PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Growth and Form in Lower Plants and the Occurence of Meristems

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Growth and form in lower plants and the occurrence of meristems

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Fungi and streptomycetes have a similar morphology and in both groups branching appears to be regulated in a similar manner. Both types of hyphae grow by tip extension but streptomycete hyphae never attain the extension rates commonly observed for fungi. Fungal hyphae are able to attain high rates of extension because a very large volume of protoplasm contributes to tip growth and because a vesicular growth system facilitates the rapid assembly of the tip wall. Growth of fungal and streptomycete mycelia involves the duplication of a physiological unit of growth which consists of a tip and a portion of hypha whose average length remains constant. However, it is not clear that growth of such mycelia is truly modular. Although hyphal fusions within a mycelium are common in higher fungi their significance in the organism's life style is not known. Growth in lower green plants, especially algae, is considered and the question of whether coenocytic algae are modular or not is discussed.

Introduction

Although hyphae of fungi (3–10 μm in diameter, but occasionally 100 μm) and streptomycetes (0.5–1.5 μm in diameter) differ in diameter, these eukaryotic and prokaryotic organisms produce mycelia which have a very similar morphology (Oliver & Trinci 1985). In both groups, hyphae grow radially outwards from the centre of the colony, branch regularly and execute 'avoidance' reactions. Mycelial growth appears to be an adaptation to life in heterogeneous environments, particularly soil and, as mentioned by Pfenning (1984), this view is supported by the example of convergent evolution in the life styles of the prokaryotic streptomycetes and the eukaryotic fungi. This contribution will describe the growth of hyphae and the formation of mycelia and, where possible, comparisons will be made between fungi and streptomycetes, and algae. Some aspects of growth in the algae and other lower green plants will also be compared.

Apical wall growth of fungal and streptomycete hyphae

It is well established that fungal hyphae only increase in length by apical extension. The length of the zone involved in extension may be determined either by depositing markers onto the tip and following their subsequent displacement (Castle 1959), or by measuring the distance from the tip to the point at which the hypha first attains its maximum diameter (Trinci & Halford 1975). The latter method was used to show that the extension rate of *Phycomyces* sporangiophores (figure 1) was directly related to extension zone length (Trinci & Halford 1975).

Several attempts have been made to explain tip growth in fungi. Reinhardt (1892) suggested that the tip wall is rigid and that a gradient in the rate of intussusception of new wall material determines the specific growth rate of the surface area of the wall and hence determines tip shape.

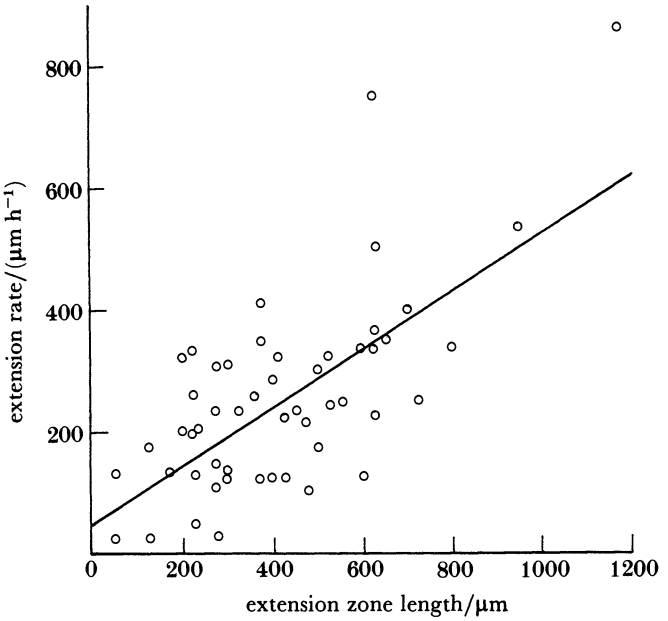

FIGURE 1. Relation between the extension rate of stage I sporangiophores of *Phycomyces blakesleeanus* and the length of their extension zones. The correlation coefficient (+0.70) between the rate of extension and extension zone length is highly significant ($p < 0.001$). (Redrawn from Trinci & Halford 1975.)

The 'unitary' growth hypothesis of Bartnicki-Garcia (1973) provided a mechanism to explain how new wall material might be inserted into the rigid tip wall. He proposed that tip extension involves the combined action of enzymes that hydrolyse wall polymers, for example, chitinases and glucanases, and enzymes that synthesize wall polymers, for example, chitin synthase and glucan synthases. However, unlike Reinhardt's model, Bartnicki-Garcia's hypothesis suggests that turgor pressure plays a role in hyphal extension by forcing apart the ends of microfibrils severed by lytic enzymes. Evidence that lytic enzymes may be involved in wall growth is provided by Rosenberger (1979) and Humphreys & Gooday (1984). The latter workers isolated a microsomal chitinase from *Mucor mucedo* that was apparently membrane-bound and which, like chitin synthase in the same organism, was activated by proteases. Humphreys & Gooday suggest that chitinase and chitin synthase may be regulated in concert during hyphal growth. However, as with most previous reports of the possible involvement of lytic enzymes in tip extension, no evidence is presented that allows a discrimination to be made between the action of chitinase in tip growth and its action in branch initiation. This evidence is provided by Kritzman *et al.* (1978) who used an immunofluorescent technique to demonstrate the presence of β-(1,3)-glucanase at the tips of hyphae of *Sclerotium rolfsii*. In contrast to the models of Reinhardt and Bartnicki-Garcia, Robertson (1959) proposed that tip extension involves a balance between wall synthesis and wall rigidification. The involvement of lytic enzymes in tip growth is not implicit in Robertson's model.

Radioautography can be used to determine the sites at which hyphae form wall polymers. *N*-[^{3}H]acetyl D-glucosamine is a precursor which is rapidly incorporated into chitin in fungal hyphae and into peptidoglycan in streptomycete hyphae; hyphae need only be exposed to this compound for 1 min before being processed for radioautography. The highly polarized nature of the synthesis of these structural polymers is shown in figure 2. Extraction of hyphae (with

water at 100 °C or 1 M KOH at 60 °C) of *Schizophyllum commune*, which had been exposed to *N*-[^{3}H]acetyl D-glucosamine for 10 min before preparing radioautographs, demonstrated that chitin formed at the tip was present in an insoluble form (Sonnenberg 1984). It had previously been shown that the tips of fungal hyphae were always covered with chitin microfibrils (Hunsley & Burnett 1970). By contrast, when growing hyphae of *S. commune* were exposed to [^{3}H]glucose for 10 min, most of the label at the hyphal tip was present as water- or alkali-soluble glucan and very little was present as alkali-insoluble glucan (Sonnenberg 1984). Sonnenberg (1984) used pulse-chase experiments to demonstrate that glucans synthesized at the hyphal apex of *S. commune* moved subapically at a rate (7 μm h^{-1}) that was approximately the same as the rate of hyphal extension.

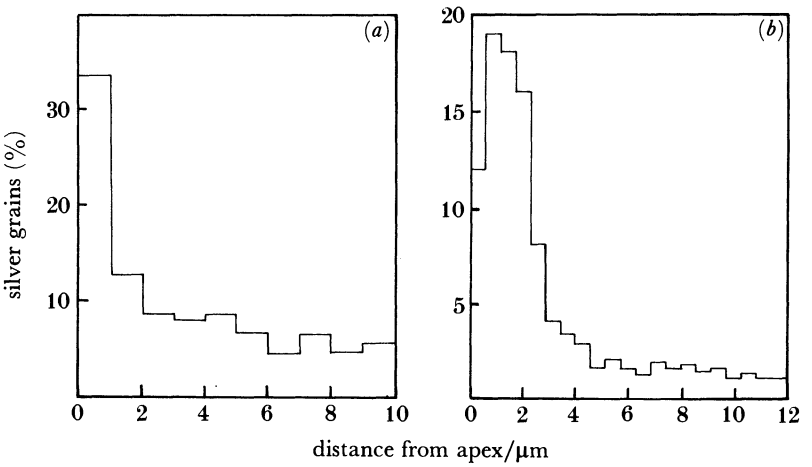

FIGURE 2. Wall synthesis in hyphae of (*a*) *Streptomyces antibioticus* (redrawn from Braña *et al.* 1982) and (*b*) *Schizophyllum commune* (redrawn from Sonnenberg 1984). The *Streptomyces antibioticus* hypha was incubated in *N*-[^{3}H]acetylglucosamine for 1 min. The *S. commune* hypha was incubated in *N*-[^{3}H]acetylglucosamine for 10 min and the radioactivity was then chased with non-radioactive medium.

Braña *et al.* (1982) used electron microscopy to determine the distribution of label along *Streptomyces antibioticus* hyphae and found that after exposure to *N*-[^{3}H]acetyl D-glucosamine for 1 min the highest frequency (30 % of the total silver grains counted) occurred within the first 1 μm of the hypha (figure 2). The pattern of apical wall growth found in *Streptomyces antibioticus* was very similar to that observed in fungi (figure 2 and Gooday 1971) and this indicates that primary wall growth in both groups occurs preferentially in apical regions of hyphae.

WALL RIGIDIFICATION IN FUNGAL HYPHAE

The shape and length of the extension zone of a hypha growing at a linear rate remain constant with time, as does the radius of the hypha at the base of the extension zone. Steele & Trinci (1975) proposed that the period (called the extension zone expansion time) required for a hypha to expand from its minimum to its maximum radius is an inverse measure of the rate at which newly formed wall becomes rigidified. They found that although the extension zones of *Neurospora crassa* hyphae varied from 2.2 to 29.2 μm in length, all hyphae had approximately the same extension zone expansion time (42–54 s). They concluded that wall material inserted into the tip remains plastic for only a relatively short period of time. Although

Robertson (1968) suggested that wall rigidification might involve wall thickening, Trinci & Collinge (1975) failed to detect any difference between the thickness of the wall within and just below the extension zone.

Wessels *et al.* (1983) and Sonnenberg (1984) have advanced a model for tip growth in *S. commune* hyphae which is based upon the hypothesis of Robertson (1959). An important feature of this hypothesis is that synthesis and assembly of wall polymers are separate processes. In particular, it is suggested that (1,3)-β-glucan and chitin are inserted into the plastic extension zone wall as separate components which rapidly become cross-linked, and that wall rigidification is associated with this cross-linking process. The enzyme or enzymes that effect the coupling of the β-glucan to chitin must operate outside the protoplasmic membrane because one of the substrates (chitin) is not formed within the cytoplasm. Cross-linking probably occurs concurrently with crystallization of the chitin and glucan chains to produce the β-glucan–chitin complex that is found in the 'rigid' subapical parts of the wall (Sietsma & Wessels 1977, 1979); both components tend to form hydrogen bonds among themselves. Chitin is present at the tip in an insoluble form. However, since nascent chitin is particularly sensitive to chitinase and acid (Gooday & Trinci 1980; Lopez-Romero *et al.* 1978; Schneider & Seaman 1982; Vermeulen & Wessels 1984), at least some of the chitin in the extension zone may be present in an amorphous condition and thus may be available for cross-linking with other wall polymers.

The conclusion that wall growth and wall rigidification are independent processes is supported by Robertson's (1958) observation that when extension growth of a hypha of *Fusarium oxysporum* was stopped by an osmotic shock, the entire extension zone became rigidified within about 40 s. Similarly, Sonnenberg (1984) found that cross-linking between chitin and β-glucan occurred at the apex of non-growing hyphae of *S. commune* so that eventually the whole apex became rigidified. The results of Robertson, Sonnenberg and Steele & Trinci suggest that wall rigidification is a time-dependent rather than a growth-dependent process.

Extension rates of fungal and streptomycete hyphae

Streptomycete hyphae never attain the high rates of extension attained by fungal hyphae. For example, hyphae of *Streptomyces coelicolor* extend at 30 °C at a maximal rate of *ca.* 30 μm h^{-1} (E. J. Allan & J. I. Prosser, personal communication) while hyphae of *N. crassa* SY7A extend at 25 °C at a maximal rate of *ca.* 2300 μm h^{-1} (Steele & Trinci 1975). Thus, although convergent evolution has resulted in the production by streptomycetes and fungi of mycelia that are morphologically similar, the constituent hyphae of these mycelia extend at quite different rates. This difference cannot be accounted for in terms of a difference in specific growth rate, since *S. coelicolor* has a specific growth rate of 0.26 h^{-1} (Allan & Prosser 1983) and *N. crassa* SY7A has a specific growth rate of 0.27 h^{-1} (Trinci 1973*a*). The difference in the extension rates of streptomycete and fungal hyphae can be attributed to two aspects of their growth. First, the volume of protoplasm contributing to hyphal extension in fungi is much greater than that contributing to the extension of streptomycete hyphae; the volume of the peripheral growth zone (the region of a hypha contributing to tip extension (Trinci 1971)) of a leading hypha of *N. crassa* SYR-17-3A extending at a linear rate is about 260×10^3 μm^3 (calculated from data in Trinci (1973*a*)) while the equivalent zone in *S. coelicolor* is only about 80 μm^3. The terminal 6.8 mm (Trinci 1973*a*) of an *N. crassa* hypha can contribute to tip extension because protoplasm formed in intercalary compartments can migrate towards the hyphal apex via pores

present in the septa. Such migration is not possible in streptomycete hyphae because their septa lack pores; in streptomycetes the maximum length of hypha contributing to tip extension is less than *ca.* 100 μm. Some fungi (Fiddy & Trinci 1976*a*; Valla 1984) resemble streptomycetes in that tip extension is largely or wholly supported by the apical compartment. In other fungi the volume of protoplasm in a growing hypha remains virtually constant and migrates forward at the same rate as tip extension (Robinow 1963; Heath & Heath 1978; Gow & Gooday 1984). The second important difference is that extension of a fungal hypha, unlike that of a streptomycete hypha, involves the fusion of vesicles with the tip wall. Thus, extension rates of up to 100 μm min^{-1} can be achieved in fungi because a large volume of protoplasm synthesizes membrane and cell wall precursors that are transported to the hyphal tip where they are rapidly integrated with the existing protoplasmic membrane and wall. Vesicular systems of tip growth may be a way of ensuring that expansion of membrane and wall are closely integrated and similar systems are involved in apical growth of root hairs (Bonnett & Newcomb 1966), pollen tubes (Rosen 1964) and algal filaments (Sievers 1965; Otto & Brown 1974).

In algae such as *Vaucheria*, in which typical tip growth occurs (Kataoka 1975*a*), new regions of wall growth can be induced by unilateral light, leading to bending (Kataoka 1975*b*), or by illumination with blue light just below the apex, leading to branching (Kataoka 1975*b*; Aberg 1978). The lobes of desmids such as *Micrasterias* also exemplify tip growth (Kallio & Lehtonen 1981). By using a fluorescent indicator, it has recently been shown that each area of growth that will give rise to a lobe can be identified by accumulation of membrane-bound calcium (Meindl 1982). These results indicate that the template for the complex pattern of the *Micrasterias* cell lies in the plasma membrane, and support Kiermayer's (1981) view that the incorporation of vesicles into the plasma membrane depends on a membrane-recognition process between vesicle and plasma membranes. Meindl considers that this mechanism may be mediated by membrane-bound calcium.

Microvesicles, called chitosomes, have been isolated from a wide range of fungi (Bracker *et al.* 1976; Bartnicki-Garcia *et al.* 1979). Chitosomes are 40–70 nm in diameter and serve as conveyors of chitin synthase zymogen from its point of synthesis to the region of apical extension. The observation that a chitin microfibril can be formed by a single chitosome indicates that a chitosome must contain sufficient chitin synthase to synthesize the many chains that make up a microfibril. Each chitin chain is presumably made by a separate chitin synthase unit and the chains collectively crystallize into a microfibril as they are synthesized. Various reports (Archer 1977; Braun & Calderone 1978; Wessels & Sietsma 1979) suggest that active chitin synthase is predominately located in the protoplasmic membrane where it would be accessible to substrates and effectors from the cytoplasm and, providing that it spanned the membrane, could 'spin out' the growing chitin chain to the wall. An aggregate of enzyme monomers could thus give rise directly to the microfibrils in the wall. Similarly, (1,3)-β-glucan synthase has been shown to be associated with the protoplasmic membrane of *Saprolegnia monoica* (Girard & Fèvre 1984). However, Bartnicki-Garcia *et al.* (1984) have shown that the protoplasmic membrane fraction isolated from the slime mutant of *N. crassa* contained only a small portion of the chitin synthase of this fungus. This group of workers believe that the bulk of chitin synthase in growing fungi is in chitosomes rather than in the protoplasmic membrane and they think that chitosomes may be released into the wall (Bartnicki-Garcia *et al.* 1979).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Polarity

Transcellular electrical currents have been shown to occur in many eukaryotes and electrical currents have been demonstrated around the tips of extending hyphae and rhizoids of various fungi (Kropf *et al.* 1983; Armbruster & Weisenseel 1983; Gow 1984; Stump *et al.* 1980; Horwitz *et al.* 1984) and algal filaments (Weisenseel & Kichereer 1981). The direction of flow of the electrical current (*ca.* 0.25–0.30 μA cm^{-2} at the tip) in *Achlya bisexualis* was normally inward for the apical 350 μm of the hypha and outward further back, and the net inward and outward currents were approximately equal (figure 3). These measurements were made approximately

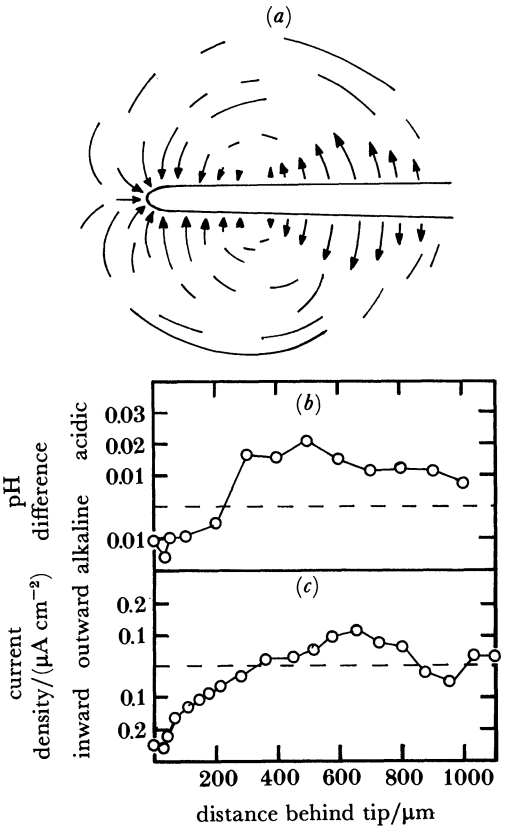

Figure 3. Transhyphal electrical current in hyphae of *Achlya bisexualis* and longitudinal pH gradient in the surrounding medium. (*a*) Pattern of transhyphal ion–electric current around the hypha; (*b*) extracellular pH profile in medium adjacent to the hypha; (*c*) electrical current profile of the same hypha. (Redrawn from Gow (1984) and Gow *et al.* (1984).)

30 μm from the hyphae, thus current density at the membrane surface may be considerably greater than the recorded values. The pH of the medium immediately external to the tips of extending hyphae of *A. bisexualis* was always alkaline (on average by about 0.022 pH units) with respect to the bulk, unbuffered culture medium, but the pH of the medium adjacent to segments of hyphae located 250–1000 μm from the tip was on average about 0.028 pH units more acidic than the bulk medium. The average location of maximum alkalinization (*ca.* 38 μm behind the apex) around an extending hypha corresponded approximately to the region (*ca.* 30–60 μm behind the apex) of maximum inward current, and the extent of alkalinization of

the medium around a hyphal tip was quantitatively consistent with an influx of protons at a rate given by the observed inward electrical current (Gow *et al.* 1984). However, although the zone of alkalinity around the tip matched that of inward current, the distal acidic zone extended beyond the region of outward current. Gow *et al.* (1984) suggest that some of this distal acidity results from the production of acidic metabolites such as lactic or succinic acids. Hyphae have been seen to drive currents even though they were not extending, and some hyphae have been seen to extend in the absence of electrical current flow (Kropf *et al.* 1984).

Figure 4 illustrates the hypothesis proposed by Kropf *et al.* (1984) to account for the profiles of pH and current observed around hyphae of *A. bisexualis*. They suggest that electrical current is driven through the hypha because protons are expelled by distally located proton-translocating ATPase and flow back into the tip by symport (co-transport) with amino acids. Thus, this hypothesis suggests that a fungal hypha is a spatially extended chemiosmotic system, with the nutrient symporter preferentially located in the hyphal tip. The actual distribution of symporters and ATPases is not known: symporters may be concentrated at the tip, or ATPases may be excluded from the tip, or both. Kropf *et al.* (1983) showed the transhyphal electrical current required the presence of amino acids and was abolished by raising the external pH from 6.5 to 8.5.

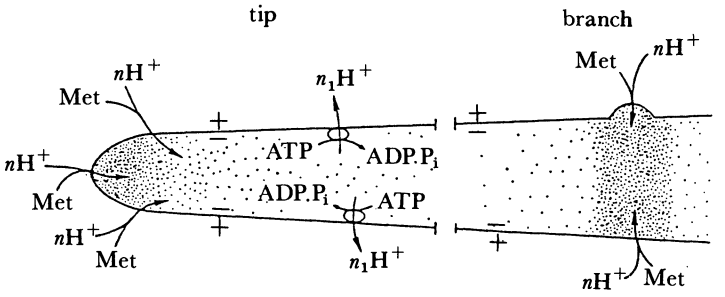

FIGURE 4. Model proposed to account for the generation of transcellular ion currents by *Achlya bisexualis*. The inward current results from symport of n protons with one methionine molecule (Met). The outward current is thought to be generated by an electrogenic ATPase that extrudes n_1 protons per ATP hydrolysed (from Kropf *et al.* 1984).

Support for the hypothesis shown in figure 4 is provided by the work of Slayman and others. Slayman (1965*a*, *b*) inserted microelectrodes into hyphae of *N. crassa* and found that they maintained a membrane potential of approximately −200 mV. Most of the potential was indifferent to K^+ diffusion and extremely sensitive to inhibition of mitochondrial respiration. The potential was subsequently shown to be generated by an electrogenic, proton-translocating ATPase which was plugged through the protoplasmic membrane (Slayman *et al.* 1973; Scarborough 1976). In *N. crassa* the proton current generated by the ATPase drives the accumulation of various nutrients such as glucose (Slayman & Slayman 1974), phosphate and ammonium ion (Slayman 1977). The proton-translocating ATPase of the protoplasmic membrane of eukaryotes appears to be distinct from that of mitochondria (Harold 1982).

Other eukaryotic organisms that form tip growing filaments have also been shown to drive transcellular currents. These include germinating algal eggs (Nuccitelli & Jaffe 1974), root hairs (Weisenseel *et al.* 1979) and pollen tubes (Weisenseel *et al.* 1975). The transcellular currents generated in these systems are similar to those observed in fungal hyphae, that is, the current is oriented in the same axis of polarity as the cells, with positive current entering the growing apex and leaving further back.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

H. MacKinnon & N. A. R. Gow (personal communication) have shown that *N. crassa*, like zygotes of *Fucus* (Peng & Jaffe 1976), spores of *Equisetum* and *Funaria* (Bentrup 1968; Chen & Jaffe 1979) and pollen grains (Marsh & Beams 1945), became oriented in external electrical fields. They found that when grown in electrical fields of 20–40 mV per cell, spores and mycelia of *N. crassa*, respectively, formed germ tubes and branches preferentially on sides facing the anode, and growing hyphae became preferentially oriented towards the anode. MacKinnon & Gow suggest that (since under normal conditions the hyphal tip is electropositive) growth tends to occur preferentially towards the positive poles of both endogenous and applied fields.

Jaffe (1977) has suggested that transcellular currents in filamentous systems may constitute a force that directs cell constituents (vesicles?), either by electrophoresis of charged vesicles within the cytoplasm or of particles within the fluid phase of the membrane, to sites of wall synthesis. Evidence for self-electrophoresis as a mechanism of growth localization is provided by studies of the oocyte-nurse cell syncytium of *Hyalophora cecropia* (Woodruff & Telfer 1980; DeLoofe 1983).

By using eggs of *Pelvetia* and labelled calcium, Robinson & Jaffe (1975) showed that a substantial calcium current passed through the cells while they were being polarized by unilateral light. Fields of 0.1 V cm^{-1} or more could be produced. During the induction of rhizoids by light, membrane components that are involved in the inward movement of ions are translocated to the dark side of the cell, leading to a localized increase in intracellular Ca^{2+} (Quatrano 1978). One means of controlling the redistribution of macromolecules, etc., might be by electrophoretic segregation of particles (Quatrano 1978).

Local application of a calcium ionophore tends to establish the rhizoid on that side. The drug is thought to act by rendering the membrane leaky to calcium ions. Enhanced localized entry of calcium ions may be part of the positive feedback loop that establishes the rhizoid pole (see Jaffe 1982).

Polarity may be established, however, not just in tip-growing regions, but in every cell of a multicellular filament. By refinement of earlier work on *Griffithsia* and *Cladophora*, Duffield *et al.* (1972) excised and cultured single intercalary cells of the filamentous red alga, *Griffithsia*, and showed that within a few days such cells regenerated a shoot cell at the original apical end, and a rhizoidal cell at the base. These experiments suggest that each cell of a filament is polarized, perhaps because of its position with respect to a longitudinal gradient.

Growth of mycelia formed by fungi and streptomycetes

When cultured on a solid medium under conditions that support unrestricted growth (Righelato 1975), a spore of *Geotrichum candidum* or a hyphal fragment of *Coprinus cinereus* forms a mycelium whose total hyphal length and number of branches increase exponentially at approximately the same specific rate (Trinci 1974; Butler 1984). The ratio between the total hyphal length of a *G. candidum* mycelium and its number of branches eventually attains a value (G, the hyphal growth unit) which remains approximately constant (*ca.* 110 μm) as the mycelium increases in size. Hyphal growth unit length is strain- (figure 5) and species-specific and in fungi values ranging from 35 μm (*Cunninghamella* sp.) to 602 μm (*Fusarium avenaceum*) have been observed (Bull & Trinci 1977).

In *Achlya bisexualis*, a new zone of inward electrical current precedes the emergence of a branch by about 20 min and predicts its location, and during branch initiation the intensity of the

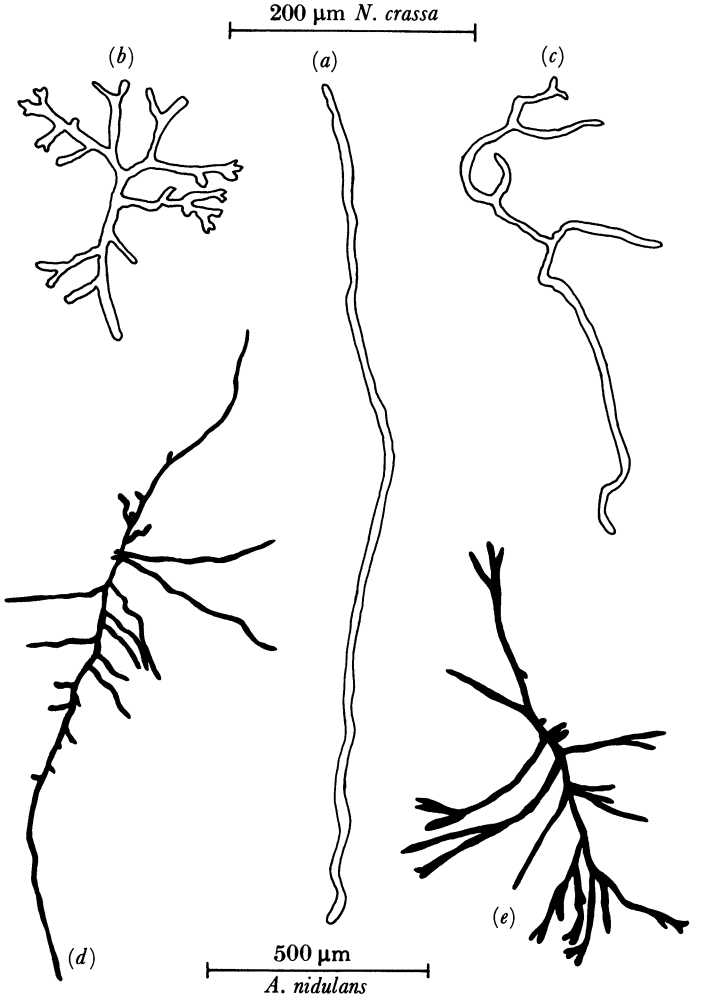

FIGURE 5. Mycelia of *Neurospora crassa* ((*a*)–(*c*)) and *Aspergillus nidulans* ((*d*) and (*e*)) grown at 25 and 37 °C, respectively. (*a*) *N. crassa* SYR-17-3A; wild type strain; (*b*) *N. crassa spco* 12; spreading colonial mutant; (*c*) *N. crassa spco* 1; spreading colonial mutant; (*d*) *A. nidulans* parental strain; lateral branches; (*e*) *A. nidulans sep* A2; lateral and dichotomous branches. (*a*)–(*c*) Redrawn from Trinci (1973*b*); (*d*) and (*e*) redrawn from Trinci & Morris (1979).)

inward current at the tip of the parent hypha diminishes and sometimes reverses. Despite these changes in current intensity and current direction before and during branch emergence, the tip of the parent hypha continues to elongate at a constant rate (Kropf *et al.* 1983). By contrast, there is no detectable alteration in the pH profile of medium adjacent to hyphae which are undergoing branching (Gow *et al.* 1984); it is possible that metabolic acid (see above) swamps the small proton influx produced as a result of branch initiation. Kropf *et al.* (1983) suggest that reversal of the electrical current during branching of *A. bisexualis* may be due to the presence of other ion fluxes that mask the inward current carried by protons. Kropf *et al.* (1984) propose that symporters for methionine and other amino acids are concentrated at a new branch point (figure 4). Future sites of out-growth of rhizoids from *Pelvetia* zygotes (Nuccitelli 1978) and pollen tubes from pollen grains (Weisenseel *et al.* 1975) are also preceded and predicted by a localized influx of electrical current, suggesting that the generation of a transcellular current is also a very early event in the establishment of cell polarity in these organisms; in *Pelvetia*

and in pollen tubes the inward current is carried in part by calcium and potassium ions, respectively (Nuccitelli 1978; Weisenseel & Jaffe 1976).

Although mycelial growth of *Streptomyces hygroscopicus* (Schuhmann & Bergter 1976) and *S. coelicolor* (Allan & Prosser 1983) is biphasic, their growth kinetics resemble those of *G. candidum*, namely, the total hyphal length of the mycelium and its number of branches increase exponentially at approximately the same specific rate. However, hyphal growth unit lengths recorded for streptomycete mycelia lie at one extreme of the range observed for fungi, for example, *S. coelicolor* has a hyphal growth unit of *ca.* 32 μm (Allan & Prosser 1983). Thus, when all nutrients are present in excess and no inhibitors are formed, growth of fungal and streptomycete mycelia may be considered in terms of the duplication of a hypothetical growth unit which consists of a tip and a certain length of hypha. However, the portion of hyphae actually supporting growth of a tip will vary from a very short length just after branch initiation to a maximum length when the hypha attains the linear growth rate characteristic of the conditions and strain.

If the mean radius of the constituent hypha of a mycelium remains constant as the mycelium increases in size, the volume (and mass?) as well as the length of the hyphal growth unit will remain constant. However, although the mean radius of hyphae in young mycelia of most fungi does remain approximately constant, the radius of hyphae of the temperature-sensitive mutant, *A. nidulans sep* A2 varies with temperature (Trinci & Morris 1979). At permissive temperatures septa are formed and lateral branches are produced by *sep* A2. However, at 37 °C (the restrictive temperature) no septa are formed, hyphal radius is increased and dichotomous as well as lateral branches are formed (figure 5). Although mycelia of *sep* A2 have a shorter hyphal growth unit at 37 °C than at permissive temperatures, the volume of protoplasm per tip is approximately the same at 37 °C as at permissive temperatures (Trinci 1984). This result suggests that branching is regulated by the changes in protoplasmic volume (mass?) that accompany growth. When Robinson & Smith (1979) grew *G. candidum* in glucose-limited chemostat culture, they found that hyphal diameter increased with increase in dilution rate, hyphal growth unit length decreased with increase in dilution rate but hyphal growth unit volume remained approximately constant (figure 6). Thus, under conditions of restricted growth in chemostat culture, growth of *G. candidum* involves the duplication of a growth unit whose volume (and mass?) remains constant but whose length and diameter vary with dilution rate.

Riesenberger & Bergter (1979) grew *Streptomyces hygroscopicus* at different dilution rates in glucose-limited chemostat culture and obtained results that were similar to those reported by Robinson & Smith (1979) for *G. candidum* (figure 6). Thus, branch initiation in fungi and streptomycetes is regulated by a mechanism which involves the organism monitoring its own volume (mass?). A similar 'sizer' mechanism has been invoked by Nurse (1981) to explain the regulation of DNA synthesis and mitosis in *Schizosaccharomyces pombe*, and by Fiddy & Trinci (1976*b*) to explain the regulation of mitosis in *A. nidulans*.

Colony growth

Exponential growth of a mycelium will only continue provided that all nutrients (including oxygen) are present in excess, the pH of the medium does not become inhibitory for growth and no inhibitory substances are produced as a result of growth. Growth on a solid substrate will eventually result in conditions at the centre of the colony becoming less favourable for growth than was initially the case. Figure 7 shows that a glucose gradient develops beneath

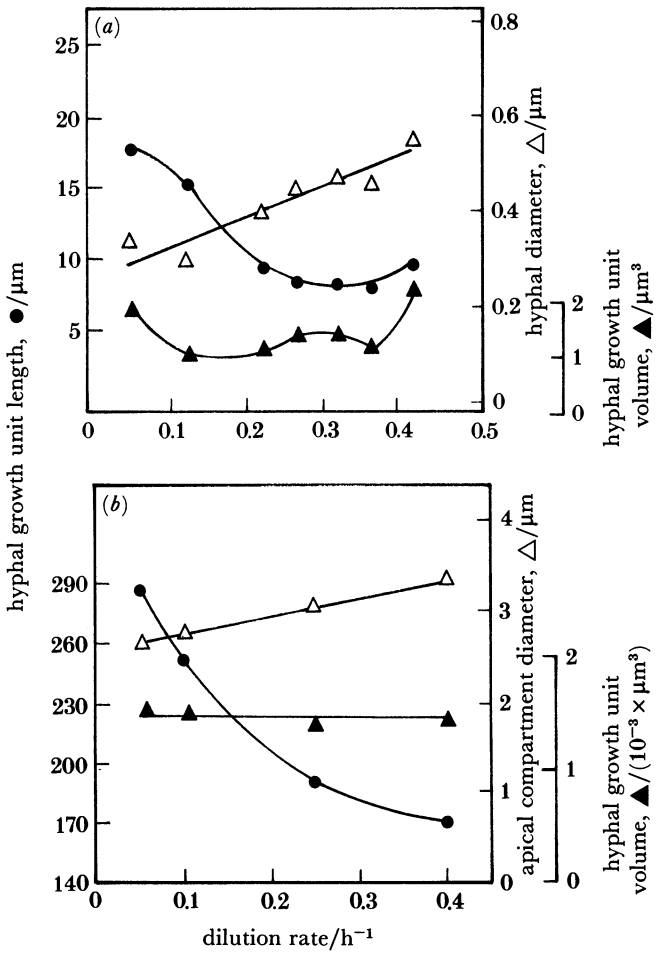

FIGURE 6. Effect of dilution rate on hyphal growth unit length, hyphal growth unit volume and hyphal diameter of mycelia of (*a*) *Streptomyces hygroscopicus* (redrawn from Riesenberg & Bergter (1979)) and (*b*) *Geotrichum candidum* (redrawn from Robinson & Smith (1979)).

and around *Rhizoctonia* colonies cultured on medium containing 10 μM glucose. This gradient is established in response to uptake of glucose by the mycelium and diffusion of glucose from uncolonized to colonized parts of the substrate. The development of conditions below the centre of a colony that are unfavourable for growth will inevitably result first in a deceleration in growth rate and eventually in a cessation of growth (Trinci & Thurston 1976). For most fungal colonies, however, the marginal hyphae will continue to extend at a linear rate so that eventually the colony may attain a diameter of several metres, for example, as in 'fairy rings'. The development of unfavourable conditions for growth at the centre of a colony is usually associated with plugging of septal pores (Trinci & Collinge 1973). This event reduces 'communication' within the colony and is associated with the induction of sporulation.

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. Hyphal fusions between two different strains may result in the formation of a heterokaryon, and subsequent operation

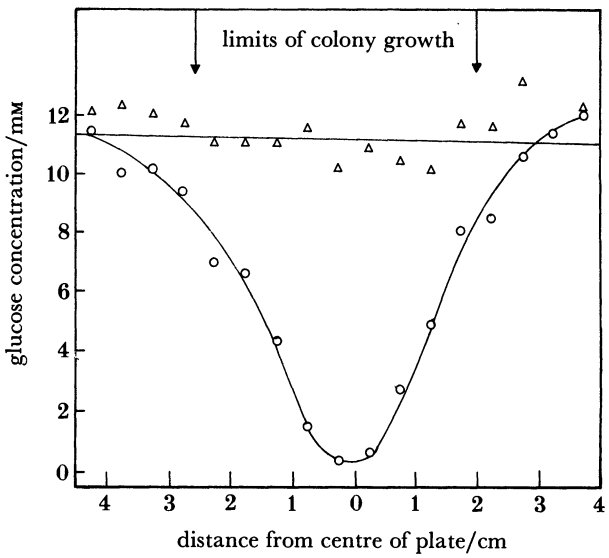

FIGURE 7. Glucose concentration in the medium below a colony of *Rhizoctonia cerealis* (○) and in the medium of an uninoculated plate of the same medium (△). (Previously unpublished data of G. D. Robson and A. P. J. Trinci.)

of the parasexual cycle (Pontecorvo 1956) in this mycelium may lead to genetic recombination. Consequently, heterokaryosis has generally been considered to be an important component of the genetic systems of fungi, particularly for fungi lacking sexual reproduction.

Vegetative hyphal fusions are common in the Ascomycotina, Basidiomycotina and Deuteromycotina, but are completely lacking in 'lower' fungi including the Zygomycotina, although gametangial fusions do occur in the latter group during sexual reproduction. 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 (Galun *et al.* 1981) where conditions are usually considered to be favourable for vegetative growth (Trinci 1971). Buller (1931, 1953) suggested that vegetative hyphal fusions only occur between growing hyphal tips, but Aylmore & Todd (1984) observed occasional fusions between the tip of one hypha of *Coriolus versicolor* and the side wall of another. When two dikaryotic hyphae of *C. versicolor* fused, the participating hyphal compartments exhibited a donor–recipient relationship with respect to the subsequent behaviour of their nuclei; the two nuclei in the recipient compartment degenerated and were replaced by daughter nuclei formed by division of the pair of nuclei in the donor compartment. After completion of this 'nuclear disintegration–replacement reaction' both compartments were able to initiate branches and undergo mitosis. Fusions of the above type were observed between genetically identical (self-fusions) and between genetically different (non-self-fusions) hyphae of *C. versicolor*.

CELL FUSION IN ALGAE

If an intercalary cell of the filamentous red alga *Griffithsia* is punctured and killed, the cell above (distal to) it develops a rhizoid, and the subjacent cell gives rise to an atypical (repair) shoot cell, with some rhizoidal characters, such as colour banding. The two new cells grow towards one another within the wall of the punctured cell, and eventually fuse, forming a functional intercalary cell (Waaland & Cleland 1974; Waaland 1984). Later, Waaland

& Watson (1980) showed that decapitated filaments would only form repair shoots when a rhizoid was present within a period of 4–7 h after decapitation. They obtained evidence of the occurrence of an endogenous hormone, rhodomorphin; this was subsequently partly purified, and identified as a glycoprotein (Watson & Waaland 1983). Since the hormone acted as an attractant between rhizoids and repair shoots, it was suggested that a comparable substance might regulate the series of complex cell fusions that occur during sexual reproduction in the red algae (Waaland & Watson 1980).

This hormone is species-specific, since fusion did not occur between cells of different species of *Griffithsia* (Waaland 1975). However, fusions were obtained between cells of male and female filaments of the same species. Most of the hybrid filaments gave rise to tetrasporangial branches typical of the diploid plant, but eventually male or female branches were formed. This suggested that these male–female hybrids functioned as a dikaryon (Waaland 1978).

Vegetative incompatibility

As mentioned above, heterokaryosis and the parasexual cycle are often considered as important components of the genetic systems of fungi. However, for several fungi it has been shown that genetically determined incompatibility systems restrict the formation of heterokaryons within a species and hence restrict genetic recombination. In *Podospora anserina* the macroscopic manifestation of a vegetative incompatibility reaction is the appearance between neighbouring mycelia of a 'barrage' or a demarcation zone, which is pigmented and consists of hyphal debris (Esser & Kuenen 1967). In *C. versicolor*, vegetative incompatibility reactions prevent the formation of the type of unit mycelium described by Burnett & Partington (1957). Instead a single piece of wood may be colonized by several genetically distinct mycelia of *C. versicolor* which do not fuse (Rayner & Todd 1977; Todd & Rayner 1978). Thus, vegetative incompatibility mechanisms help to maintain the 'individuality' of mycelia.

Rhizoctonia solani has been divided into nine anastomosis groups on the basis of hyphal fusions (anastomoses) (Parameter *et al.* 1969). Hyphae of one *R. solani* isolate will fuse with hyphae of all other isolates in the same anastomosis group, but will not fuse with the hyphae of isolates belonging to other anastomosis groups. However, successful fusions usually result in the vacuolation and death of five or six hyphal compartments on either side of the fusion compartments (Flentje *et al.* 1967). Thus, a vegetative incompatibility reaction prevents the formation of a heterokaryon even between isolates belonging to the same anastomosis group. DNA homology studies by Kuninga & Yokosawa (1984*a*, *b*) have shown that an incompatibility reaction can even occur between strains which have very high (97.6–100%) DNA homology values.

Caten (1971) showed that the frequency of intrastrain incompatibility in *Aspergillus versicolor* and *Aspergillus terreus*, two imperfect species, was similar to that observed in four perfect species (*Aspergillus amstelodami*, *Aspergillus glaucus* group, *Aspergillus heterothallicus* and *A. nidulans*). Thus, despite the added genetic significance of heterokaryosis in the absence of sexual reproduction, vegetative incompatibility is apparently no less extensive in fungi lacking sexual reproduction than in fungi with sexual reproduction.

The natural functions of vegetative incompatibility within a fungal species remain uncertain. Two possibilities exist. First, vegetative incompatibility could act as a genetic isolation mechanism so that natural populations consist of a mixture of asexually reproducing clones

(Grindle 1963*a*, *b*). Second, vegetative incompatibility may reduce the spread within a species of harmful cytoplasmic mutations and viral infections. Caten (1972) showed that the cytoplasmically inherited mutant character 'vegetative death' transferred freely between pairs of vegetative compatible isolates of *A. amstelodami* but not at all, or with a very reduced frequency, between pairs of incompatible isolates. Similarly, it has been shown that vegetative incompatibility prevents the transfer of dsRNA between isolates of *Endothia parasitica*; the dsRNA affects the morphology and virulence of this fungus (Anagnostakis & Waggoner 1981). A possible physiological benefit of hyphal fusions is that they may facilitate the transport of food reserves between various parts of a mycelium (provided that septal pores are not blocked (Trinci & Collinge 1973)) and hence they may enable the whole or large parts of the mycelium to cooperate in the formation of fruit bodies.

Meristems in lower plants

In many of the simpler algae, growth is rather diffuse and is not related to a specialized region of growth such as a meristem. Sometimes growth may be concentrated at the base of the filament, as in *Rivularia*, or at the apex, where there may or may not be a distinctive apical cell. In the filamentous brown alga *Sphacelaria*, which has an evident apical cell, the subapical cell can replace a damaged apical cell within 24 h (Ducreux 1977); this must involve considerable cytoplasmic reorganization. *Sphacelaria* also exhibits a kind of apical dominance; both the angle of the branches, and their determinate growth, are altered by damage to the apical cell. *Chara* and *Nitella* both have distinctive apical cells that give rise to cells of different function, which themselves divide to give rise to the short nodal and long internodal cells so characteristic of these organisms. The apical cell of *Dictyota* and similar algae may occasionally divide equally to give rise to equal branches of the thallus, as well as unequally to give rise to the other cells of the thallus. Larger brown algae also have apical cells that exert apical dominance (Moss 1965, 1967, 1970). Apical cells are characteristic of most bryophytes and ferns.

A type of marginal meristem occurs in the flat plate-like algae *Coleochaete* and *Padina*, and a type of intercalary meristem in the stipes of *Laminaria* and related algae. The meristoderm of these algae contributes to growth in thickness.

Apart from studies of apical meristems, however, relatively little work has been carried out on the mode of growth or its control in these meristems.

Modular organisms

To what extent do these various modes of growth in lower plants give rise to modular constructions? Mycelial organisms possess some of the properties normally associated with modular organisms. First, during unrestricted growth they are constructed from growth units that consist of a hyphal tip associated with a certain amount of protoplasm, the average volume of which remains approximately constant. Second, except during the initial stages of growth, a mycelium does not behave as an integrated whole, but instead responds to the local environmental conditions to which its constituent parts are exposed. In so doing, 'communication' within a colony becomes progressively reduced. Finally, in most mycelial organisms senescence is a local event. Provided that suitable cultural conditions are maintained, a colony

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

can continue to expand in diameter for an indefinite period of time, as witnessed by the formation of 'fairy rings'. If modules are defined as units of construction (Harper 1977), rather than as the developmental products of a single apex (White 1984), ferns, *Equisetum*, *Selaginella*, *Lycopodium* and many mosses could be regarded as modular, in various ways, in much the same way as higher plants. Some of the colonial green algae, even, might be considered to be truly modular. For example, *Pandorina* consists of similar cells all of which can form daughter colonies. However, Chapman (1981) considers that a module is a multicellular unit. By this definition, the simplest modular photosynthetic organisms might be algae such as *Nitella* or the more complex *Chara*, constructed of units comprising a node and an internode. Growth of such modules would be limited, however. On the other hand, organisms of apparently modular construction occur among the coenocytic algae, for example, *Bryopsis* and *Caulerpa*. In such cases, far from being multicellular, the module could comprise only part of a cell.

In higher plants there are usually correlative effects between modules, but this might be less likely in the case of *Caulerpa*, in which, for example, it has been shown that indoleacetic acid is uniformly distributed rather than forming a gradient (Brennan & Jacobs 1980).

The extent to which lower plants can be equated with higher plants as modular organisms thus remains debatable.

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