5 Hyphal cell biology

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The hyphal mode of growth

The dominance of filamentous fungi within the ecosystem is attributed to their common mode of growth, extending as branched filaments (hyphae) which can rapidly spread across uncolonized substrates. The success of this growth habit for exploiting the natural environment can be judged on a number of factors: the extraordinary diversity of fungal species (estimated at three million, second only to the insects), their distribution in virtually every habitat on the planet and the parallel evolution of a similar growth habit by another important class of soil microorganisms, the prokaryotic streptomycetes. Clearly the ability of a microbe to rapidly colonize new substrates by concentrating growth at its apex, is well suited for life as a heterotroph in a heterogenous environment.

Spore dormancy and germination

Spores are products of both sexual and asexual reproduction and act as units of dispersal in fungi. The majority of spores germinate to produce one or more germ tubes and a new fungal mycelium when the spore settles on an appropriate substrate under favourable environmental conditions. When a spore is faced with unfavourable conditions such as lack of nutrients, low temperature, an unfavourable pH or the presence of an inhibitor (e.g. on a plant surface), the spore remains dormant. Spores under these conditions are *exogenously dormant* and will only germinate when the environmental conditions become favourable. Some fungal species produce spores that fail to germinate immediately, even under favourable conditions because of factors within the spore such as nutrient impermeability or the presence of endogenous inhibitors. Spores

exhibiting these characteristics are termed *endogenously dormant.* Dormancy within these spores is usually broken by ageing, when nutrients can begin to enter or the endogenous inhibitors leach out, reducing the concentration within the spore.

Prior to the emergence of a germ tube, fungal spores undergo a process of swelling during which spores increase in diameter up to fourfold due the uptake of water. During this phase, the metabolic activity increases greatly and protein, DNA and RNA production all increase. This is followed by the emergence of one or more germ tubes that extend outwards from the spore in a polarized manner (Figure 5.1).

Colony formation

Following germination, the extension rate of the germ tube increases exponentially towards a maximum rate at which point the hypha attains a linear extension rate. The maximum rate of hyphal extension varies greatly between different fungal species and is also dependent on environmental conditions such as temperature, pH and nutrient availability. Before the maximum rate of extension is attained, a lateral branch is formed to produce a new growing hypha which also accelerates towards a maximum rate. As the germling continues to grow, new lateral branches are formed at an exponential rate. Although individual hyphae in the developing mycelium eventually attain a maximum linear rate, the overall growth of the mycelium is therefore exponential (Figure 5.2). During early growth, nutrients surrounding the young mycelium are in excess and the mycelium is *undifferentiated.* During undifferentiated growth, the mean rate of hyphal extension is dependent on the specific growth rate of the organism (the rate of growth per unit time) and the degree of branching. This is quantified as follows;

$$E = \mu G$$

Where E = mean hyphal extension rate ($\mu m\ h^{-1}$), μ = specific growth rate ($h - 1$) and G = hyphal growth unit (μm, the mean length of hypha associated with each branch).

As the mycelium develops further, nutrients in the centre are increasingly utilized and a zone of nutrient depletion begins to form

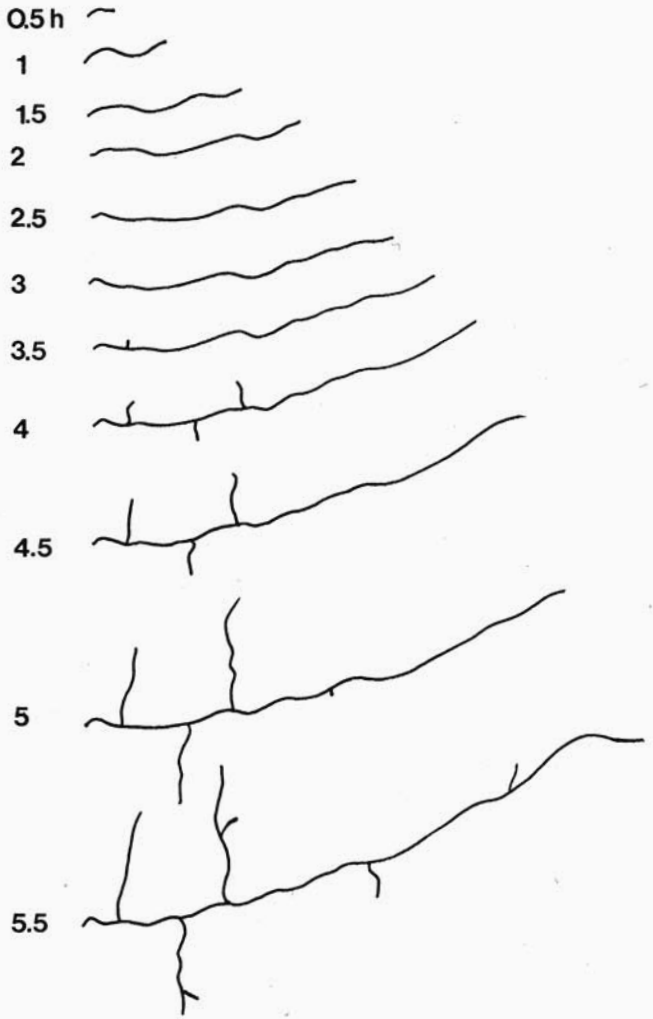


Figure 5.1 Development of a young germling over time.

beneath the colony. At this point growth at the centre of the young colony begins to decrease and as the colony expands further, ceases altogether. Therefore as the colony develops, growth becomes restricted to its periphery where nutrients are still available and the mycelium is *differentiated.* This peripheral ring, which is growing at the maximum rate, is the *peripheral growth zone.* The maximum rate of extension of

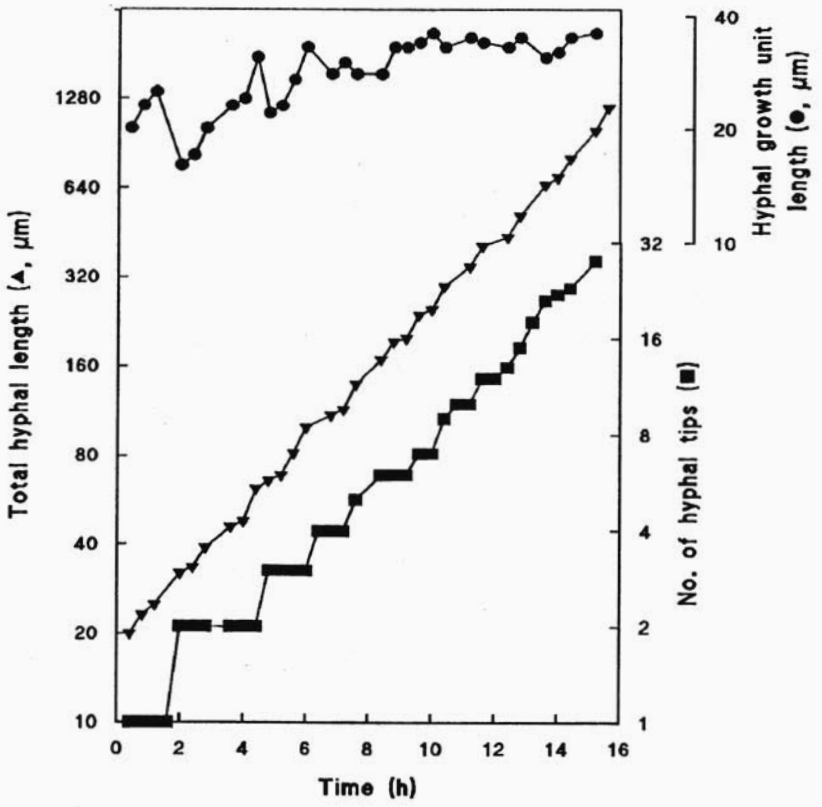


Figure 5.2 Total hyphal length, number of branches and the hyphal growth unit of a developing fungal mycelium. Notice that initially the hyphal growth unit fluctuates as new branches are formed but quickly becomes constant after more than five branches have been formed.

hyphae at the peripheral growth zone can be measured by the rate at which the colony expands. This rate or *colony radial growth rate* is dependent on the specific growth rate and the width of the peripheral growth zone and is defined as:

$$Kr = w\mu$$

where Kr is the colony radial growth rate ($\mu m\ h^{-1}$), w is the width of the peripheral growth zone (μm) and μ is the specific growth rate (h^{-1}). The fungal colony therefore grows outward radially at a linear rate, continually

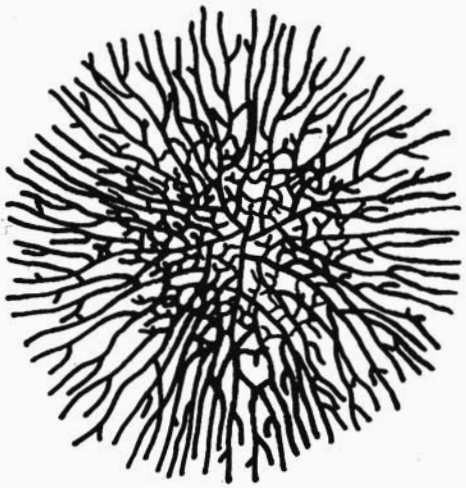


Figure 5.3 A developing young fungal colony. Notice how the growing hyphae are orientated outward into uncolonized regions whilst the production of branches ensures the mycelium efficiently colonizes available substrate.

growing into unexploited substratum. As it does so, the production of new branches ensures the efficient colonisation and utilization of the substratum (Figure 5.3). The hyphae of many fungi can alter their direction of growth to avoid growing into each other and move into uncolonized regions of the substratum. The avoidance mechanism or *autotropism* (Figure 5.4) is particularly evident at low hyphal densities, such as the margin of the growing colony. The ability of hyphae to sense the presence of another hypha is thought to be due either to a localized depletion of oxygen around the hypha, a higher concentration of carbon dioxide or the presence of a secreted metabolite.

As nutrients become depleted at the centre of the colony and metabolic products accumulate, spore production is often initiated. Therefore, different parts of the colony are at different physiological ages, with the youngest actively extending hyphae at the edge of the fungal colony and the oldest, non-growing, sporulating mycelium at the centre (Figure 5.5).

Unlike colonies formed by unicellular bacteria and yeast, where colony expansion is the result of the production of daughter cells and

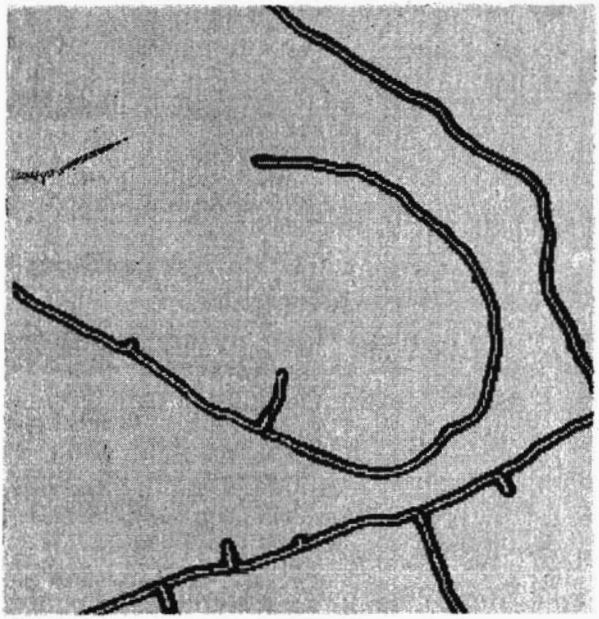


Figure 5.4 A fungal hypha has detected the presence of an adjacent hypha and reorientated its growth in an autotropic response.

Figure 5.5 Schematic representation of a growing fungal colony illustrating the gradient in physiological age from the youngest growth at the margin to the oldest mycelium at the colony centre. As the colony continues to grow, the oldest mycelium at the colony centre may produce staling products and secondary metabolites which diffuse into the agar medium and differentiate to form spore-bearing structures.

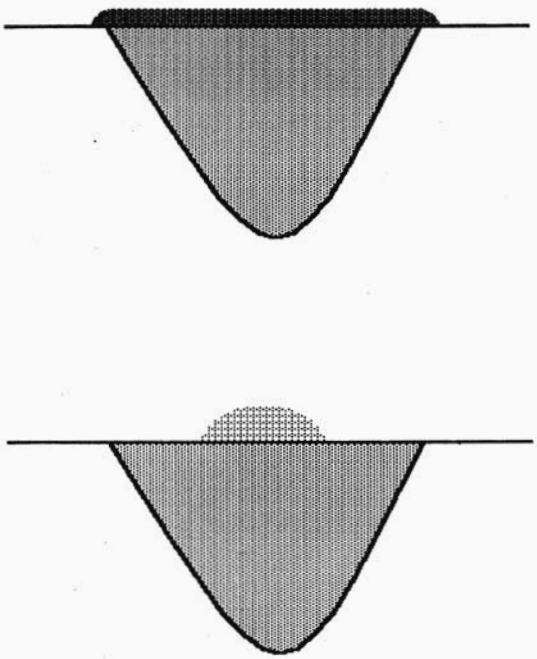


Figure 5.6 A comparison of nutrient utilization beneath a bacterial and a fungal colony. In the upper picture, the edge of the growing fungal colony advances beyond the nutrient-depleted region beneath the colony. By contrast, in the lower panel, the slower rate of expansion of a bacterial colony results in the nutrient-limited diffusion of the colony which limits the size it can attain.

occurs only slowly, the ability of filamentous fungi to concentrate all their growth capacity at the hyphal apex allows the colony to expand far more rapidly. The fungal colony therefore expands at a rate which exceeds the rate of diffusion of nutrients from the surrounding substratum. As a consequence, nutrients under the colony are rapidly exhausted whereas the hyphae at the edge of the colony have only a minor effect on the substrate concentration. By contrast, the rate of expansion of bacterial and yeast colonies is extremely slow and less than the rate of diffusion (Figure 5.6). Colonies of unicellular organisms quickly become diffusion limited and therefore, unlike fungal colonies, can only attain a finite size.

Hyphal growth

Polarized growth of fungal hyphae is achieved by restricting growth to the hyphal apex. The cell wall at the hyphal tip has viscoelastic properties and yields to the internal turgor pressure within the hypha. Further behind the tip, the wall is rigid and resistant to the turgor forces. Turgor pressure generated within the hypha therefore acts as the driving force for hyphal extension. Hyphal growth is supported by the continuous flow of vesicles generated within the cytoplasm behind the tip. The vesicles are derived from the endoplasmic reticulum and processed through Golgi bodies before migrating towards the extending apex. The vesicles are readily seen in longitudinal sections of fixed hyphae under electron microscopy and consist of two main size classes, the smallest ranging from 20 to 80 nm and the largest from 80 to 200 nm. The smaller group, which has been isolated from hyphae and shown to contain an inactive form of the major wall biosynthetic enzyme chitin synthase, are known as *chitosomes*. Fusion of the vesicles with the membrane at the hyphal apex releases the biosynthetic machinery for wall assembly and also adds new membrane to the growing hypha. Synthesis of the hyphal wall and plasma membrane are therefore coordinately regulated. The development of such a highly polarized vesicular pathway not only supports rapid hyphal extension, but also acts as a transport mechanism for extracellular enzymes. Extracellular enzymes are secreted into the environment at the hyphal tip catalysing the degradation of complex polymers such as lignocellulose. It is possible to estimate the number of vesicles that must fuse with the extending tip to support tip extension. For *Neurospora crassa*, the maximum hyphal extension rate of 25 $\mu m\ min^{-1}$ requires 38 000 vesicles to fuse with the apical membrane per minute! Despite the presence of such a highly polarized secretory system in filamentous fungi, remarkably little is known about the mechanisms by which vesicles flow in a polarized fashion to the tip, or their means of transportation. Recently, detailed studies of growing hyphae at high magnification by video-enhanced microscopy have revealed that extension is not strictly constant, but occurs as a series of cyclic pulses. The duration of the pulses is dependent upon the fungal species, varying from 2 to 15 sec (Figure 5.7).

In many, but not all filamentous fungi, an electron dense body is present in the cytoplasm just distal to the growing apex and appears to be

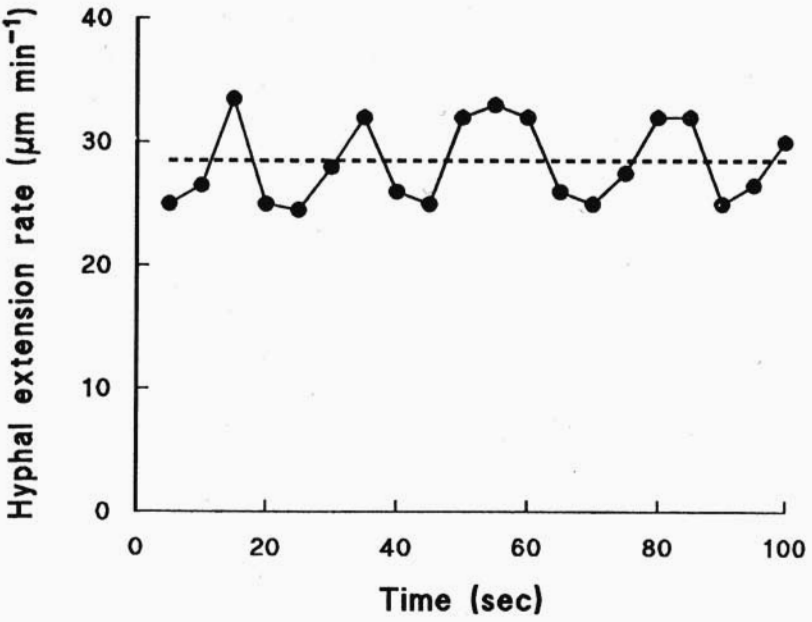


Figure 5.7 The extension rate of fungal hyphae does not attain a maximum linear rate as previously thought, but oscillates regularly around a mean giving rise to 'pulsed' growth.

intimately associated with hyphal extension and growth. This body, or *Spitzenkörper* is composed of a complex of vesicles when observed in longitudinal sections of the hypha under electron microscopy. Recent evidence has shown that the position of the *Spitzenkörper* controls both the direction of growth of the growing hyphae and the rate of extension. Satellite *Spitzenkörpers*, which appear to separate from the main body and migrate to the lateral cell wall, have been shown to precede the initiation of new branches. This has led to the concept of the *Spitzenkörper* as a *vesicle supply centre*, which acts as a focal point for the migrating apical vesicles. In this model, vesicles migrate to the *Spitzenkörper* and then radiate outwards equally in all directions. Using a computer model based on this concept, a tube with a tapering tip analogous to a growing hypha could be generated. Moreover, the direction of growth could be altered by moving the vesicle supply centre upwards or downwards, mimicking the movement observed of the *Spitzenkörper* during changes in the

direction of hyphal growth. The *Spitzenkörper* is therefore thought to play a critical role in the mechanism of polarized growth in these fungi, controlling not only hyphal extension rate and direction of growth, but also in generation of new lateral branches. Despite the evidence that the *Spitzenkörper* is involved in tip growth, it is clearly not the only way in which polarized growth can occur as lower fungi, for example *Saprolegnia ferax,* and other polarized systems, for example pollen tubes, lack a *Spitzenkörper* or equivalent body. To maintain its position behind the growing hypha, the *Spitzenkörper* must be anchored in some manner to the hyphal apex. The precise nature of this anchor is currently unknown but two classes of cytoskeletal proteins, the *microtubulins* and *actin* appear likely candidates. The microtubulins are heteropolymers of tubulin dimers containing peptides derived from different genes to form a tubulin protofilament. Thirteen protofilaments are connected laterally to form microtubule filaments, which are found in the hypha as single elements and as bundles. Microtubules are involved in nuclear division, forming the spindle pole bodies involved in chromosome separation during mitosis and meiosis and are also involved in flagellar movement of motile zoospores of fungi of the order Mastigomycotina. There is also evidence to support the role of microtubulins in the motility of organelles, including nuclei, mitochondria and vesicles within the hypha. Disruption of the microtubules with drugs such as colchicine and taxol, inhibits organelle motility and positioning and results in an abnormal distribution of microvesicles. Actin filaments are composed of an array of polymerized actin monomers that, in fungi, may be the product of a single gene. In hyphae, actin has been found located at growing tips as a fibrillar network radiating from the apex as slender cables and in localized areas as actin plaques within the cytoplasm (Figures 5.8 and 5.9). Disruption of actin also affects organelle motility and to an accumulation of microvesicles.

Recent evidence from other polarized tip growing systems such as pollen tubes, has revealed the presence of an apical gradient of *calcium* at the hyphal tip, with the concentration highest immediately below the tip. The maintenance of such a gradient is thought to be due to the presence of Ca^{2+} ion channels at the tip, allowing the flow of calcium ions down a concentration gradient from the outside to the inside of the cell. The

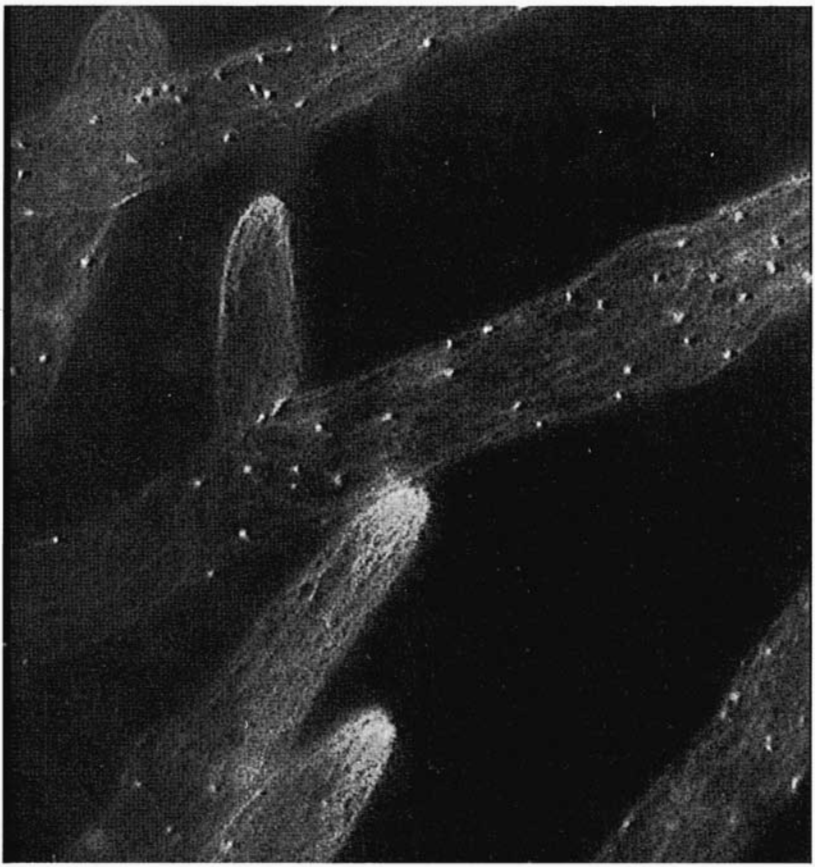


Figure 5.8 Fluorescence micrograph showing actin microfibrils concentrated at the hyphal tips and actin plaques in older regions. (From Heath, I. B. (1987) *Eur. J. Cell Biol.* 44:10–16.)

concentration of Ca^{2+} in eukaryotic cells is highly regulated and maintained at low levels in the cytoplasm by *calcium pumping ATPases.* Calcium-pumping ATPases are located on the plasma membrane, pumping calcium out of the cell and the vacuolar membranes allows sequestration and storage of the ion inside vacuoles within the hypha. Although the role and function of a calcium gradient in polarized cell growth has yet to be established, the presence of a tip high calcium gradient is likely to be an important factor in establishing cell polarity. Ca^{2+}

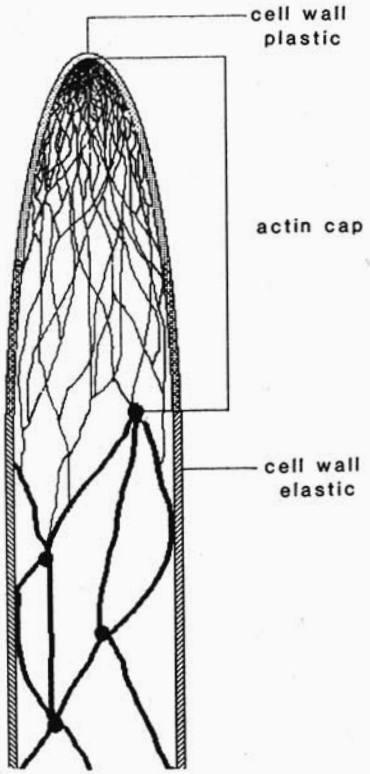


Figure 5.9 Model of actin distribution in growing hyphae. The proliferation of actin microfibrils at the tip is thought to play an essential structural role at the growing apex.

ions are known to regulate the assembly of the cytoskeleton and to aid in vesicle fusion with membranes.

Yeast mycelial dimorphism

The majority of fungi grow either as round or spherical cells (yeasts) or as a polarized branching mycelium (filamentous). However, some fungi are capable of growing as either form, depending on the environmental conditions surrounding them. Such fungi are found throughout the fungal kingdom and are termed *dimorphic*. Dimorphism is common amongst several human and animal pathogens (e.g. *Candida albicans*,

Paracoccidiodes brasilensis, Histoplasma capsulatum and *Blastomyces dermatitidis*) as well as a number of plant pathogens (e.g. *Ustilago maydis, Ophiostoma ulmi* and *Rhodosporidium sphaerocarpum*). Whilst most dimorphic fungi are members of the Ascomycotina, dimorphism also occurs in other classes (e.g. *Mucor rouxii*, a member of the Zygomycotina). *Candida albicans* is an important opportunistic pathogen of humans and the majority of research has focused on understanding the mechanisms involved in dimorphism in this organism. Normally, infections are relatively superficial and restricted to mucosal membranes causing both oral and vaginal candidosis (thrush). However, for individuals who are immunocompromised, for example following immunosuppressive therapies or the advent of AIDS, candida infections often become systemic and invasive and have a high rate of morbidity. Invasive candidosis has been associated with the presence of the hyphal form of the organism, whereas superficial infections are generally associated with the yeast phase, so implicating the transition from yeast to hypha as an important event in the pathology of the organism. However, the human pathogens *Histoplasma capsulatum*, and *Paracoccidiodes brasiliensis* are pathogenic only in the yeast phase and a mutant strain of *C. albicans* was still able to cause infections and death in mice, though the extent and spread of the organism was reduced. A wide range of environmental parameters have been shown to induce a yeast to hypha transition in candida, including serum, *N*-acetylglucosamine, proline and a shift from an acidic to a more alkaline medium. This suggests that a number of independent signal transduction systems exist within candida for each factor, particularly as mutants which have lost the ability to undergo a yeast to mycelium transition by one stimulus are still capable of forming germ tubes when exposed to other stimuli.

The fungal cell wall

The fungal *wall* defines the shape of the fungal hypha and provides the mechanical strength to resist the internal *turgor pressure*. The hyphal wall of most filamentous fungi consists of an inner primary wall composed of *chitin* microfibrils (a polymer of *N*-acetylglucosamine) and an outer or secondary wall composed largely of β-1-3 and β-1-6 *glucans* (a polymer of glucose). The exceptions are members of the Oomycotina,

Figure 5.10 Scanning electron microscopy image of a chemically treated fungal hypha revealing the chitin microfibrillar network. (From Gooday, G. W. and Trinci, A. P. J. (1980). *The Eukaryotic Microbial Cell.* Society for General Microbiology Symposium 30. Cambridge University Press, Cambridge.)

where cellulose rather than chitin forms the inner microfibrillar component of the wall. β-1-3 glucans and mannoproteins are also present as components of the outer wall matrix. Removal of the outer wall with lytic enzymes has revealed the architecture of the inner chitin wall which is composed of microfibrils formed by the aggregation of the chitin polymers by hydrogen bonding (Figure 5.10). The chitin inner

wall is cross-linked to the outer β-glucan components and forms a major component of the walls of most fungi. Synthesis of the cell wall occurs at the plasma membrane of the growing hyphal tip. *Chitin synthase* catalyses the formation of chitin from the precursor UDP-*N*-acetylglucosamine and appears as both the active enzyme and an inactive zymogen, requiring activation by cleavage of a peptide by an endogenous protease. Recently, three genes, chitin synthase I, II and III have been cloned from the yeast *Saccharomyces cerevisiae* and serve different functions in the cell. CHS1 acts as a repair enzyme and is involved in synthesizing chitin at the point where the daughter and mother cells separate, CHS2 is involved in septa formation and CHS3 is involved in chitin synthesis of the cell wall. In filamentous fungi the situation appears to be even more complex. At least six chitin synthase genes have been isolated from *Aspergillus fumigatus* and it seems likely that each may play a role in specific stages of fungal growth. However, the precise functions of the individual genes remains to be determined.

β-1-3 glucan, like chitin, is synthesized by a membrane-associated β-1-3 *glucan synthase* which utilizes UDP-glucose as the substrate, inserting glucose into the β-glucan chains. β-1-3 glucan synthase activity is found both in the membrane and cytosolic fractions of fungal mycelia and is stimulated by GTP. Although genes encoding a β-1-3 glucan synthase have been isolated from a number of fungi, it is not clear whether a gene family for β-1-3 glucan synthase exists in fungi as it does for chitin synthase.

As chitin and β-glucans are not found in mammalian cells (chitin is present in the exoskeleton of many insects and some molluscs) and so is a potentially powerful target for antifungal development, surprisingly few antifungal agents are used which are directed against wall biosynthesis. Two classes of nucleoside peptides, the *polyoxins* and the *nikkomycins* act as potent and specific inhibitors of chitin synthesis, competing as analogues of UDP-*N*-acetylglucosamine. However, their toxicity has prevented them from being exploited clinically as antifungal agents and their use as agrochemical fungicides hampered by the rapid emergence of resistant fungal strains. Another class of naturally occurring antibiotics, the *echinocandins*, are specific inhibitors of β-glucan biosynthesis. Initially, this class of compounds showed only a narrow spectrum of

activity toward fungi and was useful only when administered intravenously. More recently however, semisynthetic echinocandins have been synthesized with a broader antifungal spectrum, higher potency, and in a form which can be taken orally. This class of compounds is likely to become extremely important in the treatment of human fungal infections and has stimulated researchers to reappraise the cell wall as an important target for antifungal development.

Wall biosynthesis

The mature wall below the extension zone is rigid and resistant to internal turgor pressure, whereas the wall at the tip where growth occurs is plastic and malleable, yielding to the internal turgor pressure of the hypha and driving extension. Previously, the simultaneous delivery of both wall synthetic and lytic enzymes at the growing hyphal tip was proposed to account for the malleable nature of the hyphal tip, with the lysins continuously breaking the newly formed polymers at the tip and serving both to loosen the polymers at the tip and to create new sites for polymer extension. More recently, a new model has been formed based on experimental work which examined the fate of radiolabelled glucosamine and glucose incorporation into the wall at the growing tip. By exposing growing hyphae to radiolabelled glucosamine and glucose for short intervals followed by 'chasing' with unlabelled substrate, it was possible to determine the time taken for newly incorporated material at the tip to become incorporated into the wall. Initially, both glucosamine and glucose were readily extracted from the tip; however, as the radiolabelled components were displaced by the continuous supply of new precursors to the tip, they became progressively more difficult to extract from the hypha. These studies have revealed that wall precursors released at the tip, become progressively cross-linked and more rigidified as they are displaced down the hyphal tip (Figure 5.11). In this model, there is no requirement for the presence of lytic enzymes in order to produce a plastic, malleable hyphal tip.

The fungal membrane

The membranes of fungi, like those of other eukaryotic cells function to provide a barrier between the hypha and its environment, and are

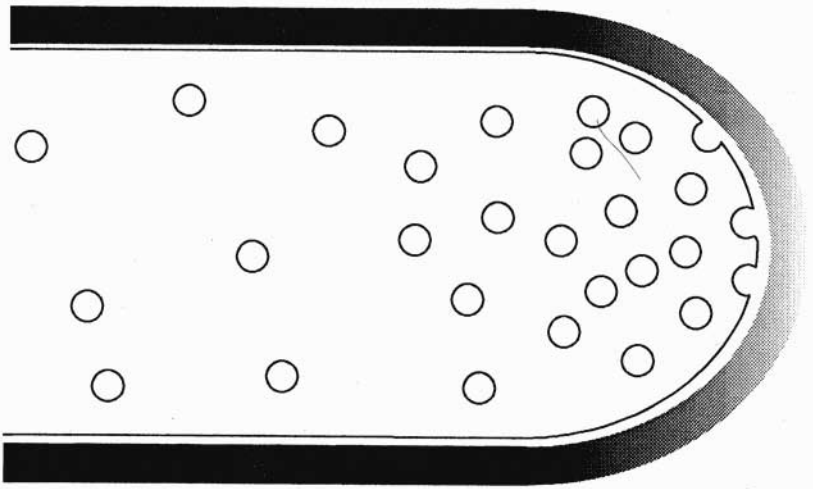


Figure 5.11 Schematic diagram of a growing fungal hypha illustrating the gradient in wall plasticity. Vesicles migrating to the tip fuse with the plasmalemma releasing wall precursors at the apex. Newly formed chitin becomes progressively cross-linked to the β-1-3 glucan component of the wall resulting in increasing wall rigidification away from the apex.

composed of a phospholipid bilayer into which are anchored various proteins. Sterols are also a critical component of the fungal membrane and serve to regulate membrane fluidity and the activity of membrane-associated enzymes and transport mechanisms. In mammalian cells, cholesterol is the chief sterol in the membrane, whilst in the majority of fungi it is *ergosterol*, the exceptions being the Chytridiomycotina and Oomycotina, where the dominant sterol is also cholesterol. This difference in the primary sterol component of fungal and mammalian cells has allowed the development of two classes of antifungal agents, the *polyenes* and the *azoles*.

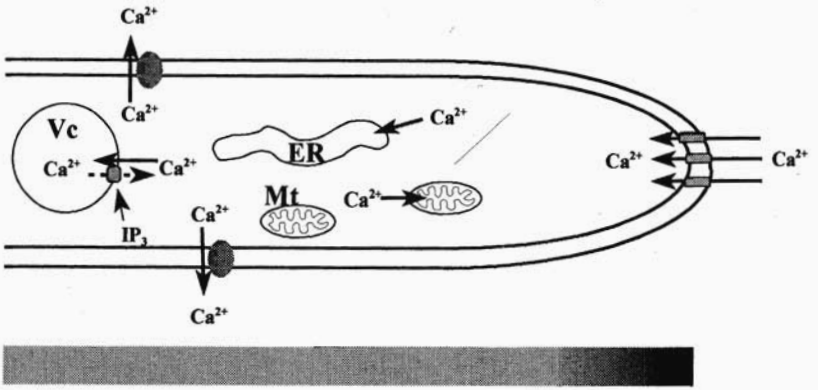
The polyene antifungals, which includes nystatin and amphotericin, bind hydrophobically to the ergosterol component of the membrane forming pores leading to a loss of cell membrane integrity. The azole antifungals, which include the triazoles and imidazoles, act primarily by inhibiting 14-α-sterol demethylase which is involved in ergosterol biosynthesis, resulting in ergosterol depletion in the membrane and the accumulation of 14-α-methylsterols. The subsequent alteration in the

sterol composition of the membrane results in changes in membrane fluidity, transport processes and wall biosynthesis and ultimately in cell death.

Nutrient uptake

In order for the fungi to grow, external nutrients must be assimilated across the plasma membrane. To successfully achieve the absorption of nutrients from the surrounding environment, the fungi possess a diverse range of specific transport proteins in the plasma membrane. Three main classes of nutrient transport occur in fungi, facilitated diffusion, active transport and ion channels. Fungi usually contain two transport mechanisms for the assimilation of solutes such as sugars and amino acids. The first is a constitutive *low affinity transport* system which allows the accumulation of solutes when present at a high concentration outside of the hypha. This process of facilitated diffusion is not energy dependent and does not allow accumulation of solutes against a concentration gradient. When the solute concentration is low (as is often the case in the environment), a second class of carrier proteins is induced that have a *higher affinity* for the solute and mediate the energy-dependent uptake of the solute against a concentration gradient at the expense of ATP. In order to assimilate nutrients at low concentrations, fungi create an electrochemical proton gradient by pumping out hydrogen ions from the hyphae at the expense of ATP via proton pumping *ATPases* in the plasma membrane. The proton gradient created provides the electrochemical gradient which drives nutrient uptake as hydrogen ions flow back down the gradient.

Thus fungi are capable of adapting their transport mechanisms according to the external solute concentration, ensuring continued uptake under different nutrient concentrations. *Ion channels* are highly regulated pores in the membrane which allow influx of specific ions into the cell when open. A number of ion channels have been described in fungi by patch clamping studies analogous to studies conducted on mammalian cells. Patch clamping involves measuring the current flowing across the cell membrane, which can be used to study the flow of various ions across the membrane. In fungi, the cell wall has first to be removed by incubating mycelium in an osmotic stabilizer and a mixture of lytic



Gradient in intracellular calcium

Figure 5.12 Model of calcium homeostasis in the hyphal tip. The high calcium gradient is thought to be generated by stretch-activated calcium channels located at the apex. Further back, calcium levels are maintained at a lower, constant level by the concerted action of calcium-pumping ATPases which pump calcium out of the hypha and sequestration into organelles. In addition, the intracellular messenger inositol trisphosphate (IP_3) has been shown to mobilize calcium from the vacuole where the resulting transient rise in intracellular calcium levels may trigger a number of downstream events. Vc, vacuole; Mt, mitochondria; ER, endoplasmic reticulum.

enzymes which digest away the cell wall producing naked sphaeroplasts or protoplasts. Two Ca^{2+} stimulated K^+ channels have been identified in *Saprolegnia ferax* which carry an inward flux of K^+ ions and are thought to be involved in regulating the internal turgor pressure of the hypha. More recently, the presence of a mechanosensitive or stretch-activated Ca^{2+} channel has also been described in this organism. Stretch-activated channels are opened when the membrane is under mechanical stress and may play an important role in the generation of the tip high calcium gradient observed in this organism (Figure 5.12).

Nutrient sensing

When readily utilizable sugars, such as glucose or fructose are added to fungi which are derepressed (starved) for glucose or other sugars, there follows a number of rapid metabolic responses which are mediated by a

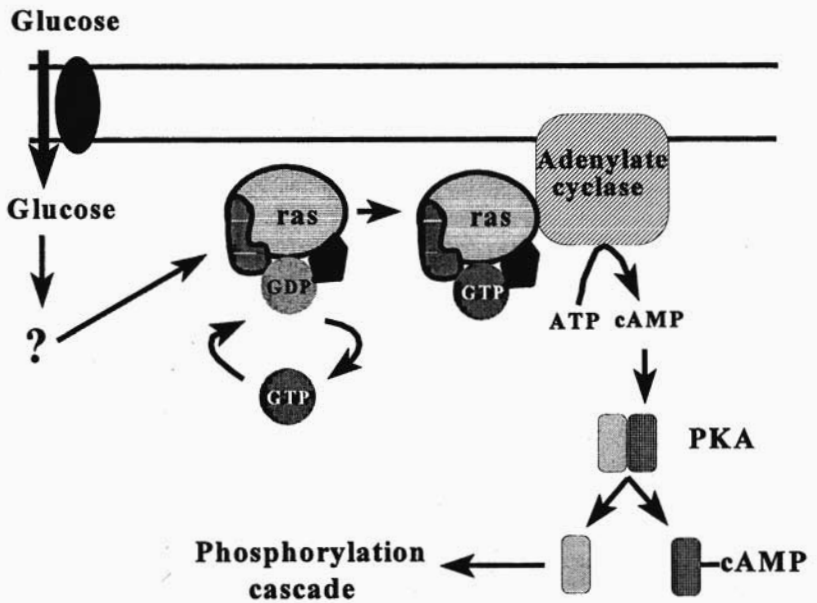


Figure 5.13 Schematic diagram showing the activation of the ras-cAMP pathway in fungi. The influx of glucose catalyses the exchange of GDP for GTP leading to a conformation change in the ras complex which then activates membrane-bound adenylate cyclase. The rise in cAMP activates cAMP-dependent protein kinase (PKA) by binding to the regulatory domain of the enzyme, which releases the active catalytic subunit. PKA activation triggers the phosphorylation and activation of a number of downstream targets.

transient rise in the levels of *cAMP*. These responses include the inhibition of gluconeogenesis and the activation of glycolysis and trehalase, which is involved in trehalose degradation, and are mediated by the activation of *cAMP-dependent protein kinase* (PKA) (Figure 5.13). The increase in cAMP is due to the activation of the membrane-bound enzyme adenylate cyclase as a result of the activation of a class of small GTP-binding proteins, the RAS proteins. Activation of RAS leads to the exchange of RAS-bound GDP for GTP, causing a conformational change leading to the stimulation of adenylate cyclase activity. The RAS protein complex includes an intrinsic GTPase which converts GTP-bound RAS to GDP-bound RAS, thus returning RAS to its resting state in the absence of

an activator. Currently, the mechanism by which glucose stimulates the RAS complex is unknown but the available evidence strongly suggests that the RAS pathway forms part of a global mechanism for nutrient sensing.

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Molecular fungal biology

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