1

Introduction

1.1 What are fungi?

About 80 000 to 120 000 species of fungi have been described to date, although the total number of species is estimated at around 1.5 million (Hawksworth, 2001; Kirk *et al*., 2001). This would render fungi one of the least-explored biodiversity resources of our planet. It is notoriously difficult to delimit fungi as a group against other eukaryotes, and debates over the inclusion or exclusion of certain groups have been going on for well over a century. In recent years, the main arguments have been between taxonomists striving towards a phylogenetic definition based especially on the similarity of relevant DNA sequences, and others who take a biological approach to the subject and regard fungi as organisms sharing all or many key ecological or physiological characteristics – the 'union of fungi' (Barr, 1992). Being interested mainly in the way fungi function in nature and in the laboratory, we take the latter approach and include several groups in this book which are now known to have arisen independently of the monophyletic 'true fungi' (**Eumycota**) and have been placed outside them in recent classification schemes (see Fig. 1.25). The most important of these 'pseudofungi' are the Oomycota (see Chapter 5). Based on their lifestyle, fungi may be circumscribed by the following set of characteristics (modified from Ainsworth, 1973):

1. *Nutrition*. Heterotrophic (lacking photosynthesis), feeding by absorption rather than ingestion.
2. *Vegetative state*. On or in the substratum, typically as a non-motile mycelium of hyphae showing internal protoplasmic streaming. Motile reproductive states may occur.
3. *Cell wall*. Typically present, usually based on glucans and chitin, rarely on glucans and cellulose (Oomycota).
4. *Nuclear status*. Eukaryotic, uni- or multinucleate, the thallus being homo- or heterokaryotic, haploid, dikaryotic or diploid, the latter usually of short duration (but exceptions are known from several taxonomic groups).
5. *Life cycle*. Simple or, more usually, complex.
6. *Reproduction*. The following reproductive events may occur: sexual (i.e. nuclear fusion and meiosis) and/or parasexual (i.e. involving nuclear fusion followed by gradual de-diploidization) and/or asexual (i.e. purely mitotic nuclear division).
7. *Propagules*. These are typically microscopically small spores produced in high numbers. Motile spores are confined to certain groups.
8. *Sporocarps*. Microscopic or macroscopic and showing characteristic shapes but only limited tissue differentiation.
9. *Habitat*. Ubiquitous in terrestrial and freshwater habitats, less so in the marine environment.
10. *Ecology*. Important ecological roles as saprotrophs, mutualistic symbionts, parasites, or hyperparasites.
11. *Distribution*. Cosmopolitan.

With photosynthetic pigments being absent, fungi have a heterotrophic mode of nutrition. In contrast to animals which typically feed by ingestion, fungi obtain their nutrients by extracellular digestion due to the activity of secreted enzymes, followed by absorption of the solubilized breakdown products. The combination of extracellular digestion and absorption can be seen as the ultimate determinant of the fungal lifestyle. In the course of evolution, fungi have conquered an astonishingly wide range of habitats, fulfilling important roles in diverse ecosystems (Dix & Webster, 1995). The conquest of new, often patchy resources is greatly facilitated by the production of numerous small spores rather than a few large propagules, whereas the colonization of a food source, once reached, is achieved most efficiently by growth as a system of branching tubes, the **hyphae** (Figs. 1.1a,b), which together make up the **mycelium**.

Hyphae are generally quite uniform in different taxonomic groups of fungi. One of the few features of distinction that they do offer is the presence or absence of cross-walls or **septa**. The Oomycota and Zygomycota generally have aseptate hyphae in which the nuclei lie in a common mass of cytoplasm (Fig. 1.1a). Such a condition is described as **coenocytic** (Gr. *koinos* = shared, in common; *kytos* = a hollow vessel, here meaning cell). In contrast, Asco- and Basidiomycota and their associated asexual states generally have septate hyphae (Fig. 1.1b) in which each segment contains one, two or more nuclei. If the nuclei are genetically identical, as in a mycelium derived from a single uninucleate spore, the mycelium is said to be **homokaryotic**, but where

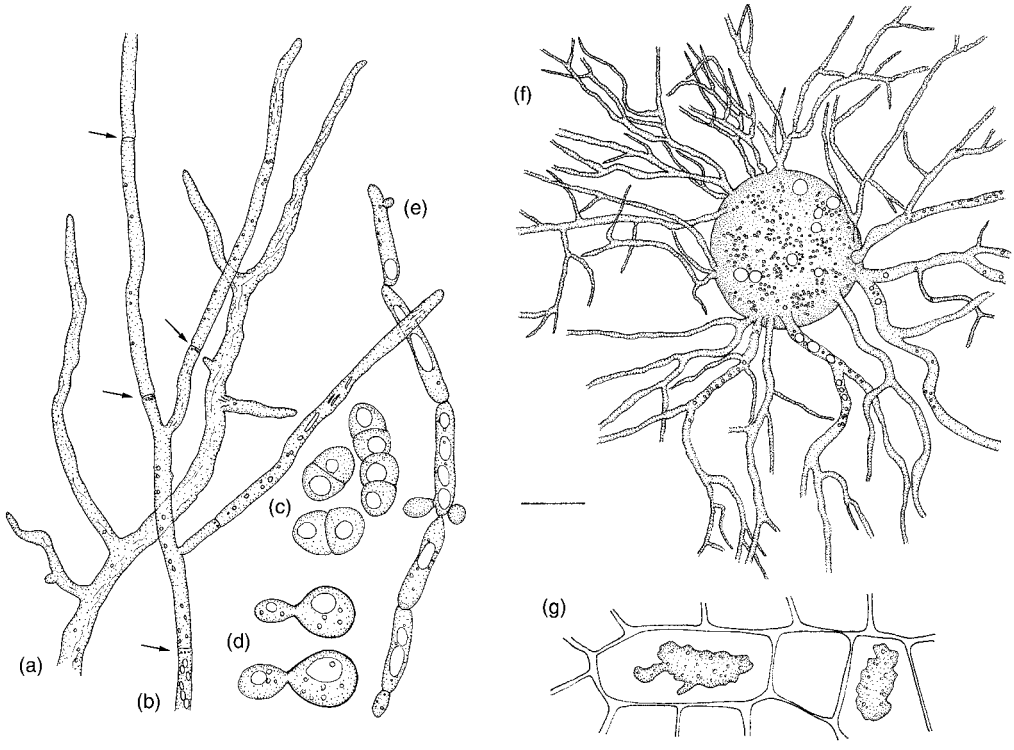


Fig 1.1 Various growth forms of fungi. (a) Aseptate hypha of *Mucor mucedo* (Zygomycota). The hypha branches to form a mycelium. (b) Septate branched hypha of *Trichoderma viride* (Ascomycota). Septa are indicated by arrows. (c) Yeast cells of *Schizosaccharomyces pombe* (Ascomycota) dividing by binary fission. (d) Yeast cells of *Dioszegia takashimae* (Basidiomycota) dividing by budding. (e) Pseudohypha of *Candida parapsilosis* (Ascomycota), which is regarded as an intermediate stage between yeast cells and true hyphae. (f) Thallus of *Rhizophlyctis rosea* (Chytridiomycota) from which a system of branching rhizoids extends into the substrate. (g) Plasmodia of *Plasmodiophora brassicae* (Plasmodiophoromycota) inside cabbage root cells. Scale bar = 20 μm (a,b,f,g) or 10 μm (c–e).

a cell or mycelium contains nuclei of different genotype, e.g. as a result of fusion (**anastomosis**) of genetically different hyphae, it is said to be **heterokaryotic**. A special condition is found in the mycelium of many Basidiomycota in which each cell contains two genetically distinct nuclei. This condition is **dikaryotic**, to distinguish it from mycelia which are **monokaryotic**. It should be noted that septa, where present, are usually perforated and allow for the exchange of cytoplasm or organelles.

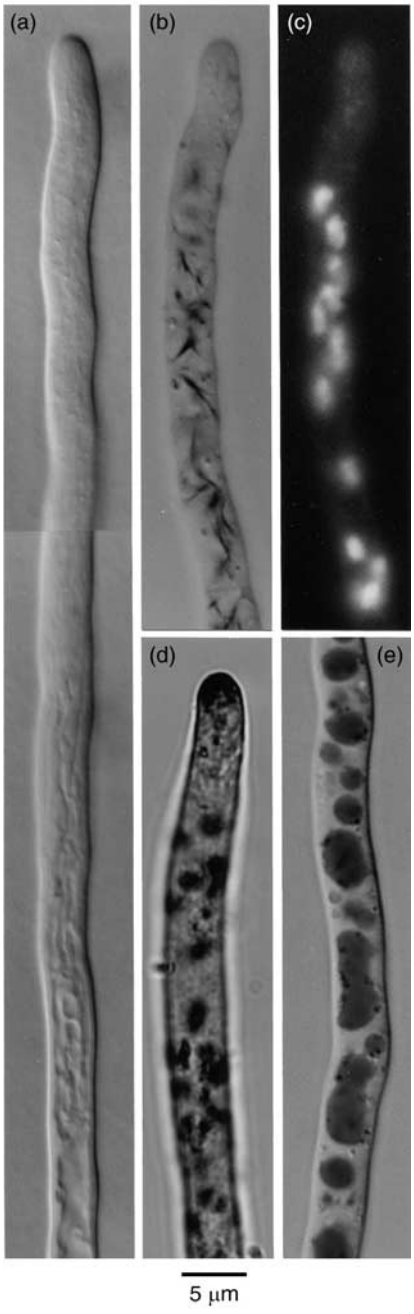
Not all fungi grow as hyphae. Some grow as discrete **yeast** cells which divide by fission (Fig. 1.1c) or, more frequently, budding (Fig. 1.1d). Yeasts are common, especially in situations where efficient penetration of the substratum is not required, e.g. on plant surfaces or in the digestive tracts of animals (Carlile, 1995). A few species, including certain pathogens of humans and animals, are **dimorphic**, i.e. capable of switching between hyphal and yeast-like growth forms (Gow, 1995). Intermediate stages between yeast cells and true hyphae also occur and are termed **pseudohyphae** (Fig. 1.1e). Some lower fungi grow as a **thallus**, i.e. a walled structure in which the protoplasm is concentrated in one or more centres from which root-like branches (**rhizoids**) ramify (Fig. 1.1f). Certain obligately plant-pathogenic fungi and fungus-like organisms grow as a naked **plasmodium** (Fig. 1.1g), a uni- or multinucleate mass of protoplasm not surrounded by a cell wall of its own, or as a **pseudoplasmodium** of amoeboid cells which retain their individual plasma membranes. However, by far the most important device which accounts for the typical biological features of fungi is the hypha (Bartnicki-Garcia, 1996), which therefore seems an appropriate starting point for an exploration of these organisms.

1.2 Physiology of the growing hypha

1.2.1 Polarity of the hypha

By placing microscopic markers such as small glass beads beside a growing hypha, Reinhardt (1892) was able to show that cell wall extension, measured as an increase in the distance between two adjacent markers, occurred only at the extreme apex. Four years earlier, H.M. Ward (1888), in an equally simple experiment, had collected liquid droplets from the apex of hyphae of *Botrytis cinerea* and found that these 'ferment-drops' were capable of degrading plant cell walls. Thus, the two fundamental properties of the vegetative fungal hypha – the polarity of both growth and secretion of degradative enzymes – have been known for over a century. Numerous studies have subsequently confirmed that 'the key to the fungal hypha lies in the apex' (Robertson, 1965), although the detailed mechanisms determining hyphal polarity are still obscure.

Ultrastructural studies have shown that many organelles within the growing hyphal tip are distributed in steep gradients, as would be expected of a cell growing in a polarized mode (Girbardt, 1969; Howard, 1981). This is visible even with the light microscope by careful observation of an unstained hypha using phase-contrast optics (Reynaga-Peña *et al*., 1997), and more so with the aid of simple staining techniques (Figs. 1.2a–d). The cytoplasm of the extreme apex is occupied almost exclusively by secretory vesicles and microvesicles (Figs. 1.2a, 1.3). In the higher fungi (Asco- and Basidiomycota), the former are arranged as a spherical shell around the latter, and the entire formation is called the **Spitzenkörper** or 'apical body' (Fig. 1.4c; Bartnicki-Garcia, 1996). The Spitzenkörper may be seen in growing hyphae even with the light microscope. Hyphae of the Oomycota and some lower Eumycota (notably the Zygomycota) do not contain a recognizable Spitzenkörper, and the vesicles are instead distributed more loosely in the apical dome (Fig. 1.4a,b). Hyphal growth can be simulated by means of computer models based on the assumption that the emission of secretory vesicles is coordinated by a 'vesicle supply centre', regarded as the mathematical equivalent of the Spitzenkörper in higher fungi. By modifying certain parameters, it is even possible to generate the somewhat more pointed apex often found in hyphae of Oomycota and Zygomycota (Figs. 1.4a,b; Diéguez-Uribeondo *et al*., 2004).



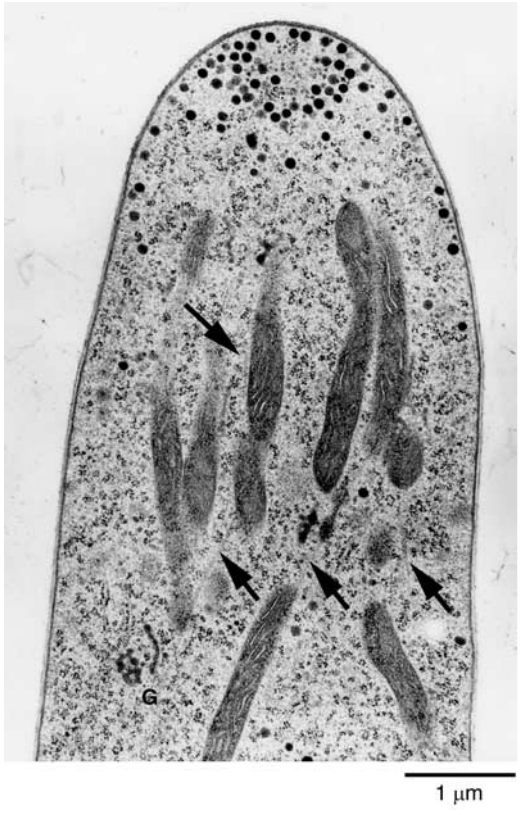


Fig I.3 Transmission electron microscopy of a hyphal tip of *Fusarium acuminatum* preserved by the freeze-substitution method to reveal ultrastructural details. The vesicles of the Spitzenkörper as well as mitochondria (dark elongated organelles), a Golgi-like element (G) and microtubules (arrows) are visible. Microtubules are closely associated with mitochondria. Reproduced from Howard and Aist (1980), by copyright permission of The Rockefeller University Press.

Fig I.2 The organization of vegetative hyphae as seen by light microscopy. (a) Growing hypha of *Galactomyces candidus* showing the transition from dense apical to vacuolate basal cytoplasm. Tubular vacuolar continuities are also visible. (b–e) Histochemistry in *Botrytis cinerea.* (b) Tetrazolium staining for mitochondrial succinate dehydrogenase. The mitochondria appear as dark filamentous structures in subapical and maturing regions. (c) Staining of the same hypha for nuclei with the fluorescent DNA-binding dye DAPI. The apical cell contains numerous nuclei. (d) Staining of acid phosphatase activity using the Gomori lead-salt method with a fixed hypha. Enzyme activity is localized both in the secretory vesicles forming the Spitzenkörper, and in vacuoles. (e) Uptake of Neutral Red into vacuoles in a mature hyphal segment. All images to same scale.

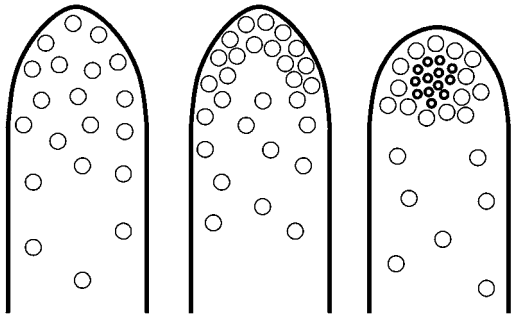


Fig 1.4 Schematic drawings of the arrangement of vesicles in growing hyphal tips. Secretory vesicles are visible in all hyphal tips, but the smaller microvesicles (chitosomes) are prominent only in Asco- and Basidiomycota and contribute to the Spitzenkörper morphology of the vesicle cluster. (a) Oomycota. (b) Zygomycota. (c) Ascomycota and Basidiomycota.

A little behind the apical dome, a region of intense biosynthetic activity and energy generation is indicated by parallel sheets of endoplasmic reticulum and an abundance of mitochondria (Figs. 1.2b, 1.3). The first nuclei usually appear just behind the biosynthetic zone (Fig. 1.2c), followed ultimately by a system of ever-enlarging vacuoles (Fig. 1.2d). These may fill almost the entire volume of mature hyphal regions, making them appear empty when viewed with the light microscope.

1.2.2 Architecture of the fungal cell wall

Although the chemical composition of cell walls can vary considerably between and within different groups of fungi (Table 1.1), the basic design seems to be universal. It consists of a structural scaffold of fibres which are cross-linked, and a matrix of gel-like or crystalline material (Hunsley & Burnett, 1970; Ruiz-Herrera, 1992; Sentandreu *et al.*, 1994). The degree of cross-linking will determine the plasticity (extensibility) of the wall, whereas the pore size (permeability) is a property of the wall matrix. The scaffold forms the inner layer of the wall and the matrix is found predominantly in the outer layer (de Nobel *et al.*, 2001).

In the Ascomycota and Basidiomycota, the fibres are **chitin** microfibrils, i.e. bundles of linear β-(1,4)-linked *N*-acetylglucosamine chains (Fig. 1.5), which are synthesized at the plasma membrane and extruded into the growing ('nascent') cell wall around the apical dome. The cell wall becomes rigid only after the microfibrils have been fixed in place by cross-linking. These cross-links consist of highly branched **glucans** (glucose polymers), especially those in which the glucose moieties are linked by β-(1,3)- and β-(1,6)-bonds (Suarit *et al.*, 1988; Wessels *et al.*, 1990; Sietsma & Wessels, 1994). Such β-glucans are typically insoluble in alkaline solutions (1 M KOH). In contrast, the alkali-soluble glucan fraction contains mainly α-(1,3)- and/or α-(1,4)-linked branched or unbranched chains (Wessels *et al.*, 1972; Bobbitt & Nordin, 1982) and does not perform a structural role but instead contributes significantly to the cell wall matrix (Sietsma & Wessels, 1994). Proteins represent the third important chemical

Table 1.1. The chemical composition of cell walls of selected groups of fungi (dry weight of total cell wall fraction, in per cent). Data adapted from Ruiz-Herrera (1992) and Griffin (1994).

Group	Example	Chitin	Cellulose	Glucans	Protein	Lipid
Oomycota	*Phytophthora*	0	25	65	4	2
Chytridiomycota	*Allomyces*	58	0	16	10	?
Zygomycota	*Mucor*	9*	0	44	6	8
Ascomycota	*Saccharomyces*	1	0	60	13	8
	Fusarium	39	0	29	7	6
Basidiomycota	*Schizophyllum*	5	0	81	2	?
	Coprinus	33	0	50	10	?

*Mainly chitosan.

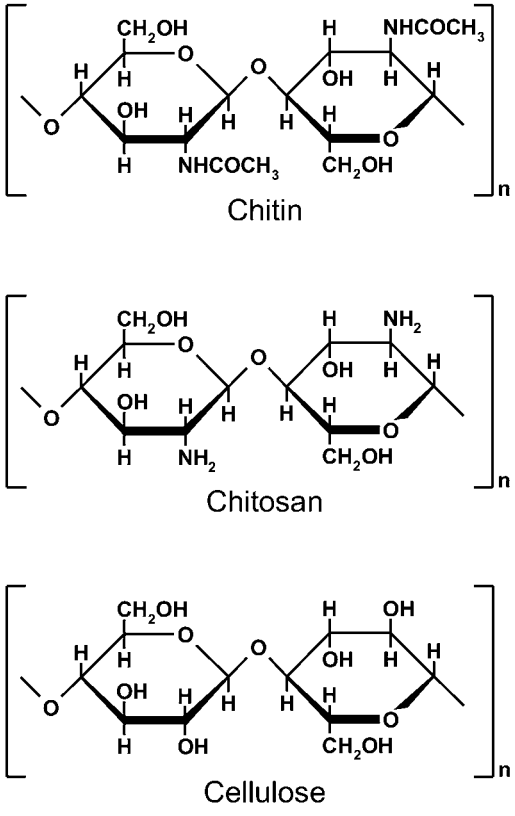


Fig 1.5 Structural formulae of the principal fibrous components of fungal cell walls.

constituent of fungal cell walls. In addition to enzymes involved in cell wall synthesis or lysis, or in extracellular digestion, there are also structural proteins. Many cell wall proteins are modified by glycosylation, i.e. the attachment of oligosaccharide chains to the polypeptide. The degree of glycosylation can be very high, especially in the yeast *Saccharomyces cerevisiae*, where up to 90% of the molecular weight of an extracellular protein may be contributed by its glycosylation chains (van Rinsum *et al*., 1991). Since mannose is the main component, such proteins are often called **mannoproteins** or mannans. In *S. cerevisiae*, the pore size of the cell wall is determined not by matrix glucans but by mannoproteins located close to the external wall surface (Zlotnik *et al*., 1984). Proteins exposed at the cell wall surface can also determine surface properties such as adhesion and recognition (Cormack *et al*., 1999). Structural proteins often contain a glycosylphosphatidylinositol anchor by which they are attached to the lumen of the rough endoplasmic reticulum (ER) and later to the external plasma membrane surface, or a modified anchor which covalently binds them to the β-(1,6)-glucan fraction of the cell wall (Kollár *et al*., 1997; de Nobel *et al*., 2001).

In the Zygomycota, the chitin fibres are modified after their synthesis by partial or complete deacetylation to produce poly-β-(1,4)-glucosamine, which is called **chitosan** (Fig. 1.5) (Calvo-Mendez & Ruiz-Herrera, 1987). Chitosan fibres are cross-linked by polysaccharides containing glucuronic acid and various neutral sugars (Datema *et al*., 1977). The cell wall matrix comprises glucans and proteins, as it does in members of the other fungal groups.

One traditional feature to distinguish the Oomycota from the 'true fungi' (Eumycota) has been the absence of chitin from their cell walls (Wessels & Sietsma, 1981), even though chitin is now known to be produced by certain species of Oomycota under certain conditions (Gay *et al*., 1993). By and large, however, in Oomycota, the structural role of chitin is filled by **cellulose**, an aggregate of linear β-(1,4)-glucan chains (Fig. 1.5). As in many other fungi, the fibres thus produced are cross-linked by an alkali-insoluble glucan containing β-(1,3)- and β-(1,6)-linkages. In addition to proteins, the main matrix component appears to be an alkali-soluble β-(1,3)-glucan (Wessels & Sietsma, 1981).

1.2.3 Synthesis of the cell wall

The synthesis of chitin is mediated by specialized organelles termed **chitosomes** (Bartnicki-Garcia *et al*., 1979; Sentandreu *et al*., 1994) in which inactive chitin synthases are delivered to the apical plasma membrane and become activated upon contact with the lipid bilayer (Montgomery & Gooday, 1985). Microvesicles, visible especially in the core region of the Spitzenkörper, are likely to be the ultrastructural manifestation of chitosomes (Fig. 1.6). In contrast, structural proteins and enzymes travel together in the larger secretory vesicles and are discharged into the environment when the vesicles fuse with the plasma membrane

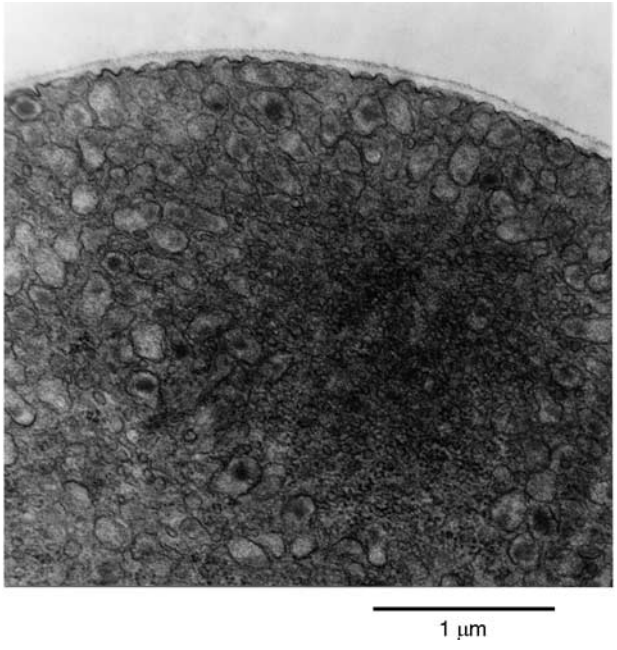


Fig 1.6 The Spitzenkörper of *Botrytis cinerea* which is differentiated into an electron-dense core consisting of microvesicles (chitosomes) and an outer region made up of larger secretory vesicles, some of which are located close to the plasma membrane. Reprinted from Weber and Pitt (2001), with permission from Elsevier.

(Fig. 1.6). Whereas most proteins are fully functional by the time they traverse the plasma membrane (see p. 10), the glucans are secreted by secretory vesicles as partly formed precursors (Wessels, 1993a) and undergo further polymerization in the nascent cell wall, or they are synthesized entirely at the plasma membrane (Sentandreu *et al*., 1994; de Nobel *et al*., 2001). Cross-linking of glucans with other components of the cell wall takes place after extrusion into the cell wall (Kollár *et al*., 1997; de Nobel *et al*., 2001).

Wessels *et al*. (1990) have provided experimental evidence to support a model for cell wall synthesis in *Schizophyllum commune* (Basidiomycota). The individual linear β-(1,4)-*N*-acetylglucosamine chains extruded from the plasma membrane are capable of undergoing self-assembly into chitin microfibrils, but this is subject to a certain delay during which cross-linking with glucans must occur. The glucans, in turn, become alkali-insoluble only after they have become covalently linked to chitin. Once the structural scaffold is in place, the wall matrix can be assembled. Wessels (1997) suggested that hyphal growth occurs as the result of a continuously replenished supply of soft wall material at the apex, but there is good evidence that the softness of the apical cell wall is also influenced by the activity of wall-lytic enzymes such as chitinases or glucanases (Fontaine *et al*., 1997; Horsch *et al*., 1997). Further, when certain Oomycota grow under conditions of hyperosmotic stress, their cell wall is measurably softer due to the secretion of an *endo*-β-(1,4)-glucanase, thus permitting continued growth when the turgor pressure is reduced or even absent (Money, 1994; Money & Hill, 1997). Since, in higher Eumycota, both cell wall material and synthetic as well as lytic enzymes are secreted together by the vesicles of the Spitzenkörper, the appearance, position and movement of this structure should influence the direction and speed of apical growth directly. This has indeed been shown to be the case (López-Franco *et al*., 1995; Bartnicki-Garcia, 1996; Riquelme *et al*., 1998).

Of course, cell wall-lytic enzymes are also necessary for the formation of hyphal branches, which usually arise by a localized weakening of the mature, fully polymerized cell wall. An *endo*-β-(1,4)-glucanase has also been shown to be involved in softening the mature regions of hyphae in the growing stipes of *Coprinus* fruit bodies, thus permitting intercalary hyphal extension (Kamada, 1994). Indeed, the expansion

of mushroom-type fruit bodies in general seems to be based mainly on non-apical extension of existing hyphae (see p. 22), which is a rare exception to the rule of apical growth in fungi.

The properties of the cell wall depend in many ways on the environment in which the hypha grows. Thus, when *Schizophyllum commune* is grown in liquid submerged culture, a significant part of the β-glucan fraction may diffuse into the liquid medium before it is captured by the cell wall, giving rise to mucilage (Sietsma *et al.*, 1977). In addition to causing problems when growing fungi in liquid culture for experimental purposes, mucilage may cause economic losses when released by *Botrytis cinerea* in grapes to be used for wine production (Dubourdieu *et al.*, 1978a). On the other hand, secreted polysaccharides, especially of Basidiomycota, may have interesting medicinal properties and are being promoted as anti-tumour medication both in conventional and in alternative medicine (Wasser, 2002).

Another difference between submerged and aerial hyphae is caused by the **hydrophobins**, which are structural cell wall proteins with specialized functions in physiology, morphogenesis and pathology (Wessels, 2000). Some hydrophobins are constitutively secreted by the hyphal apex. In submerged culture, they diffuse into the medium as monomers, whereas they polymerize by hydrophobic interactions on the surface of hyphae exposed to air, thereby effectively impregnating them and rendering them hydrophobic (Wessels, 1997, 2000). When freeze-fractured hydrophobic surfaces of hyphae or spores are viewed with the transmission electron microscope, polymerized hydrophobins may be visible as patches of rodlets running in parallel to each other. Other hydrophobins are produced only at particular developmental stages and are involved in inducing morphogenetic changes of the hypha, leading, for example, to the formation of spores or infection structures, or aggregation of hyphae into fruit bodies (Stringer *et al.*, 1991; Wessels, 1997).

Some fungi are wall-less during the assimilative stage of their life cycle. This is true especially of certain plant pathogens such as the Plasmodiophoromycota (Chapter 3), insect pathogens (Entomophthorales; p. 202) and some members of the Chytridiomycota (Chapter 6). Since their protoplasts are in direct contact with the host cytoplasm, they are buffered against osmotic fluctuations. The motile spores (zoospores) of certain groups of fungi swim freely in water, and bursting due to osmotic inward movement of water is prevented by the constant activity of water-expulsion vacuoles.

1.2.4 The cytoskeleton

In contrast to the hyphae of certain Oomycota, which seem to grow even in the absence of measurable turgor pressure (Money & Hill, 1997), the hyphae of most fungi extend only when a threshold turgor pressure is exceeded. This can be generated even at a reduced external water potential by the accumulation of compatible solutes such as glycerol, mannitol or trehalose inside the hypha (Jennings, 1995). The correlation between turgor pressure and hyphal growth might be interpreted such that the former drives the latter, but this crude mechanism would lead to uncontrolled tip extension or even tip bursting. Further, when hyphal tips are made to burst by experimental manipulation, they often do so not at the extreme apex, but a little further behind (Sietsma & Wessels, 1994). It seems, therefore, that the soft wall at the apex is protected internally, and there is now good evidence that this is mediated by the cytoskeleton.

Both main elements of the cytoskeleton, i.e. microtobules (Figs. 1.7a,b) and actin filaments (Fig. 1.7c), are abundant in filamentous fungi and yeasts (Heath, 1994, 1995a). Intermediate filaments, which fulfil skeletal roles in animal cells, are probably of lesser significance in fungi. Microtubules are typically orientated longitudinally relative to the hypha (Fig. 1.7a) and are involved in long-distance transport of organelles such as secretory vesicles (Fig. 1.7b; Seiler *et al.*, 1997) or nuclei (Steinberg, 1998), and in the positioning of mitochondria, nuclei or vacuoles (Howard & Aist, 1977; Steinberg *et al.*, 1998). They therefore maintain the polarized distribution of many organelles in the hyphal tip.

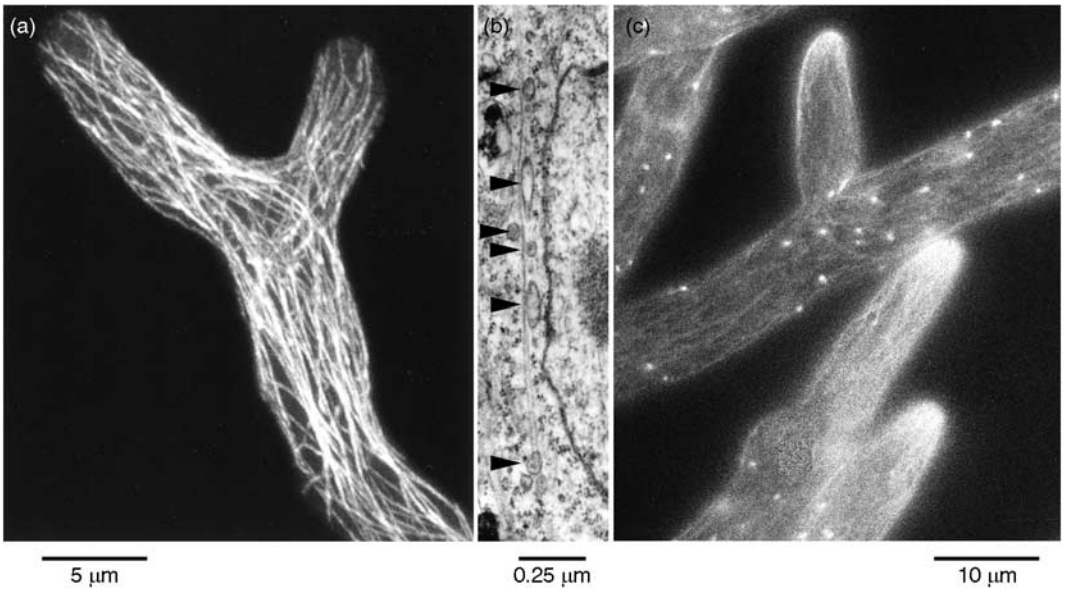


Fig I.7 The cytoskeleton in fungi. (a) Microtubules in *Rhizoctonia solani* (Basidiomycota) stained with an α-tubulin antibody. (b) Secretory vesicles (arrowheads) associated with a microtubule in *Botrytis cinerea* (Ascomycota). (c) The actin system of *Saprolegnia ferax* (Oomycota) stained with phalloidin—rhodamine. Note the dense actin cap in growing hyphal tips. (a) reproduced from Bourett *et al.* (1998), with permission from Elsevier; original print kindly provided by R. J. Howard. (b) reproduced from Weber and Pitt (2001), with permission from Elsevier. (c) reproduced from I. B. Heath (1987), by copyright permission of Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart; original print kindly provided by I. B. Heath.

Actin filaments are found in the centre of the Spitzenkörper, as discrete subapical patches, and as a cap lining the inside of the extreme hyphal apex (Heath, 1995a; Czymmek *et al.*, 1996; Srinivasan *et al.*, 1996). The apical actin cap is particularly pronounced in Oomycota such as *Saprolegnia* (Fig. 1.7c), and it now seems that the soft wall at the hyphal apex is actually being assembled on an internal scaffold consisting of actin and other structural proteins, such as spectrin (Heath, 1995b; Degousée *et al.*, 2000). The rate of hyphal extension might be controlled, and bursting prevented, by the actin/spectrin cap being anchored to the rigid, subapical wall via rivet-like integrin attachments which traverse the membrane and might bind to wall matrix proteins (Fig. 1.8; Kaminskyj & Heath, 1996; Heath, 2001). Indeed, in *Saprolegnia* the cytoskeleton is probably responsible for pushing the hyphal tip forward, at least in the absence of turgor (Money, 1997), although it probably has a restraining function under normal physiological conditions. Heath (1995b) has proposed an ingenious if speculative model to explain how the actin cap might regulate the rate of hyphal tip extension in the Oomycota. Stretch-activated channels selective for Ca^{2+} ions are known to be concentrated in the apical plasma membrane of *Saprolegnia* (Garrill *et al.*, 1993), and the fact that Ca^{2+} ions cause contractions of actin filaments is also well known. A stretched plasma membrane will admit Ca^{2+} ions into the apical cytoplasm where they cause localized contractions of the actin cap, thereby reducing the rate of apical growth which leads to closure of the stretch-activated Ca^{2+} channels. Sequestration of Ca^{2+} by various subapical organelles such as the ER or vacuoles lowers the concentration of free cytoplasmic Ca^{2+}, leading to a relaxation of the actin cap and of its restrictive effect on hyphal growth.

In the Eumycota, there is only indirect evidence for a similar role of actin, integrin and other structural proteins in protecting the apex and restraining its extension (Degousée *et al.*, 2000; Heath, 2001), and the details of

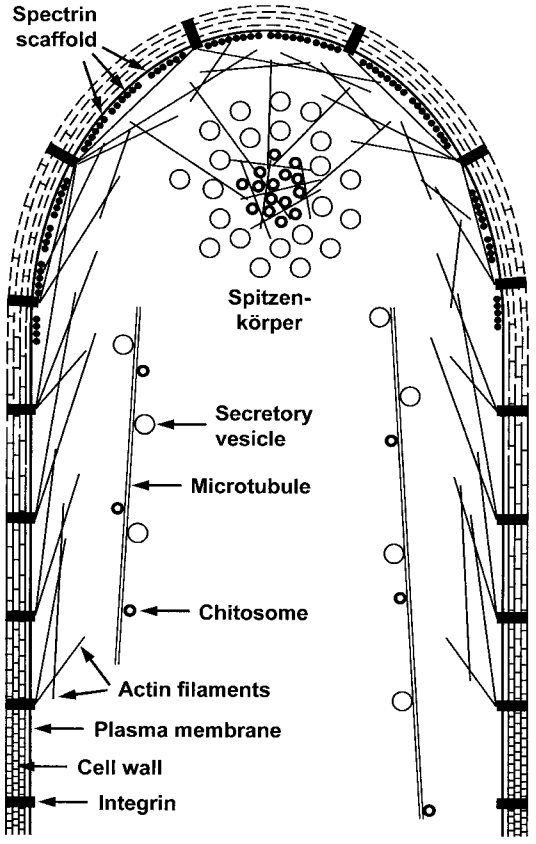


Fig 1.8 Diagrammatic representation of the internal scaffold model of tip growth in fungi proposed by Heath (1995b). Secretory vesicles and chitosomes are transported along microtubules from their subapical sites of synthesis to the growing apex. The Spitzenkörper forms around a cluster of actin filaments. An actin scaffold inside the extreme apex is linked to rivet-like integrin molecules which are anchored in the rigid subapical cell wall. The apex is further stabilized by spectrin molecules lining the cytoplasmic surface of the plasma membrane. Redrawn and modified from Weber and Pitt (2001).

regulation are likely to be different. Whereas a tip-high Ca^{2+} gradient is present and is required for growth, stretch-activated Ca^{2+} channels are not, and the apical Ca^{2+} seems to be of endogenous origin. Silverman-Gavrila and Lew (2001, 2002) have proposed that the signal molecule inositol-(1,4,5)-trisphosphate (IP_3), released by the action of a stretch-activated phospholipase C in the apical plasma membrane, acts on Ca^{2+}-rich secretory vesicles in the Spitzenkörper region. These would release Ca^{2+} from their lumen, leading to a contraction of the apical scaffold. As in the Oomycota, sequestration of Ca^{2+} occurs subapically by the ER from which secretory vesicles are formed. These therefore act as Ca^{2+} shuttles in the Eumycota (Torralba *et al.*, 2001). Although hyphal tip growth appears to be a straightforward affair, none of the conflicting models accounts for all aspects of it. A good essay in hyphal tip diplomacy has been written by Bartnicki-Garcia (2002).

Numerous inhibitor studies have hinted at a role of the cytoskeleton in the transport of vesicles to the apex. Depolymerization of microtubules results in a disappearance of the Spitzenkörper, termination or at least severe reduction of apical growth and enzyme secretion, and an even redistribution of secretory vesicles and other organelles throughout the hypha (Howard & Aist, 1977; Rupeš *et al.*, 1995; Horio & Oakley, 2005). In contrast, actin depolymerization leads to uncontrolled tip extension to form giant spheres (Srinivasan *et al.*, 1996). Long-distance transport of secretory vesicles therefore seems to be brought about by microtubules, whereas the fine-tuning of vesicle fusion with the plasma membrane is controlled by actin (Fig. 1.8; Torralba *et al.*, 1998). The integrity of the Spitzenkörper is maintained by an interplay between actin and tubulin. Not surprisingly, the yeast *S. cerevisiae*, which has a very short vesicle transport distance between the mother cell and the extending bud, reacts more sensitively to disruptions of the actin component than the microtubule component of its cytoskeleton; continued growth in the absence of the latter can be explained by Brownian motion of secretory vesicles (Govindan *et al.*, 1995; Steinberg, 1998).

1.2.5 Secretion and membrane traffic

One of the most important ecological roles of fungi, that of decomposing dead plant matter, requires the secretion of large quantities of hydrolytic and oxidative enzymes into the environment. In liquid culture under optimized experimental conditions, certain fungi