. Median optical sections of a growing hyphal tip containing elongated mitochondria focussed on the tip. In this hypha, the tip stopped growing transiently, and the mitochondria moved back (1:26), then they reformed and focussed to the right of centre as growth resumed to the right (4:59–5:59) and refocussed to the left prior to growth turning left (6:29–6.54). They remained focussed to the centre during straight growth (8:31–9:05) until growth began to slow and stop as the organisation of the tip changed (44:17). Note the changes in shape of the tip during the sequence, from very tapered at 9:05 to almost hemispherical at 44:17. Growth rates for the intervals between images (top left, in μm/min) and total elapsed time (top right, in min:sec) are indicated on each print. Scale = 1 μm.

finite in the interval $\left[-\frac{\pi}{2}, \frac{\pi}{2}\right]$, $C_0=0$, and since the solutions must join continuous at $\beta=\pm\frac{\pi}{2}$, $C_1=\frac{N}{V}$. In Cartesian coordinates, the solution is then

$$\begin{cases} x^2+y^2=\left(\frac{N}{V}\right)^2, & \text{if } -\frac{\pi}{2}\leq\beta\leq\frac{\pi}{2}; \\ x=\frac{N}{V}, & \text{otherwise.} \end{cases}$$

The rate of vesicle absorption can be computed as above to be

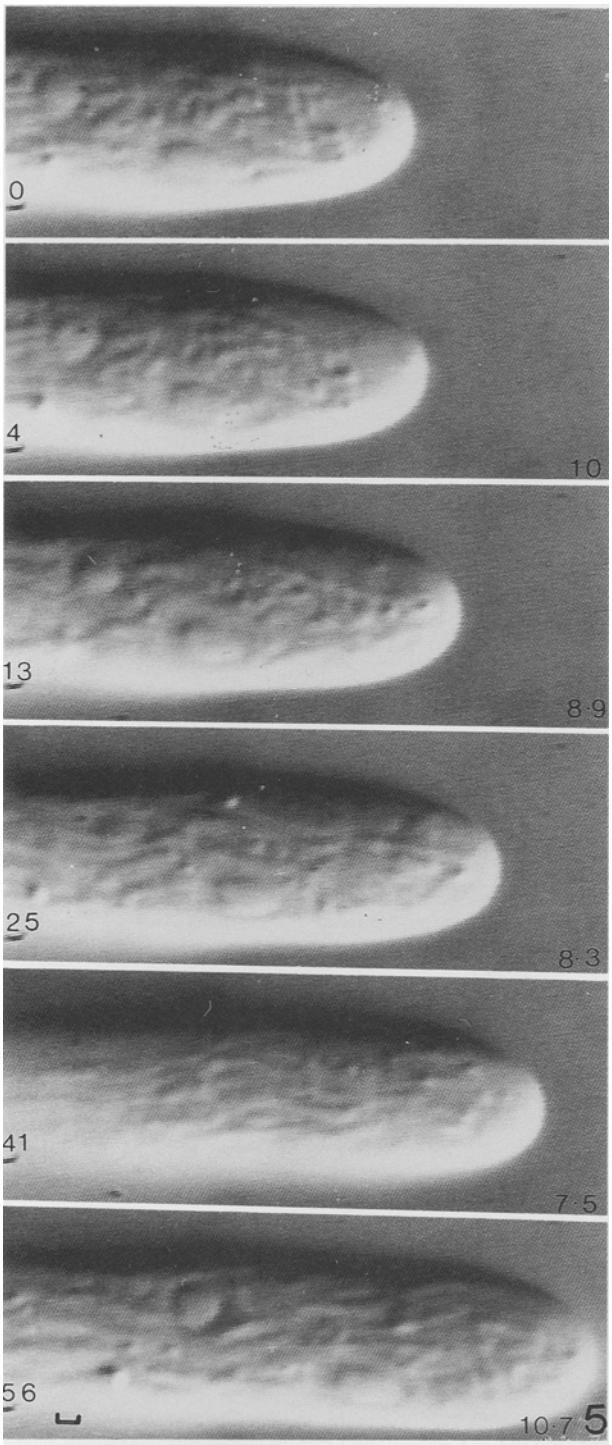


Fig. 5. Median optical sections of a growing hyphal tip in which the apical cytoplasm is primarily devoid of mitochondria. Note the difference in organisation between 4 and 56 sec, when the growth rates are similar. Growth rates for the intervals between images (lower right, in μm/min) and total elapsed time (lower left, in sec) are indicated on each print. Scale = 1 μm.

$$R(x, 0) = \frac{\sqrt{\left(\frac{N}{V}\right)^2 - x^2}}{x},$$

which is very different from the VSC model given above, and is plotted in Fig. 1.

Other more or less tapered shapes can be produced by simply assuming that

$$y = x \cot\left(\frac{Vx}{N}\right) - \varepsilon x^2$$

where ε is a third parameter in the model. This solution corresponds to a choice

$$\rho\rho_t = \frac{\csc^2(\beta)}{\varepsilon + \frac{N}{V}\csc^2\left(\frac{N}{V}\rho \sin(\beta)\right)}$$

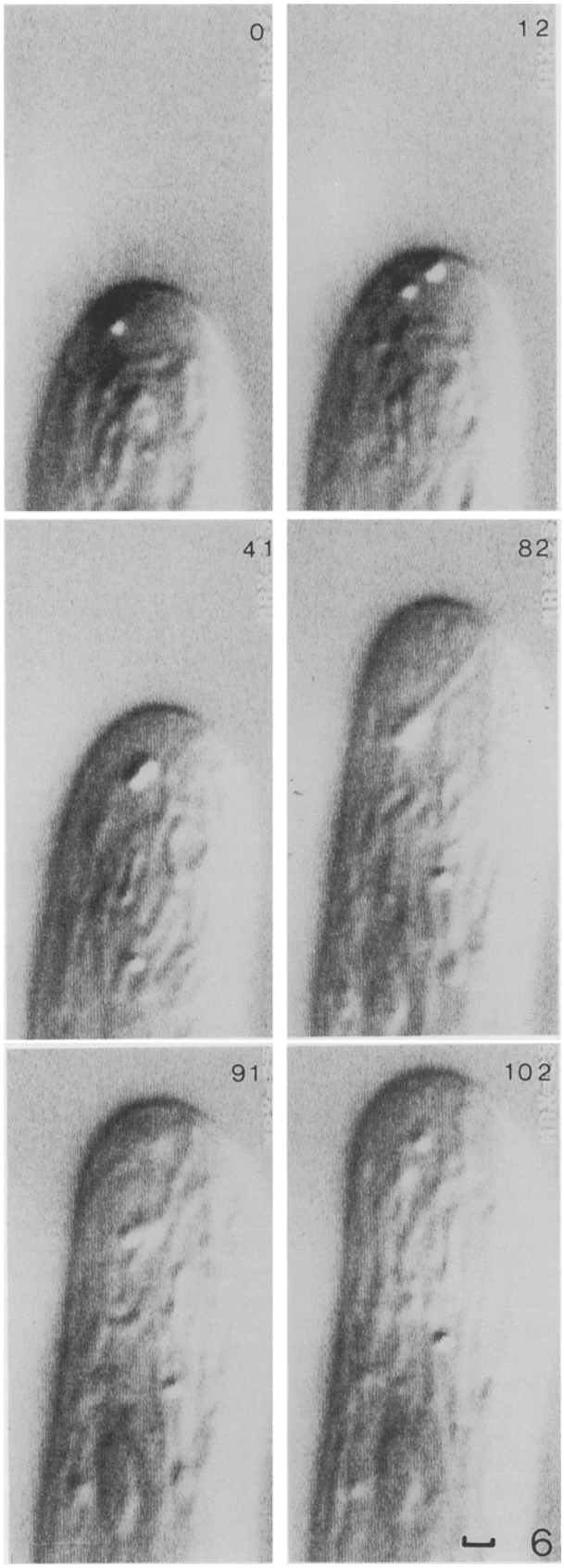
in the VSC model. By tuning ε it is possible to produce hyphae with a more or less tapered tip. The rate of vesicle absorption can be computed as

$$R(x, 0) = \frac{1}{\cot\left(\frac{Nx}{V}\right) - \frac{Nx}{V}\csc^2\left(\frac{Nx}{V}\right) - 2\varepsilon x}.$$

Positive values of ε give a more tapered hypha, whereas negative values give less tapered hyphae. An example of a tapered hyphal outline obtained by this equation is shown in Fig. 1. Hyphal tips with a more tapered shape than indicated by the "hyphoid equation" (Bartnicki-Garcia et al., 1989) are found (e.g. McKerracher and Heath, 1987). In contrast to the VSC model, the formulae for the gradients generating the tapered shapes lack a simple prediction about the mechanism of generation of the gradient. However, given the large number of factors which are likely to influence the rate of vesicle fusion with the plasma membrane, a simple element capable of describing the gradient is indeed unlikely.

Freeze substituted hyphae The VSC hypothesis predicts that wall vesicles should be transported from sub-apical Golgi bodies into the VSC, and then radially in all directions from the VSC to the apical plasma membrane. It is not specified whether the inward transport is via a single central "track" or multiple convergent "tracks." Tracings of the wall vesicles in four representative hyphae are shown in Fig. 2, together with the locations of the VSCs predicted from their hyphal diameters. Because the maximum diameters of the hyphae were not reached in the restricted regions shown in the micrographs, the locations of the VSCs are an underestimate of their true positions. However, even if the maximum diameters were double those achieved in the micrographs, the VSCs would only be double the indicated distances from the tips, well within the regions shown.

In none of the hyphae examined were there any indications of either a single "track," or multiple "tracks," focussed on the positions of the VSCs from the subapical regions of the hyphae (Fig. 2). Furthermore, neither the elongated wall vesicle profiles nor the circular profiles (indicative of either spherical vesicles or cross sections of



tubular profiles) showed any indication of a radial arrangement emanating from the VSCs towards the walls. Fortuitously, there was considerable variation in the abundance of wall vesicles between the four hyphae, presumably indicative of variations in growth rate at the instant of freezing, yet there was no evidence for any pattern in any of the hyphae, indicating that the absence of pattern cannot be attributed to density dependent factors (e.g. in a saturating density of spherical vesicles, it would be impossible to detect any distribution pattern). The only consistent "pattern" in the distributions was an abundance of elongated profiles close to, and parallel with, the plasma membrane, especially in the sub-apical regions.

Living hyphae In living, growing, hyphae, it was not possible to resolve the populations of wall vesicles, even though the longer ones may reach more than 1 μm long (Heath et al., 1985). However, the tips do clearly contain elongated mitochondria and spherical, retractile vesicles of unknown function and composition. Both of these structures are more abundant in sub-apical regions of the hyphae, but do enter the typically organelle depleted zone in which the VSC is predicted to lie. Their organisations and movements in the tips offer interesting indications of the properties of the tips which are relevant to the VSC hypothesis.

In terms of organisations, the patterns of the larger organelles can be very variable, yet the rates of growth and shapes of the tips (and thus presumed fusion patterns of the wall vesicles) are not dramatically altered (Figs. 3-7). The elongated mitochondria may (Figs. 3, 4), or may not (Figs. 5-7), be focused toward the extreme apex, but are typically oriented parallel to the long axis of the tips (Figs. 3, 4). They do not show a radial arrangement as might be expected if there were a vesicle transporting system radiating from a VSC. When present, these mitochondria are indicators of the organization of a system related to the direction of growth, because they change their focal point prior to changes in direction of growth (Fig. 4). They are also indicators of the presence of some form of cytoskeletal system, the pattern of which does not seem to mirror that predicted by the vesicle distribution system postulated by the VSC model. These observations indicate independence between the pattern of organelles in the apical central cytoplasm and the extensible behaviour of the hyphal apex.

The dynamic behaviour of the larger organelles in the central apical cytoplasm also indicates its lack of involve-

Fig. 6. Median optical sections of a growing hyphal tip in which the apical cytoplasm is primarily devoid of mitochondria. Growth rate for the duration of the sequence was consistent at 5.5 μm/min, but large spherical vesicles entered and left the tip (0-82) and a mitochondrion moved around in the tip (82-91). Total elapsed time (sec) is indicated on each print. The images are in precise register at the bottom, thus extension can be determined from the lengths of the tips. Scale = 1 μm.

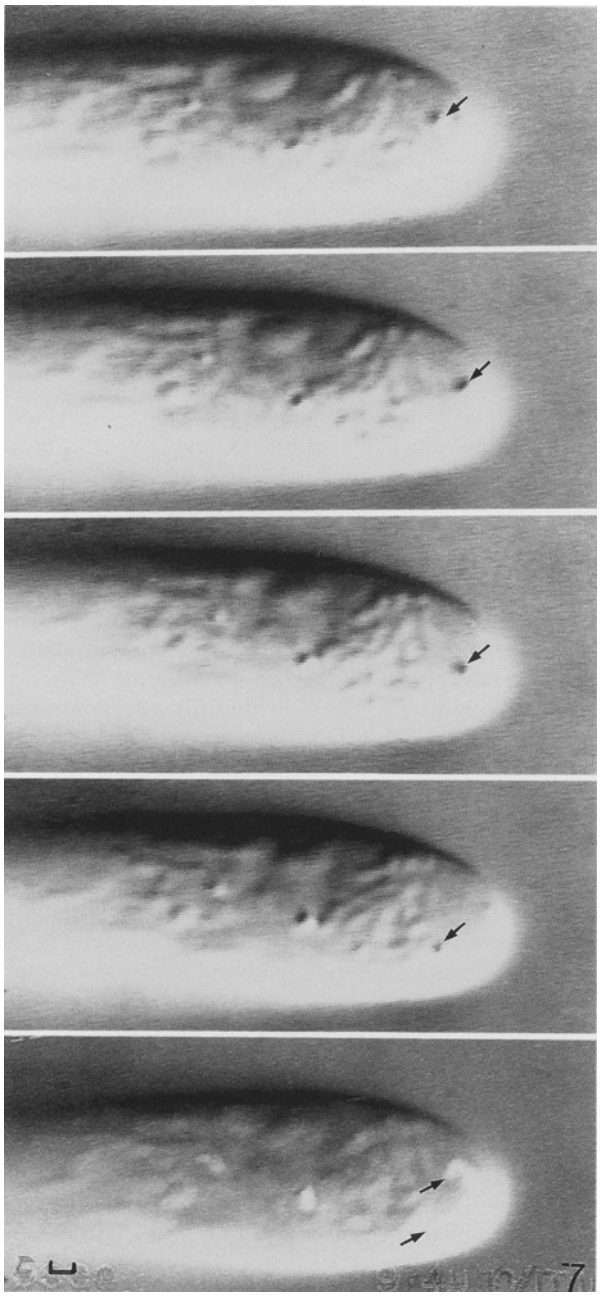


Fig. 7. Median optical sections of a growing (at a rate of 9.4 μm/min) hyphal tip in which the apical cytoplasm is primarily devoid of mitochondria, but in which a large spherical vesicle moved through the region corresponding to the postulated VSC at a rate of about 54 μm/sec. The vesicle can be traced in the first four pictures (arrows), which cover 5 sec, and its path is shown as a white line between the arrows in the 5 sec "trace" print. Scale = 1 μm.

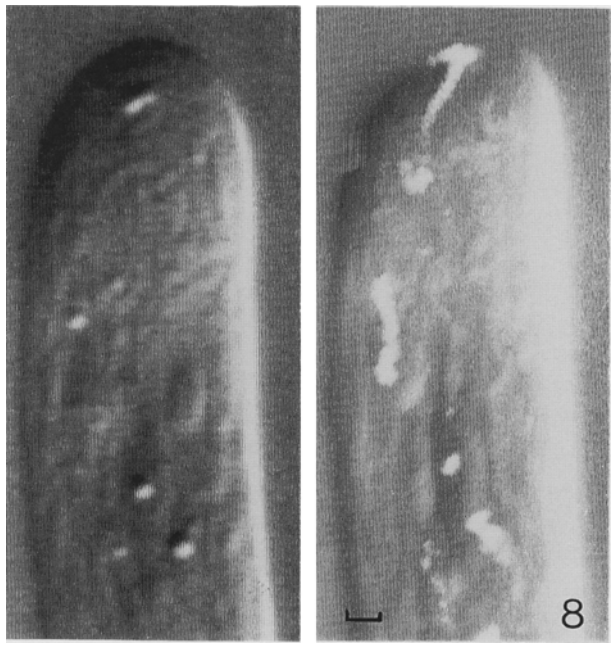


Fig. 8. Median optical sections of a growing hyphal tip (A) and a 30 sec "trace" image (B) showing the white paths of several moving organelles. This is one of the images from which the data on organelle motility in Fig. 9 were gathered. The trace of the organelle in the extreme tip was not included in the analysis because the position of the tip is not accurately indicated since it was extending during the recording of the "trace" image. Scale = 1 μm.

ment in the properties of the hyphal tip surface. As shown in Figs. 3-8, both the mitochondria and spherical vesicles enter the apices and show extensive, often rapid, migrations within the region (Figs. 6-8). These movements of the larger organelles through the apical cytoplasm of growing hyphae produce no detectable effects on the growth or shape of the hyphal tips. The migrations also show no detected pattern, nor do they indicate any areas which are inaccessible to the organelles, nor do they show regions of reduced motility (i.e. regions which the organelles enter and then slow down or stop). Perhaps most importantly, they can move rapidly right through the region of cytoplasm predicted to be occupied by the VSC (Fig. 7).

The motile behaviour of organelles in median optical sections of the tips of two growing hyphae was analyzed with the "trace" mode of the DVS 3000 system set for 30 s. All organelles which moved during this period appeared as white lines (Fig. 9), thus the region of cytoplasm in which these movements occurred could be determined by measuring the distance between the lines and their closest approach to the cell wall. These movements conspicuously did not occur in the most peripheral cytoplasm, within about 0.5 μm from the wall (Fig. 9), but were otherwise dispersed throughout the central cytoplasm. This peripheral layer of cytoplasm could also be subjectively detected by simple observation of the video sequences of median optical sections of growing tips, it appeared as a layer in which little movement could be detected and appeared to be about 0.4 μm thick. This layer is coincident with the approximate thickness (0.25 μm) of the peripheral layer of actin detected by rhodamine-phalloidin staining of hyphae (Heath, 1987) and is the primary location of the wall vesicles in slightly

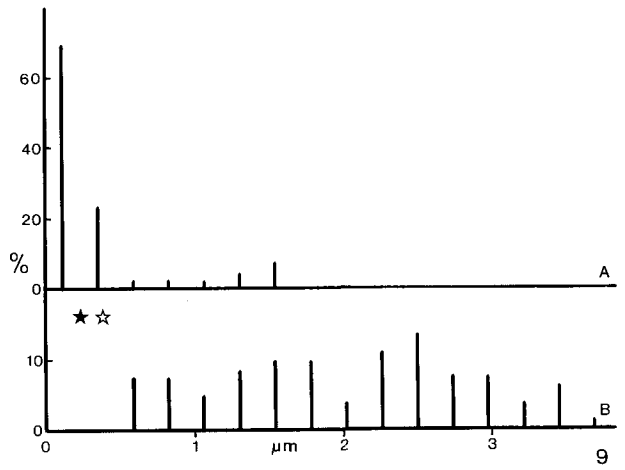


Fig. 9. Histograms showing the percentage of wall vesicles in the different regions of the cytoplasm in Fig. 1A from Heath and Kaminskyj (1989) (A) and the minimum distance between the cell wall and organelle movement traces in two hyphal tips (B). In A, the distance between the centre of each vesicle profile (average diameter -0.07 μm) and the plasma membrane was measured. The section was taken at about 14 μm behind the tip. In B, the tips were growing at a rate of -5 μm/min and all traces were within the most apical 16 μm of the tip and were printed at a magnification of 4200x. Also indicated are the approximate thickness of the peripheral actin network determined from Heath (1987) (solid star) and the thickness of the outer layer of cytoplasm (open star) which was subjectively judged to show little organelle motility in median optical video images of growing tips viewed at 6350x on the screen. Abscissa is the distance from the plasma membrane.

subapical regions (Fig. 9).

Discussion

As indicated in the introduction, the VSC hypothesis is unique among hypotheses designed to explain tip growth in that it can explain and predict numerous facets of the process and makes testable predictions about the intracellular behaviour of the wall vesicles. The ideal test of the model would involve the description of the movements of the wall vesicles themselves, but such has not yet been published for any tip growing system. Thus the present indirect tests of correlates of the hypothesis are all that is currently possible.

Mathematically, we have shown that if one assumes a point source for the origin of vesicles (the VSC), then the postulated uniform distribution of vesicles per unit angle from the VSC per unit time is the only one which will give a physical shape to the hypha, other patterns give unphysical shapes. A mechanism to achieve such uniformity remains undescribed, and may be hard to achieve in biological systems noted for variability. We have also shown that, as is implicit in the VSC model, a specific gradient of vesicle fusions, however achieved, generates a "hyphoid" shape. More importantly, variations of such a gradient can also generate physical shapes for hyphae. These variations can generate an infinite diversity of shapes, from the simple hemisphere atop a cylinder to a range of tapered tips. In reality, the shape of hyphal tips is more varied than the uniformity predicted by the "hyphoid equation," as illustrated in Fig. 4 and McKerracher and Heath (1987). The formulae for the diverse gradients lack the simple elegance and prediction of a singular structure of the VSC model but may be expected to more closely match reality in the complexity which characterizes the biological realm!

We have also been able to experimentally test predictions of the VSC model. These tests show that:- a) the predicted patterns of vesicles entering or leaving the region of cytoplasm predicted to be the VSC do not occur, b) the site of the predicted VSC does not exist in terms of presenting any detectable impediment to the movement of large organelles, c) the movement of large organelles through the region in which the critical uniform radial distribution of wall vesicles is predicted to occur had no detectable effect on the distribution of vesicles, as witnessed by the absence of change in the shape or growth rate of the hyphal tip, d) variations in the pattern and degree of occupation of the apical cytoplasm (through which the postulated uniform distribution of wall vesicles must occur) by large organelles had no detectable effect on tip shape or growth rate. Collectively, these observations, which are contrary to predictions of the VSC model, indicate that the model does not apply to hyphae of *Saprolegnia ferax*. Bartnicki-Garcia (1990) has indicated that the hypothesis fits the growth of oomycete hyphae as well as any other fungi, this extrapolating of the present negative results to other species is reasonable. At the least, the present observations show that the hypothesis, as stated, cannot be universal for tip growing cells.

While the present results question the universality of the fundamental feature of the VSC hypothesis, the VSC itself, they do not in any way negate a corollary feature of the model, that tip morphogenesis is the result of an appropriate gradient of vesicle fusions with the plasma membrane. A gradient in fusion of wall vesicles with the plasma membrane, however it may be achieved, will also fit the computer simulations upon which the VSC model is based. Thus, the ability of the model to explain the diversity of morphogenic forms encountered in the fungi, and other tip growing cells, can be taken as support for a vesicle fusion gradient (VFG) model as well as the VSC model.

A VFG model for tip growth is conceptually more compatible with our general understanding of cell biology than the VSC model. There are no other systems described which show the existence of such a precise vesicle directing structure as postulated by the VSC. Certainly cells have the ability to direct vesicles to different parts of a cell, but not with the level of precision implied by the VSC. Perhaps the closest equivalent is the cytocentre of epidermal melanophores and erythrophores, but they are very much larger, equivalent to the size of the entire hyphal tip (e.g. Bray, 1992). In contrast, mechanisms for determining the sites of vesicle fu-

sions can be very fine. For example, in the endomembrane system, vesicles are directed to individual cisternae of Golgi bodies and during exocytosis at the plasma membrane of structures such as synapses, targeting is precise, to the μm level (e.g. Lodish et al., 1995). The factors responsible for the regulation of the postulated VFG in hyphal tips remain unknown, but presumably involve the postulated membrane skeleton of the plasma membrane (Heath, 1995). Evidence for the existence of a membrane skeleton is presented in this paper in the form of the peripheral region in which organelle movements are lacking. This is the same region occupied by the peripheral actin arrays shown in previous work (Heath, 1987). That it has a role in the transport and possible fusion of the wall vesicles is shown by the concentration of these vesicles in this region in freeze substituted hyphal tips (Heath and Kaminskyj, 1989).

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