# 10 The Mycelium as an Integrated Entity

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CONTENTS



## I. Introduction

Hyphal and mycelial growth appears to be an adaptation to life in inhomogeneous environments with solid surfaces, primarily soils.

Norbert Pfennig (1984)

The morphology of a mycelium is determined by mechanisms which regulate the polarity and the direction of growth of hyphae and the frequency with which they branch. As implied by Pfennig (1984), these regulatory mechanisms make a significant contribution to the efficiency with which fungi colonise solid surfaces. Observation of a colony developing on a solid medium shows that hyphae grow radially outward from the inoculum with leading hyphae at the colony margin growing approximately parallel to one another and at approximately the same distance apart. The growth kinetics observed during the development of mycelia on solid media appear to be common to all molds, and even extend to filamentous streptomycetes (Allan and Prosser 1983, 1985). However, although the analogy may be valid, at least qualitatively for certain aspects of growth, recent studies have increased our appreciation of the distinct cellular mechanisms involved in filamentous growth of prokaryotic streptomycetes and eukaryotic fungi (Prosser 1994). One major difference is that streptomycetes lack the vesicular system of membrane and wall growth observed in fungi.

This chapter concentrates on growth of mycelial or filamentous fungi and links cellular aspects of growth to growth kinetics of the mycelium. Not surprisingly, many aspects of the cell biology of fungal growth are similar to those of unicellular eukaryotic microorganisms and, where possible, these similarities will be identified.

## II. Unrestricted Growth of Fungal Mycelia

### A. The Hyphal Growth Unit

When a microorganism is grown in batch culture on a medium containing an excess of nutrients, growth is exponential and proceeds at the or-

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ganism's maximum specific growth rate ($\mu_{max}$) for the prevailing conditions (type of nutrients, temperature, pH etc.). Because $\mu_{max}$ is limited by the type of nutrients and not by their concentration, Schaechter et al. (1958) referred to this situation as "unrestricted growth", assuming that the growth rate observed under such conditions is the highest which can be attained with the nutrients available to the microorganism. In a batch culture, growth will continue at $\mu_{max}$ until a nutrient is depleted sufficiently to become growth-limiting (Monod 1949), or until some other cultural condition, e.g. pH, is altered sufficiently to affect growth rate. By contrast to growth in batch culture, growth in a chemostat is "restricted" (i.e. it is always less than $\mu_{max}$) from the outset by the concentration of a particular nutrient (Pirt 1975).

Figure 1 shows unrestricted growth of a mycelium of *Fusarium graminearum* A3/5 from a macroconidium (a multiseptate spore). The total hyphal length of the mycelium increased exponentially at a specific growth rate of $0.26\,h^{-1}$. Figure 1 also shows that, initially, hyphal tips were produced discontinuously by the mycelium, but that over the observation period their number increased exponentially at a specific rate of $0.27\,h^{-1}$. If, as here, hyphal length and tip number increase exponentially at essentially the same specific rate, the ratio (hyphal length: tip number) between these parameters will approximate to a constant, as indeed is confirmed by the data in Fig. 1. This ratio is called the hyphal growth unit (G) and represents the average length of hypha which supports the extension of each tip in the mycelium (Caldwell and Trinci 1973; Trinci 1974). Thus, growth of a mycelium can be considered in terms of the duplication of a physiological unit of growth which consists of a tip and a specific length of hypha ($391 \pm 18\,\mu m$ for the mycelium in Fig. 1). Further, if the mean hyphal radius remains constant during mycelial growth, the hyphal growth unit will be of constant volume as well as of constant length. It follows from this that branch formation in fungi can be considered as being analogous to cell division in a unicellular microorganism such as *Schizosaccharomyces pombe*. However, in *S. pombe* the cell is both a physiological and a morphological unit of growth, but in fungi the hyphal growth unit is a physiological unit of growth only. Thus, branch initiation is regulated by the changes in cytoplasmic volume which accompany growth, i.e. when the mean hyphal growth unit (= volume of cytoplasm per tip) exceeds a critical length (= critical volume of cytoplasm) a new branch is initiated somewhere in the mycelium.

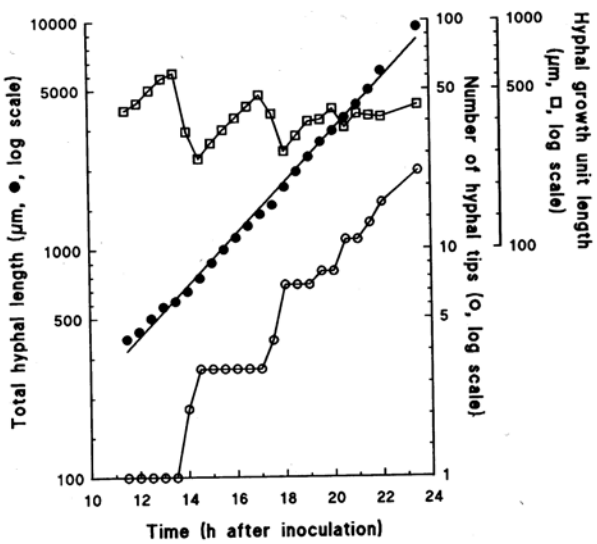



**Fig. 1.** Unrestricted growth of a mycelium of *Fusarium graminearum* A 3/5 at 25 °C on a glucose-mineral salts agar medium

For fungi, the length of the hyphal growth unit is species- and strain-specific (Trinci 1984). Trinci (1973) described the variation in hyphal growth unit length observed for mutants of *Neurospora crassa* and Wiebe et al. (1992) recently isolated spontaneous mutants of *F. graminearum* A3/5 which in liquid batch culture varied in G from 21 (strain CC1-1) to 174 (CC1-3) $\mu$m; the value of G for the parental strain was $232\,\mu m$. Thus, for a given species, G can vary over wide limits. The physiological changes responsible for this variation are poorly understood, although enzymes such as glucose-6-P-dehydrogenase (Brody and Tatum 1966) and phosphoglucomutase (Brody and Tatum 1967) have been identified as being involved in the altered morphology. The implication, then, is that the mutant morphology is probably part of a pleiotropic response to some alteration in enzyme activity.

## B. Mean Hyphal Extension Rate

During unrestricted growth of a mycelium, some hyphae extend at the linear rate characteristic for the strain and cultural conditions but most are accelerating towards this rate (Trinci 1974). For example, Fig. 2 shows that of 20 tips of a *Mucor*

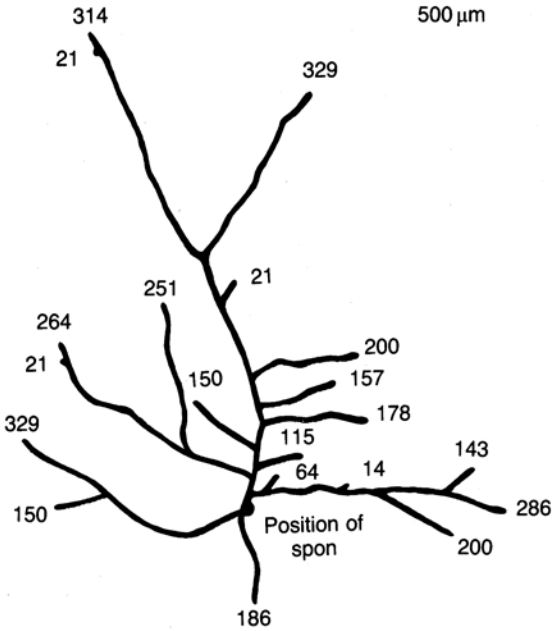



**Fig. 2.** Extension rates (measured during the previous 1 h) of hyphae during unrestricted growth of a mycelium of *Mucor hiemalis* cultured at 25 °C on malt extract agar. Under the prevailing conditions, hyphae of *M. hiemalis* extend at a linear rate of ca. 325 $\mu m\,h^{-1}$. (Trinci 1974)

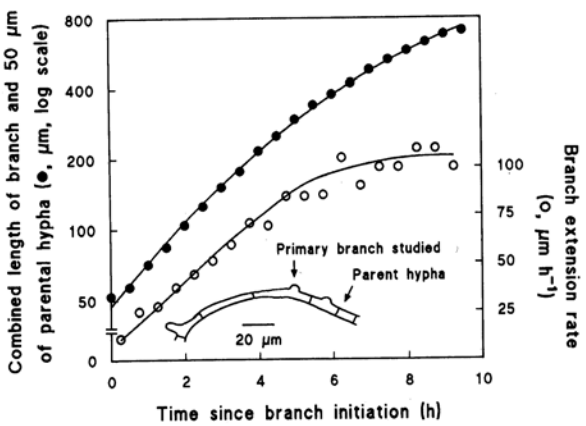



**Fig. 3.** Length (●) of a primary branch +50 $\mu m$ portion of the parental hypha of *A. nidulans*, and the extension rate (○) of this branch. (Fiddy and Trinci 1976)

*hiemalis* mycelium, only three were extending at the linear rate (ca. 325 $\mu m\,h^{-1}$) characteristic of the particular cultural conditions, whilst the remainder were presumably accelerating towards this value. Figure 3 shows the growth of a (primary) branch formed during unrestricted growth of a mycelium of *Aspergillus nidulans*. The branch was produced by an intercalary compartment which was 34 $\mu m$ long, but in Fig. 3 it is assumed that growth of the branch was supported by a 50 $\mu m$ length of the parental hypha (septa in *A. nidulans* have central pores and therefore growth of a branch may be supported by a portion of parental hypha longer than the compartment from which it is formed). When this assumption was made, the kinetics of branch growth during the first 5 h were adequately described by the equation:

$$\ln L_1 = \ln L_0 + \mu(t_1 - t_0), \tag{1}$$

where $L_0$ is the combined length of the supporting parental hypha and the branch at time $t_0$; $L_1$ is the combined length of the supporting parental hypha and the branch at time $t_1$; and $\mu$ is the specific growth rate of the branch plus the supporting parental hypha. Thus, initially, the supporting parental hypha and the branch grew exponentially at the same specific rate ($\mu = 0.32\,h^{-1}$) as the whole mycelium. The extension rate of the branch became linear when the branch (together with the supporting parental hypha) was ca. 375 $\mu m$ long; this length, therefore, represents the peripheral growth zone of the hypha [see Eq. (5)].

Using Eq. (2), Trinci (1974) determined the mean rate of extension (E) of hyphae during unrestricted growth of mycelia:

$$E = \frac{2(H_t - H_o)}{B_0 + B_t}, \tag{2}$$

where $H_0$ is the total hyphal length of the mycelium at zero time; $H_t$ is the total length 1 h later; $B_0$ is the number of tips at zero time, and, $B_t$ is the number of tips 1 h later. Instantaneous measurements of E would be preferable to the 1 h period used by Trinci (1974) but unfortunately it is not possible to make such measurements. Steele and Trinci (1975) showed that E was a function of the length of the hyphal growth unit and the organism's specific growth rate (during unrestricted growth, $\mu = \mu_{max}$). Thus,

$$E = G\mu. \tag{3}$$

However, for a particular mycelium the actual length of hypha supporting growth of a tip varies over wide limits, from a minimum length just after branch initiation to a maximum length when the tip is extending at a linear rate (Fig. 3).

By converting the biomass supporting a hyphal tip into a volume, Eq. (3) can be rewritten (Trinci 1984) as follows:

$$E = \frac{V_g}{\pi r^2} \cdot \mu, \quad (4)$$

where $V_g$ is hyphal growth unit volume; and r is hyphal radius. If the volume of the hyphal growth unit remains constant during mycelial growth, it follows from Eq. (4) that relatively small changes in r will have appreciable effects on hyphal growth unit length (since G will be inversely related to $r^2$) and hyphal extension rate. That $V_g$ may indeed be a constant was demonstrated by Robinson and Smith (1979) for glucose-limited chemostat cultures of *Geotrichum candidum*.

From Eqs. (3) and (4), it can be predicted that, in the absence of a change in r, changes in specific growth rate will only affect hyphal growth unit length (i.e., mycelial morphology) if mean hyphal extension rate is not linearly related to specific growth rate, i.e. if the ratio $E/\mu$ is not a constant. The observation that hyphal growth unit length of *N. crassa spco 1* is not affected by temperature (Trinci 1973) suggests that $E/\mu$ remains constant when $\mu$ is changed by varying temperature, and this conclusion has been confirmed experimentally (Trinci 1974). Further, Robinson and Smith (1979) demonstrated that temperature had no appreciable effect on hyphal growth unit volume of *G. candidum* mycelia grown in glucose-limited chemostat culture. Thus, temperature affects the rate at which the hyphal growth unit of a mycelium is duplicated, not its length or volume.

### C. Compounds (Paramorphogens) Which Inhibit Hyphal Extension But Not Specific Growth Rate

Many compounds have been identified which inhibit hyphal extension but apparently have no effect on specific growth rate. Such compounds are called paramorphogens (Tatum et al. 1949). Because paramorphogens alter the ratio $E/\mu$, they

**Table 1.** Compounds (paramorphogens) which inhibit hyphal extension but not specific growth rate and therefore cause mycelia to branch more profusely [Eq.(3)]. Cultures were grown on agar medium containing the paramorphogen

| Paramorphogens | Lowest concentration at which effect was observed | Mode of action of paramorphogens | Reference |
|---|---|---|---|
| Sugars | | | |
| Cellobiose | 58 mM | Unknown | Wilson and Niederpruem (1967) |
| Glucosamine | 4 mM | Unknown | Jejelowo and Trinci (1988) |
| 3-*O*-methyglucose | 1 mM | Unknown | Galpin et al. (1977); Jejelowo and Trinci (1988) |
| L-sorbose | 1 mM | Inhibition of $\beta$-1-3 glucan synthetase | Mishra and Tatum (1972); Howell (1978); Jejelowo and Trinci (1988) |
| Fungicides | | | |
| Edifenphos | 10 $\mu$M | Inhibition of phosphatidylcholine and chitin biosynthesis | Wiebe et al. (1990); Binks et al. (1993) |
| Validamycin A | 1 $\mu$M | Inhibition of phosphatidylinositol biosynthesis | Wakae and Matsuura (1975) Robson et al. (1988) Robson et al. (1989) |
| Cilofungin | 5 $\mu$M | Inhibition of $\beta$-1,3-glucan synthetase | Vaughan (1993) |
| Echinocandin B | 5 $\mu$M | Inhibition of $\beta$-1,3-glucan synthetase | Vaughan (1993) |
| Tetrahydro-echinocandin B | 5 $\mu$M | Inhibition of $\beta$-1,3-glucan synthetase | Vaughan (1993) |
| Other | | | |
| Cyclosporin A | 40 nM | Inhibition of calmodulin-dependent protein phosphatase | A. Morten and G.D. Robson (unpubl. result) |
| Lysocellin | 100 nM | Inhibition of phosphatidylinositol turnover | Imoto et al. (1990); G.D. Robson (unpubl. result) |
| Sclareol | 50 nM | Unknown | Bailey et al. (1974, 1975); G.D. Robson (unpubl. result) |

reduce hyphal growth unit length, i.e. they cause increased branching [Eq. (3)]. Table 1 lists various paramorphogens; they include sugars such as cellobiose, 3-*O*-methylglucose, L-sorbose and glucosamine; fungicides such as cilofungin, echinocandin B, tetrahydroechinocandin B, edifenphos and validamycin A; the phosphoinositide inhibitor, lysocellin, and other compounds such as sclareol and cyclosporin A.

Many of the paramorphogens listed in Table 1 inhibit membrane and/or wall biosynthesis. Such compounds would be expected to reduce hyphal extension and therefore cause increased branching because of effects on the ratio $E/\mu$ [Eq. (3)]. It seems likely that in paramorphogen-treated-hyphae, hyphal extension is limited by the rates at which membrane and wall precursors can be incorporated into the tip wall, not the rate of supply of these precursors. Certainly, Binks et al. (1993) found a relationship between the effect of edifenphos on chitin synthesis and its effect on colony radial growth rate.

### D. Linear Hyphal Extension

Although it is widely considered that leading hyphae extend at a linear rate, computer-enhanced video microscopy has been used recently to show pulsed (at 3–25-s intervals), not linear, growth of fungal hyphae (R.M. López-Franco and C.E. Bracker, pers. comm.). However, this result awaits confirmation by others and in any case hyphal extension is linear when measurements are made over longer periods. Trinci (1971) showed that the extension rate ($K_r$) of a leading hypha of a mature colony is a function of the length of hypha (w) contributing to tip growth and the organism's specific growth rate ($\mu$). Thus,

$$K_r = w\mu. \quad (5)$$

It is generally considered that by regulating the proportions of biomass directed towards hyphal extension and branching, a fungal mycelium can colonise solid medium (and presumably soil and plant surfaces) much more efficiently than unicellular microorganisms (Prosser 1993; Trinci 1969). In nutrient-poor conditions, branch growth is "suppressed" to "maximise" extension of leading hyphae while, under more favourable nutrient conditions, branch growth continues to optimise utilisation of substrates behind the leading hypha. Consequently, it might be anticipated that evolution would favour the selection of strains whose hyphae extend rapidly, i.e. strains with long peripheral growth zones [Eq. (5)]. Indeed, this may be an important reason for the presence of pores in septa, as such an arrangement allows many subapical hyphal compartments to contribute to tip extension (Trinci 1978). Until recently, this hypothesis was supported by the observation that, although mutants more highly branched (shorter G values) than their parental strains have frequently been isolated, mutants more sparsely branched (longer G values) than their parental strains have never been reported. However, recently, Withers et al. (1994) and M.G. Wiebe (unpubl. results) isolated strains of *Aspergillus oryzae* and *F. graminearum* A3/5, respectively, which are significantly more sparsely branched (longer G values and therefore presumably longer w values; Jejelowo and Trinci 1988) than their respective parental strains. It is not known if the increased G values of these mutants were due to increases in $V_g$ (with r remaining constant) or decreases in r (with $V_g$ remaining constant).

Under conditions of restricted growth, linear extension of a hypha may be limited either by the rate of supply of cell wall precursors from the peripheral growth zone to the tip or by the rate at which the tip wall can be assembled from these precursors. Since the extension zone wall always contains chitin or cellulose (Gooday and Trinci 1980), the rate of formation of wall microfibrils may be the factor which limits hyphal extension. However, the observations that hyphae of *N. crassa* extend at rates in excess of $100\,\mu m\,min^{-1}$, and that chitin synthase from diverse fungi have similar kinetic properties (Gooday and Trinci 1980) suggest that (when there is an abundance of the enzymes involved in wall biosynthesis at the tip) hyphal extension may be limited by the rate of supply of precursors to the tip rather than by the rate at which the tip wall can be assembled.

Although convergent evolution has given rise to fungi and streptomycetes which produce morphologically similar hyphae, fungal hyphae extend at 1 to $100\,\mu m\,min^{-1}$, whereas streptomycete hyphae extend at rates of ca. $0.5\,\mu m\,min^{-1}$ or less (Prosser and Tough 1991; Prosser 1994). This difference in hyphal extension rate is not correlated with a difference in specific growth rate as streptomycetes generally have higher $\mu_{max}$ values than fungi (Prosser and Tough 1991). Instead, it can be attributed to differences in peripheral growth zone length and to differences in cell bio-

logy. Firstly, the length and volume of the peripheral growth zone of fungal hyphae are much larger than those of streptomycete hyphae, e.g. hyphae of *N. crassa SYR-17-3A* have a w of ca. 6.8 mm and a $V_g$ of ca. $2.6 \times 10^5\,\mu m^3$, whereas hyphae of *Streptomyces coelicolor* have a w of $<100\,\mu m$ and a $V_g$ of ca. $80\,\mu m^3$. Secondly, hyphal growth of fungi differs from that of streptomycetes in that a large number of vesicles are generated in the peripheral growth zone and transported to the tip, where they fuse with the protoplasmic membrane. Thus, wall synthesis at the tips of fungal hyphae involves exocytosis of vesicles that contribute both protoplasmic membrane and cell wall polymers to apical growth. This vesicular system ensures that expansion of protoplasmic membrane and wall at the hyphal tip are closely integrated, and similar vesicular systems are observed in other filamentous eukaryotes (Bonnet and Newcomb 1966; Otto and Brown 1974; Rosen 1964).

Vesicles are present at the tips of fungal hyphae to the exclusion of most other organelles. Figure 4 shows their distribution at the tip of a hypha of *N. crassa cot 3*; ca. 80% of the volume of the apical $1\,\mu m$ of this hypha was occupied by vesicles but their concentration fell sharply to ca. 10%, $10\,\mu m$ from the tip, and to ca. 5% at the base of the 27-$\mu$m-long extension zone. Collinge and Trinci (1974) suggested that most or all of the vesicles observed at the hyphal tip are generated in the peripheral growth zone behind the extension zone. This hypothesis was supported by the observation of a correlation between the observed vesicle concentration in the hyphal tip of *N. crassa cot 3* and (1) the predicted concentration based on the assumption that the number of vesicles per unit length of extension zone is constant (Fig. 4), and (2) relative silver grain density over hyphal tips incubated for 1 min in $^3H_1$-*N*-acetylglucosamine (Gooday 1971; Gooday and Trinci 1980).

## E. Mathematical Models of Hyphal Growth

A novel mathematical model has been developed by Bartnicki-Garcia et al. (1989) to explain how a fungal hypha can be generated by a tip-growing cell. The model assumes that: (1) the cell surface expands as a result of vesicles fusing with the protoplasmic membrane, (2) the vesicles move

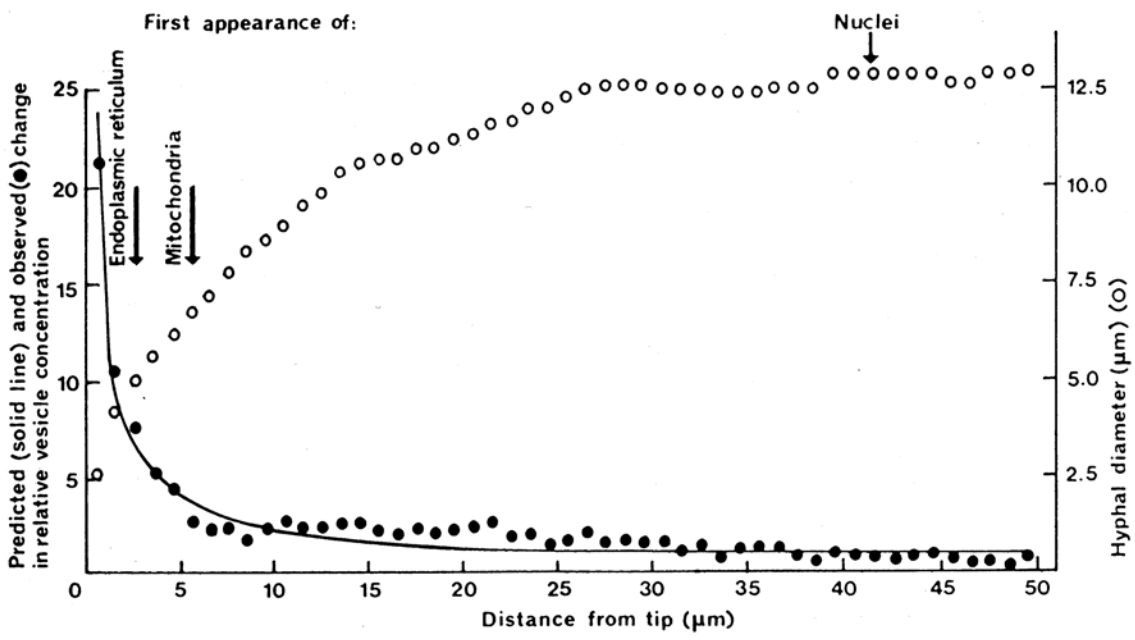



**Fig. 4.** Hyphal diameter (○) and vesicle concentration (●) of a hypha of *N. crassa cot 3* grown at 25 °C. The *solid line* is vesicle concentration predicted from $V_P = R^2/r^2$, where $V_P$ is the predicted vesicle concentration, $R^2$ is the square of the radius below the extension zone, and $r^2$ is the radius of the hyphae at any point; in predicting vesicle concentration, it was assumed that the number of vesicles per unit length of hypha remained constant. (Collinge and Trinci 1974)

from a vesicle supply centre, and (3) vesicles move from this supply centre to the surface of the cell in a random direction. In this model, the position and movement of the vesicle supply centre is the critical determinant of hyphal morphogenesis.

Mathematical models have also been used to relate the growth kinetics of mycelial fungi to the vesicular system of hyphal growth proposed by Collinge and Trinci (1974). For example, Prosser and Trinci (1979) modelled mycelial growth by considering hyphal tip extension, resulting from incorporation of vesicles produced distally, septation and branching. This model has been modified by Yang et al. (1992) to incorporate variable rates of production of material required for tip synthesis and transport by diffusion. They also include a stochastic component for branch angle, branch position and tip growth direction, and their approach is similar to that of Hutchinson et al. (1980). It involves experimental determination of mean values for each parameter, and associated variability. Mycelia are then reconstructed by calculating, at each of a series of time steps, growth direction and branch formation based on experimentally observed means and variability.

## III. Balanced Growth of Fungal Mycelia

Growth is said to be balanced when all extensive properties of the biomass increase at the same rate (Campbell 1957). In mycelial fungi, these extensive properties include morphological features such as mycelial length, number of tips and number of septa (Trinci 1978), organelles such as nuclei and mitochondria, macromolecules such as DNA, RNA, protein and wall polymers, and dry weight. In the slime mound, *Physarum polycephalum*, vigorous protoplasmic streaming ensures that organelles are distributed more or less uniformly throughout the coenocyte and that events such as mitosis are highly synchronised. However, because growth in fungi is highly polarised and because protoplasmic streaming in fungi is apparently less vigorous than in slime moulds, other mechanisms must exist to ensure that growth in fungi remains balanced.

### A. Regulation of Mitosis

Balanced growth of a fungal mycelium can be illustrated by reference to the generation and distribution of nuclei. The large germling in Fig. 5a contains 100 nuclei which are distributed more or less uniformly throughout the cytoplasm despite the highly polarised nature of hyphal growth. This figure suggests that mechanisms exist in fungi to regulate both the generation and spatial distribution of nuclei. After germination (the conidium is uninucleate), nuclei in germlings of *A. nidulans* divide more or less synchronously until they contain 8 or 16 nuclei (Fig. 5b) but thereafter mitotic synchrony decays, possibly because plugged septa restrict cytoplasmic mixing (Fiddy and Trinci 1976; Rosenberger and Kessel 1967). Thus, eventually there is intra- but not (extensive) intercompartmental mitotic synchrony and consequently growth in large germlings (Fig. 5a) is analogous to an asynchronous population of a unicellular microorganism which is growing exponentially.

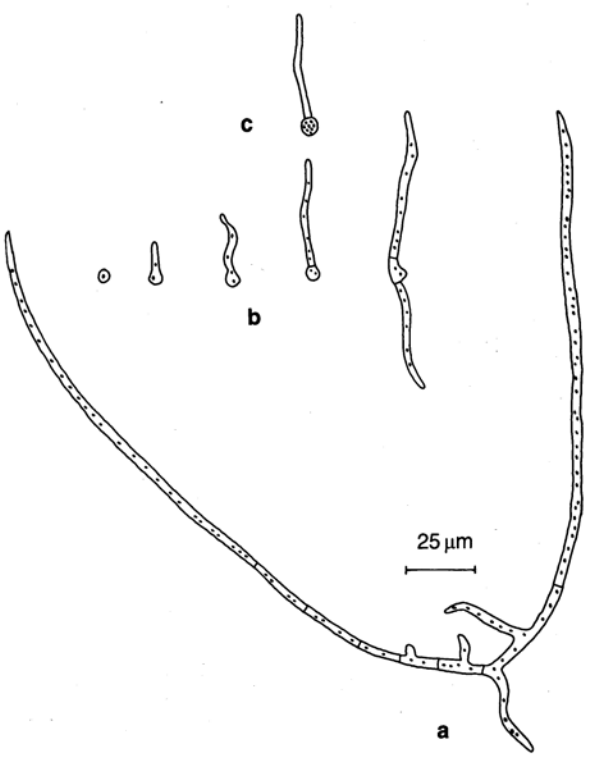



**Fig. 5a–c.** Distribution of nuclei in germlings of *A. nidulans* stained with Giemsa. **a** Germling with 100 nuclei. **b** Conidium and germlings with 1 to 16 nuclei. **c** A nud (nuclear distribution) mutant grown at the restrictive temperature – the nuclei divide but do not migrate

### B. Regulation of Nuclear Migration

The spatial distribution of nuclei in a mycelium (Fig. 5) is determined by their migratory behaviour, the most detailed description of which is that provided by Girbardt (1968) for dikaryotic (two nuclei per apical compartment) hyphae of the basidiomycete, *Polystictus versicolor*. Girbardt divided the migratory cycle of nuclei of *P. versicolor* into four phases (Fig. 6). During the prolonged, first phase, most of the cell contents (nuclei, mitochondria, vacuoles, granules) move forward at about the same rate as the extending hyphal tip and therefore the distance between the tip and the nuclei remains approximately constant. However, in the second phase, the nuclei stop migrating although other components of the cytoplasm continue to move forward. Thus, during the second phase, the distance between the hyphal tip and the nuclei increases (Fig. 6). Synchronous mitosis of the two nuclei occurs during the third phase, and during the fourth and final phase, one pair of daughter nuclei moves rapidly (at a faster rate than tip extension) forward through the hypha to assume the phase 1 migratory distance behind the hyphal tip, whilst the second pair of nuclei moves slowly in the opposite direction, away from the tip. Nuclei in apical compartments of uninucleate (Robinow 1963) and multinucleate (Koenig and Howard 1962) apical compartments of fungal hyphae apparently behave in a manner similar to *P. versicolor*, e.g., at a particular point in the mitotic cycle, the nuclei stop migrating and undergo mitosis, but, subsequently, the daughter nuclei re-occupy their normal position behind the hyphal tip.

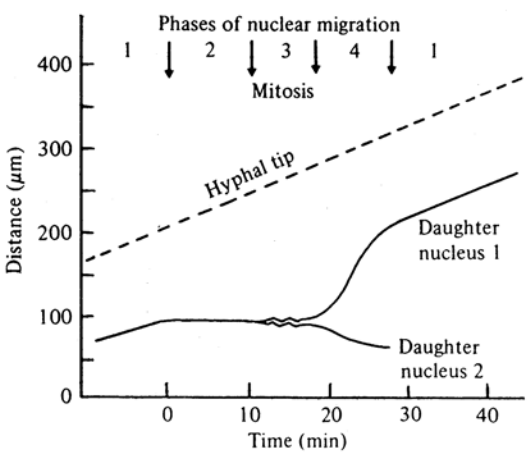



**Fig. 6.** Hyphal extension and nuclear migration in dikaryotic apical compartments of *Polystictus versicolor* grown at 24 °C. The migration of one of the pair of nuclei is shown and the *curves* are the mean of 34 observations. (After Girbardt 1968 by Trinci 1979)

The behaviour of nuclei in germlings of *A. nidulans* (Fig. 5) and in *P. versicolor* (Fig. 6) demonstrates that not only is the timing of mitosis in a hypha regulated, but so is the positioning of nuclei. A functional microtubule system is necessary for normal nuclear migration in *A. nidulans* (Oakley and Morris 1980, 1981), but not for mitochondrial movements (Oakley and Rinehart 1985). Osmani et al. (1990) showed that a 22 kDa nud protein is involved in the migration of nuclei in *A. nidulans* but this protein is not required for mitosis, movement of other subcellular organelles, or short-term growth of germlings (Fig. 5c). The observation that nud mutants are lethal (Morris 1976) illustrates the importance of balanced growth.

## IV. Roles of Phosphoinositides, Calcium, Calmodulin and Cyclic Nucleotides in the Regulation of Hyphal Extension and Branching

### A. Role of Phosphoinositides

In mammalian cells, the stimulation of the phosphoinositide-signalling pathway leads to an increase in breakdown of phosphatidylinositol bisphosphate by phospholipase C, and a subsequent increase in the second messenger, inositol 1,4,5 trisphosphate ($IP_3$). Transient increases in $IP_3$ levels inside the cell stimulate the release of calcium from intracellular stores. In fungi, elements of the phosphoinositide cycle have been identified (Hanson 1991; Prior et al. 1993; Robson et al. 1991a) and $IP_3$ has been reported to stimulate the release of calcium from vacuoles of *N. crassa* (Cornelius et al. 1989), suggesting that phosphoinositide turnover may influence intracellular calcium concentrations in fungi. Lithium ions, which inhibit inositol phosphatase and perturb the phosphoinositide cycle, induce branching in *N. crassa* (Hanson 1991), although the high lithium concentrations used by Hanson may have induced many pleiotropic effects. A number of inhibitors of mammalian phosphoinositide turn-

over have been identified including lysocellin, piericidin B and vinaxanthone (Aoki et al. 1991; Imoto et al. 1990; Nishioka et al. 1991). Lysocellin and piericidin B induce branching (reduced G) in both *F. graminearum* and *N. crassa* at micromolar concentrations without altering specific growth rate (S. Prior, G.D. Robson and A.P.J. Trinci, unpublished result). In addition, validamycin A, an antifungal antibiotic used to control the rice sheath blight pathogen *Rhizoctonia solani*, inhibits phosphoinositide synthesis in *R. cerealis* and increases branching (Robson et al. 1989). Further, inositol auxotrophs of *N. crassa* display a highly branched phenotype when grown on suboptimal concentrations of inositol and this effect is associated with a decrease in phosphoinositide synthesis (Hanson and Brody 1979; Shatkin and Tatum 1961). Therefore, the present evidence suggests that phosphoinositide turnover (and presumably calcium release from intracellular stores) may play a role in regulating hyphal extension and branching in filamentous fungi. However, changes in the phosphoinositide levels in membranes induced by the various treatments described above may exert their effects on hyphal extension and branching by directly influencing the activity of membrane-bound, wall-synthesising enzymes (Montgomery and Gooday 1985; Vermeulen and Wessels 1983).

### B. Role of Calcium

Calcium plays a key role in the regulation of cellular functions in eukaryotic cells, but, although our knowledge of the regulatory roles of this ion in mammalian cells has increased considerably over the past decade, comparatively little is known about its function in filamentous fungi (also see Chaps. 3 and 5). Nevertheless, there is evidence that calcium is involved in maintaining tip growth of fungal hyphae. For example, reduction in the concentration of extracellular calcium to between $10^{-2}$ and $10^{-8}$ M by chelating agents (principally EGTA) decreases hyphal extension and also affects hyphal morphology (Jackson and Heath 1989; Robson et al. 1991b; Schmid and Harold 1988). In non-fungal systems, calcium plays an essential role in tip extension and appears to exist as a tip-to-base gradient maximal apically (Picton and Steer 1983; Reiss and Herth 1979; Reiss et al. 1985) but evidence for the presence of such gradients in fungi remains largely circumstantial. In pollen tubes, apical gradients of free intracellular calcium have been demonstrated using the calcium fluorochromes Quin-2 (Nobiling and Reiss 1987) and dextran-linked fura-2 (Miller et al. 1992) but it has proved difficult to load fungal hyphae with fluorochromes even by microinjection (Read et al. 1992a,b). Thus, much of the current evidence for the existence of calcium gradients in fungal hyphae relies on the use of chlortetracycline (CTC), a compound which chelates calcium and fluoresces strongly when bound to cell membranes. Hence this compound reports membrane-associated calcium, not intracellular free calcium. In *Saprolegnia ferax*, apical CTC fluorescence is dependent on the extracellular concentration of calcium and is diminished as this is reduced, as is hyphal extension (Jackson and Heath 1989). However, Yuan and Heath (1991) showed that the majority of the CTC fluorescence was associated with mitochondria and that, immediately behind the tip (where there are no mitochondria and the cytoplasm is dominated by wall vesicles), little fluorescence was visible. A similar observation with CTC has been reported for germlings of *N. crassa* (Barja and Turian 1992). These observations suggest that mitochondria play an important role in regulating calcium in fungi, at least at extending hyphal tips. Previously, the vacuole was thought to be the primary organelle involved in regulating the intracellular levels of calcium in fungi (Cornelius and Nakashima 1987).

If a gradient of calcium exists at the hyphal apex, the questions of how it is produced and the nature of its role need to be addressed. In pollen tubes, calcium is thought to enter preferentially at the tip, possibly through apically located calcium channels similar to those present in mammalian cells (Schramm and Towart 1985). Treatment with the calcium channel blocker, nifedipine, results in inhibition of normal pollen-tube extension, the loss of CTC fluorescence, and inhibition of apical branching (Reiss and Herth 1985). Similarly, treatment of *N. crassa* and *F. graminearum* with calcium channel blockers reduces hyphal extension and leads to abnormal hyphal morphologies (Dicker and Turian 1990; Robson et al. 1991c). In *N. crassa*, treatment with verapamil leads to a rapid loss of CTC fluorescence at the hyphal apex and a reduction in hyphal extension, but normal fluorescence and extension are restored when the external calcium concentration is raised to 50 mM. Intriguingly, hyphae of two

highly branched mutants of *N. crassa*, "frost" and "spray" display little CTC fluorescence at their tips but normal fluorescence and wild-type branching are observed when these mutants are grown on medium supplemented with 50 mM calcium (Dicker and Turian 1990). The above observations implicate $Ca^{2+}$ in hyphal extension and branching but the distribution of intracellular free calcium at the hyphal apex has yet to be established, as has the role of specific $Ca^{2+}$ channels. Significantly, a new class of stretch-activated ion channels which specifically gate anions or cations and are opened by membrane stress has recently been detected in *S. ferax* (Garrill et al. 1992). One of these is permeable to calcium and located preferentially at the hyphal apex and, therefore, may be involved in generating and maintaining a calcium gradient at the tip.

### C. Role of Calmodulin and Other Calcium-Binding Proteins

In many eukaryotic cells, calcium acts as a second messenger. Transduction of an external stimulus (e.g. from a hormone) elevates intracellular calcium levels either by stimulating the breakdown of phosphatidylinositol bisphosphate, the generation of $IP_3$ and the triggering of the release of calcium from intracellular stores, and/or by the opening of voltage-dependent calcium channels in the membrane (Berridge 1993). The effects of increases in the level of intracellular calcium are mediated via calcium-binding proteins of which calmodulin (CAM) is ubiquitous in eukaryotes; CAM plays an essential role in fungi, and disruption of the *CAM* gene in *A. nidulans* leads to a null phenotype due to cell cycle arrest (Lu and Means 1993). Calmodulin has been found in a wide range of taxonomically diverse fungi and the CAMs of *Aspergillus nidulans* and *N. crassa* share >75% sequence homology with mammalian CAM (Melnick et al. 1993; Rasmussen et al. 1990). $Ca^{2+}$-CAM complexes activate a number of CAM-dependent proteins, including CAM-dependent protein kinase and phosphatase (calcineurin) which regulate the activity of a further class of proteins by phosphorylation/dephosphorylation. CAM-dependent protein kinases have been detected in *A. nidulans* (Bartelt et al. 1988) and *N. crassa* (Ulloa et al. 1991) and calcineurin from *N. crassa* shares a 75% sequence homology with mammalian calcineurin (Higuchi et al. 1991). Studies on inhibition of CAM using pharmacological agents suggest that CAM plays a role in hyphal growth. In *N. crassa* and *F. graminearum*, calmodulin antagonists cause a marked decrease in hyphal extension and an increase in branching (Ortega-Perez and Turian 1987; Robson et al. 1991c). Bovine brain CAM has been shown to activate chitin synthase in *Phycomyces blakesleeanus* (Martinez-Cadena and Ruiz-Herrera 1987), suggesting that chitin synthesis at the hyphal tip may be regulated by calcium. Further, in *F. graminearum*, the calmodulin antagonist, trifluoperazine increases branching (reduces G), and reduces hyphal extension and the rate of incorporation of [$^{14}$C] *N*-acetylglucosamine into the cell wall in vivo (Fig. 7). The effects of CAM inhibition on mycelial morphology may be mediated by CAM binding proteins; however, although several CAM binding proteins have been demonstrated in *Saccharomyces cerevisiae* (Liu et al. 1990), it is not known if they are present in filamentous fungi.

In addition to calmodulin, other calcium-binding proteins are thought to play important roles in cell function. They form two major classes, the EF-hand family and the annexins (Heizmann and Hunziker 1991). Members of the EF-hand family exhibit a characteristic structural motif present in multiple copies and bind calcium selectively with a high affinity. These proteins, which include calmodulin, are generally cytosolic. The annexins are a family of proteins which interact with phospholipids and membranes in a

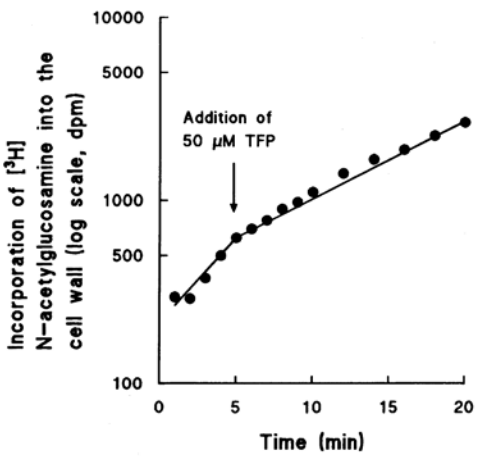
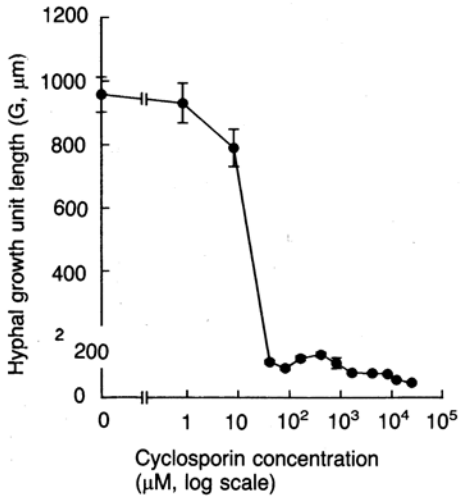



**Fig. 7.** Inhibition by trifluoperazine of in vivo incorporation of [$^{14}$C]-*N*-acetylglucosamine into the cell wall of *Fusarium graminearum* grown at 25 °C on Vogel's medium. (G.D. Robson and A.P.J. Trinci, previously unpublished result)



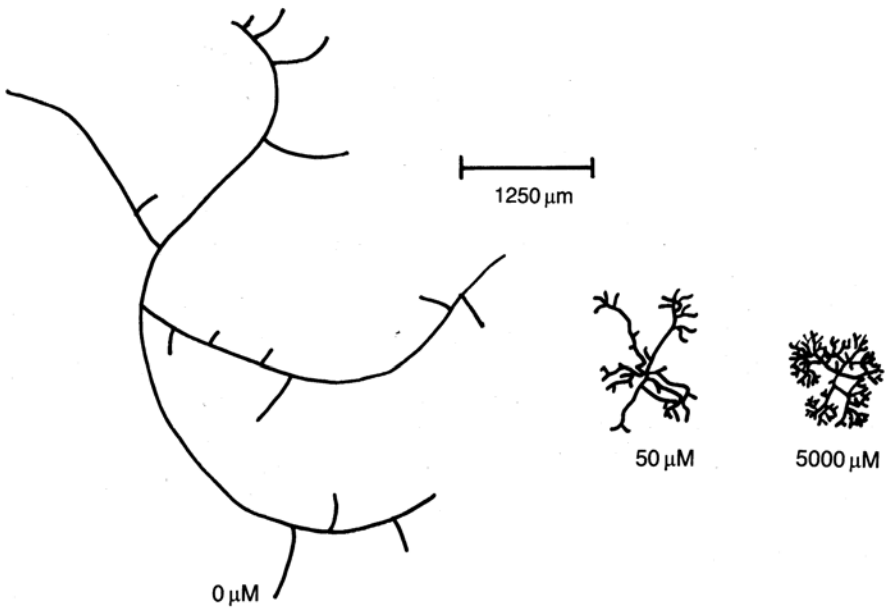




**Fig. 8a,b.** Effect of cyclosporin A on **a** the hyphal growth unit, and **b** the morphology of *Neurospora crassa* grown at 25 °C on glucose-mineral-salts medium. (A. Morton, A.J. Dickson, A.P.J. Trinci and G.D. Robson, previously unpublished data)

calcium-dependent manner and display a high degree of sequence homology. Although the function of annexins is still unclear, they are thought to be involved in mediating calcium-dependent fusion of vesicles to membranes (Burgoyne and Morgan 1992) and have recently been identified in yeast (Creutz et al. 1991). In addition, annexins bind to F-actin and may modulate cytoskeletal/membrane interactions. Importantly, actin is thought to play an important role in hyphal extension in fungi; the actin cap at the hyphal apex of *S. ferax* disappears along with the calcium gradient as external calcium is reduced (Jackson and Heath 1989). Thus, annexins may play a role in hyphal extension by modulating calcium-dependent vesicle fusion and/or actin assembly at the tip. Putative calcium-binding proteins have been isolated from *F. graminearum*, and these may include annexins from the membrane (G.D. Robson and A.P.J. Trinci, unpubl. data).

Studies of the effect of the immunosuppressant drug, cyclosporin A, on fungi suggest that protein phosphorylation/dephosphorylation may play an important role in hyphal extension and branching. Cyclosporin A binds to an abundant class of proteins, the cyclophilins. In eukaryotic cells, including *N. crassa*, the cyclosporin A-cyclophilin complex specifically binds to and inhibits calcineurin (Tropschug et al. 1989). In *N.*

*crassa* and *F. graminearum*, inhibition of calcineurin by cyclosporin A decreases hyphal extension and increases branching (reduces G) without affecting specific growth rate (Fig. 8a,b). These observations suggest that calmodulin-dependent protein phosphatase plays an important role in regulating mycelial morphology and further implicates calcium and calmodulin in regulating tip extension. Little is known about calcineurin substrates in mammalian cells, but one of the few substrates studied is a regulatory subunit of cAMP-dependent protein kinase (Blumenthal et al. 1986), suggesting a possible interaction between the calcium and cAMP pathways.

### D. Role of Cyclic Nucleotides

In mycelial fungi, cAMP has been implicated in a number of diverse functions including conidiation, dimorphism, blue-light responses, spore germination, circadian rhythms and hyphal extension and branching (Pall 1981; Pall and Robertson 1986; Terenzi et al. 1976; Tomes and Moreno 1990). In *N. crassa*, a number of pleiotropic effects are caused by the *crisp* mutant which lacks a functional adenylate cyclase and consequently has a low endogenous cAMP content compared to the wild type. Two effects of *crisp* are a marked reduction in hyphal extension and an increase in branching (reduced G); characteristics which can be largely reversed by the addition of exogenous cAMP. Although, compared to the wild type, the yield of *N. crassa crisp* on many carbon sources is reduced, its specific growth rate, appears, at least initially, to be only slightly affected. It seems, therefore, that cAMP may not be essential for growth, but is required for normal mycelial development. Addition of exogenous cAMP to wild-type *N. crassa* and *F. graminearum* also decreases hyphal extension and increases branching (Mishra 1976; Robson et al. 1991d). Thus, both a decreased cAMP level (*crisp*) and an increased cAMP level (addition of exogenous cAMP) induce a colonial phenotype indicating a role for cAMP in regulating hyphal extension and branching in mycelial fungi. cAMP-dependent protein kinase (kinase A) has been identified in a number of fungi, although the identity of the proteins phosphorylated has yet to be established and the function of kinase A in fungi is unclear. However, when the gene encoding a temperature-sensitive colonial (highly branched) mutant of *N. crassa*, *cot-1*, was isolated and sequenced, it was found to be homologous with the catalytic subunit of mammalian kinase A (Yarden et al. 1993). Thus, at non-permissive temperatures, kinase A activity is reduced, suggesting that the requirement for cAMP for normal mycelial growth observed in *crisp* may reflect a requirement for active kinase A. A number of suppressor mutants of *crisp* and *cot-1* have been isolated (Garnjobst and Tatum 1970; Perkins et al. 1982), and studies of these mutants should provide information about the regulatory roles of cAMP and kinase A in hyphal extension and branching.

In mammalian systems, the calcium and cAMP pathways interact (Rasmussen 1981), and this may also occur in fungi. Both CAM-dependent adenylate cyclase and phosphodiesterase have been detected in *N. crassa* (Ortega-Perez et al. 1983; Reig et al. 1984; Tellez-Inon et al. 1985) and, as mentioned above, in mammalian cells, the CAM-dependent protein phosphatase, calcineurin, is known to act on the regulatory subunit of protein kinase A. In yeast, activation of protein kinase A by cAMP activates both phosphatidylinositol kinase and phosphatidylinositol-4-phosphate kinase, suggesting that the cAMP pathway interacts with an early step in the calcium pathway (Hiroyaki et al. 1989).

If little is known about the function of cAMP in fungi, even less is known about cGMP. This nucleotide has been detected in a number of fungi but neither guanylate cyclase nor guanylate kinase activities have been reported. cGMP has been shown to increase hyphal extension and reduce branching (increase G) in *F. graminearum* (Robson et al. 1991a). In mammalian cells, guanylate cyclase is stimulated by nitric oxide, leading to vasodilation and muscle relaxation. However, addition of pharmacological agonists and antagonists of mammalian guanylate cyclase, including sodium nitroprusside and nitro-L-argine, has no effect on the morphology of *F. graminearum* (G.D. Robson and A.P.J. Trinci, unpubl. result).

## V. Restricted Growth of Fungal Mycelia

### A. Reasons for Restricted Growth

Unrestricted growth of a mycelium in batch culture will only continue provided all nutrients (including oxygen) are present in excess and pro-

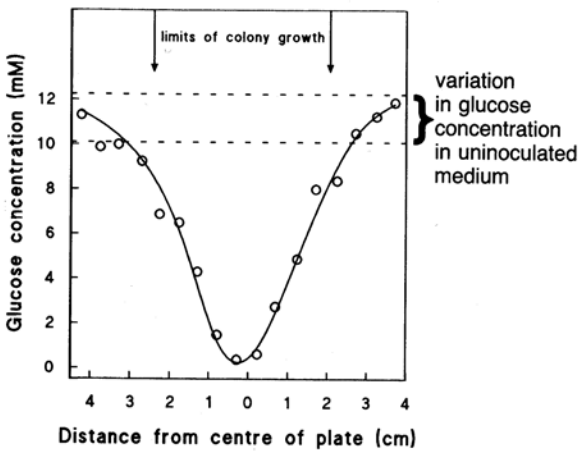



**Fig. 9.** Glucose concentration in the medium below a colony of *Rhizoctonia cerealis* grown at 25 °C on 20 ml of agar medium in a 9-cm Petri dish. (After Robson et al. 1987)

vided other factors such as pH are not altered sufficiently to affect $\mu_{max}$. Growth of a fungus on a solid medium will eventually result in the establishment of conditions below the centre of the colony which are less favourable for growth than was initially the case. Such unfavourable conditions include nutrient depletion, changes in pH, and the production of secondary metabolites inhibitory to growth. Figure 9 shows the gradient in glucose concentration established in the medium beneath and around a colony of *Rhizoctonia cerealis* grown on solid medium. The gradient results from glucose uptake by the fungus and diffusion of glucose from uncolonized to colonized parts of the substrate. Similar gradients are established for other nutrients, $O_2$ (Peters et al. 1987) and pH. The development of conditions which are less favourable for growth than those present prior to inoculation cause a deceleration from $\mu_{max}$ until eventually growth ceases (Trinci and Thurston 1976). The rate of change in conditions below a colony will be related to the density (biomass per unit surface area) of the fungal biomass supported. It follows from this that a profusely branching mycelium (low G value) will develop unfavourable conditions in the medium below the colony more rapidly than a sparsely branching mycelium (high G value). Consequently, a relationship between G and w would be anticipated, and essentially this is what is observed (Jejelowo and Trinci 1988; Trinci 1973). In *N. crassa*, the development of these unfavourable conditions is correlated with plugging of septal pores (Trinci and Collinge 1973), an event which reduces "communication" within the colony. Lack of "communication" between hyphal compartments separated by plugged septal pores is one explanation of why light-induced carotenogenesis and conidiophore growth in *Aspergillus giganteus* (Trinci and Banbury 1969) and light-induced conidiation in *Trichoderma viride* (Galun 1971) are restricted to illuminated regions of colonies and not communicated to adjacent, non-illuminated regions. Restricted growth is also brought about in some fungi by genetically programed senescence (see Chap. 11).

## B. Formation of Colonies

Hutchinson et al. (1980) used simulation of a stochastic model to demonstrate that in a fungal colony natural variability in hyphal extension rate, interbranch distance and branch angle were sufficient to produce a circular colony. Such variability will also ensure that the mean distance between leading hyphae at the colony margin remains approximately constant, allowing branch hyphae to catch up with and fill gaps between leading hyphae as the colony increases in radius.

## C. Linear Extension of Colonies

Mature colonies of fungi increase in radius at a linear rate ($K_r$). Thus,

$$R = R_0 + K_r(t_1 - t_0), \quad (6)$$

where $t_0$ is the time of onset of linear extension and $R_0$ is colony radius at time $t_0$. The relationship between $K_r$ and the organism's specific growth rate was developed by Pirt (1967) who suggested that initially, a colony would increase in radius exponentially, but that, for the reasons given above, growth at the centre of the colony would eventually decelerate and then cease. Thereafter, expansion of the colony would be the result of unrestricted growth in a ring of biomass (known as the peripheral growth zone) at the colony margin. Growth in the peripheral growth zone would occur at or close to $\mu_{max}$ for the prevailing conditions and therefore expansion (linear increase in radius) of the colony is described by the same equation [Eq. (5)] used to describe linear growth of individual hyphae (Trinci 1971). Growth may occur in the colony

centre (i.e. inside the peripheral growth zone), but this would not contribute to radial expansion of the colony. In practise, growth probably proceeds at $\mu_{max}$ only in the outer margin (with respect to the colony centre) of the peripheral growth zone.

It follows from Eq. (5) that $K_r$ can only be used to determine the effect of an environmental variable on $\mu$, provided that the variable studied does not affect w (Bull and Trinci 1977). For example, $K_r$ varies with glucose concentration because of an effect on $w$, not $\mu$ (Bull and Trinci 1977; Trinci 1969), and many compounds (Table 1) act by reducing w and extension rate, rather than affecting $\mu$. This important point is still ignored by some workers who persist in using $K_r$ to study the effects of environmental variables on fungal growth.

### D. The Duplication Cycle in Leading Hyphae at the Colony Margin

As mentioned above, mechanisms are present in moulds which regulate and integrate the synthesis of the various constituents of protoplasm, and these mechanisms are likely to be similar to those present in unicellular organisms. However, since cell separation does not occur in moulds, the term "duplication cycle" has been used to describe events during fungal growth which are analogous to those which comprise the cell cycle of uninucleate cells (Trinci 1978, 1979). Duplication cycles have been described in leading hyphae of monokaryotic (one nucleus per compartment), dikaryotic (two nuclei per compartment) and multinucleate (up to ca. 75 nuclei per compartment) apical compartments. The duration (2.1 h) of a duplication cycle in apical compartments of leading hyphae of *A. nidulans* is identical to the doubling time of the organism in liquid culture (Fiddy and Trinci 1976), and this suggests that growth of apical compartments is unrestricted. In *A. nidulans*, which has about 50 nuclei per apical compartment, the main morphological events (Fig. 10) in the duplication cycle are as follows:

1. The apical compartment is reduced to about half its original length by the formation of two to six septa at the rear of the compartment.
2. The newly formed apical compartment continues to increase in length at a linear rate and the nuclei migrate towards the hyphal tip at a slightly slower rate.
3. The volume of cytoplasm per nucleus increases until, at a critical ratio, the nuclei are induced to divide more or less synchronously – a single mitosis takes 5 min (Doonan 1992) compared with 12 min for mitosis of all 50 nuclei in the apical compartment.
4. Mitosis is followed by the formation of several (two to six) septa in the rear of the apical compartment which reduces its length by half: a round of septation occupies the final 7% of the duplication cycle. Thus, in *A. nidulans*, there is a temporal but not quantitative or spatial relationship between dividing nuclei and subsequent septation.

Fiddy and Trinci (1976) suggest that synchronous mitosis in apical hyphal compartments of

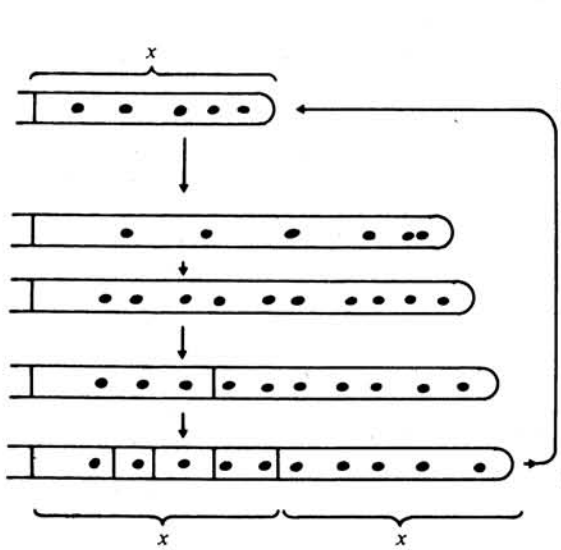



**Fig. 10.** Diagrammatic representation of the duplication cycle in a leading hypha of *A. nidulans* extending at a linear rate on solid medium. On average, completion of a duplication cycle takes 2.1 h, therefore a 0.01 fraction of the duplication cycle is equivalent to ca. 1.25 min. (Trinci 1979)

leading hyphae is regulated by a sizer mechanism similar to that observed in *Schizosaccharomyces pombe* (Nurse 1990), i.e. mitosis is triggered when the cytoplasmic volume:number of nuclei ratio exceeds a critical value (for a discussion on cell division in yeast, see Chap. 1). In *S. pombe*, the interphase to mitosis transition is dependent on a protein phosphorylation cascade, a central component of which is the $p34^{cdc2}$ protein kinase (Nurse 1990). As in *S. pombe*, temperature-sensitive mutants have been isolated (Morris 1976) to investigate the mitotic cycle of *A. nidulans*. Observations on these mutants shows that the interphase to mitosis transition in *A. nidulans* requires at least two kinase activities, one of which resembles the $p34^{cdc35}$ kinase of *S. pombe* and reaches a maximum as cells enter mitosis (Osmani et al. 1991). Genes encoding two of the regulatory subunits of this kinase have been identified, nimT which encodes a phosphotyrosine phosphatase, similar to cdc25 phosphatase of *S. pombe*, and nimE which encodes a cyclin B subunit, similar to the cdc13 cyclin of *S. pombe*. The other kinase activity, nimA, appears to act alongside the p34 kinase as a positive regulator of mitosis, as return of nimA hyphae to the permissive temperature results in synchronous mitosis within a few minutes (Oakley and Morris 1983). When multiple copies of nimA are overexpressed, hyphae of *A. nidulans* persist in mitosis, which suggests that low levels of nimA kinase is a prerequisite for exit from mitosis (Doonan 1992). Thus, the present results indicate that active nimA kinase and active p34 kinase are required for mitosis in *A. nidulans*. During mitosis, many proteins become phosphorylated, but at anaphase these phosphoproteins are dephosphorylated. *BimG* mutants fail to complete anaphase correctly and the wild-type gene has been shown to encode a protein over 80% identical to mammalian protein phosphatase (PPI). However, surprisingly, there is no apparent change in the level of PPI activity through the cell cycle (Doonan 1992).

## VI. Extension Rate of Heterokaryons

### A. Vegetative Hyphal Fusions

In fungi, fusions may occur between adjacent hyphae belonging to a single mycelium or, more rarely, between hyphae of two different strains of the same species. The latter event may result in the formation of a heterokaryon. Vegetative hyphal fusions usually develop in the old (slowly growing?) parts of a mycelium, but they have also been observed at the margin of some colonies where conditions are favourable for vegetative growth (Galun et al. 1981). Gregory (1984) has reviewed the early work on hyphal fusions and describes how they convert the mycelium into a "three-dimensional transport system". In this section we consider heterokaryons which affect hyphal extension.

### B. Growth of Heterokaryons Formed Between Highly Branched (Colonial) Mutants

Barratt and Garnjobst (1949) confirmed that in heterokaryons formed between *N. crassa* wild-type and a colonial (highly-branched) mutant, where the colonial strain contributed more nuclei than the wild-type, the extension rate of the resultant heterokaryon was slower than that of the wild-type, and was dependent on the ratio of the wild-type and mutant conidia in the inoculum. Wiebe et al. (1992) characterized 20 highly branched (colonial) mutants which arose spontaneously in glucose-limited, chemostat cultures of *Fusarium graminearum*. All the morphological mutants were recessive to the wild type, and produced colonies which expanded in radius more slowly than the wild type. Figure 11 shows the colony radial growth rates observed for heterokaryotic colonies formed from spore inocula of two of these colonial mutants (CC1-3 and MC3-2). The initial ratio of the two parental macroconidia in the mixed inoculum (to form the heterokaryon) is indicated on the x-axis and the final ratio of the two parents in conidia harvested from the heterokaryon is indicated above each datum point. The final ratio of CC1-3 to MC3-2 in the heterokaryon which had the fastest colony radial growth rate was greater than 1:800. Beadle and Coonradt (1944) suggested that the ratio of nuclei contributed to a heterokaryon from each mutant would determine whether the heterokaryon exhibited a wild-type phenotype or only a partially wild-type phenotype. Shifts in nuclear ratio have sometimes been observed in heterokaryons of *N. crassa*, and generally these changes were in a direction which resulted in the fastest extension rate (Pittenger and Atwood 1954). However, in crosses between CC1-3 and MC3-2, shifts in the nuclear ratio did not always result in

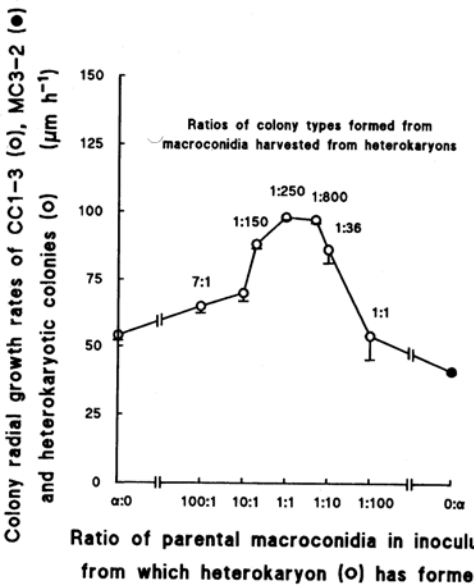



**Fig. 11.** Radial growth rates (±SE) of: CC1-3 (○) and MC3-2 (●) and heterokaryons (○) formed when macroconidia of both strains of *Fusarium graminearum* were mixed in the inoculum in the ratio of CC1-3 to MC3-2 indicated *along the x axis*. Ratios *above* the heterokaryon colony radial growth rates indicate the ratio of macroconidia of each produced from 7-day-old heterokaryons. (Wiebe et al. 1992)

optimal extension rates, although, if observations for these heterokaryons had been continued, they might eventually have reached optimal nuclear ratios, and hence maximal or wild-type $K_r$ values.

## VII. Conclusions

The mechanisms which regulate hyphal extension and direction of hyphal growth play crucial roles in ensuring that fungi colonise solid substrates efficiently and effectively. Indeed, the highly polarized nature of hyphal extension may be the main reason why fungi are so efficient at colonizing solid substrates such as soil and plants. Results have been obtained which suggest that phosphoinositides, calcium, calmodulin and cyclic nucleotides are involved in the mechanisms which regulate hyphal extension and branching but the precise nature of these mechanisms has yet to be elucidated. The existence in mycelial fungi of mechanisms to regulate and integrate the synthesis of organelles, macromolecules and enzymes is essential to the maintenance of balanced growth. That at least some of these mechanisms in filamentous fungi are similar to those present in yeast and other unicellular microorganisms is now beyond doubt. In the first Benefactors' Lecture of the British Mycological Society, P.H. Gregory (1984) wrote:

"It is now clear that biologically, the vegetative mycelium is the most important part of the fungus."

Most mycologists agree with Gregory and continue to be fascinated by the mycelium.

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# The Mycota

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## I *Growth, Differentiation and Sexuality*

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