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The dynamics of hyphal growth

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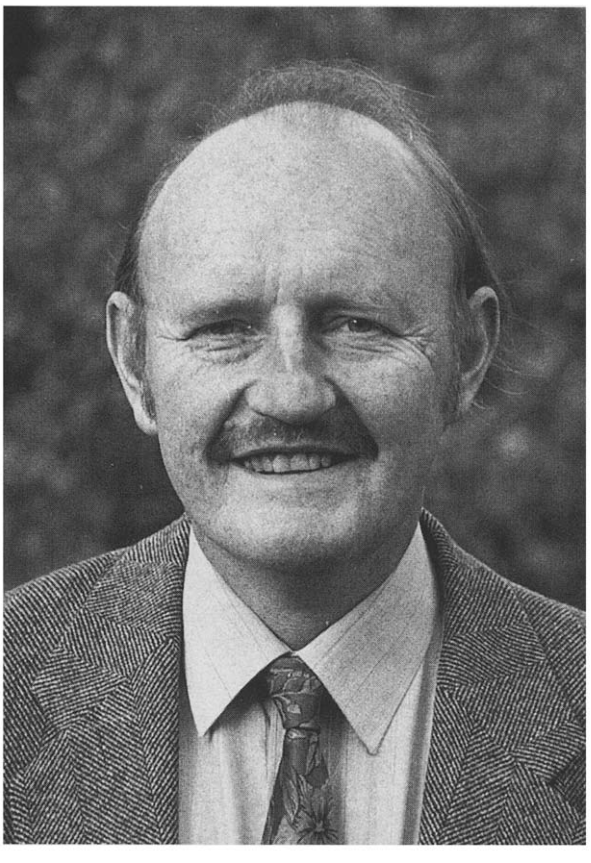
This account reflects diverse aspects of hyphal growth and development that I have encountered since first becoming interested in fungi. Apical growth is discussed, with particular reference to the phenomena of hyphal maturation and the formation of helical hyphae. It is contrasted with cases of intercalary growth and reversed growth of hyphae. Localization of chitin synthesis at the apex and at other particular sites in the wall is described in detail. It is suggested that chitin microfibrils are synthesized at hyphal tips and at other sites by a transmembrane enzyme complex of several polypeptides allosterically regulated by effector molecules but also perhaps by local physical stress of the membrane. Behaviour of hyphae is discussed with examples of mating zygophores, of contact sensing of surfaces and of responses to applied electric fields.

The hypha is the characteristic growth form of fungal cells. Its most important feature is that it enables the fungus to explore and exploit new environments and substrates. It can also differentiate to form a variety of structures, such as sporangia, conidiophores, rhizoids and fruit bodies. A major interest of mine for many years has been the many guises of hyphal growth, with the aim of understanding the mechanisms of these dynamic processes. I have worked with a range of fungi, in each case trying to get a feeling for the organism. All have been rewarding in their own ways.

With few exceptions, hyphae elongate strictly by apical deposition of wall skeletal polysaccharides, especially chitin and β-glucans. This phenomenon of apical growth was surmised over a hundred years ago by Ward (1888) and Reinhardt (1892) from their observations of growing hyphae. Thus 'the key to the fungal hypha lies in the apex' (Robertson, 1965). Confirmation of the specific apical deposition of wall polysaccharides has come from microscopic autoradiography with tritiated *N*-acetylglucosamine and glucose (Bartnicki-Garcia & Lippman, 1969; Gooday, 1971), and also by the rapid bursting of apices of growing hyphae by antibiotics, such as nikkomycin and echinocandin, specific inhibitors of chitin and glucan synthesis, respectively (Gooday, 1990; Zhu & Gooday, 1992). What follows is an account of some investigations into the mechanisms and dynamics of hyphal growth.

MECHANISMS INVOLVED IN APICAL HYPHAL GROWTH

Fig. 1 is a representation of some of the processes involved in apical growth. The wall at the apex must be plastic to allow its extension by insertion of new material. Thus it must be progressively rigidified as it is transformed to become the lateral wall of the hypha. Chiefly from studies of Wessels and



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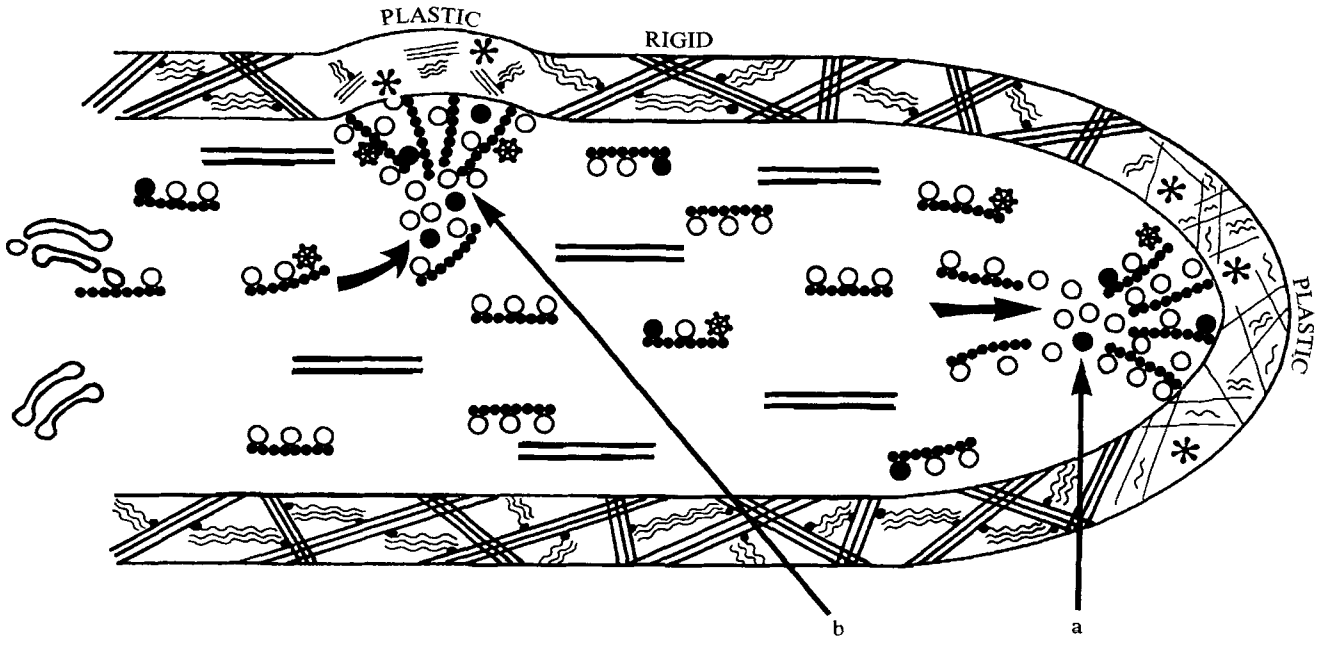

Fig. 1. Model of some suggested features of apical growth and branching of a hypha. Microvesicles of various types (empty, solid and starred circles) are shown being produced by Golgi bodies and being transported to two sites, the Spitzenkörper of the apex (a) and of the new branch (b). The microvesicles are associated with cytoskeleton, particularly microfilaments (beaded lines), but also perhaps with microtubules (parallel lines). The apical arrangement of microfilaments also gives some physical support to the tip. The plastic wall at the apex is shown with nascent fibrils of chitin (straight lines) and glucan (wavy lines). The wall becomes progressively rigidified by their crystallization, and cross-linked by covalent bonds (black dots). At the branch site, the action of lytic enzymes (stars) has created a soft spot in the wall. It is likely that lytic enzymes are also secreted at the apex, but their role there is unclear. Modified from Gooday (1994*a*).

colleagues (Wessels, 1990), it is clear that two processes involved in this rigidification are the covalent cross-linking of wall materials, especially chitin and β-(1 → 3)-glucans, and the hydrogen-bonding of adjacent polysaccharide chains, especially chitin, to give microfibrils (Fig. 1). There is good evidence that these skeletal polysaccharides are inserted into the wall vectorially by transmembrane synthetic enzymes. Also occurring during maturation of the wall is the deposition of matrix materials, notably mannoproteins in most fungi. These matrix materials are probably chiefly secreted into the developing wall by exocytosis, having been synthesized by the endomembrane system, involving endoplasmic reticulum and Golgi bodies.

Inherent in apical growth is the forward transport of a range of types of vesicles. These provide new cell membrane material. Some carry membrane-bound enzymes, such as zymogenic chitin synthase in chitosomes (Bartnicki-Garcia, Ruiz-Herrera & Bracker, 1979); others carry material for secretion, such as wall mannoproteins and extracellular enzymes. The vesicles accumulate in the Spitzenkörper, 'apical body' (Girbardt, 1969; Grove & Bracker, 1970; Howard, 1981; Roberson & Fuller, 1988). From there they move to the apical dome. This process has been mathematically modelled, with the accumulation of vesicles being termed the vesicle supply centre (Bartnicki-Garcia, Hergert & Gierz, 1989). Steady movement forward of the vesicle supply centre gives rise to an apically extending hypha.

There is good evidence that cytoskeletal elements play key roles in the maintenance of polarity, by transporting the vesicles and thus controlling the orderly deposition of wall and membrane material at the apex (Heath, 1994). Most evidence centres on the active role of actin microfilaments. Microtubules are also present, but their role is less clear; they may play a more indirect role than microfilaments. As well as having roles in transport of vesicles, elements of cytoskeleton, chiefly actin microfilaments, probably give some structural support to the apical dome.

Also shown in Fig. 1 is the formation of a branch, by formation of a new Spitzenkörper at a sub-apical site, to give rise to a new hyphal apex.

DEVELOPMENT OF MATURE HYPHAE

When a spore germinates, typically it produces one or more germ-tubes which elongate exponentially. This results from the autocatalytic effect of the increasing rate of uptake and metabolism of nutrients from the medium (Prosser, 1994). The extension rate eventually reaches a nearly constant value, probably when transport of material from the sub-apical region becomes limiting. Exponential growth of the colony is achieved by formation of sub-apical branches, each of which becomes an apically elongating hypha. Hyphae in a young colony, however, are juvenile in behaviour and undergo a poorly understood slow process of maturation. Juvenile hyphae of many species are slower growing and narrower

Table 1. Variations of apical growth rate and diameter with age of leading hyphae

	Juvenile			Mature			
	Age (h)	E (μm h^{-1})[a]	D (μm)[b]	Age (h)	E (μm h^{-1})	D (μm)	Reference
Botrytis cinerea	20	85	5·7	44	330	8·7	Zhu & Gooday (1992)
Mucor rouxii	12	315	5·0	26	340	8·4	Zhu & Gooday (1992)
Neurospara crassa	25	410	6·8	40	1380	12·8	McLean & Prosser (1987)
Candida albicans	12	19	2·6	72	46	3·4	Gow & Gooday (1982*a*)
Coprinus cinereus	—	16	3·0	—	269	5·9	Butler (1984)

[a] E, Mean extension rate. [b] D, Mean diam.

than are mature hyphae. Thus, in experiments with *Botrytis cinerea*, the mean extension rate of leading hyphae increased from 85 μm h^{-1} at 20 h to 330 μm h^{-1} at 44 h after inoculation (Table 1; Zhu & Gooday, 1992), and remained constant thereafter. Over the same period the mean diameter of the hyphae increased linearly from 5·7 to 8·7 μm. There was a direct relationship between extension rate and square of diameter. In contrast, for *Mucor rouxii*, in the same study, extension rate increased dramatically from spore germination at 5·5 h to reach a value of over 300 μm h^{-1} at 10 h, after which it increased little over the next 36 h. Meanwhile, hyphal diameter increased more slowly, so that from 5·8 μm at 15 h it did not reach its maximum value of 8·4 μm until 26 h (Table 1). There was no direct relationship between extension rate and square of diameter. Other examples of these phenomena of hyphal maturation include *Neurospora crassa* (McLean & Prosser, 1987) and *Coprinus* species (Butler, 1984). A positive relationship between hyphal diameter and extension rate for a particular species has been reported for many fungal colonies: for both variables, values for leading hyphae are greater than those for primary branches, which in turn are greater than those for secondary branches (Prosser, 1994).

Working with *N. crassa*, Steele & Trinci (1975) distinguish between 'undifferentiated' and 'differentiated' hyphae; the former being juvenile hyphae found in young colonies on solid media or in submerged culture, and the latter being wider and faster extending mature hyphae found at the edges of older colonies on solid media. Similar observations for *Geotrichum candidum* are discussed by Robinson & Smith (1979) and Smith & Robinson (1980), who also report that this hyphal differentiation only occurred in colonies growing on solid media with a sufficiently high initial glucose concentration.

A different style of growth is shown by hyphae of the human pathogen, *Candida albicans*. Although these hyphae still exhibit a maturation process, becoming faster growing and wider with age (Gow & Gooday, 1982*a*; Table 1), differences are seen when yeast cells form germ-tubes and when hyphae branch. The germ-tubes elongate at a constant rate, not in an autocatalytic fashion. This was explained by the observation that the germ-tubes are formed at the expense of vacuolation of the parent yeast cells. Thus germ-tube formation can be regarded as resulting from migration of the protoplasm from the yeast cell to the apex of the hypha (Gow & Gooday, 1982*b*, 1984, 1987). This phenomenon of extensive vacuolation of individual cells continues during hyphal growth, so that there is a delay, sometimes very extended, between septation (to give uninucleate hyphal cells) and production of branches behind the septa (Gow, Henderson & Gooday, 1986; Gow & Gooday, 1987). It also occurs during formation of pseudomycelium of *C. albicans* (Yokoyama & Takeo, 1983).

Juvenile hyphae, as well as being slower growing and thinner than mature ones, also differ in other ways. For example, young hyphae of *B. cinerea* and *M. rouxii* are much less susceptible than mature ones to antifungal agents, such as nikkomycin and echinocandin, inhibitors of cell wall biosynthesis (Zhu & Gooday, 1992). Thus leading hyphae of a mature colony of *B. cinerea* (44 h and older) burst within one minute when treated with 10 μM nikkomycin or 93·5 μM echinocandin. In contrast, juvenile hyphae (e.g. aged 16 and 24 h), treated with 100 μM nikkomycin took more than 5 min to swell and burst while those treated with 935 μM echinocandin showed little bursting, although they did stop growing.

Juvenile hyphae of many fungi have a limited repertoire of differentiation. Thus those of *Mucor mucedo* do not produce the sex hormone, trisporic acid, or respond to it to produce zygophores, as do mature hyphae (Gooday, 1968). Competence for sporulation of *Aspergillus nidulans* is acquired after 18–20 h growth of mycelium (Pastushok & Axelrod, 1976; Champe *et al.*, 1981). Acquisition of this competence for sporulation appears to be controlled genetically rather than environmentally, as it is unaffected by continuous replacement of the medium or by concentrations of limiting nutrients, and precocious mutants have been described that can sporulate earlier.

The mechanism of maturation of a vegetative hypha is unclear, but it may prove that growth regulating chemicals have to reach critical concentrations to allow expression of particular genes. Such a situation is seen in colonies of some bacteria, where signalling molecules, such as homoserine lactone, need to accumulate to allow expression of particular genes, such as those for luminescence. The observation in many cases that differentiation does not occur in submerged cultures is consistent with this idea, as gradients of putative effectors could not then accumulate. One aspect of metabolic control of hyphal development is demonstrated by experiments with *cr-1* mutants of *N. crassa* (Pall & Robertson, 1986). These mutants are deficient in cyclic AMP. They form mycelia with a single size class of hyphae, 3–5 μm, in contrast to the hyphal hierarchy of their wild-type parent, which has leading hyphae of 14–20 μm, primary branches of 8–12 μm and secondary branches of 3–5 μm. When grown with 2–3 mM 8-bromocyclic AMP (an analogue of cyclic AMP), after 18–20 h

some hyphal hierarchy was observed, with hyphal diameters of 3–13 μm. Thus it may be concluded that metabolism involving cAMP plays some part in the process of hyphal maturation.

HELICAL HYPHAL GROWTH

Hyphae rarely grow completely straight. They usually meander to some extent. This may be the result of surface irregularities or of tropic responses to neighbouring hyphae or to oxygen gradients, or it may be the inherent result of random movements of the Spitzenkörper relative to the axis of the apex. Direct observations of living hyphae have shown that the Spitzenkörper is indeed a dynamic structure, undergoing random changes in shape and position (C. E. Bracker & R. M. López-Franco, pers. comm). As well as this stochastic feature, a helical element appears to be inherent in the apical growth of hyphae. This is manifested by many fungi growing on agar media of very weak nutrient concentrations, when they exhibit spiral growth resulting from interactions between rotating hyphal tips and the substrata (Madelin, Toomer & Ryan, 1978; Trinci *et al.*, 1979). Beever (1980) has described *coil-1* mutants of *N. crassa* which showed very pronounced spiral growth so that complete circles were frequently formed.

We have observed that hyphae of *C. albicans* frequently grow to form helices on surfaces such as cellophane membranes (Sherwood-Higham *et al.*, 1994). Helical growth was shown by a range of strains of *C. albicans* and with different media supporting hyphal growth. The rigidity of the substrate was important: helical hyphae occurred on 1·5% agar and above but not at 1% and below, and on 0·8% Gellan gel and above but not at 0·7% and below. Hyphae growing embedded in the agar grew straight but often became helical after emerging on the surface. In all cases where differential focusing of live hyphae was possible, the helicity was right-handed.

There are many examples of helical hyphae of aerial and sporulating structures. The most well-known is the developing sporangiophore of *Phycomyces blakesleeanus* which shows marked helical growth, the direction of which reverses during development but the final structure is a straight vertical hypha (Castle, 1942). Other examples are aerial hyphae of saprotrophically growing *Trichophyton* species (Davidson & Gregory, 1937) and sporophores of *Spirodactylon aureum* (Benjamin, 1959).

Thus it may be that there is always a rotatory element in apical hyphal growth, which is only permanently manifested when a surface provides an opposing reaction (as in *C. albicans* on solid media) or in particular cases of aerial hyphae (e.g. *Trichophyton* and *S. aureum*). The mechanism for tip rotation remains obscure, as there is no obviously radially anisotropic component in hyphal tips. Hyphae of some fungal sporophores have helical arrangements of microfibrils in their walls (Middlebrook & Preston, 1952; Kamada *et al.*, 1991), but chitin microfibrils in hyphal walls of vegetative hyphae appear to be arranged randomly (Burnett, 1979; Gow & Gooday, 1983) and there is no relationship between helicity of microfibrils and sporangiophore growth in *P. blakesleeanus* (Middlebrook & Preston, 1952). Chemical control of helical growth has been proposed for the oomycete, *Saprolegnia ferax*, by Kaminskyj & Heath (1992). They reported formation of helical hyphae when *S. ferax* was grown on Gellan gel and suggested that this could be in response to morphogenetic regulators in the Gellan. There is no evidence, however, for such chemical control in our experiments with *C. albicans*, or in other reports of helical or spiral growth. This difference in behaviour may be explained by the fact that hyphae of Oomycetes respond chemotropically to nutrients such as sugars but those of Eumycota other than chytrids do not (Gooday, 1976). Touzé-Soulet *et al.* (1978) describe the formation of helical hyphae by *Boletus edulis* in response to culture filtrate from its mycoparasite, *Hypomyces chlorinus*. They ascribe this to the action of lytic enzymes weakening the wall and allowing an underlying helical element of growth to be manifested.

That helical growth of the hyphal apex is regulated at a fundamental intracellular level is suggested by the invariant sense of helicity (right- or left-handed) of any particular fungal strain; by the occurrence of mutants for spiral growth; and by the developmental control of helicity, e.g. in the aerial propagules of *Trichophyton* species. Helical growth would ensue if there were an asymmetry in the apical cytoplasm leading to a lateral displacement of the growing tip. The cytological mechanism of this displacement is unclear, but in other systems, such as algal cells, axial rotation of cytoskeletal elements, especially microtubules and microfilaments, has been implicated in determining cytomorphogenesis (Jarosch, 1990). In a similar fashion rotation of microtubules and microfilaments in a growing hyphal tip may be a primary cause of its axial rotation. As described earlier, direct observations of living hyphae have shown that the Spitzenkörper is a dynamic structure, undergoing changes in shape and position (C. E. Bracker & R. M. López-Franco, pers. comm.). In some cases, rotational motion of the Spitzenkörper can be observed, up to 20° from the central axis. On a suitable surface, resulting axial rotation of the tip would result in two-dimensional spiral growth or three-dimensional helical growth, depending on the physical interactions between apex and substrate. In the case of *C. albicans*, this 'cork-screwing' growth, acting together with the contact sensing of the hyphae described later, could facilitate penetration of host tissue.

NON-APICAL ELONGATION OF HYPHAE

Although hyphae typically elongate in a strictly apical fashion, exceptions are found in sporulating structures, in which some hyphae show intercalary elongation. One example is provided by the major stipe cells *Coprinus cinereus* (Gooday, 1985). Their walls have a high chitin content, about 11% of total stipe dry weight throughout elongation. Autoradiography shows that chitin is synthesized uniformly throughout the length of the cells. The chitin in these walls is in the form of shallow helices of microfibrils, as shown by polarized light microscopy of living cells, and light microscopy and shadow-cast electron microscopy of chitin ghosts of cells from which all other components have been extracted (Gooday, 1979; Kamada *et al.*, 1991). Throughout the phase of rapid stipe

elongation, the chitin microfibrils are at an angle of about 87° to the vertical, but may be as left- or right-handed helices, in the ratio about 2:1. New chitin microfibrils must be inserted uniformly between existing ones, presumably by continual breaking and reforming of interchain bonds (Kamada *et al.*, 1991).

An intriguing example of non-apical growth is shown by thalli of *Allomyces macrogynus*. Naked zoospores of this chytrid settle, immediately form a chitin-rich wall, and germinate to form narrow ramifying rhizoids and wide apically growing hyphae, which eventually will form sporangia in appropriate environmental conditions. When grown in a microaerobic environment, these hyphae are narrow. If they are then exposed to normal atmospheric oxygen concentration, they revert to normal width (Youatt, 1986). This does not occur, however, by the apex swelling and growing forwards. Rather, the apex swells and then 'grows backwards', with swelling of the preformed narrow hyphae. Then, when this process is complete, the now-wide apex resumes growth. This backward development was inhibited by polyoxin B, a specific inhibitor of chitin synthesis, and so must be due to active growth (Youatt *et al.*, 1988). Narrow hyphae treated with polyoxin showed swelling at various sites along their length, in contrast to apically growing hyphae, which typically swell at their apices. The occurrence of these swellings towards the base of narrow hyphae suggests that wall softening had been occurring there ahead of where chitin synthesis would have been expected to take place in the absence of polyoxin.

REGULATION OF SYNTHESIS OF CHITIN IN HYPHAE

The most characteristic shape-determining component of fungal walls is chitin, the β-(1 → 4)-linked homopolymer of *N*-acetylglucosamine. Chitin microfibrils in the wall are usually randomly arranged, appearing as a skeletal network (Hunsley & Burnett, 1970; Burnett, 1979). Shadow-cast electron microscopic preparations of microfibrils, although involving drastic chemical treatments and drying of the specimens, with accompanying artefacts, nevertheless show a range of different architectures. Thus those from hyphae of *Candida albicans* were short and stubby, at 9–15 μm in diam., averaging 33 μm in length, with 109 μm being the longest seen, while those from *Coprinus cinereus* were 7–25 μm in diam., with the longest that could be traced being 1310 μm (Gow & Gooday, 1983).

As discussed earlier, apical hyphal extension involves very localized apical deposition of newly synthesized wall skeletal material, notably chitin. A branch forms where a new localized site of chitin deposition occurs in the lateral wall. Thus chitin synthesis plays a key role in hyphal growth and differentiation, and its orderly deposition in space and time must be under tight regulation. It is synthesized by the enzyme chitin synthase, utilizing the nucleotide sugar, uridine diphospho-*N*-acetylglucosamine (UDPGlcNAc), as substrate:

$$(GlcNAc)_n + 2UDPGlcNAc \rightarrow (GlcNAc)_{n+2} + 2UDP.$$

This equation is written with the enzyme adding two molecules of *N*-acetylglucosamine at one time as the unit cell of chitin is diacetylchitobiose, the β-(1 → 4) linked dimer, in which the two monomers of *N*-acetylglucosamine are arranged 180° to each other. It thus seems likely that they are added together, in very rapid succession, rather than the synthetic complex rotating through 180° after each addition. Currently we have the anomalous situation that there is rapidly increasing knowledge of the molecular biology of chitin synthases, but very little knowledge of their activity in the cell. It is clear, however, that individual fungi have several chitin synthase genes (Bulawa, 1993). Chitin synthases are integral membrane-bound enzymes, requiring phospholipids for activity (Duran & Cabib, 1978; Vermeulen & Wessels, 1983; Montgomery & Gooday, 1985; Binks *et al.*, 1990; Machida *et al.*, 1994). They accept substrate molecules on their inner faces from the cytoplasm, and extrude the growing nascent chains through the membrane into the wall. As yet nothing is known of the arrangement of enzyme polypeptides in the membrane. It seems likely, however, that chitin microfibrils are synthesized by enzyme complexes, consisting of a group of polypeptides in the membrane. This multi-subunit assemblage could consist of identical chitin synthase polypeptides or perhaps (as for many allosteric proteins) of chitin synthase polypeptides from more than one structural gene. In addition the synthetic complexes could contain other polypeptides, with regulatory or enzymic properties. Enzymes that have been suggested to work in consort with chitin synthase include chitinases (Rast *et al.*, 1991) and UDPase (Gooday, 1979).

When investigated in detail, chitin synthase preparations usually show cooperative kinetics, being activated allosterically by the substrate, UDPGlcNAc. Thus for solubilized preparations of enzyme from *Coprinus cinereus* the Hill number was close to 2 and 4 at concentrations above and below 0·1 mM, respectively (Rousset-Hall & Gooday, 1975; Gooday, 1977), and for *Agaricus bisporus* it was close to 2 (Hanseler *et al.*, 1983). Chitin synthase preparations typically also are activated allosterically by chitin monomer and dimer, GlcNAc and $(GlcNAc)_2$ (Rousset-Hall & Gooday, 1975; Gooday, 1977; Horsch & Rast, 1993), and by Mg^{2+} (Gooday, 1979), and inhibited by the co-product, UDP (Rousset-Hall & Gooday, 1975) (Table 2).

Thus chitin synthesis probably occurs via a synthase complex in the fungal cell membrane, composed of a group of polypeptide subunits able to respond allosterically to effectors at their cytoplasmic faces. There is no evidence, however, that substrate UDPGlcNAc is limiting. On the contrary, estimates of its cytoplasmic concentration in a range of fungi are about 1 mM, in the same order as estimates of Km or $S_{[0.5]}$ for UDPGlcNAc for a range of chitin synthases (Table 2; Gooday & Trinci, 1980).

There is evidence that inactive chitin synthase is distributed throughout the hyphal plasma membrane. Thus treatment of hyphae of *Aspergillus nidulans* with cycloheximide, a specific inhibitor of protein synthesis, led to an uncontrolled increase in chitin synthesis approximately uniformly along the hyphae (Katz & Rosenberger, 1971; Sternlicht *et al.*, 1973). This must have been due to activation of pre-existing enzyme molecules rather than synthesis of new ones. Uncoordinated increases in

Table 2. Some aspects of chitin synthase preparations from a range of fungi

	$K_{0.5}$ for UDPGlcNAc[a] (mM)	K_a' for GlcNAc[a] (mM)	K_i' for UDP[a] (mM)	Cytoplasmic [UDPGlcNAc] (mM)
Allomyces macrogynus	1·2	—	—	—
Blastocladiella emersonii	1·8–4·1	3–4	—	1·8–3·8
Mucor rouxii	0·5	0·7–12·5	0·4	—
Candida albicans	0·7–4·5	—	0·2–2·0	—
Neurospora crassa	1·4	4·5	2·2	0·4–1·5
Agaricus bisporus	0·9	1·0	—	0·5
Coprinus cinereus	0·9	0·6	0·5	—

Compiled from Gooday & Trinci (1980); Gooday (1990); Horsch & Rast (1993).

[a] Values for kinetic parameters vary with different preparations and different conditions. $K_{0.5}$ values are for substrate concentrations giving half maximal activity. K_a' and K_i' values are apparent equilibrium constants for activation and inhibition, respectively. Preparations probably contain mixtures of different gene products.

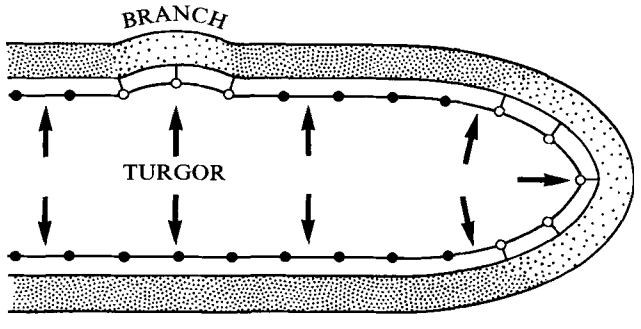

Fig. 2. Model of activation/inactivation of enzymes and/or ion channels by stress on the cell membrane. Heavy stippling signifies rigid walls; light stippling signifies plastic walls. Solid circles represent particular membrane proteins in their resting states; open circles with stalks signify their changed state under physical stress from the outward force of the cell turgor pressure. Modified from Gooday (1994*b*).

chitin deposition also result from treatment with a range of other antifungal compounds, such as Calcofluor white and Congo red, dyes interfering with chitin microfibril assembly (Roncero & Duran, 1985; Pancaldi *et al.*, 1988); monensin, a monovalent cation ionophore (Poli *et al.*, 1986); and azole derivatives inhibiting ergosterol biosynthesis (Vanden Bossche, 1986, 1990; Hector & Braun, 1987). A mutant strain of *C. albicans*, with lowered ergosterol content, also shows increased chitin synthase activity (Pesti, Campbell & Peberdy, 1981). Chitin synthesis in the growing zone of elongating sporangiophores of *P. blakesleeanus* is stimulated by light (Ruiz-Herrera, 1992). At the same time the wall in this region is weakened (Ortega, Gamow & Ahlquist, 1975) and this may act as a stimulus for chitin synthesis.

Although these observations suggest that chitin synthase is present throughout the cell membrane, it is only active at the apex and at an incipient branch site. The mechanism(s) of localized activation remain unclear. One possibility, discussed later, is proteolytic activation. We have investigated an alternative hypothesis: that local physical stress of the membrane may activate the chitin synthase. The existence of stretch-activated ion channels in fungal cell membranes is well documented. Zhou *et al.* (1991) describe them in germ-tubes of *Uromyces appendiculatus*, and implicate them in contact sensing by hyphal apices on leaf surfaces. Garrill, Lew & Heath (1992) describe the concentration of such channels in hyphal tip membranes of *Saprolegnia ferax*. The sites of chitin synthesis, i.e. apex and branch points, are where the cell's turgor pressure may stretch the membrane outwards against the overlying plastic wall (Wessels, 1990; Figs 1, 2). Results of our experiments show that cells that have been subjected to hypo-osmotic stress have raised native chitin synthase activities (Gooday & Schofield, 1995; Table 3). It is possible that stressing the membrane causes a conformational change in chitin synthase molecules in the membrane, or changes in interactions between them and associated polypeptides, or in phosphorylation of the enzyme, leading to activation. This hypothesis is consistent with observations described earlier which are summarized in Table 4.

Most chitin synthase preparations can be activated, sometimes very dramatically, by treatment with proteolytic enzymes, i.e. they are zymogenic (Cabib, 1987). Thus chitin synthase solubilized and partly purified from mycelium of *C.*

Table 3. Effect of osmotic stress on chitin synthase activity in fungal cells

		Chitin synthase activity [pkat (mg protein)$^{-1}$] ± S.D. (3)	
	Treatment	Low O.P. (mOSM)	High O.P. (mOSM)
C. albicans hyphae	1 h, 37 °C	0·94 ± 0·17 (114)	0·32 ± 0·16 (1238)
C. albicans yeasts	1 h, 25 °C	0·62 ± 0·21 (221)	0·26 ± 0·07 (1528)
C. cinereus	1 h, 25 °C	21·0 ± 1·9 (422)	9·7 ± 1·9 (1430)
S. cerevisiae α/a	1 h, 30 °C	0·47 ± 0·02 (461)	0·24 ± 0·01 (1462)

Cells were incubated for 1 h in growth media of low or high osmotic pressure (values given in parentheses; obtained by addition of appropriate amounts of sorbitol). Mixed membrane preparations were made and assayed with appropriate conditions for native chitin synthase activities. From Gooday & Schofield (1995).

Table 4. Possible examples of increased chitin synthesis with membrane stress

Site/treatment	Reason for membrane stress
Wall	Wall softening owing to
Apex	Nascent wall
Branch point	Autolysins
C. cinereus stipe cell	Autolysins
P. blakesleeanus sporangiophore	Light via autolysins
Calcofluor white, Congo red	Inhibition of H-bonding
Membrane	Membrane stress owing to
Osmotic shock	Increased turgor
Lowered ergosterol content	
Azole derivatives	Reduced fluidity
Mutant strain	Reduced fluidity
Monensin ionophore	?
Cycloheximide	?

References are given in text.

albicans could be activated 18-fold by treatment with trypsin (Hardy & Gooday, 1983). On incubation at 30 °C, these preparations self-activated several fold over 20 h, and then slowly lost activity. Currently, however, the mechanism(s) of proteolytic activation *in vitro* and their significance *in vivo* remain unclear. It is possible that the proteases are destroying an endogenous proteinaceous inhibitor present in the preparations, or are indirectly causing a change in conformation of chitin synthase in the membrane. These questions may be answered when amino acid sequences of active enzymes are known.

INVOLVEMENT OF LYTIC ENZYMES

The involvement of lytic enzymes in apical hyphal growth was suggested over 100 years ago by H. Marshall Ward, who observed apical extension of *Botrytis* hyphae and wrote: 'I imagine that... the ferment-substance at the apex keeps the cellulose of the hypha at that place in a soft, extensible condition, and the pressure from behind stretches it and drives the tip forwards' (Ward, 1888). This idea was formalized in a modern context by Bartnicki-Garcia (1973), with his unitary model of cell wall growth, involving a dynamic balance between enzymes lysing walls and those synthesizing them. In contrast, Wessels (1990) presents a steady-state model, which does not involve lytic enzymes. Instead, it views the plastic apex as being nascent wall, being physically supported by an underlying cytoskeleton, based on an array of actin microfibrils. This is an attractive proposition, but wherever investigated, wall lytic enzymes, such as chitinases and glucanases, have been found in actively growing fungal mycelia (Rosenberger, 1979; Gooday & Gow, 1990). For example, in both *Mucor mucedo* and *Mucor rouxii*, chitinase activities can be detected in parallel with those of chitin synthase throughout exponential growth (Gooday, Humphreys & McIntosh, 1986; Pedraza-Reyes & Lopez-Romero, 1989; Rast *et al.*, 1991; Gooday, Zhu & O'Donnell, 1992). What are they doing? It is generally accepted that they are involved in branching, i.e. in locally softening the wall to allow a localized herniation, but do they have a role in apical growth? That they are under specific regulation is suggested by the characterization of zymogenic chitinase activities (Humphreys & Gooday, 1984*a*; Rast *et al.*, 1991). Humphreys & Gooday (1984*a*, *b*) further describe latent membrane-bound activity from exponentially growing *M. mucedo*, that was regulated by its phospholipid environment.

A valuable tool in unravelling roles of chitinase in fungal growth seemed to be provided by the discovery of a specific inhibitor, the antibiotic allosamidin (Sakuda *et al.*, 1987). This is a potent competitive inhibitor of chitinase activities of *C. albicans* and *M. rouxii* (Gooday, Zhu & O'Donnell, 1992; Milewski, O'Donnell & Gooday, 1992). However, while it inhibits daughter cell separation of budding yeast cells of *C. albicans* and slows germination of sporangiospores of *M. rouxii*, it has no clearly discernible effect on apical growth or branching of hyphae (Gooday, Zhu & O'Donnell, 1992). Further experiments with *M. mucedo* suggested, however, that chitinase activities *in situ* may be in a privileged state, being protected in some way from inhibition, perhaps by being sequestered in microsites in the wall. Thus, using a soluble fluorogenic substrate, 95 and 99% inhibition was achieved of cell free activity from homogenized cells by 10 and 100 μM allosamidin, respectively, as opposed to values of 58 and 63% inhibition of periplasmic activity measured *in situ*. A much higher concentration, 2·5 mM, was required to inhibit spore germination. The suggestion of a protected state for the enzyme in the intact cell is reinforced by studies of the effect of pH on activity. Cell free activity showed a sharp optimum at pH 6, with 3 and 15% activity at pH 3 and 8, respectively, whereas pH had little effect on activity measured in cells *in situ*, with an optimum at pH 5, and 63 and 93% activity at pH 3 and 8, respectively (Gooday, Zhu & O'Donnell, 1992). An observation that may be related is that mixed membrane preparations from hyphae of *Mucor mucedo* made from cultures grown on agar had a powerful chitinolytic activity, so that chitin synthase assays gave *N*-acetylglucosamine as product from the efficient degradation of nascent chitin; in contrast, preparations from submerged cultures gave, as expected, chitin as product (Gooday *et al.*, 1986). This difference in hyphae between the two modes of growth may be a consequence of different amounts or types of hydrophobic cell surface proteins, the hydrophobins (Wessels, 1992), which in turn may give rise to different microenvironments and thus different properties of enzymes associated with the walls.

THE BEHAVIOUR OF HYPHAE

Hyphae respond to a variety of environmental stimuli, by directed growth and by differentiation. They can have a range of senses. Probably all hyphae respond to volatile chemicals, soluble chemicals, to the physical nature of surfaces and to temperature gradients. In addition many hyphae respond to light and to applied electrical fields. Some respond to gravity. I will describe three examples of hyphal behaviour that colleagues and I have studied, which illustrate different aspects of these phenomena.

Mating between (+) and (−) strains of *Mucor mucedo* and other mucoraceous fungi involves two levels of chemical signalling between the two partners. As two compatible cultures grow towards each other, initial contact is the exchange via diffusion of mating-type-specific prohormones, chiefly methyl-4-dihydrotrisporate from (+) and trisporol from (−). These are metabolized by (−) and (+), respectively, to trisporic acid (Bu'Lock, Jones & Winskill, 1976; Gooday, 1978; Gooday & Adams, 1993). Both (+) and (−) cells then respond to trisporic acid by switching from vegetative growth to sexual differentiation. Aerial zygophores are produced which grow towards each other in mated pairs, from distances up to 2 mm (equivalent to 60 m on a human scale). This zygotropic response is probably mediated by volatile diffusion of the two specific prohormones. As soon as a (+) zygophore fuses with a (−) zygophore, it ceases to be attractive to other (−) zygophores, and vice versa, which would be explained by metabolism of the prohormone attractants being metabolized to trisporic acid instead of being released.

Hyphae of the human pathogen *C. albicans* can sense the physical nature of their substrate. When grown on Nuclepore or other perforated membranes overlying nutrient agar the

hyphal apices grow down through pores that they encounter (Sherwood *et al.*, 1992). Then, growing between agar and membrane, they will grow back up any further pores as they come to them. The result is that the hyphae weave in and out of the membrane. Together with the helical rotation of the tip described earlier, this behaviour is likely to aid penetration of host tissue, by enabling the hyphae to grow down surface discontinuities such as gaps between epithelial cells, membrane invaginations or local wounds.

Many fungal hyphae grow in a particular direction in response to an applied electric field. The direction of this galvanotropic response varies for different fungal strains, for different hyphal types, and with the pH of the medium. Thus rhizoids of *A. macrogynus* grew towards the anode, while hyphae grew towards the cathode (Youatt *et al.*, 1988; De Silva *et al.*, 1992). Effect of pH was shown strongly for *N. crassa*: at pH 4·8 and below hyphae grew towards the cathode, while at pH 5·8 and above they grew towards the anode (Lever *et al.*, 1994). The extent of galvanotropism depends on the field strength (Crombie, Gow & Gooday, 1990; Lever *et al.*, 1994). For *Aspergillus fumigatus*, it also depends on the exogenous Ca^{2+} concentration (Lever *et al.*, 1994). These observations suggest that galvanotropism could result from interplays between changes in the distribution of certain proteins in the hyphal cell membrane and polarized responses of some ion channels (Lever *et al.*, 1994).

CONCLUSION

This has been an account of some aspects of the growth of hyphae that have occupied me over the years. During this time there have been enormous advances in our understanding of biological systems, in particular through the development of molecular biology (in which fungi have been major players). Despite these advances, we remain largely ignorant about key features of hyphal growth that taxes the minds of mycologists such as Ward and Reinhardt over one hundred years ago. In the light of the account given here, some of the fundamental questions can be rephrased. Polarity of the extending hypha is a consequence of the organization of the cytoskeleton, but how is this established and maintained? Shape and diameter of the hyphal apex are results of the relative rates of synthesis and rigidification of wall components, but what controls these processes? Leading hyphae in mature colonies have many distinctive properties in comparison to juvenile hyphae, but what are the mechanisms of maturation? Apically growing hyphae can change their growth pattern to intercalary or 'backward' growth, but how do they do this? What gives rise to regular helical growth? We know a lot about fungal chitin synthase genes, but have to answer many questions about the regulation of chitin synthesis *in vivo*. Growing hyphae have appreciable activities of chitinases and other lytic enzymes, but the cases for and against their direct involvement in apical growth remain unproven. We have discovered that hyphae sense and respond to a range of chemical and physical stimuli, but only in a few cases are we starting to understand the mechanisms by which they do this. Hyphae respond in different ways to applied electrical fields, but what does this tell us about the establishment and maintenance of apical polarity?

I thank the many people who have inspired my interest in fungi over the years, from Lilian Hawker onwards, and all the colleagues, collaborators and students who have helped me in so many ways. As they are so numerous, I am sure that they will forgive me for not mentioning them individually. Many are co-authors on papers cited here. I also thank Ann Hoyle, University of Otago, for drawing the Figures.

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