Computer modelling of hyphal tip growth in fungi

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Starting from simple morphological considerations and a hypothesis involving 'unset' and 'set' wall, a mathematical model simulating the growth of the hyphal tip is derived, and the results displayed by plotter.

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A partir de considérations morphologiques simples et d'une hypothèse impliquant une paroi 'non fixée' et 'fixée,' les auteurs dérivent un modèle mathématique simulant la croissance de l'extrémité de l'hyphe. Les résultats sont présentés par une traçeuse. [Traduit par le journal]

Introduction

The hypha is the architectural module of all higher fungi. The average agaric is made up of miles of mycelium. And yet we know remarkably little about how hyphae grow. We do know that, in most cases, growth is restricted to the hyphal tip, and a few workers (e.g. Reinhardt 1892; Robertson 1965; Burnett 1968) have thought about and experimented with hyphal apices, but we still do not know how a hypha maintains a constant diameter as it grows, or just how and where new wall material is interpolated at its apex. Our present interest in the mechanism and the regulation of hyphal tip growth sprang from the fact that it is often the hyphal apex that gives rise to the conidia of the Hyphomycetes: conidia whose mode of origin is currently one of our prime concerns, and whose shape or configuration has always been regarded as an important taxonomic character. Before one can understand the special case of conidium production, one must clearly understand the normal apical growth pattern of the hypha.

One of us (Kendrick 1971), while attempting to explain the derivation of some spore shapes, wrote that at the hyphal tip: ".... one fluid (protoplasm) is bounded by a membrane (the cell wall) beyond which is another fluid (air or water). As far as the cell wall is concerned, I assume for the sake of simplicity in model-building that it may exist in two conditions, which I will call 'unset' and 'set'. For the purposes of this discussion, the 'set' wall is inelastic and incapable of further deformation.... The 'unset' wall is elastic and can yield and stretch in response to pressure differentials. One further assumption is necessary for simplicity's sake: that there is at any given moment a sharp line of demarcation between set and unset wall (in reality there must usually be a zone rather than a line).

The first and simplest case is the growing hyphal tip.... which can be represented as a cylinder of set wall, with a hemisphere of unset wall (the shape dictated by surface tension) at its end. As hyphal growth proceeds, fluid pressure generated within the hypha finds expression at the unset tip, tending to blow it out like a balloon. If the diameter of the hypha is to remain relatively constant, then wall setting must move forward at exactly the same rate as the tip is blowing out."

The work reported here is an attempt to explore this hypothesis by producing, within its guidelines, a mathematical model (by a digital computer) of a growing hyphal tip. The model allows us to examine certain physical aspects of growth, and, as our mathematical procedures are modified by the dictates of accurate modelling, to modify our hypothesis accordingly.

Preliminary Considerations

If we think of the hyphal tip as being represented by a geometrical surface of the same configuration, we can analyze the process of growth into a series of surfaces successively displaced with time in a common direction. Each surface must be derived by an appropriate expansion of the previous one. In constructing our model

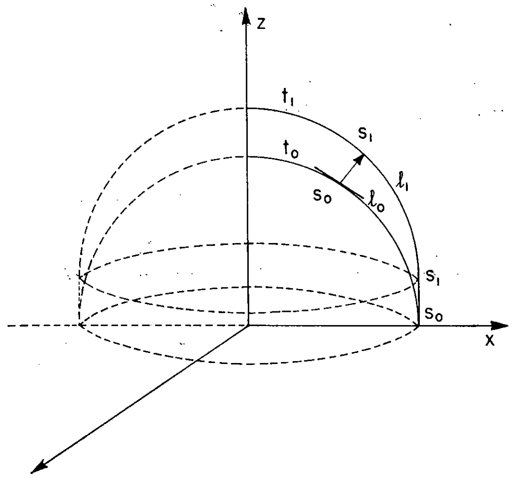

FIG. 1.

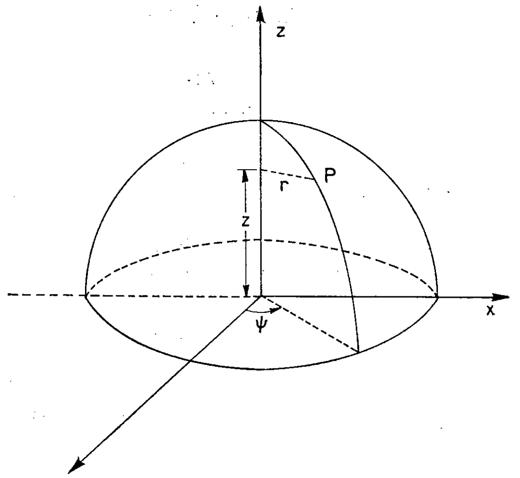

FIG. 2.

we will begin by studying the geometrical evolution of a small element of the surface in a small interval of time, and integrate from that to derive a picture of the behavior of the whole hyphal apex.

We assume that the increment in area of a small element of surface in time is proportional to the original area. (See equation 1 below.)

We also assume that in each small interval of time a point on the growing surface moves perpendicularly to the plane tangent to the surface at that point. This means that there is no sliding movement of points across the surface, but a simple outward swelling.

We can represent the shape of the outer surface of our hypha (cylinder topped by a hemisphere) by a geometrical surface of rotation. This is a surface which is, by definition, symmetrical about the axis of growth. Knowing this, we can restrict our attention to the points on any meridian line of this surface, beginning at the absolute apex or distal point of the hyphal tip (through which its long axis runs) and extending to the point where the hypha attains its full diameter. We need go no further, to begin with, because the wall below this point, according to our hypothesis, is the set, inelastic, inert, periclinal wall of the mature hypha. Any operations carried out on a meridian can, because of the symmetry involved, be easily extended to the entire surface of the apex.

If the surface of rotation derived from the line l_0 in Fig. 1 represents the configuration of the growing tip (unset wall) at time t_0, then at a time t_1 very close to time t_0, the surface of rotation of a new line, l_1, will represent the new configuration assumed by the hyphal apex, and the short cylinder obtained by rotation of the segment S_0S_1 about the axis of growth will represent the new set wall. Repeating this process and drawing lines l_2, l_3, $l_4 \ldots l_n$, at successively higher levels at times t_2, t_3, $t_4, \ldots t_n$, we can build up a picture of the process of growth: the movement of the tip in the direction z and the formation of a steadily elongating cylinder of set wall behind it. The problem of studying the behavior of the whole surface can thus be reduced to a planar problem dealing with the outline of half of a median section of the hypha.

Formalization of the Assumptions

In a three-dimensional model of the hyphal tip we can specify any point on its surface by polar coordinates. In Fig. 2, at a given instant, a point P is specified by its coordinates $P = P(r,z,\psi)$. In any given median section, P can be specified by $P = P(r,z)$. If p is the coordinate on the chosen meridian, then this expression becomes $P = P(p) = P(r(p), z(p))$; and if we introduce the parameter, time, it becomes $P = P(p,t) = P(r(p,t),z(p,t))$.

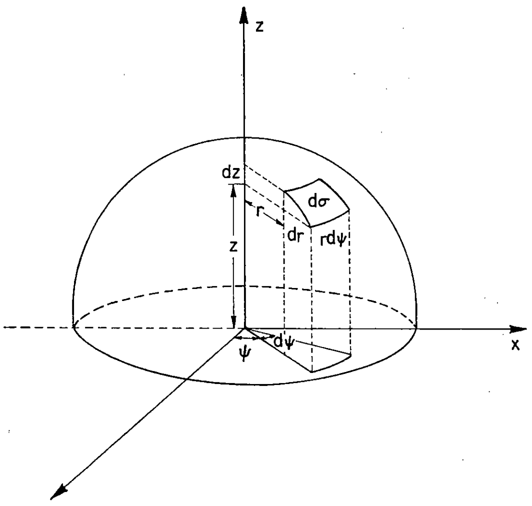

FIG. 3.

If dσ is the element of surface under consideration, the hypothesis of proportionality in areal increase can be expressed

$$[1] \qquad \partial \mathrm{d}\sigma/\partial t = \theta \mathrm{d}\sigma$$

where θ is a coefficient of proportionality. This is basic, and the acceptability of our whole model system hinges upon it. Now we can introduce quantitative data to our consideration of area expansion by assigning a value to θ. We will consider θ as a function of point P on the surface (or of coordinate p on the meridian) and of the time, t:

$$[2] \qquad \theta = \theta\,(p,t).$$

We shall call θ our expansion function.

Let d_pP be the spatial displacement of point P, and let d_tP be the variation of point P in a small interval of time. The hypothesis of motion for the points described can now be written with vector notation:

$$[3] \qquad \mathrm{d}_pP \times \mathrm{d}_tP = 0.$$

Considering expression [1] and applying expression [2] we obtain

$$(\partial/\partial t) \log \mathrm{d}\sigma = \theta\,(p,t)$$

and, integrating,

$$\log \mathrm{d}\sigma = \int_0^t \theta\,(p,q)\mathrm{d}q + \log k(p)$$

where $\log k(p)$ is the constant of integration. So we have

$$\mathrm{d}\sigma = k\,(p) \exp \int_0^t \theta\,(p,q)\mathrm{d}q.$$

At time $t = 0$, this will be

$$[\mathrm{d}\sigma]_{t=0} = k(p) \exp 0 = k\,(p),$$

from which we derive

$$[4] \qquad \mathrm{d}\sigma = [\mathrm{d}\sigma]_{t=0} \exp \int_0^t \theta\,(p,q)\mathrm{d}q.$$

Consider the element of surface dσ as in Fig. 3. For this surface we have the equation

$$[5] \qquad \mathrm{d}\sigma = \sqrt{(\mathrm{d}r^2 + \mathrm{d}z^2)}\, r\mathrm{d}\psi$$

where dr and dz are infinitesimal spatial displacements. Equation 5 can be rewritten as

$$[6] \qquad \mathrm{d}\sigma = \sqrt{\left\{\left(\frac{\partial r}{\partial p}\right)^2 + \left(\frac{\partial z}{\partial p}\right)^2\right\}}\, \mathrm{d}p\, r\, \mathrm{d}\psi.$$

We must now evaluate $[\mathrm{d}\sigma]_{t=0}$, the expression for dσ on the initial hemispherical[1] surface, which produces the expression

$$[\mathrm{d}\sigma]_{t=0} = R \sin\,(p/r)\, \mathrm{d}p\, \mathrm{d}\psi,$$

R being the radius of the hypha. Interpolating this statement in the right-hand side of expression 4, and then substituting the right-hand side of the modified [4] for the left-hand side of expression 6, we obtain

$$\sqrt{\left\{\left(\frac{\partial r}{\partial p}\right)^2 + \left(\frac{\partial z}{\partial p}\right)^2\right\}}\ \mathrm{d}p\, r\, \mathrm{d}\psi = R \sin\,(p/R)\, \mathrm{d}p\, \mathrm{d}\psi \exp \int_0^t \theta\ (p,q)\mathrm{d}q,$$

which may be stated

$$\left(\frac{\partial r}{\partial p}^2\right) + \left(\frac{\partial z}{\partial p}\right)^2 = \left(\frac{R}{r} \sin \frac{p}{R}\right)^2 \exp 2 \int_0^t \theta\ (p,q)\mathrm{d}q.$$

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[1] In our model, any other initial shape could be chosen, and would necessitate only very simple modifications in the program. If any shape other than a simple hemisphere is considered, then either the organism is doing work to maintain that shape, or else our assumptions about set/unset wall would have to be modified. In fact, hyphal tips are sometimes deeply paraboloid in outline, and either 'degrees of setness' increasing toward the periphery, or some strengthening feature such as the concentrations of transverse microfibrils postulated by Burnett (1967) may explain this. Nevertheless, there is little reason to doubt the general truth of the hypothesis.

Consider now eq. 3. Since

$$\mathrm{d}_pP \times \mathrm{d}_tP = \frac{\partial z}{\partial p}\frac{\partial z}{\partial t} + \frac{\partial r}{\partial p}\frac{\partial r}{\partial t},$$

from eqs. 1 and 3 we obtain the following system of differential equations in two unknowns.

$$\begin{aligned} &\left(\frac{\partial r}{\partial p}\right)^2 + \left(\frac{\partial z}{\partial p}\right) = \left(\frac{R}{r}\sin\frac{p}{R}\right)^2 \exp 2\int_0^t \theta\ (p,q)\,\mathrm{d}q, \\ &\frac{\partial z}{\partial p}\frac{\partial z}{\partial t} + \frac{\partial r}{\partial p}\frac{\partial r}{\partial t} = 0, \end{aligned} \tag{7}$$

with the initial and boundary conditions:

$$\begin{aligned} z(p,o) &= R\cos(p/R), \\ r(p,o) &= R\sin(p/R), \\ z\{(\pi/2)R,t\} &= 0, \\ r\{(\pi/2)R,t\} &= 1. \end{aligned} \tag{8}$$

The solution of the system (eq. 7) with the conditions (eq. 8) will give, for an opportune choice of the expansion function, θ, a description of the process of growth.

The Expansion Function

Equations 7 and 8 are the motion equations for the evolving surface of the hypha. Once a choice of θ has been made for the first of the equations (7), the pattern of development is given, it should be possible to derive θ. On the other hand, a process of differentiation such as that we use in defining θ usually yields information about the causes of a phenomenon (its intrinsic properties) and it is possible to examine a wide variety of biophysical hypotheses by the use of θ.

In practice this derivation is difficult if we start only from experimental data. In fact, all the data we have concern only a few superficial parameters such as diameter, and the amount of longitudinal growth with time; or they are simply qualitative observations of such things as the 'set' quality of the periclinal wall; thus we cannot derive θ from our observations—we must, in fact, assign it arbitrarily.

From each arbitrary choice of θ we can derive a pattern of growth, and check our calculated against the observed pattern. Thus, our approach to a working model has been through successive approximations. If other biophysical hypotheses than that postulated in this study are developed in future, it should be possible to test them by our procedure.

It could be useful to consider θ not simply as a function of the position and of the time, but also as a function of other quantities related to the growth itself.

Integration of the Equations

As we said earlier, the process of growth can be represented as a series of lines progressing in space with the passage of time. Our method is to derive each line from knowledge of the previous one, each considered point on the new line being derived from knowledge of its predecessor on the same line, and of the point on the preceding line from which that was derived.

We could derive the points of line l_0 in two ways: by proceeding from the center outwards, starting at the highest value for the z coordinate and deriving successive points until we reach the lowest value of the z coordinate at the periphery; or by proceeding from the periphery inwards. We found it simpler to work out from the center. Boundary situations at the center (on the z axis) are easy to compute, while error is easy to measure at the periphery.

Determination of the Starting Point

Each point (c) on a line (Fig. 4) is normally calculated from a knowledge of two other points: the most recently calculated point (b) on the same line and the point (a) on the previous line.

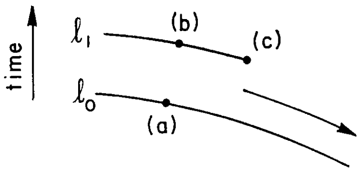

FIG. 4.

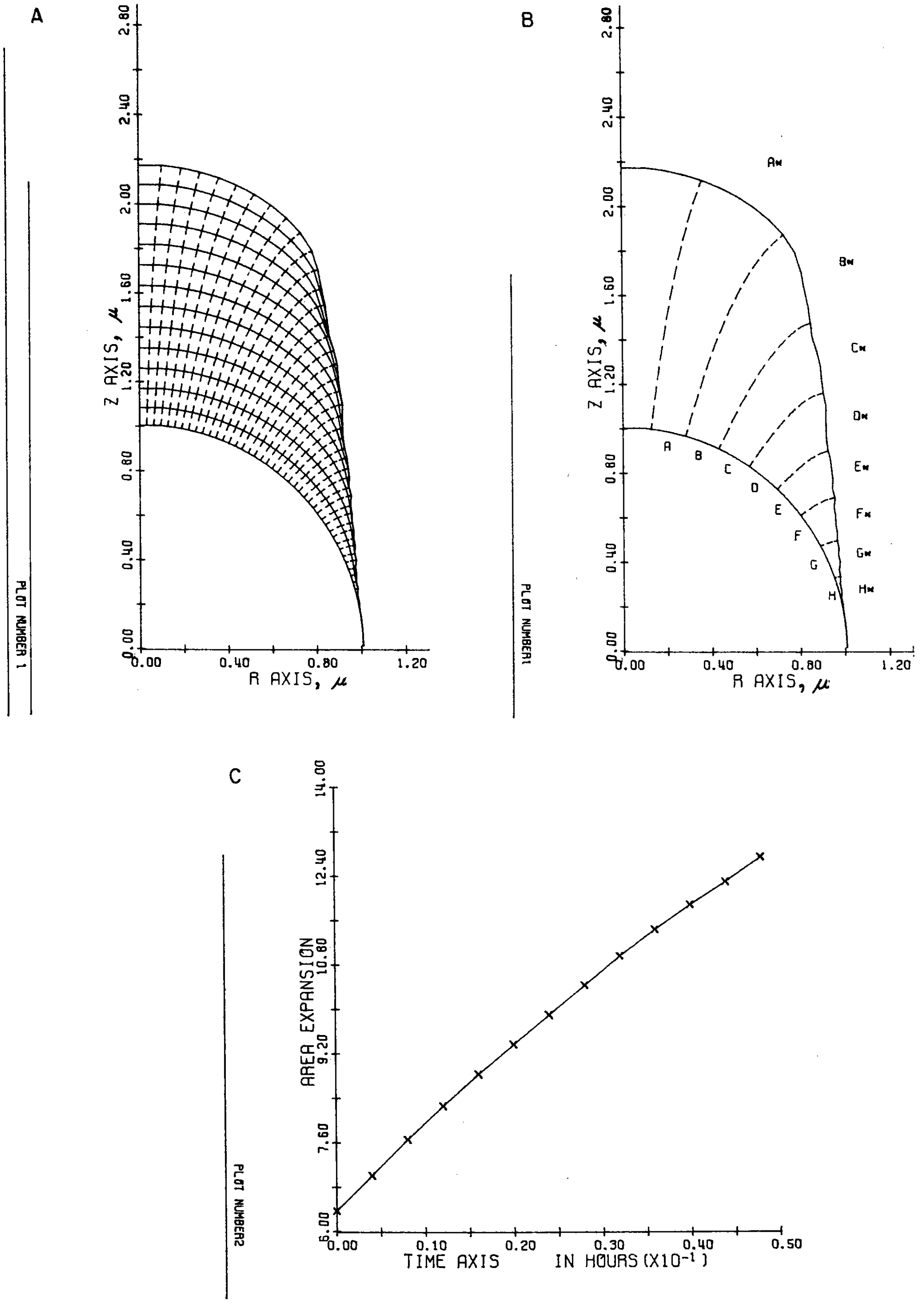

FIG. 5. (A) Evolution of shape (constant E.F.). Time of growth, 0.052 h; growth rate, 22.45 μ/h. (B) Segment expansion (constant E.F.). (C) Area expansion (constant E.F.).

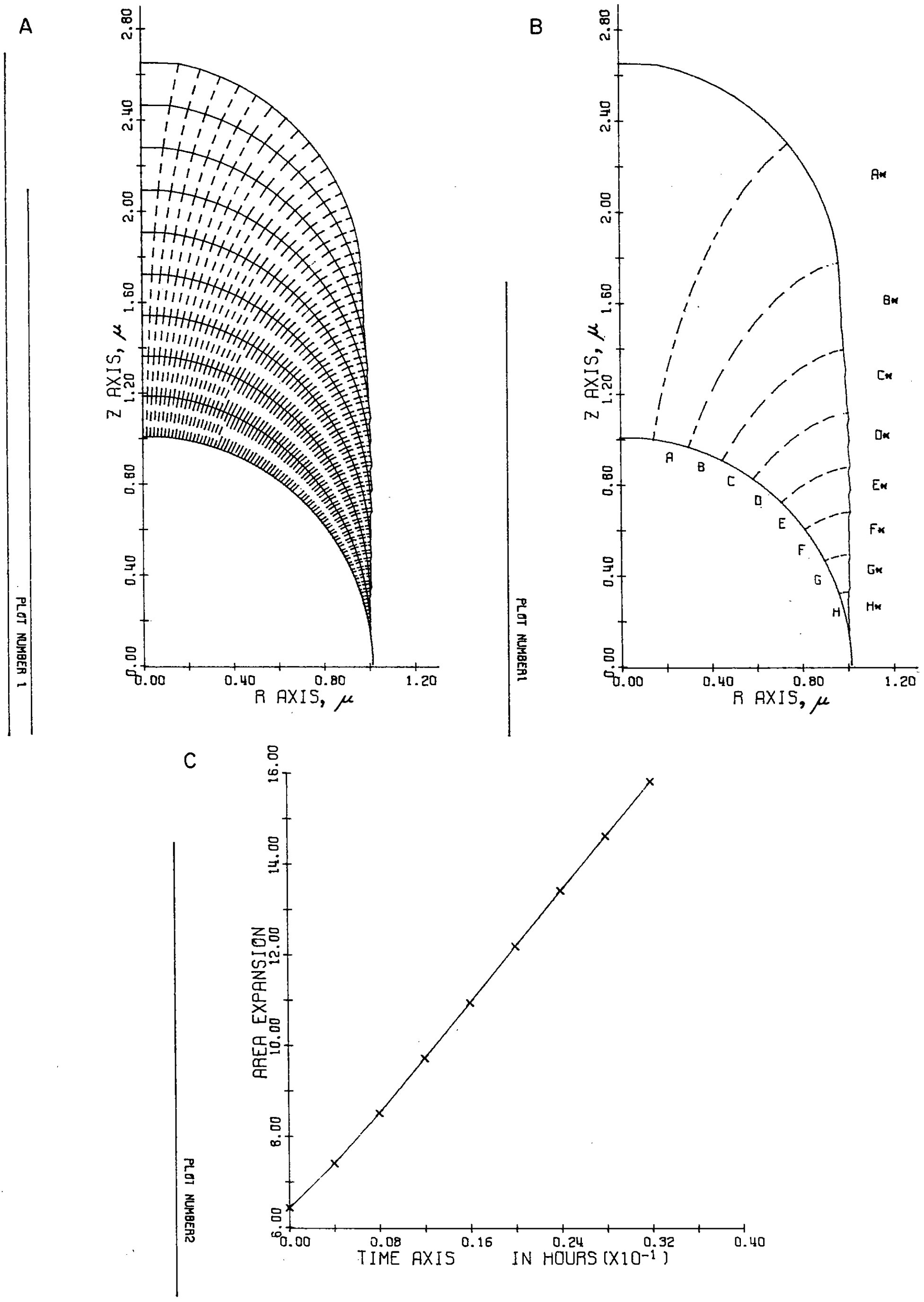

FIG. 6. (A) Evolution of shape (decreasing E.F.). Time of growth, 0.036 h; growth rate, 45.5 μ/h. (B) Segment expansion (decreasing E.F.). (C) Area expansion (decreasing E.F.).

At the beginning of each line (a point along the Z axis) since there are no preceding points to work from, the amount of advance must initially be chosen arbitrarily and supplied to the computer. The computer then calculates points and constructs a new line which, if it satisfies certain criteria of fit, is accepted. However, more usually, the first line does not fit, and another starting point must be chosen. The computer is instructed to proceed as follows. If the new line is too high, the machine chooses a new starting point half way between the last acceptable line and the unacceptably high line. If the new line proves to be too low, that is, it intercepts the previous acceptable line before the hypothetical hyphal margin, the machine regards this incorrect line as the previous line, and chooses another starting point at a standard increment higher. This target-finding or ranging process continues until an acceptable starting point and line are arrived at. When the line being calculated reaches the predetermined periphery, only a few further points are calculated, since it is assumed that such points now form part of the set wall. The main criterion for 'fit' is that the line should reach the set wall at a very small distance above the point where the previous line intersected set wall.

The lines actually plotted by the Calcomp plotter are so chosen as to give a suitable display of the development of the hyphal tip with time (Figs. 5A and 6A). The continuous lines represent the successive outlines of the hyphal tip, while the broken lines indicate the paths followed through time by a number of points on the initial line, l_0.

The choice of expansion function (E.F.) is the chief factor in determining the shape assumed by the model. If we choose an E.F. that has higher values at points near the uppermost part of the tip than those near the set wall, we produce a model in which the expansion will take place mainly toward the top of the tip (Fig. 6A). If, on the other hand, we choose an E.F. of constant value, the expansion at the center and that near the periphery will be much more similar (Fig. 5A).

The total area of the three-dimensional surface of the hypha increases with time. After each calculated step, the old area, the new area, and the expansion involved, are printed out (Tables

TABLE 1

Segment expansion

Old segment	New segment	Ratio
A = 0.15999	A* = 0.44955	R = 2.80982
B = 0.15999	B* = 0.41835	R = 2.61478
C = 0.15999	C* = 0.32516	R = 2.03233
D = 0.15999	D* = 0.25809	R = 1.61310
E = 0.15999	E* = 0.21660	R = 1.35382
F = 0.15999	F* = 0.19085	R = 1.19289
G = 0.15999	G* = 0.16662	R = 1.04139
H = 0.15999	H* = 0.16172	R = 1.01082

TABLE 2

Area expansion

	Old area	New area	Expansion %
Time = 0.0040 h	6.3844	7.0260	10.049810
Time = 0.0080 h	7.0260	7.6715	9.186935
Time = 0.0120 h	7.6715	8.2752	7.870579
Time = 0.0160 h	8.2752	8.8468	6.906796
Time = 0.0200 h	8.8468	9.3854	6.087589
Time = 0.0240 h	9.3854	9.9186	5.681229
Time = 0.0280 h	9.9186	10.4526	5.383587
Time = 0.0320 h	10.4526	10.9804	5.049801
Time = 0.0360 h	10.9804	11.4594	4.362392
Time = 0.0400 h	11.4594	11.9080	3.914452
Time = 0.0440 h	11.9080	12.3260	3.509998
Time = 0.0480 h	12.3260	12.7685	3.590393
Time = 0.0520 h	12.7685	13.1984	3.366566

TABLE 3

Segment expansion

Old segment	New segment	Ratio
A = 0.16000	A* = 0.57886	R = 3.61794
B = 0.16000	B* = 0.37889	R = 2.36808
C = 0.16000	C* = 0.28456	R = 1.77851
D = 0.16000	D* = 0.23609	R = 1.47558
E = 0.16000	E* = 0.20645	R = 1.29034
F = 0.16000	F* = 0.18428	R = 1.15178
G = 0.16000	G* = 0.17001	R = 1.06260
H = 0.16000	H* = 0.16248	R = 1.01549

TABLE 4

Area expansion

	Old area	New area	Expansion %
Time = 0.0040 h	6.4522	7.4248	15.073490
Time = 0.0080 h	7.4248	8.5282	14.860720
Time = 0.0120 h	8.5282	9.7421	14.234350
Time = 0.0160 h	9.7421	10.9621	12.522880
Time = 0.0200 h	10.9621	12.2049	11.336420
Time = 0.0240 h	12.2049	13.4314	10.049530
Time = 0.0280 h	13.4314	14.6315	8.935070
Time = 0.0320 h	14.6315	15.8255	8.160496
Time = 0.0360 h	15.8255	17.0223	7.562351

2 and 4), and the expansion of the global area increase illustrated in Figs. 5C and 6C. The expansion of chosen segments is illustrated in Figs. 5B and 6B, and the ratios of final to original lengths are given in Tables 1 and 3.

Conclusions

Our final results are illustrated in part in Figs. 5 and 6. Many trial runs were needed to find values of the function θ corresponding to growth rates of 22.45 μ/h and 45.5 μ/h respectively for a hypha of diameter 2 μ.

It appears that under our first hypothesis of uniform growth all over the tip (constant θ) we arrive at a rate of renewal of the membrane such that after an hour only 1/40 of the original membrane material remains, and is incorporated in the set wall, the rest having been replaced in the growing tip by new material. Under the second hypothesis (θ decreasing from center to margin) only 1/120 of the original wall material remains after an hour if we consider only the top of the tip (the region immediately adjacent to the *z* axis).

This model gives a very realistic configuration, with constant diameter, and we think it may mean that the degree of 'unsetness' and intussusception is at a maximum at the *z* axis, and decreases steadily until it reaches a point at the margin where 'setness' is complete.

From the morphological point of view both hypotheses are rather simple, but may reflect very different possible biophysical assumptions. This study has shown how it may be possible to derive quantitative working models of a specific dynamic biological system from a combination of scanty qualitative data and a few quantitative parameters.

Acknowledgments

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