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

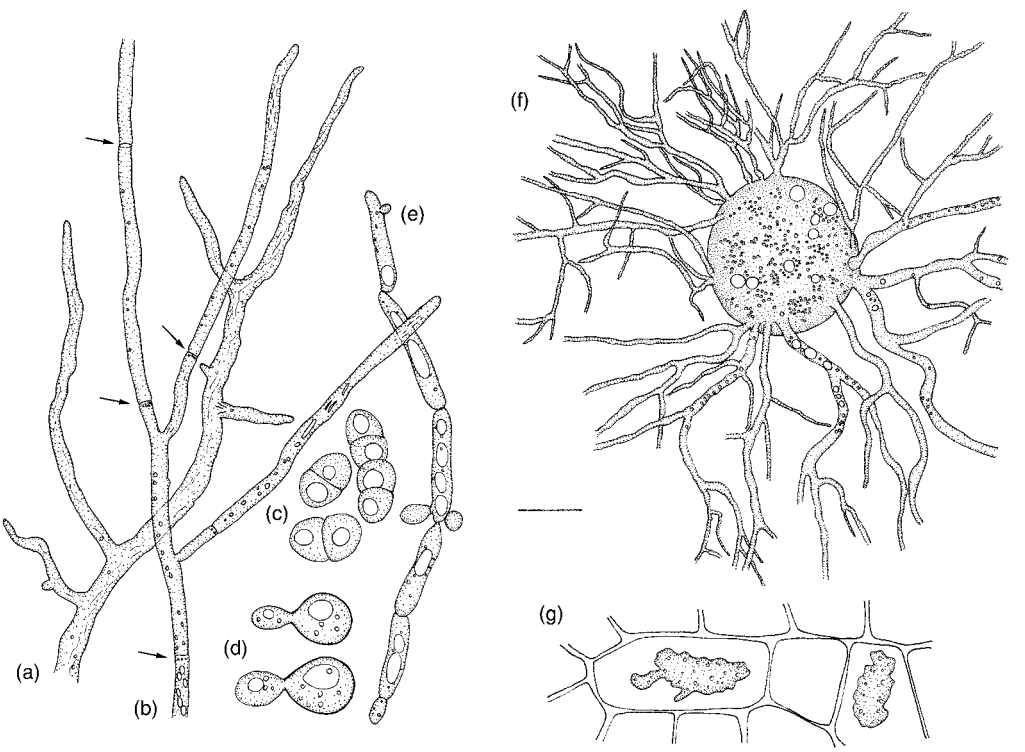

Fig 1.1 Various growth forms of fungi. (a) Aseptate hypha of *Mucor mucedo* (Zygomycota). The hypha branches to form a mycelium. (b) Septate branched hypha of *Trichoderma viride* (Ascomycota). Septa are indicated by arrows. (c) Yeast cells of *Schizosaccharomyces pombe* (Ascomycota) dividing by binary 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).

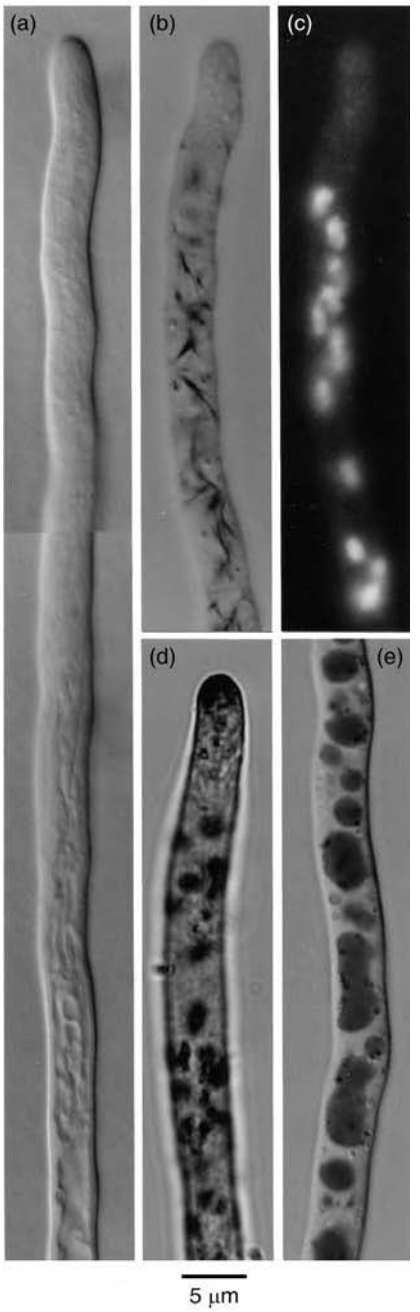

Fig I.2 The organization of vegetative hyphae as seen by light microscopy. (a) Growing hypha of *Galactomyces candidus* showing the transition from dense apical to vacuolate basal cytoplasm. Tubular vacuolar continuities are also visible. (b–e) Histochemistry in *Botrytis cinerea.* (b) Tetrazolium staining 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.

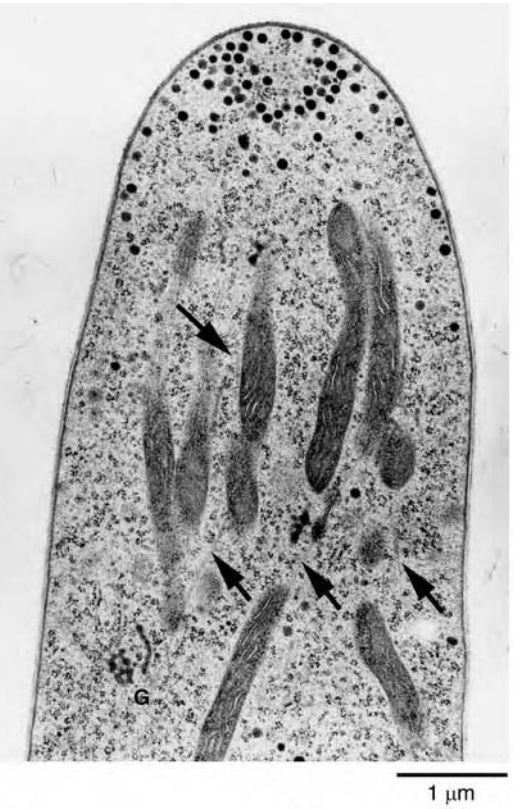

Fig I.3 Transmission electron microscopy of a hyphal tip of *Fusarium acuminatum* preserved by the freeze-substitution method to reveal ultrastructural details. The vesicles of the Spitzenkörper as well as mitochondria (dark elongated organelles), a Golgi-like element (G) and microtubules (arrows) are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

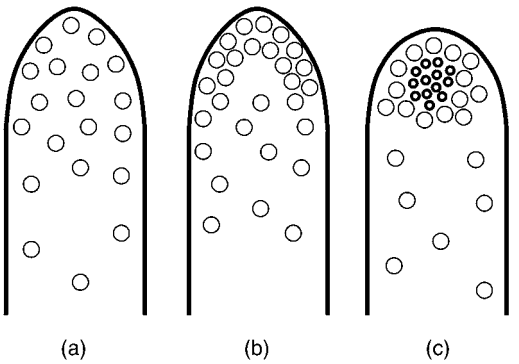

Fig 1.4 Schematic drawings of the arrangement of vesicles in growing hyphal tips. Secretory vesicles are visible in all hyphal tips, but the smaller microvesicles (chitosomes) are prominent only in Asco- and Basidiomycota and contribute to the Spitzenkörper morphology of the vesicle cluster. (a) Oomycota.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

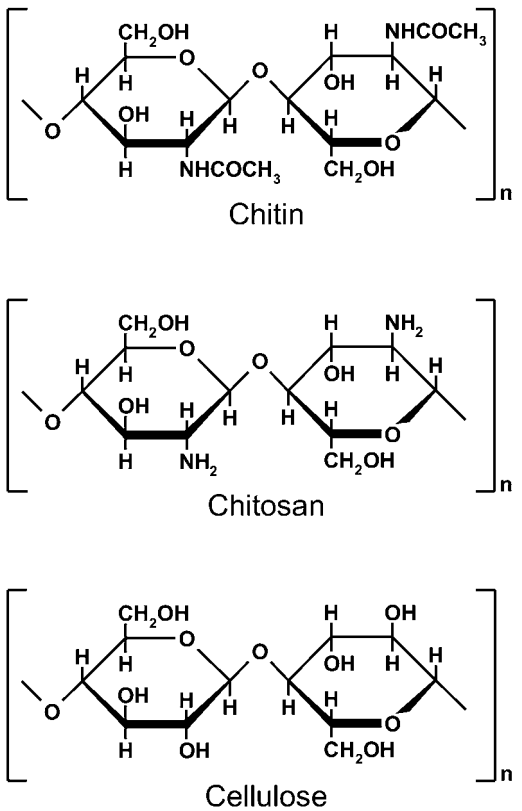

Fig 1.5 Structural formulae of the principal fibrous components of fungal cell walls.

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

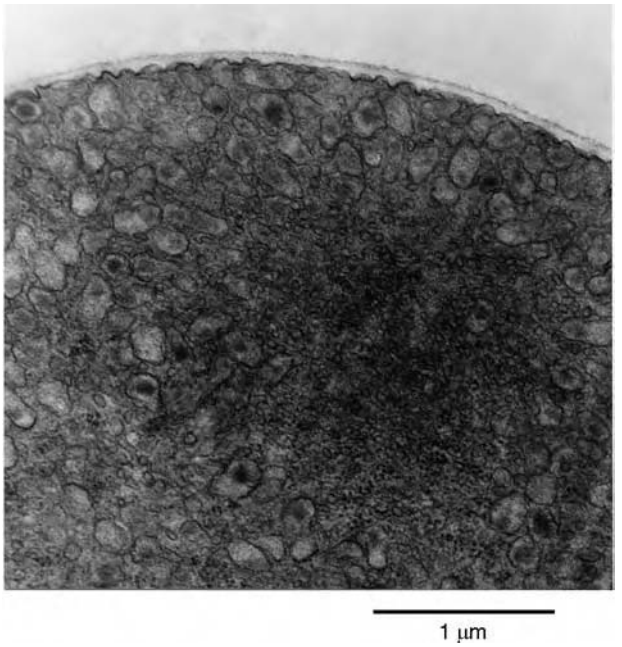

Fig 1.6 The Spitzenkörper of *Botrytis cinerea* which is differentiated into an electron-dense core consisting of microvesicles (chitosomes) and an outer region made up of larger secretory vesicles, some of which are located close to the plasma membrane. Reprinted from Weber and Pitt (2001), with permission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

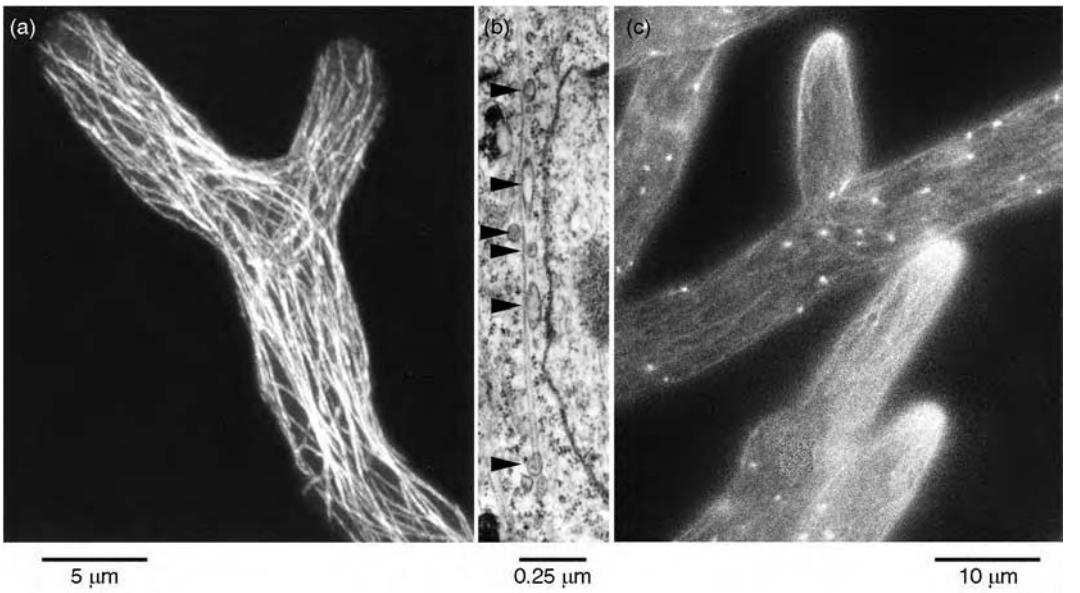

Fig I.7 The cytoskeleton in fungi. (a) Microtubules in *Rhizoctonia solani* (Basidiomycota) stained with an α-tubulin antibody. (b) Secretory vesicles (arrowheads) associated with a microtubule in *Botrytis cinerea* (Ascomycota). (c) The actin system of *Saprolegnia ferax* (Oomycota) stained with phalloidin–rhodamine. 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

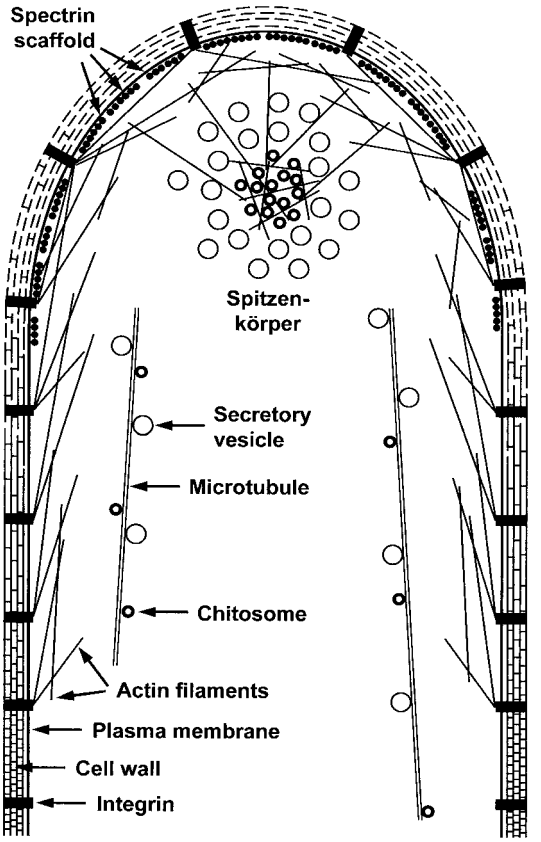

Fig 1.8 Diagrammatic representation of the internal scaffold model of tip growth in fungi proposed by Heath (1995b). Secretory vesicles and chitosomes are transported along microtubules from their subapical sites of synthesis to the growing apex. The Spitzenkörper forms around a cluster of actin filaments. 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

are capable of secreting more than 20 g of a single enzyme or enzyme group per litre culture broth within a few days' growth (Sprey, 1988; Peberdy, 1994). Clearly, this aspect of fungal physiology holds considerable potential for biotechnological or pharmaceutical applications. However, for reasons not yet entirely understood, fungi often fail to secrete the heterologous proteins of introduced genes of commercial interest to the same high level as their own proteins (Gwynne, 1992). There are still great deficits in our understanding of the fundamental mechanisms of the secretory route in filamentous fungi, although much is known in the yeast *S. cerevisiae*. An overview is given in Fig. 1.10.

As in other eukaryotes, the secretory route in fungi begins in the ER. Ribosomes loaded with a suitable messenger RNA dock onto the ER membrane and translate the polypeptide product which enters the ER lumen during its synthesis unless specific internal signal sequences cause it to be retained in the ER membrane. As soon as the protein is in contact with the ER lumen, oligosaccharide chains may be added onto selected amino acids. These glycosylation chains are subject to successive modification steps as the protein traverses the secretory route, whereby the chains in *S. cerevisiae* become considerably larger than those in most filamentous fungi (Maras *et al*., 1997; Gemmill & Trimble, 1999). Paradoxically, even though filamentous fungi possess such powerful secretory systems, morphologically recognizable Golgi stacks have not generally been observed except for the Oomycota, Plasmodiophoromycota and related groups (Grove *et al*., 1968; Beakes & Glockling, 1998). In all other fungi, the Golgi apparatus seems to be much reduced to single cisternae (Howard, 1981; see Fig. 1.3), with images of fully fledged Golgi stacks only published occasionally (see e.g. Fig. 10.1). In *S. cerevisiae* and probably also in filamentous fungi, the transport of proteins from the ER to the Golgi system occurs via vesicular carriers (Schekman, 1992), although continuous membrane flow is also possible (see p. 272). Membrane lipids seem to be recycled to the ER by a different mechanism relying on tubular continuities (Rupeš *et al*., 1995; Akashi *et al*., 1997).

In the Golgi system, proteins are subjected to stepwise further modifications (Graham & Emr, 1991), and proteins destined for the vacuolar system are separated from those bound for secretion (Seeger & Payne, 1992). Both destinations are probably reached by vesicular carriers, the secretory vesicles moving along microtubules to reach the growing hyphal apex (Fig. 1.7b), which is the site for secretion of extracellular enzymes as well as new cell wall material (Peberdy, 1994). Collinge and Trinci (1974) estimated that 38 000 secretory vesicles per minute fuse with the plasma membrane of a single growing hypha of *Neurospora crassa*. Microvesicles (chitosomes) probably arise from a discrete population of Golgi cisternae (Howard, 1981).

There is mounting evidence that fungi, like most eukaryotes, are capable of performing endocytosis by the inward budding of the plasma membrane at subapical locations. Endocytosis may be necessary to retrieve membrane material in excess of that which is required for extension at the growing apex, i.e. endocytosis and exocytosis may be coupled (Steinberg & Fuchs, 2004). The prime destination of endocytosed membrane material or vital stains is the vacuole (Vida & Emr, 1995; Fischer-Parton *et al*., 2000; Weber, 2002). In fungi, large vacuoles (Figs. 1.2e, 1.9) represent the main element of the lytic system and are the sink not only for endocytosed material but also for autophagocytosis, i.e. the sequestration and degradation of organelles or cytoplasm. Autophagocytosis is especially prominent under starvation conditions (Takeshige *et al*., 1992). Careful ultrastructural studies have revealed that adjacent vacuoles may be linked by thin membranous tubes, thereby providing a potential means of transport (Rees *et al*., 1994). These tubes can extend even through the septal pores and show peristaltic movement, possibly explaining why especially mycorrhizal fungi are capable of rapid translocation of solutes over long hyphal distances (Fig. 1.9; Cole *et al*., 1998; Ashford *et al*., 2001).

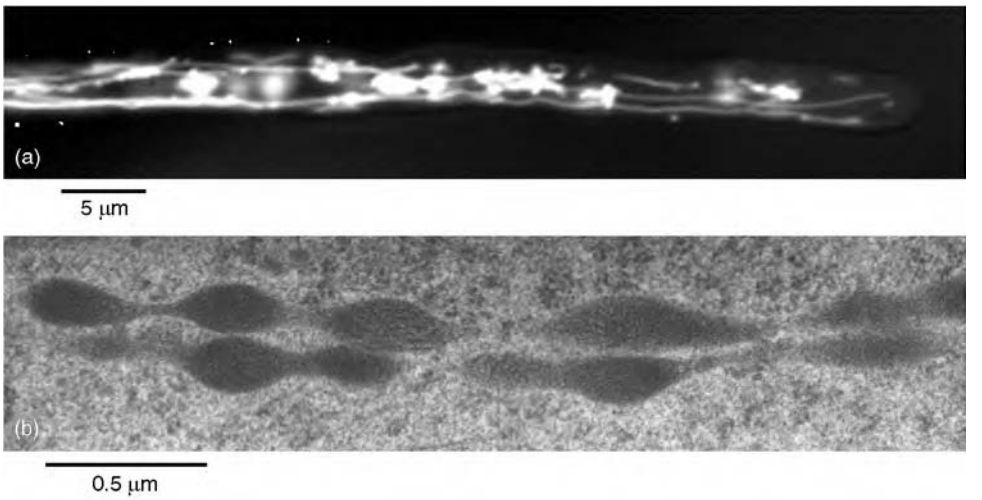

Fig 1.9 Tubular continuities linking adjacent vacuoles of *Pisolithus tinctorius*. (a) Light micrograph of the vacuolar system of *Pisolithus tinctorius* stained with a fluorescent dye. (b) TEM image of a freeze-substituted hypha. Reproduced from Ashford *et al*. (2001), with kind permission of Springer Science and Business Media. Original images kindly provided by A. E. Ashford.

1.2.6 Nutrient uptake

One of the hallmarks of fungi is their ability to take up organic or inorganic solutes from extremely dilute solutions in the environment, accumulating them 1000-fold or more against their concentration gradient (Griffin, 1994). The main barrier to the movement of water-soluble substances into the cell is the lipid bilayer of the plasma membrane. Uptake is mediated by proteinaceous pores in the plasma membrane which are always selective for particular solutes. The pores are termed **channels** (system I) if they facilitate the diffusion of a solute following its concentration gradient whilst they are called **porters** (system II) if they use metabolic energy to accumulate the solute across the plasma membrane against its gradient (Harold, 1994). Fungi often possess one channel and one porter for a given solute. The high-affinity porter system is repressed at high external solute concentrations such as those found in most laboratory media (Scarborough, 1970; Sanders, 1988).

In nature, however, the concentration of nutrients is often so low that the porter systems are active. Porters do not directly convert metabolic energy (ATP) into the uptake of solutes; rather, ATP is hydrolysed by ATPases which pump protons (H^+) to the outside of the plasma membrane, thus establishing a transmembrane pH gradient (acid outside). It has been estimated that one-third of the total cellular ATP is used for the establishment of the transmembrane H^+ gradient (Gradmann *et al*., 1978). The inward movement of H^+ following its electrochemical gradient is harnessed by the porters for solute uptake by means of solute–porter–H^+ complexes (Slayman & Slayman, 1974; Slayman, 1987; Garrill, 1995). Different types of porter exist, depending on the charge of the desired solute. Uniport and symport carriers couple the inward movement of H^+ with the uptake of uncharged or negatively charged solutes, respectively, whereas antiports harness the outward diffusion of cations such as K^+ for the uptake of other positively charged solutes. Charge imbalances can be rectified by the selective opening of K^+ channels. Porters have been described for NH_4^+, NO_3^-, amino acids, hexoses, orthophosphate and other solutes (Garrill, 1995; Jennings, 1995).

The ATPases fuelling active uptake mechanisms are located in subapical or mature regions of the plasma membrane, whereas the porter systems are typically situated in the apical membrane (Harold, 1994), closest to the site where the solutes may be released by the activity of extracellular enzymes. Thus, mature hyphal segments make a substantial direct contribution

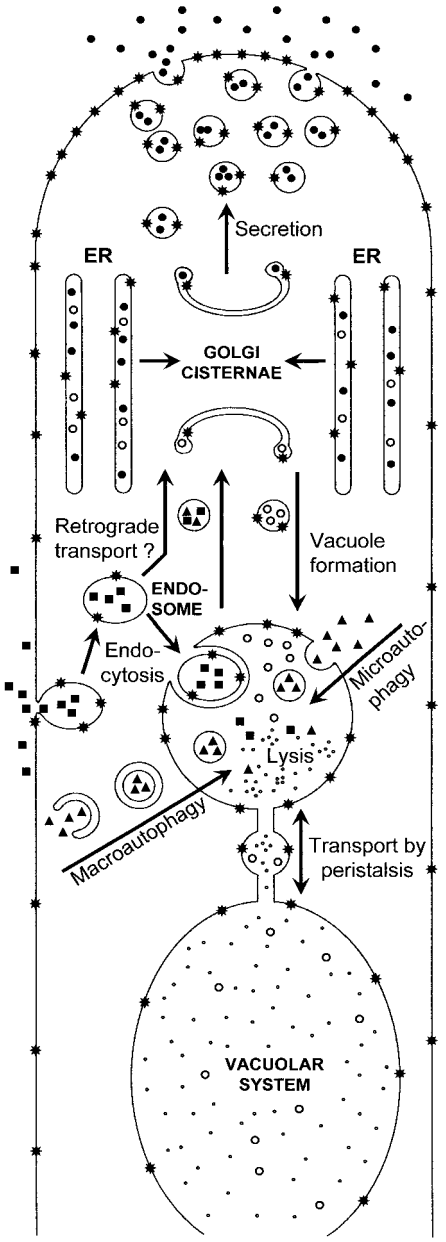

Fig 1.10 Schematic summary of the pathways of membrane flow in a growing hypha. Secretory proteins (•), vacuolar luminal proteins (○), membrane-bound proteins (✱), endocytosed (■) and autophagocytosed (▲) material is indicated, as are vacuolar degradation products (•). Redrawn and modified from Weber (2002).

to the growth of the hypha at its tip. The spatial separation of H^+ expulsion and re-entry generates an external electric field carried by protons (Fig. 1.11), which was at one time thought to be a causal factor of hyphal tip polarity but is now regarded as a consequence of it (Harold, 1994).

Proton pumps fuelled by ATP are prominent also in the vacuolar membrane, the tonoplast (Fig. 1.11), and their activity acidifies the vacuolar lumen (Klionsky *et al*., 1990). The principle of proton-coupled solute transport is utilized by the vacuole to fulfil its role as a system for the storage of nutrients, for example phosphate (Cramer & Davis, 1984) or amino acids such as arginine (Keenan & Weiss, 1997), or for the removal of toxic compounds from the cytoplasm, e.g. Ca^{2+} or heavy metal ions (Cornelius & Nakashima, 1987).

1.2.7 Hyphal branching

Assimilative hyphae of most fungi grow monopodially by a main axis (**leading hypha**) capable of potentially unlimited apical growth. Branches arise at some distance behind the apex, suggesting some form of apical dominance, i.e. the presence of a growing apex inhibits the development of lateral branches close to it. Dichotomous branching is rare, but does occur in *Allomyces* (see Fig. 6.20d) and *Galactomyces geotrichum*. In septate fungi, branches are often located immediately behind a septum. Branches usually arise singly in vegetative hyphae, although whorls of branches (i.e. branches arising near a common point) occur in reproductive structures. Branching may thus be under genetic or external control (Burnett, 1976). An even spacing between vegetative hyphae results from a combination of chemotropic growth towards a source of diffusible nutrients, and growth away from staling products secreted by other hyphae which have colonized a substratum. The circular appearance of fungal colonies in Petri dish cultures arises because certain lateral branches grow out and fill the space between the leading radial branches, keeping pace with their rate of growth. This **invasive growth** is the most efficient way to spread throughout a substratum. In nature, it may be obvious even to the naked eye, for example, in the shape of fairy rings (see Figs. 19.18a,b).

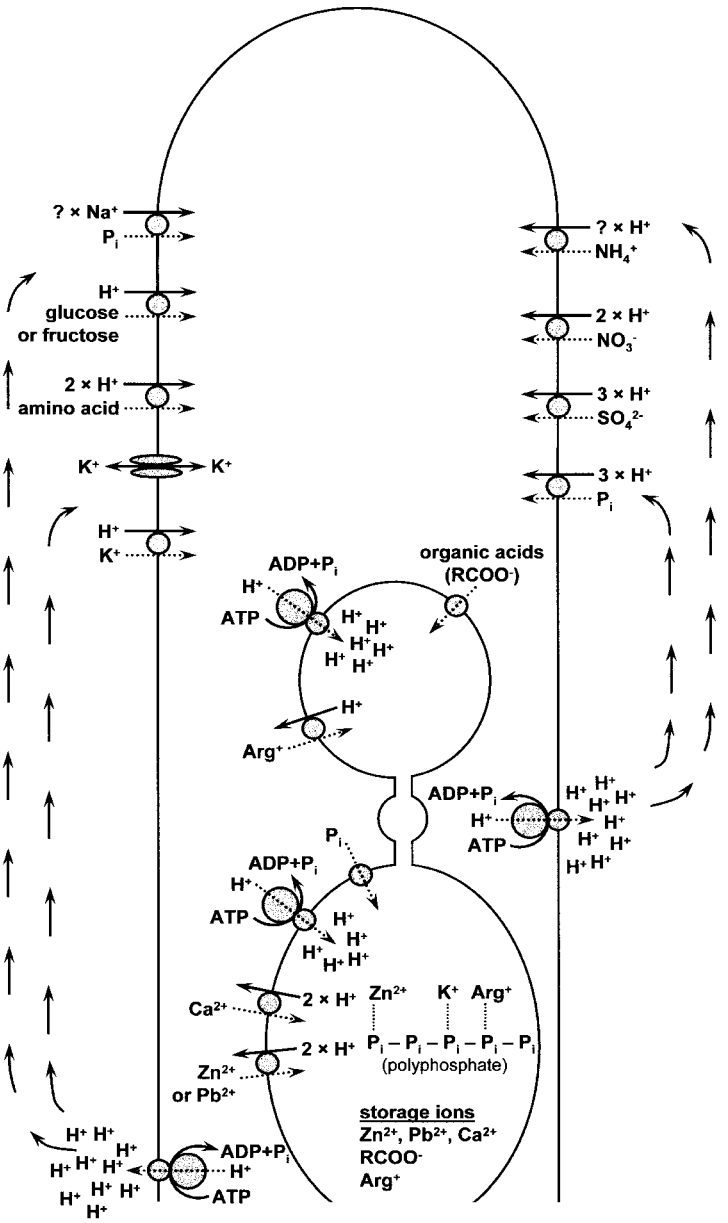

Fig 1.11 Ion fluxes in a growing hypha. The proton (H^+) gradient across the plasma membrane is generated by subapical ATP-driven expulsion of protons. It is used for the active uptake of nutrients by porters. Channels also exist for most of the nutrients but are not shown here, except for the K^+ channel which operates to compensate for charge imbalances. Dotted arrows indicate movement of a solute against its concentration gradient; solid arrows indicate movement from concentrated to dilute. For details, see Garrill (1995).

1.3 Hyphal aggregates

Whereas plants and animals form genuine tissues by their ability to perform cell divisions in all directions, fungi are limited by their growth as one-dimensional hyphae. None the less, fungi are capable of producing complex and characteristic multicellular structures which resemble the tissues of other eukaryotes. This must be controlled by the positioning, growth rate and growth direction of individual hyphal branches (Moore, 1994). Further, instead of spacing themselves apart as during invasive growth, hyphae must be made to aggregate. Very little is known about the signalling events

leading to the synchronized growth of groups of hyphae. However, it may be speculated that the diffusion of signalling molecules takes place between adjacent hyphae, i.e. that a given hypha is able to influence the gene expression of adjacent hyphae by secreting chemical messengers. This may be facilitated by an extrahyphal glucan matrix within which aggregating hyphae are typically embedded (Moore, 1994). Such matrices have been found in rhizomorphs (Rayner *et al*., 1985), sclerotia (Fig. 1.16c; Willetts & Bullock, 1992) and fruit bodies (Williams *et al*., 1985). The composition of proteins on the surface of hyphal walls may also play an important role in recognition and adhesion phenomena (de Nobel *et al*., 2001).

1.3.1 Mycelial strands

The formation of aggregates of parallel, relatively undifferentiated hyphae is quite common in the Basidiomycota and in some Ascomycota. For instance, mycelial strands form the familiar 'spawn' of the cultivated mushroom *Agaricus bisporus*. Strands arise most readily from a well-developed mycelium extending from an exhausted food base into nutrient-poor surroundings (Fig. 1.12a). When a strand encounters a source of nutrients exceeding its internal supply, coherence is lost and a spreading assimilative mycelium regrows (Moore, 1994). Alternatively, mycelial strands may be employed by fungi which produce their fructifications some distance away from the food base, as in the stinkhorn, *Phallus impudicus*. Here the mycelial strand is more tightly aggregated and is referred to as a **mycelial cord**. The tip of the mycelial cord, which arises from a buried tree stump, differentiates into an egg-like basidiocarp initially upon reaching the soil surface (Fig. 1.12b).

The development of *A. bisporus* strands has been described by Mathew (1961). Robust leading hyphae extend from the food base and branch at fairly wide intervals to form finer laterals, most of which grow away from the parent hypha. A few branch hyphae, however, form at an acute angle to the parent hypha and tend to grow parallel to it. Hyphae of many fungi occasionally

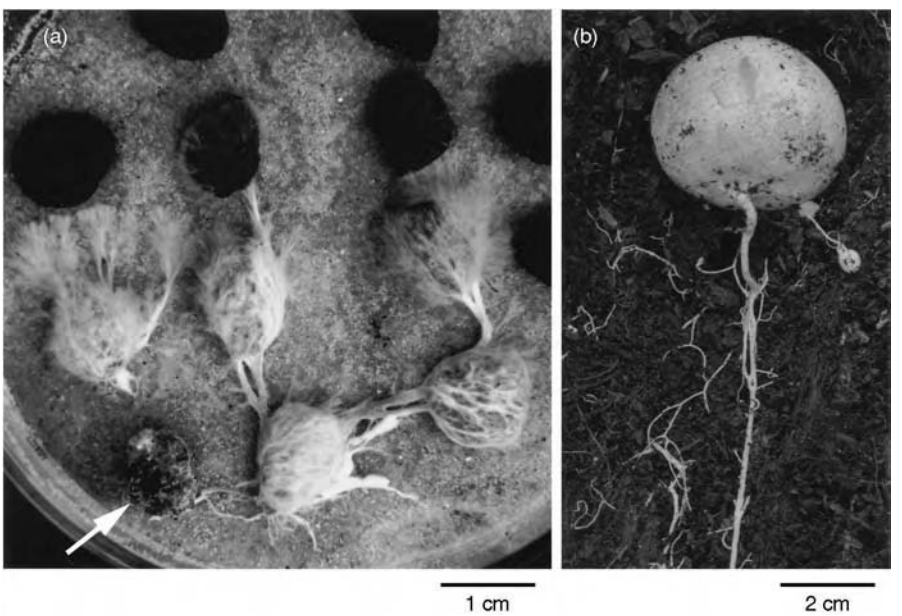

Fig 1.12 Mycelial strands. (a) Strands of *Podosordaria tulasnei* (Ascomycota) extending from a previously colonized rabbit pellet (arrow) over sand. Note the dissolution of the strand upon reaching a new nutrient source, in this case fresh sterile rabbit pellets. (b) Excavated mycelial cords of the stinkhorn *Phallus impudicus*, which can be traced back from the egg-like basidiocarp primordium to the base of an old tree stump (below the bottom of the picture, not shown).

grow alongside each other or another physical obstacle which they chance to encounter. A later and specific stage in strand development is characterized by the formation of numerous fine, aseptate 'tendril hyphae' as branches from the older regions of the main hyphae. The tendril hyphae, which may extend forwards or backwards, become appressed to the main hypha and branch frequently to form even finer tendrils which grow round the main hyphae and ensheath them. Major strands are consolidated by anastomoses between their hyphae, and they increase in thickness by the assimilation of minor strands. A similar development has been noted in the strands of *Serpula lacrymans*, the dry-rot fungus (Fig. 1.13), which are capable of extending for several metres across brickwork and other surfaces from a food base in decaying wood (Jennings & Watkinson, 1982; Nuss *et al*., 1991).

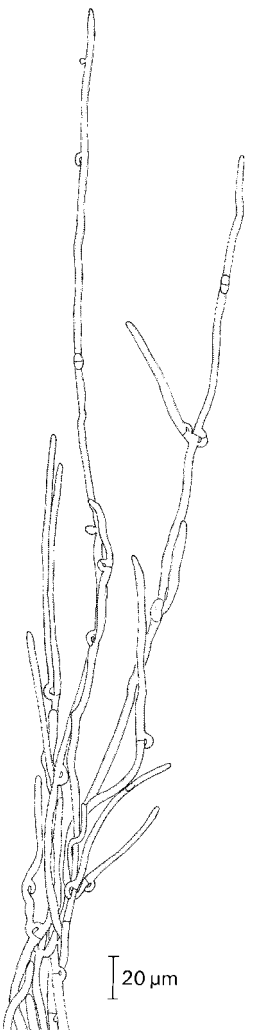

Fig 1.13 The tip of a hyphal strand of *Serpula lacrymans* (Basidiomycota). Note the formation of lateral branches which grow parallel to the direction of the main hyphae. The buckle-shaped structures at the septa are clamp connections.

By recovering the nutrients from obsolete strands and forming new strands, colonies can move about and explore their vicinity in the search for new food bases (Cooke & Rayner, 1984; Boddy, 1993). Mycelial strands are capable of translocating nutrients and water in both directions (Boddy, 1993; Jennings, 1995). This property is important not only for decomposer fungi, but also for species forming mycorrhizal symbioses with the roots of plants, many of which produce hyphal strands (Read, 1991).

1.3.2 Rhizomorphs

In contrast to mycelial strands or cords which consist of relatively undifferentiated aggregations of hyphae and are produced by a great variety of fungi, rhizomorphs are found in only relatively few species and contain highly differentiated tissues. Well-known examples of rhizomorph-forming fungi are provided by *Armillaria* spp. (Figs. 1.14 and 18.13b), which are serious parasites of trees and shrubs. In *Armillaria*, a central core of larger, thin-walled, elongated cells embedded in mucilage is surrounded by a rind of small, thicker-walled cells which are darkly pigmented due to melanin deposition in their walls. These root-like aggregations are a means for *Armillaria* to spread underground from one tree root system to another. In nature, two kinds are found – a dark, cylindrical type and a paler, flatter type. The latter is particularly common beneath the bark of infected trees (see p. 546). Rhizomorphs on dead trees measure up to 4 mm in diameter. It has been estimated that a rhizomorph only 1 mm in diameter must contain over 1000 hyphae aggregated together. The development of rhizomorphs in agar culture has been described by Garrett (1953, 1970) and Snider (1959). Initiation of rhizomorphs can first be

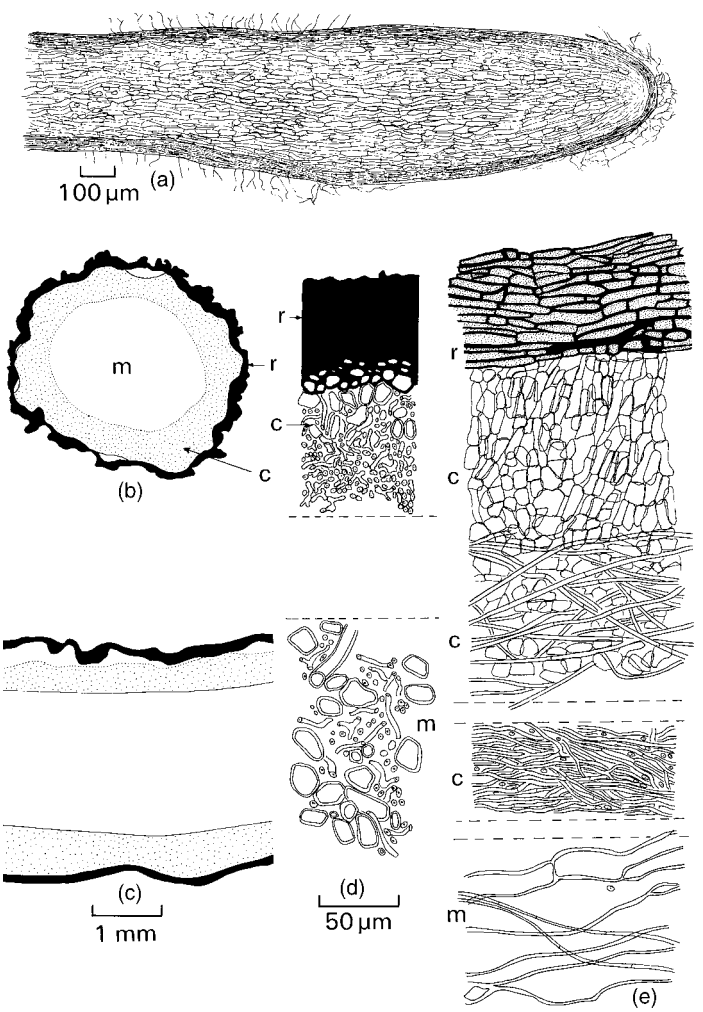

Fig 1.14 Rhizomorph structure of *Armillaria mellea* (Basidiomycota). (a) Longitudinal section. (b) Transverse section, diagrammatic. (c) L.S. diagrammatic. (d) T.S. showing details of cells in the rind (r), cortex (c) and medulla (m). (e) L.S. showing details of cells.

observed after about 7 days' mycelial growth on the agar surface as a compact mass of darkly pigmented hypertrophied cells. These pigmented structures have been termed **microsclerotia**. From white, non-pigmented points on their surface, the rhizomorphs develop. The growth of rhizomorphs can be several times faster than that of unorganized hyphae (Rishbeth, 1968). The most striking feature of the development of rhizomorphs is their compact growing point at the apex, which consists of small isodiametric cells protected by an apical cap of intertwined hyphae immersed in mucilage which they produce. Because of its striking similarity with a growing plant root, the rhizomorph tip was initially interpreted as a meristematic zone (Motta, 1967), but its hyphal nature can be demonstrated by careful ultrastructural observations (Powell & Rayner, 1983; Rayner *et al*., 1985). Behind the apex there is a zone of elongation. The centre of the rhizomorph may be hollow or solid. Surrounding the central lumen or making up the central medulla is a zone of enlarged hyphae 4–5 times wider than the vegetative hyphae (Fig. 1.14e). Possibly these **vessel hyphae** serve in translocation (Cairney, 1992; Jennings, 1995). Towards the periphery of the rhizomorph, the cells become smaller, darker, and thicker walled. Extending outwards between the outer cells of the rhizomorph, there may be a growth of vegetative hyphae somewhat resembling the root-hair zone in a higher plant. Rhizomorphs may develop on monokaryotic mycelia derived from single basidiospores, or on dikaryotic

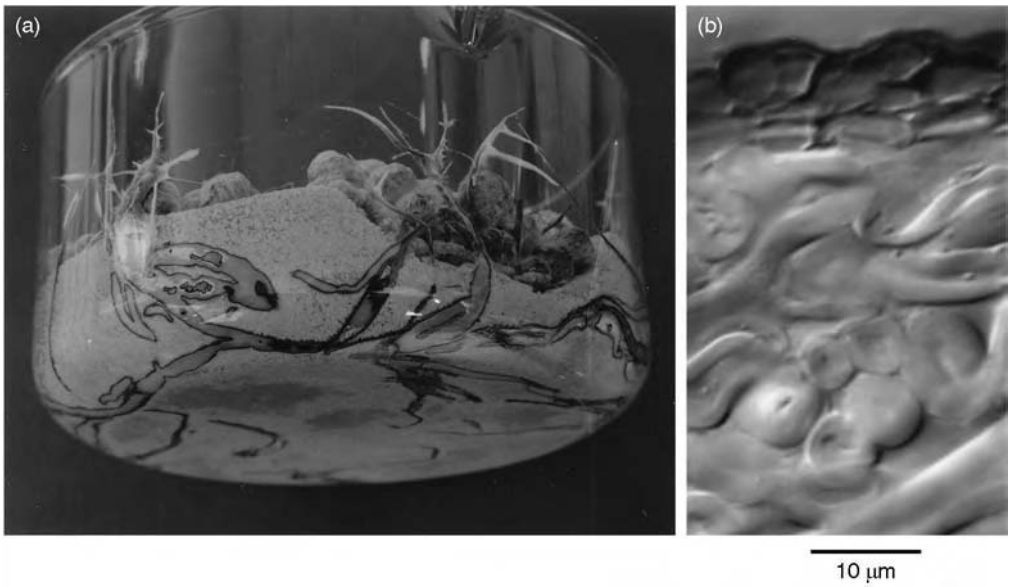

Fig 1.15 Rhizomorphs of *Podosordaria tulasnei* (Ascomycota). (a) Subterranean rhizomorphs by which the fungus spreads through the soil. (b) T.S. showing the dark rind (1–2 cells thick) and a cortex consisting of thick-walled hyaline cells.

mycelia following fusion of compatible monokaryotic hyphae. Dikaryotic rhizomorphs of *Armillaria* do not possess clamp connections (Hintikka, 1973).

Rhizomorphs are also produced by other Basidiomycota and a few Ascomycota (Fig. 1.15; Webster & Weber, 2000). They are mainly formed in soil. An interesting exception is presented by tropical *Marasmius* spp., which form a network of aerial rhizomorphs capable of intercepting falling leaves before they reach the ground (Hedger *et al*., 1993). Because these rhizomorphs have a rudimentary fruit body cap at their extending apex (Hedger *et al*., 1993), they have been interpreted as indefinitely extending fruit body stipes (Moore, 1994). Mycelial strands and rhizomorphs represent extremes in a range of hyphal aggregations, and several intergrading forms can be recognized (Rayner *et al*., 1985).

1.3.3 Sclerotia

Sclerotia are pseudoparenchymatous aggregations of hyphae embedded in an extracellular glucan matrix. A hard melanized rind may be present or absent. Sclerotia serve a survival function and contain intrahyphal storage reserves such as polyphosphate, glycogen, protein, and lipid (Willetts & Bullock, 1992). The glucan matrix, too, may be utilized as a carbohydrate source during sclerotium germination (Backhouse & Willetts, 1985). Sclerotia may also have a reproductive role and are the only known means of reproduction in certain species. They are produced by a relatively small number of Asco- and Basidiomycota, especially plant-pathogenic species such as *Rhizoctonia* spp. (p. 595), *Sclerotinia* spp. (p. 429) and *Claviceps purpurea* (p. 349). The form of sclerotia is very variable (Butler, 1966). The subterranean sclerotium of the Australian *Polyporus mylittae* (see Figs. 18.13c,d) can reach the size of a football and is known as native bread or blackfellow's bread. At the other extreme, they may be of microscopic dimensions consisting of a few cells only. Several kinds of development in sclerotia have been distinguished (Townsend & Willetts, 1954; Willetts, 1972).

The loose type

This is exemplified by *Rhizoctonia* spp., which are sclerotial forms of fungi belonging to the Basidiomycota. Sclerotia of the loose type are readily seen as the thin brownish-black scurfy scales so common on the surface of

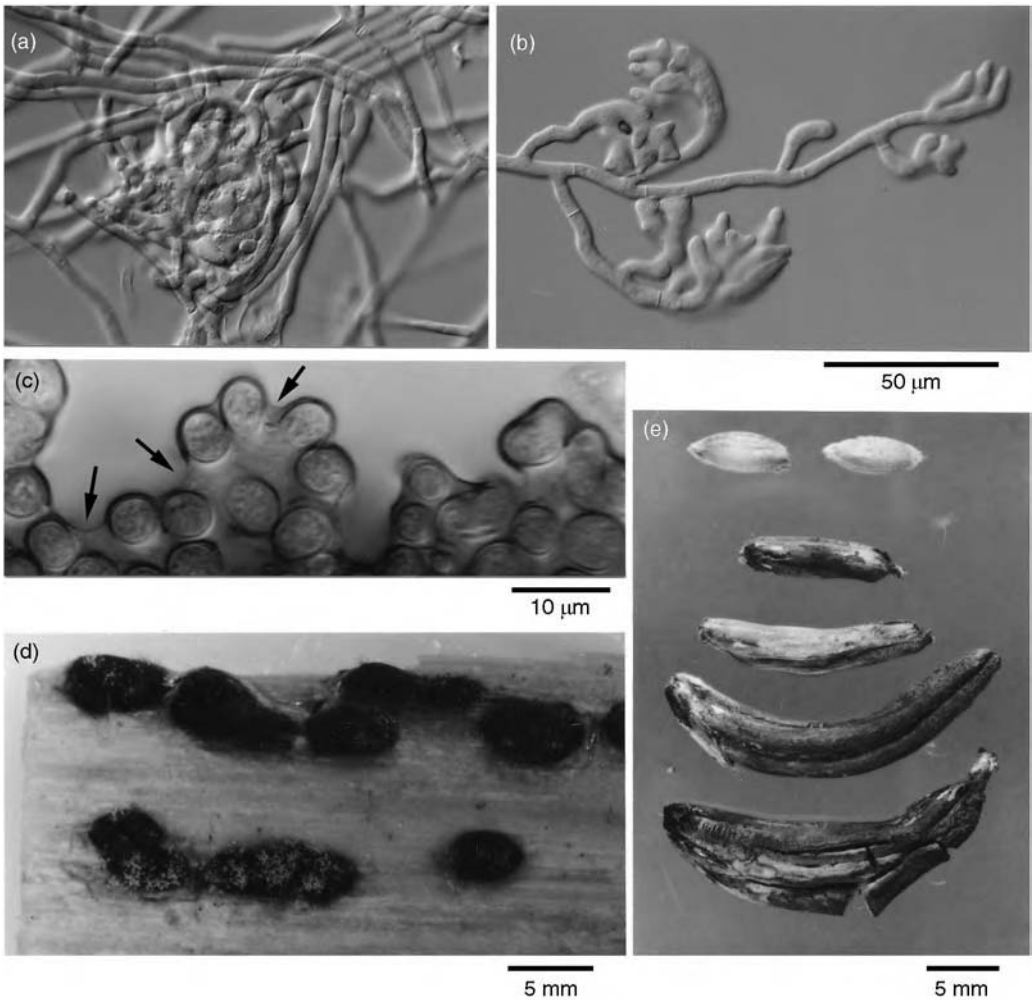

Fig 1.16 Development of sclerotia. (a) The loose type, as seen in *Rhizoctonia* (*Moniliopsis*) *solani*. (b) Hypha of *Botrytis cinerea* showing dichotomous branching on a glass coverslip to initiate the terminal type of sclerotium. (c) Later stage of sclerotium formation in *B. cinerea*. The hyphae have become melanized and are growing away from the glass surface. They are embedded in a glucan matrix (arrows). (d) Mature sclerotia of *B. cinerea* on a stem of *Conium*. Some sclerotia are germinating to produce tufts of conidiophores. (e) Sclerotia of *Claviceps purpurea* from an ear of rye (*Secale cereale*). Rye grains are shown for size comparison. (a) and (b) to same scale.

potato tubers. In pure culture, sclerotial initials arise by branching and septation of hyphae (Fig. 1.16a). These cells become filled with dense contents and numerous vacuoles, and darken to reddish-brown. The mature sclerotium does not show well-defined zones or 'tissues'. It is made up of a central part which is pseudoparenchymatous, although its hyphal nature can be seen. Towards the outside, the hyphae are more loosely arranged; a rind of thick-walled hyphae is absent (Willetts, 1969).

The terminal type

This form is characterized by a well-defined pattern of branching. It is produced, for example, by *Botrytis cinerea*, the cause of grey mould diseases on a wide range of plants, and by the saprotrophic *Pyronema domesticum* (see p. 415). Sclerotia of *B. cinerea* are found on overwintering stems of herbaceous plants, especially umbellifers such as *Angelica*, *Anthriscus*, *Conium* and *Heracleum*. They can also be induced to form in culture, especially on agar media with a high

carbon/nitrogen ratio. When growing on host tissue, the sclerotia of *Botrytis* may include host cells, a feature shared also by sclerotia of *Sclerotinia* spp. to which *Botrytis* is related (see p. 429). Sclerotia arise by repeated dichotomous branching of hyphae, accompanied by cross-wall formation (Fig. 1.16b). The hyphae then aggregate, melanize and produce mucilage, giving the appearance of a solid tissue (Fig. 1.16c). A mature sclerotium may be about 10 mm long and 3–5 mm wide, and is usually flattened, measuring 1–3 mm in thickness. It is often orientated parallel to the long axis of the host plant (Fig. 1.16d). It is differentiated into a rind composed of several layers of rounded, dark cells, a narrow cortex of thin-walled pseudoparenchymatous cells with dense contents, and a medulla made up of loosely arranged filaments. Nutrient reserves are stored in the cortical and medullary regions (Willetts & Bullock, 1992).

The strand type

Sclerotinia gladioli, the causal agent of dry rot of corms of *Gladiolus*, *Crocus* and other plants, forms sclerotia of this type. Sclerotial initials commence with the formation of numerous side branches which arise from one or more main hyphae. Where several hyphae are involved, they lie parallel. They are thicker than normal vegetative hyphae, and become divided by septa into chains of short cells. These cells may give rise to short branches, some of which lie parallel to the parent hypha, whilst others grow out at right angles and branch again before coalescing. The hyphae at the margin continue to branch, and the whole structure darkens. The mature sclerotium is about 0.1–0.3 mm in diameter, and is differentiated into a rind of small, thick-walled cells and a medulla of large, thin-walled hyphae. More complex sclerotia are found in *Sclerotium rolfsii*, the sclerotial state of *Pellicularia rolfsii* (Basidiomycota). Here the mature sclerotium is differentiated into four zones: a fairly thick skin or cuticle, a rind made up of 2–4 layers of tangentially flattened cells, a cortex of thin-walled cells with densely staining contents, and a medulla of loose filamentous hyphae with dense contents. Chet *et al*. (1969) have shown that the skin or cuticle is made up of the remnants of cell walls attached to the outside of the empty, melanized, thick-walled rind cells. All the cells of the strand-type sclerotium have thicker walls than those of vegetative hyphae. Cells of the outer cortex contain large storage bodies which consist of protein (Kohn & Grenville, 1989) and leave little room for cytoplasm or other organelles. The inner cortex is also densely packed with storage granules.

Other types

There is a great diversity of other types of sclerotia (Butler, 1966). The sclerotia of *Claviceps purpurea*, the 'ergots' of grasses and cereals (Fig. 1.16e; see also p. 349), develop from a pre-existing mass of mycelium which fills and replaces the cereal ovary, starting from the base and extending towards the apex. The outer layers form a violet, dark grey or black rind enclosing colourless, thick-walled cells. These contain abundant storage lipids which constitute 45% of the dry weight of a *C. purpurea* sclerotium (Kybal, 1964). *Cordyceps militaris*, an insect parasite, forms a dense mass of mycelium in the buried insect's body (p. 360). This mass of mycelium, from which fructifications develop, is enclosed by the exoskeleton of the host, not by a fungal rind. Many wood-rotting fungi enclose colonized woody tissue with a black zone-line of dark, thick-walled cells, and the whole structure may be regarded as a kind of sclerotium.

The giant sclerotium of *Polyporus mylittae* is marbled in structure, comprising white strata and translucent tissue. It has an outer, smooth, thin black rind. Three distinct types of hyphae make up the tissues: thin-walled, thick-walled and 'layered' hyphae. Thin- and thick-walled hyphae are abundant in the white strata but sparse in the translucent tissue, whereas the layered hyphae occur only in the translucent tissue. Detached sclerotia are capable of forming basidiocarps without wetting. It is believed that the translucent tissue functions as an extracellular nutrient and water store (Macfarlane *et al*., 1978). The structure of the sclerotium appears to be related to its ability to fruit in dry conditions, such as occur in Western Australia.

Germination of sclerotia

Sclerotia can survive for long periods, sometimes for several years (Coley-Smith & Cooke, 1971; Willetts, 1971). Germination may take place in three ways – by the development of mycelium, asexual spores (conidia) or sexual fruit bodies (ascocarps or basidiocarps). Mycelial germination occurs in *Sclerotium cepivorum*, the cause of white-rot of onion, and is stimulated by volatile exudates from onion roots (see p. 434). Conidial development occurs in *Botrytis cinerea* and can be demonstrated by placing overwintered sclerotia in moist warm conditions (Fig. 1.16d; Weber & Webster, 2003). The development of ascocarps (i.e. carpogenic germination) is seen in *Sclerotinia*, where stalked cups or apothecia, bearing asci, arise from sclerotia under suitable conditions (Fig. 15.2), and in *Claviceps purpurea*, where the overwintered sclerotia give rise to a perithecial stroma (Fig. 12.26c). Depending on environmental conditions, the sclerotia of some species may respond by germinating in different ways.

1.3.4 The mantle of ectomycorrhiza

The root tips of many coniferous and deciduous trees with ectomycorrhizal associations, especially those growing in relatively infertile soils, are covered by a **mantle**. This is a continuous sheet of fungal hyphae, several layers thick (see Fig. 19.10). The mycelium extends outwards into the litter layer of the soil, and inwards as single hyphae growing intercellularly, i.e. between the outer cortical cells of the root, to form the so-called **Hartig net**. Hyphae growing outwards from the mantle effectively replace the root hairs as a system for the absorption of minerals from the soil, and there is good evidence that, in most normal forest soils of low to moderate fertility, the performance and nutrient status of mycorrhizal trees is superior to that of uninfected trees (Smith & Read, 1997). Most fungi causing ectomycorrhizal infections are Basidiomycota, especially members of the Homobasidiomycetes (pp. 526 and 581). Within the soil or in pure culture, mycelial strands may form, but the mycelium is not aggregated into the tissue-like structure of the mantle.

1.3.5 Fruit bodies of Ascomycota and imperfect fungi

In the higher fungi, hyphae may aggregate in a highly regulated fashion to form fruiting structures which are an important and often species-specific feature of identification. In the Ascomycota, the fruit bodies produce sexual spores (i.e. as the result of nuclear fusion and meiosis) which are termed **ascospores** and are contained in globose or cylindrical cells called **asci** (Lat. *ascus* = a sac, tube). In most cases, the asci can discharge their ascospores explosively. Asci, although occasionally naked, are usually enclosed in an aggregation of hyphae termed an **ascocarp** or **ascoma**. Ascocarps are very variable in form, and several types have been distinguished (see Fig. 8.16). Their features and development will be described more fully later. Forms in which the asci are totally enclosed, and in which the ascocarp has no special opening, are termed **cleistothecia**. In contrast, **gymnothecia** consist of a loose mesh of hyphae. Both are found in the Plectomycetes (Chapter 11). A modified cleistothecium is characteristic of the Erysiphales (Chapter 13). Cup fungi (Discomycetes, Chapters 14 and 15) possess saucer-shaped ascocarps termed **apothecia**, with a mass of non-fertile hyphae supporting a layer of asci lining the upper side of the fruit body. The non-fertile elements of the apothecium often show considerable differentiation of structure. The asci in apothecia are free to discharge their ascospores at the same time. In other Ascomycota, the asci are contained within ascocarps with a very narrow opening or **ostiole**, through which each ascus must discharge its spores separately. Ascocarps of this type are termed **perithecia** or **pseudothecia**. Perithecia are found in the Pyrenomycetes (Chapter 12) whilst pseudothecia occur in the Loculoascomycetes (Chapter 17). These two types of ascocarp develop in different ways. In many of the Pyrenomycetes, the perithecia are borne on or embedded in a mass of fungal tissue termed the **perithecial stroma**, and these are

well shown by the Xylariales (p. 332), and by *Cordyceps* (p. 360) and *Claviceps* (p. 349). In some cases, in addition to the perithecial stroma, a fungus may develop a stromatic tissue on or within which asexual spores (conidia) develop. *Nectria cinnabarina* (p. 341), the coral spot fungus so common on freshly dead deciduous twigs, is such an example. It initially forms pink conidial stromata which later, under suitable conditions of humidity, become converted into perithecial stromata.

Among the imperfect (asexual) fungi, mycelial aggregations bearing conidia are seen in various genera. In some, there are tufts of parallel conidiophores termed **coremia** or **synnemata**, exemplified by *Penicillium claviforme* (see Fig. 11.19). In some imperfect fungi formerly called Coelomycetes, the conidia develop in flask-shaped cavities termed **pycnidia** (see Figs. 17.3–17.5). Various other kinds of mycelial fruiting aggregates are also known.

1.3.6 Fruit bodies of Basidiomycota

The fruit bodies of mushrooms, toadstools, bracket fungi, etc., are all examples of **basidiocarps** or **basidiomata** which bear the sexually produced spores (basidiospores) on basidia. Basidiocarps are almost invariably constructed from dikaryotic hyphae, but how vegetative hyphae aggregate to form a mushroom fruit body is still a mystery (Moore, 1994). Wessels (1997) has suggested that hydrophobins coating the surface of hyphae may confer adhesive properties, leading to their aggregation to form a fruit body initial as the first step in morphogenesis. Once an initial has been formed, its glucan matrix may provide an environment for the exchange of signalling molecules between hyphae. Moore (1994) speculated that morphogenesis might ultimately be determined by induction hyphae exerting a control over surrounding hyphae, leading to the development of morphogenetic units. This morphogenetic commitment must happen at a very early stage. For instance, in the ink-cap (*Coprinus cinereus*) an initial measuring only 1% of the final fruit body size is already differentiated into stipe and cap (Moore *et al*., 1979). Therefore, when a mushroom fruit body expands, this is due mainly to the enlargement of existing hyphae, whereas new apical growth is restricted mainly to branches filling up the space generated during expansion (Moore, 1994). Hyphae making up the mature basidiocarp may show considerable differentiation in structure and function. This is perhaps most highly developed in polypore-type basidiocarps, where a number of morphologically distinct hyphal types have been recognized (p. 517).

1.4 Spores of fungi

The reproduction by means of small spores is a cornerstone in the ecology of fungi. Although a single spore may have a negligible chance of reaching a suitable substrate, spores may be produced in such quantities that even discrete substrates can be exploited by the species as a whole. Only a few fungi make do without spores, surviving solely by means of mycelium and sclerotia. Spores may be organs of sexual or asexual reproduction, and they are involved in dispersal and survival. Gregory (1966) distinguished between **xenospores** (Gr. *xenos* = a foreigner) for spores which are dispersed from their place of origin and **memnospores** (Gr. *mémnon* = steadfast, to persist), which stay where they were formed. Some spores are violently discharged from the organs which bear them, energy for dispersal being provided by the spore itself or the structure producing it (Ingold, 1971). However, many spores are dispersed passively by the action of gravity, air or water currents, rain splash, or by animals, especially insects. Dispersal may also occur by human traffic. Spores may be present in the outdoor air at such high concentrations (e.g. 100 *Cladosporium* spores l^{-1}) that they can cause allergic respiratory diseases when inhaled (Lacey, 1996). In freshwater, the asexually produced spores (conidia) of aquatic hyphomycetes, which colonize autumn-shed tree leaves, may reach concentrations of 10 000–20 000 spores l^{-1} (see p. 685). Long-range dispersal of air-borne spores

over thousands of kilometres is known to occur in nature. For instance, the urediniospores of the coffee rust fungus, *Hemileia vastatrix*, are thought to have travelled from Africa to South America by wind at high altitudes, and the urediniospores of black stem rust of wheat (*Puccinia graminis*) undergo an annual migration from states bordering the Gulf of Mexico to the prairies of North America and Canada (Fig. 22.11). These spores are protected from the deleterious effects of UV irradiation in the upper atmosphere by pigments in the spore wall.

Some spores are not dispersed but survive *in situ*, e.g. the oospores of many soil-inhabiting Oomycota (Chapter 5), the zygospores of Zygomycota (Chapter 7) and the chlamydospores of Glomales (see p. 217) and other fungi. Fungal spores may remain dormant for many years, especially under dry and cold conditions (Sussman & Halvorson, 1966; Sussman, 1968). An extreme example of spore survival is shown by the recovery of viable spores of several fungi from glacial ice cores, including those of *Cladosporium cladosporioides* from ice samples 4500 years old (Ma *et al*., 2000).

The morphology and structure of fungal spores show great variability, from unicellular to multicellular, branched or unbranched or sometimes spirally coiled, thin- or thick-walled with hyaline or pigmented walls, dry or sticky, smooth or ornamented by mucilaginous extensions, spines, folds or reticulations. A number of general descriptive terms have been applied to characterize spores in relation to the number of cells and septa which they contain. Single-celled spores are termed **amerospores** (Gr. *a* = not, *meros* = a part; i.e. not divided), two-celled spores are **didymospores** (Gr. *didymos* = double), spores with more than one transverse septum are **phragmospores** (Gr. *phragmos* = a hedge, barricade), and spores with transverse and longitudinal septa are **dictyospores** (Gr. *dictyon* = a net). These terms may be qualified by prefixes indicating spore pigmentation such as hyalo- for colourless (hyaline) spores and phaeo- for spores with dark-coloured (melanized) walls.

Special terms have also been used to refer to spore shape. **Scolecospores** (Gr. *skolex* = a worm) are worm-shaped, **helicospores** (Gr. *helix* = twisted or wound) are spores with a two- or three-dimensional spiral shape, whilst **staurospores** (Gr. *stauros* = a cross) have arms radiating from a central point or axis. Spore septation, colour and shape, along with other criteria such as the arrangement of structures which bear the spores, have been used in classification and identification, especially in conidial fungi which do not show sexual reproduction. These criteria rarely lead to natural systems of classification, but to '**form genera**' or 'anamorph genera' made up of species unified by having similar spore forms.

Some of the more common spore types are described below. There are numerous other, less-common kinds of spore found in fungi, and they are described later, in relation to the particular fungal groups in which they occur.

1.4.1 Zoospores

These are spores which are self-propelled by means of flagella. Propulsion is often coupled with chemotactic movement, zoospores having the ability to sense chemicals diffusing from suitable substrata and to move towards them, or gametes detecting and following extremely low concentrations of hormones. In some cases oxygen or light are also stimuli for tactic movement. The fungal groups which possess flagella are mostly aquatic or, if terrestrial, rely on water for dispersal or infection. Their zoospores are of four kinds (see Fig. 1.17):

1. Posteriorly flagellate zoospores with flagella of the whiplash type are characteristic of the Chytridiomycota (Chapter 6). Each whiplash flagellum has 11 microtubules arranged in the 9 + 2 pattern typical of eukaryotes. The microtubules are enclosed in a smooth, membranous axoneme sheath continuous with the plasma membrane. In most members of the Chytridiomycota there is a single posterior flagellum (Fig. 1.17a), but in the rumen-inhabiting Neocallimastigales there may be up to 16 flagella (Fig. 1.17b). Such spores are driven forward by sinusoidal rhythmic beating of the flagellum. This type of zoospore

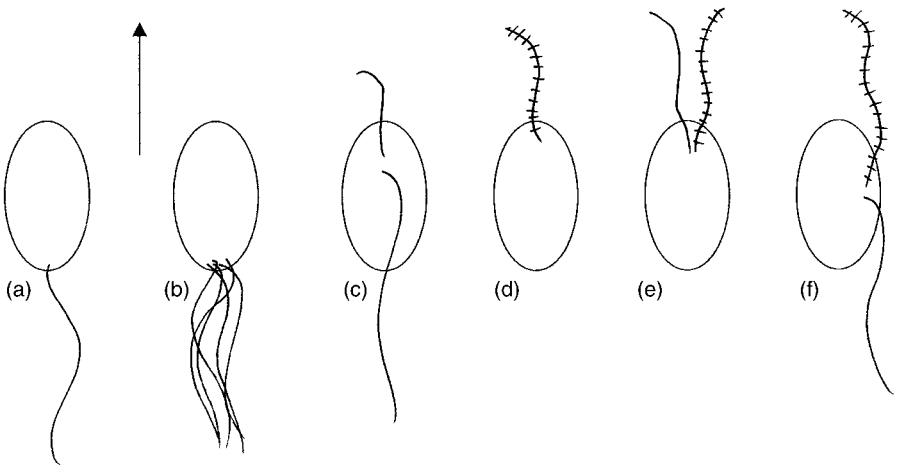

Fig 1.17 Zoospore types found in fungi, diagrammatic and not to scale. The arrow indicates the direction of movement of the zoospore. (a) Posteriorly uniflagellate (opisthokont) zoospore with a flagellum of the whiplash type found in many Chytridiomycota. (b) Posteriorly multiflagellate zoospore with numerous (up to 16) whiplash flagella which occur in certain anaerobic rumen-inhabiting Chytridiomycota (Neocallimastigales). (c) Zoospore with unequal (anisokont) whiplash flagella characteristic of the Myxomycota and the Plasmodiophoromycota. (d) Anteriorly uniflagellate zoospore with a flagellum of the tinsel type, the axoneme being clothed with rows of mastigonemes, typical of the Hyphochytriomycota. (e,f) Biflagellate zoospores with heterokont flagella, one of the whiplash and the other of the tinsel type, which are found in different groups of the Oomycota. For more details turn to the different fungal groups.

flagellation is termed **opisthokont** (Gr. *opisthen* = behind, at the back; *kontos* = a pole). Detailed descriptions of the fine structure of chytridiomycete zoospores are given on p. 129.

2. Biflagellate zoospores with two whiplash flagella of unequal length are called **anisokont** (Fig. 1.17c) and are found in some Myxomycota and the Plasmodiophoromycota, both now classified among the Protozoa (see Chapters 2 and 3).

3. Anteriorly uniflagellate zoospores with a flagellum of the tinsel type are characteristic of the Hyphochytriomycota (Chapter 4). The axoneme sheath of the tinsel or **straminipilous** flagellum (Lat. *stramen* = straw; *pilus* = hair) is adorned by two rows of fine hairs (Fig. 1.17d). These are called **tripartite tubular hairs** or **mastigonemes** (Gr. *mastigion* = a small whip; *nema* = a thread). Rhythmic sinusoidal beating of the tinsel type flagellum pulls the zoospore along, in contrast to the pushing action of whiplash flagellum. Details of the fine structure of this type of zoospore are given in Fig. 4.5.

4. Biflagellate zoospores with anteriorly or laterally attached flagella, one of which is of the whiplash type and the other of the tinsel type (Figs. 1.17e,f), are characteristic of the Oomycota (Chapter 5). Zoospores with the two different kinds of flagellum are **heterokont**. Where the two types of flagellum are attached anteriorly, as in the first-released zoospores of *Saprolegnia*, their propulsive actions tend to work against each other and the zoospore is a very poor swimmer (Fig. 1.17e). However, the secondary zoospore (termed the principal zoospore) in *Saprolegnia* and in many other Oomycota has laterally attached flagella, with the tinsel-type (pulling action) flagellum pointing forwards and the whiplash-type (pushing action) flagellum directed backwards and possibly acting as a rudder, jointly providing much more effective propulsion (Fig. 1.17f).

1.4.2 Sporangiospores

In the Zygomycota, and especially in the Mucorales (see p. 180), the asexual spores are contained in globose sporangia (Fig. 1.18) or cylindrical merosporangia. Because they are non-motile, the spores are sometimes termed **aplanospores** (Gr. *a* = not, *planos* = roaming).

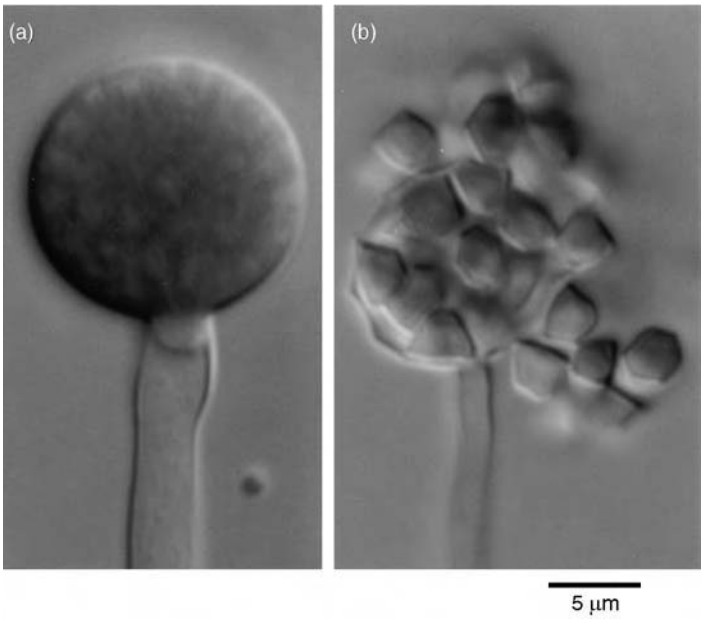

Fig 1.18 Sporangia in *Mortierella* (*Umbelopsis*) *vinacea*. (a) Maturing sporangium in which the cytoplasm is being cleaved into numerous sporangiospores. (b) Release of sporangiospores by breakdown of the sporangial wall. Unusually, in *M. vinacea* the sporangiospores are angular in shape.

The spores may be uni- or multinucleate and are unicellular. They generally have thin, smooth walls and are almost always globose or ellipsoid in shape. They are formed by cleavage of the sporangial cytoplasm. They vary in colour from hyaline (colourless) to yellow, due to carotenoid pigments in the cytoplasm. When mature, they may be surrounded by mucilage, in which case they are usually dispersed by rain splash or insects, or they may be dry and dispersed by wind currents. In some genera, e.g. *Pilobolus*, entire sporangia become detached. The number of sporangiospores per sporangium may vary from several thousand to only one. The detachment and dispersal of intact sporangia containing a few sporangiospores or a single one is indicative of the way in which conidia may have evolved from one-spored sporangia.

1.4.3 Ascospores

Ascospores are the characteristic spores of the largest group of fungi, the Ascomycota or ascomycetes. They are meiospores and are formed in the developing ascus as a result of nuclear fusion immediately followed by meiosis. The four haploid daughter nuclei then divide mitotically to give eight haploid nuclei around which the ascospores are cut out. Details of ascospore development are described in Fig. 8.11. In most ascomycetes, the eight ascospores are contained within a cylindrical ascus, from which they are squirted out together with the ascus sap when the tip of the turgid ascus breaks down and the elastic ascus walls contract. The distance of discharge may be 1 cm or more. In some cases, for example, the Plectomycetes (Chapter 11) and in ascomycetes with subterranean fruit bodies, such as the false truffles (*Elaphomyces* spp.; Fig. 11.21) and truffles (*Tuber* spp. and their allies; p. 423), ascospore release is non-violent and their asci are not cylindrical but globose. Ascospores vary greatly in size, shape and colour. In size, the range is from about $4-5 \times 1\,\mu m$ in small-spored forms such as the minute cup fungus *Dasyscyphus*, to $130 \times 45\,\mu m$ in the lichen *Pertusaria pertusa*. The shape of ascospores varies from globose to oval, elliptical, lemon-shaped, sausage-shaped, cylindrical, or needle-shaped. Ascospores are often asymmetric in form with a wider, blunter, anterior part and a narrower, more tapering posterior. This shape increases their acceleration as they are squeezed out through the opening of the ascus. Ascospores may be uninucleate or multinucleate, unicellular or multicellular, divided up by transverse or by transverse and longitudinal septa. In some

genera, e.g. *Hypocrea* (Fig. 12.15) or *Cordyceps* (Fig. 12.33), the multicellular ascospores may break up into part-spores within the ascus prior to discharge. The ascospore wall may be thin or thick, hyaline or coloured, smooth or rough, sometimes cast into reticulate folds or ornamented by ridges, and it may have a mucilaginous outer layer which is sometimes extended to form simple or branched appendages, especially in marine ascomycetes where they aid buoyancy and attachment. In many cases, ascospores are resting structures which survive adverse conditions. They may have extensive food reserves in the form of lipids and sugars such as trehalose. Because the formation of ascospores involves meiosis, they are important not only as a means of dispersal and survival but also in genetic recombination.

It is obvious that there is no such thing as a typical ascospore. *Neurospora tetrasperma* will serve as an example of an ascospore whose structure has been extensively studied (Lowry & Sussman, 1958, 1968). This fungus is somewhat unusual in that it has four-spored asci and the ascospores are binucleate. The spores are black, thick-walled and shaped rather like a rugby football, but with flattened ends. The name *Neurospora* refers to the ribbed spores, because the dark outer wall is made up of longitudinal raised ribs, separated by interrupted grooves. The structure of a spore in section is shown in Fig. 1.19. Within the cytoplasm of the spore are the two nuclei, fragments of endoplasmic reticulum (not illustrated), swollen mitochondria and vacuoles, bounded by single unit membranes. The wall surrounding the protoplast is composed of several layers. The innermost layer is the **endospore**, outside of which is the **epispore**. The ribbed layer is termed the **perispore**. Between the ribs are lighter intercostal veins containing a material which is chemically distinct from the ribs. This material is continuous over the whole surface of the spore, giving it a relatively smooth surface. The spore germinates by the extrusion of germ tubes from a preexisting **germ pore**, a thin area in the epispore at either end of the spore. In many ascomycetes a trigger is required for germination, e.g. heat shock in *Neurospora* or a chemical stimulus, for example in ascomycetes which grow and fruit on the dung of herbivorous mammals and whose spores are subjected to digestive treatment.

1.4.4 Basidiospores

Basidiospores are the sexual spores which characterize a large group of fungi, the Basidiomycota or basidiomycetes. In comparison with the morphological diversity of ascospores, basidiospores are more uniform. They also show a smaller size range, from about 3 to 20 μm, which is possibly related to their unique method of discharge. They are normally found in groups of four attached by tapering sterigmata to the cell which bears them, the **basidium**. At the time of their discharge all basidiospores

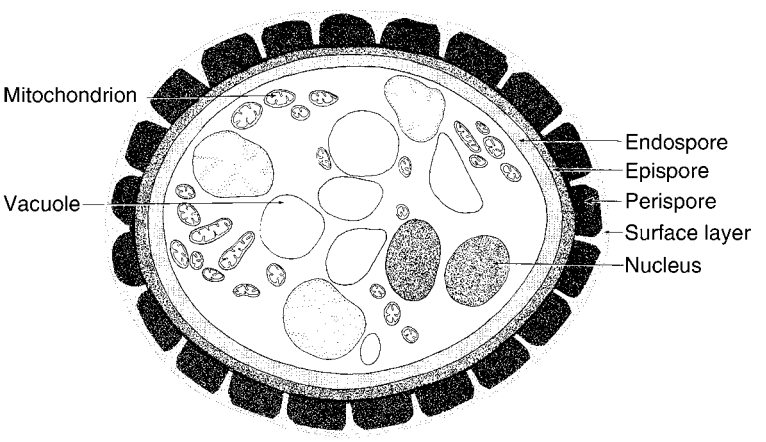

Fig 1.19 *Neurospora tetrasperma*. T.S. ascospore. Simplified diagram based on an electron micrograph by Lowry in Sussman and Halvorson (1966).

are unicellular, but they may become septate after release in some members of the Heterobasidiomycetes (Chapter 21). In shape, basidiospores are asymmetric and vary from sub-globose, sausage-shaped, fusoid, to almond-shaped (i.e. flattened), and the wall may be smooth or ornamented with spines, ridges or folds. The colour of basidiospores is important for identification. They may be colourless, white, cream, yellowish, brown, pink, purple or black. The spore colour may be due to pigments in the spore cytoplasm or in the spore wall. The appearance of pigments in the wall occurs relatively late in spore development. This explains the change of colour of the gill of a domestic mushroom (*Agaricus*) from pink, due to cytoplasmic spore pigments, to dark purplish-brown when mature, due to wall pigments.

The generalized structure of a basidiospore is illustrated in Fig. 1.20. Most basidiospores have a flatter adaxial face and a more curved abaxial face. The point of attachment of the spore to the sterigma is the **hilum**, which persists as a scar at the base of a discharged spore. Close to the hilum is a small projection, the **hilar appendix**. This is involved in the unique mechanism of basidiospore discharge, in which a drop of liquid perched on the hilar appendix coalesces with a second blob of liquid on the spore surface,

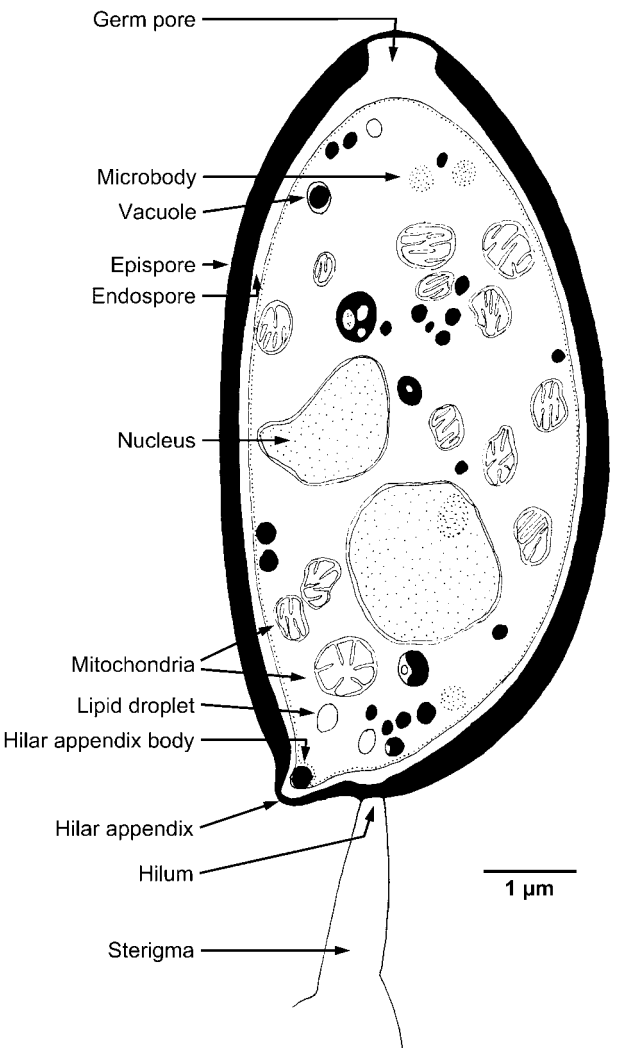

Fig 1.20 Generalized view of a median vertical section through a basidiospore as seen by transmission electron microscopy. For clarity, structures such as endoplasmic reticulum and ribosomes are not illustrated. Diagram based on *Agrocybe acericola*, after Ruch and Nurtjahja (1996).

creating a momentum which leads to acceleration of the spore (Money, 1998; see p. 493). The spore is projected for a short distance (usually less than 2 mm) from the basidium. Violently projected spores are termed **ballistospores** (Lat. *ballista* = a military engine for throwing large stones), but whilst most basidiospores are ballistospores, some are not. For example, in the Gasteromycetes (Chapter 20), which include puffballs, stinkhorns and their allies, violent spore projection has been lost in the course of evolution from ancestors which possessed it. Likewise, the basidiospores of smut fungi (Ustilaginales, Chapter 23) are not violently discharged. The term **statismospore** (Lat. *statio* = standing still) is sometimes used for a spore which is not forcibly discharged.

The cytoplasm of basidiospores usually contains a single haploid nucleus resulting from meiotic division in the basidium; sometimes a post-meiotic division gives rise to two genetically identical nuclei. The structure of the wall is complex. In *Agrocybe acericola* there are two layers, a thicker, dark-pigmented, electron-dense outer layer or epispore, and a thinner, electron-transparent inner layer, the endospore (Ruch & Nurtjahja, 1996; see Fig. 1.20). The cultivated mushroom, *Agaricus bisporus*, has a three-layered wall making up some 35% of the dry weight of the spore (Rast & Hollenstein, 1977), whereas the wall of the *Coprinus cinereus* basidiospore comprises six distinct layers (McLaughlin, 1977). A histochemical feature of the walls of some basidiospores is that they are **amyloid**, i.e. they include starch-like material which stains bluish-purple with iodine-containing stains such as Melzer's reagent. This reaction is used as a taxonomic character. The amyloid reaction is due to the presence of unbranched, short-chain amylose molecules. It has been suggested that this 'fungal starch' may aid dormancy by creating a permeability barrier to oxygen in dry spores. When the amyloid material is dissolved as water becomes available, dormancy is lost and spore germination can proceed (Dodd & McCracken, 1972). In some basidiospores, e.g. those of *Coprinus cinereus* and *Agrocybe acericola*, the basidiospore has a distinct germ pore at the end opposite to the hilum (see Fig. 1.20). In other basidiomycetes, e.g. *Oudemansiella mucida*, *Schizophyllum commune* and *Flammulina velutipes*, the basidiospores have no specialized pore.

The reserve contents of the spore may vary. In some species, lipid is the major storage product, and there is an apparent lack of insoluble polysaccharides such as glycogen (Ruch & Motta, 1987). In other spores, glycogen predominates. Where lipid is present, germination may be fuelled by its breakdown and utilization, but where it is absent spores are dependent on external nutrient supplies before germination and further development is possible. In addition to the usual organelle complement, microbodies are also prominent in basidiospores. These are single membrane-bound organelles often associated with mitochondria and lipid globules; they may function as glyoxisomes containing enzymes involved in the oxidation of lipids (Ruch & Nurtjahja, 1996).

1.4.5 Zygospores

Zygospores are sexually produced resting structures formed as a result of plasmogamy between gametangia which are usually equal in size (Fig. 1.21a). Nuclear fusion may occur early, or may be delayed until shortly before meiosis and zygospore germination. Zygospores are typical of Zygomycota (Chapter 7). They are often large, thick-walled, warty structures with abundant lipid reserves and are unsuitable for long-distance dispersal, usually remaining in the position in which they were formed and awaiting suitable conditions for further development. The gametangia which fuse to form the zygospore may be uninucleate or multinucleate, and correspondingly the zygospore may have one, two or many nuclei within it. Zygospore germination may be by a germ tube or by the formation of a germ sporangium.

1.4.6 Oospores

An oospore is a sexually produced spore which develops from unequal gametangial copulation or markedly unequal (oogamous) gametic fusion (Fig. 1.21b). It is the characteristic sexually

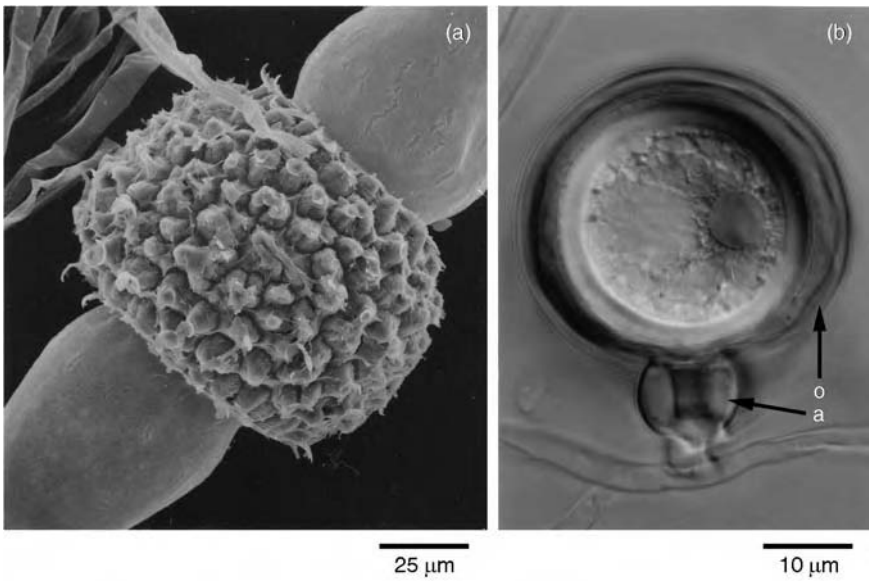

Fig 1.21 Sexual resting structures. (a) Zygospore of *Rhizopus sexualis*. The zygote has been produced by fusion of two gametangia and has laid down a thick wall with warty ornamentations. (b) Oospore of *Phytophthora erythroseptica*. The oogonium (o) has grown through the antheridium (a), and the oosphere has picked up a fertilization nucleus in the process. a kindly provided by H.-M. Ho; reprinted from Ho and Chen (1998) with permission of *Botanical Bulletin of Academia Sinica*.

produced spore of the Oomycota (Chapter 5), although oospores are also found in the Monoblepharidales (Chytridiomycota; Fig. 6.25). In the Oomycota, oospore development begins with the formation of one or more oospheres within the larger gametangium, the oogonium. After fertilization, i.e. the receipt of an antheridial nucleus by the oosphere, this lays down a thick wall and becomes the oospore. The number of oospores per oogonium may vary, and this is an important taxonomic criterion. Meiotic nuclear divisions precede oosphere and antheridial maturation in the Oomycota and nuclear fusion follows fertilization, so that the oospore is diploid. The oospore develops a thick outer wall and lays down food reserves, usually in the form of lipids. In the Peronosporales the outer wall of the oospore is surrounded by periplasm, the residual cytoplasm left in the oogonium after the oospheres have been cleaved out. Oospores are sedentary (memnospores) and are important in survival rather than dispersal. They often require a period of maturation before germination can occur and may remain dormant for long periods.

1.4.7 Chlamydospores

In most groups of fungi, terminal or intercalary segments of the mycelium may become packed with lipid reserves and develop thick walls within the original hyphal wall (Fig. 1.22). The new walls may be colourless or pigmented, and are often hydrophobic. Structures of this type have been termed chlamydospores (Gr. *chlamydos* = a thick cloak). They are formed asexually. Generally there is no mechanism for detachment and dispersal of chlamydospores, but they may become separated from each other by the collapse of the hyphae producing them. They are therefore typical memnospores, forming important organs of asexual survival, especially in soil fungi. Chlamydospores may develop within the sporangiophores of some species of the Mucorales, e.g. in *Mucor racemosus* (see Fig 7.14). The Glomales, which are fungal partners in symbiotic mycorrhizal associations with many vascular plants, reproduce primarily by large, thick-walled chlamydospores. These develop singly or in clusters (sporocarps) on coarse hyphae attached to their host plants. They are sedentary in soil but may be dispersed

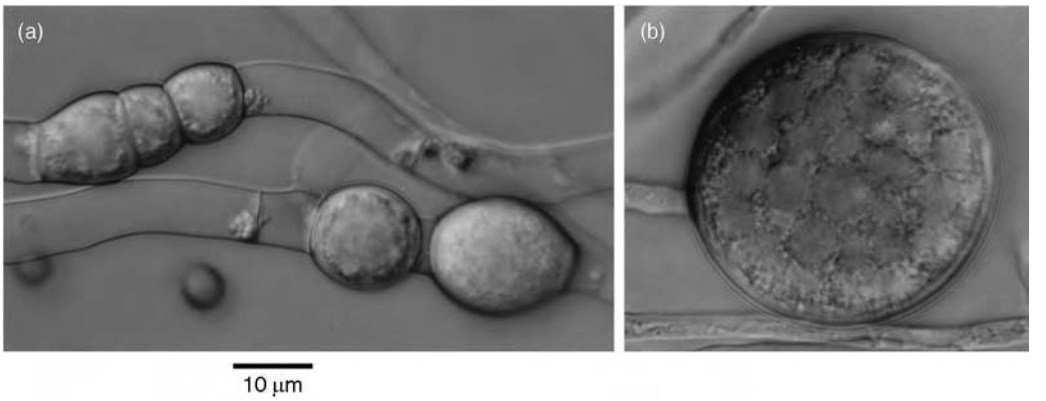

Fig 1.22 Chlamydospores formed by soil-borne fungi. (a) Intercalary hyphal chlamydospores in *Mucor plumbeus* (Zygomycota). (b) Terminal chlamydospore in *Pythium undulatum* (Oomycota). Both images to same scale.

by wind or by burrowing rodents which eat the spores. Chlamydospores may also develop within the multicellular macroconidia of *Fusarium* spp. and may survive when other, thin-walled cells making up the spore are degraded by soil micro-organisms. Similar structures are found in old hyphae of the aquatic fungus *Saprolegnia* (see Fig. 5.6g), either singly or in chains. In this genus, the chlamydospores may break free from the mycelium and be dispersed in water currents. Chlamydospores which are dispersed in this way are termed **gemmae** (Lat. *gemma* = a jewel).

The term chlamydospore is also sometimes used to describe the thick-walled dikaryotic spore characteristic of smut fungi (Ustilaginales; Chapter 23) but the term teliospore is preferable in this context. Hughes (1985) has discussed the use of the term chlamydospore.

1.4.8 Conidia (conidiospores)

Conidiospores, commonly known as conidia, are asexual reproductive structures. The word is derived from the Greek *konidion*, a diminutive of *konis*, meaning dust (Sutton, 1986). Conidia are found in many different groups of fungi, but especially within Ascomycota and Basidiomycota. The term conidium has, unfortunately, been used in a number of different ways, so that it no longer has any precise meaning. It has been defined by Kirk *et al.* (2001) as 'a specialized non-motile (cf. zoospore) asexual spore, usually caducous (i.e. detached), not developed by cytoplasmic cleavage (cf. sporangiospore) or free cell formation (cf. ascospore); in certain *Oomycota* produced through the incomplete development of zoosporangia which fall off and germinate to produce a germination tube'. In many fungi conidia represent a means of rapid spread and colonization from an initial focus of infection.

In general, conidia are dispersed passively, but in a few cases discharge is violent. For instance, in *Nigrospora* the conidia are discharged by a squirt mechanism (Webster, 1952), and in *Epicoccum* (Fig. 17.8) discharge is brought about by the rounding-off of a two-ply septum separating the conidium from its conidiogenous cell (Webster, 1966; Meredith, 1966). In the *Helminthosporium* conidial state of *Trichometasphaeria turcica*, drying and shrinkage of the conidiophore is associated with the sudden development of a gas phase, causing a jolt sufficient to project the conidium (Meredith, 1965; Leach, 1976).

There is great variation in conidial ontogeny. This topic will be dealt with more fully later when considering the conidial states of Ascomycota, and at this stage it is sufficient to distinguish between the major types of conidial development, which may be either **thallic** or **blastic**. Cells which produce conidia are conidiogenous cells. The term thallic is used to describe development where there is no enlargement of the conidium initial (Fig. 1.23a), i.e. the conidium arises by conversion of a pre-existing segment of the fungal thallus. An example of this kind is *Galactomyces candidus*, in which the conidia are

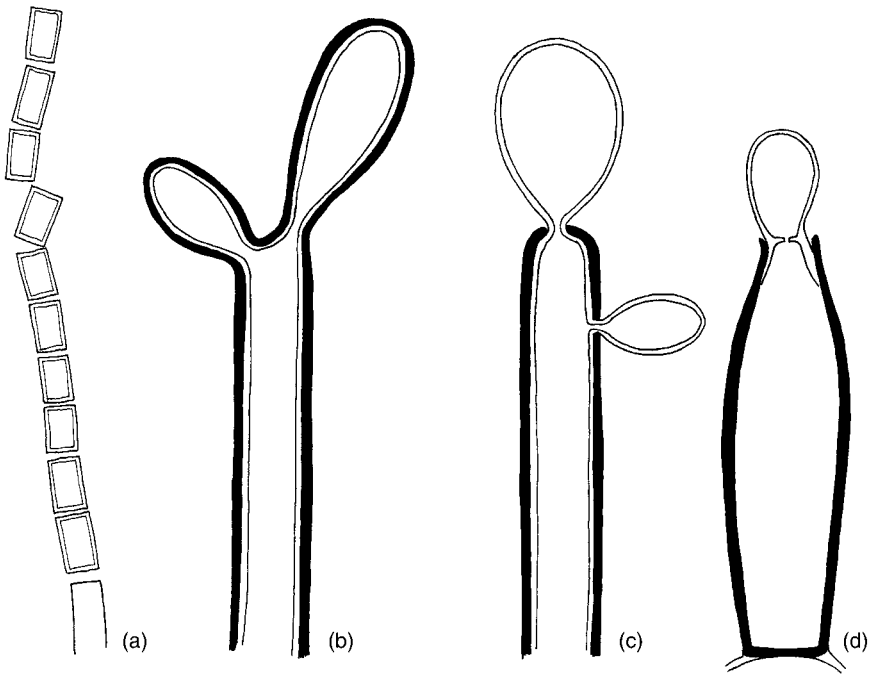

Fig 1.23 Diagrams to illustrate different kinds of conidial development. (a) Thallic development. There is no enlargement of the conidium initial. (b) Holoblastic development. All the wall layers of the conidiogenous cell balloon out to form a conidium initial recognizably larger than the conidiogenous cell. (c) Enteroblastic tretic development: only the inner wall layers of the conidiogenous cell are involved in conidium formation. The inner wall layers balloon out through a narrow channel in the outer wall. (d) Phialidic development: the conidiogenous cell is a phialide. The wall of the phialide is not continuous with the wall surrounding the conidium. The conidial wall arises de novo from newly synthesized material in the neck of the phialide. Diagrams based on Ellis (1971a).

formed by dissolution of septa along a hypha (Fig. 10.10). In most conidia, development is blastic, i.e. there is enlargement of the conidium initial before it is delimited by a septum. Two main kinds of blastic development have been distinguished:

1. **Holoblastic**, in which both the inner and outer wall layers of the conidiogenous cell contribute to conidium formation (Fig. 1.23b). An example of this kind of development is shown by the conidia of *Sclerotinia fructigena* (Fig. 15.3).

2. **Enteroblastic**, in which only the inner wall layers of the conidiogenous cell are involved in conidium formation. Where the inner wall layer balloons out through a narrow pore or channel in the outer wall layer, development is described as **tretic** (Fig. 1.23c). Examples of enteroblastic tretic development are found in *Helminthosporium velutinum* (Fig. 17.12) and *Pleospora herbarum* (Fig. 17.9d). Another important method of enteroblastic development is termed **phialidic** development. Here the conidiogenous cell is a specialized cell termed the **phialide**. During the expansion of the first-formed conidium, the tip of the phialide is ruptured. Further conidia develop by the extension of cytoplasm enclosed by a new wall layer which is laid down in the neck of the phialide and is distinct from the phialide wall. The protoplast of the conidium is pinched off by the formation of an inwardly growing flange which closes to form a septum (Fig. 1.23d). New conidia develop beneath the earlier ones, so that a chain may develop with the oldest conidium at its apex and the youngest at its base. Details of phialidic development are discussed more fully in relation to *Aspergillus* and *Penicillium* (p. 299), which reproduce by means of chains of dry phialoconidia dispersed by wind. Sticky phialospores which accumulate in slimy droplets at the tips of the phialides are common in many genera; they are usually dispersed by insects, rain splash or other agencies.

As mentioned on p. 24, the term conidium is sometimes used for structures which are probably homologous to sporangia. A series can be erected in the Peronosporales in which there are forms with deciduous sporangia which release zoospores when in contact with water (e.g. *Phytophthora*), and other forms which germinate directly, i.e. by the formation of a germ tube (e.g. *Peronospora*). A similar series can be erected in the Mucorales where in some forms the number of sporangiospores per sporangium is reduced to several or even one (see Figs. 7.24, 7.26, 7.30). One-spored sporangia may be distinguished from conidia by being surrounded by two walls, i.e. that of the sporangium and that of the spore itself.

There are numerous other kinds of spore found in fungi, and they are described later in this book in relation to the particular groups in which they occur.

1.4.9 Anamorphs and teleomorphs

Fungi may exist in a range of forms or morphs, i.e. they may be **pleomorphic**. The morph which includes the sexually produced spore form, e.g. the ascocarp of an ascomycete or the basidiocarp of a basidiomycete, is termed the **teleomorph** (Gr. *teleios*, *teleos* = perfect, entire; *morphe* = shape, form) (Hennebert & Weresub, 1977). Many fungi also have a morph bearing asexually produced spores, e.g. conidiomata. These asexual morphs are termed **anamorphs** (Gr. *ana* = throughout, again, similar to). In the older literature, the term **perfect state** was used for the teleomorph and **imperfect state** for the anamorph. This is the origin of the name of the artificial group Fungi Imperfecti or Deuteromycetes, which included fungi believed to reproduce only by asexual means. The term **mitosporic fungi** is sometimes used alternatively for such fungi. The complete range of morphs belonging to any one fungus is termed the **holomorph** (Gr. *holos* = whole, entire) (see Sugiyama, 1987; Reynolds & Taylor, 1993; Seifert & Samuels, 2000). Some fungi have more than one anamorph as in the microconidia and macroconidia of some *Neurospora*, *Fusarium* and *Botrytis* species. These distinctive states are **synanamorphs** and may play different roles in the biology of the fungus. The morph may have a purely sexual role as a fertilizing agent, e.g. in the case of spermatia of many ascomycetes and rust fungi. Such states have been termed **andromorphs** (Gr. *andros* = a man, male) (Parbery, 1996a).

The existence of different states in the life cycle of a fungus has nomenclatural consequences, because they had often been described separately and given different names before the genetic connection between them was established. Further, even after the proof of an anamorph–teleomorph relationship, usually achieved by pure-culture studies, the anamorphic name may still be in wide use, especially where it is the more common state encountered in nature or culture. For example, most fungal geneticists refer to *Aspergillus nidulans* (the name of the conidial state) instead of *Emericella nidulans* (the name for the ascosporic state; p. 308). Similarly, most plant pathologists use *Botrytis cinerea*, the name for the conidial state of the fungus causing the common grey mould disease of many plants, in preference to the rarely encountered *Sclerotinia* (*Botryotinia*) *fuckeliana*, the name given to the apothecial (ascus-bearing) state (see p. 434).

1.5 Taxonomy of fungi

Taxonomy is the science of classification, i.e. the 'assigning of objects to defined categories' (Kirk *et al*., 2001). Classification has three main functions: it provides a framework of recognizable features by which an organism under examination can be identified; it is an attempt to group together organisms that are related to each other; and it assists in the retrieval of information about the identified organism in the form of a list or catalogue.

All taxonomic concepts are man-made and therefore to a certain extent arbitrary. This is especially true of classical approaches relying on macroscopic or microscopic observations because it is a matter of opinion whether the difference in a particular character – say, a spore

or the way in which it is formed – is significant to distinguish two fungi and, if so, at which taxonomic level. The great fungal taxonomist R. W. G. Dennis (1960) described taxonomy as 'the art of classifying organisms: not a science but an art, for its triumphs result not from experiment but from disciplined imagination guided by intuition'.

Recently, great efforts have been made at introducing a seemingly more objective set of criteria based directly on comparisons of selected DNA sequences encoding genes with a conserved biological function, instead of or in addition to phenotypic characters. The results of such comparisons are usually displayed as **phylogenetic trees** (see Fig. 1.26), which imply a common ancestry to all organisms situated above a given branch. Such a grouping is ideally 'monophyletic'. However, as we shall see later, quite different phylogenies may result if different genes are chosen for comparison. Further, a decision on the degree of sequence divergence required for a taxonomic distinction is based mainly on numerical parameters generated by elaborate computerized statistical treatments, occasionally at the expense of sound judgement. An excessive emphasis on such purely descriptive studies in the recent literature has led an eminent mycologist to characterize phylogenetic trees as 'the most noxious of all weeds'. Despite their limitations, these methods have led to a revolution in the taxonomy of fungi. At present, a new, more 'natural' classification is beginning to take shape, in which DNA sequence data are integrated with microscopic, ultrastructural and biochemical characters. However, many groups of fungi are still poorly defined, and many more trees will grow and fall before a comprehensive taxonomic framework can be expected to be in place. One of the core problems in fungal taxonomy is the seemingly seamless transition between the features of two taxa, and the question as to where to apply the cut-off point. To quote Dennis (1960) again, 'a taxonomic species cannot exist independently of the human race; for its constituent individuals can neither taxonomise themselves into a species, nor be taxonomised into a species by science in the abstract; they can only be grouped into species by individual taxonomisers'.

1.5.1 Traditional taxonomic methods

Early philosophers classified matter into three Kingdoms: Animal, Vegetable, and Mineral. Fungi were placed in the Vegetable Kingdom because of certain similarities to plants such as their lack of mobility, absorptive nutrition, and reproduction by spores. Indeed, it was at one time thought that fungi had evolved from algae by loss of photosynthetic pigmentation. This was indicated by the use of such taxonomic groups as Phycomycetes, literally meaning 'algal fungi'. This grouping, approximately synonymous with the loose term 'lower fungi', is no longer used because it includes taxa not now thought to be related to each other (chiefly Oomycota, Chytridiomycota, Zygomycota). Early systems of classification were based on morphological (macroscopic) similarity, but the invention of the light microscope revealed that structures such as fruit bodies which looked alike could be anatomically distinct and reproduce in fundamentally different ways, leading them to be classified apart.

Until the 1980s, the taxonomy of fungi was based mainly on light microscopic examination of typical morphological features, giving rise to classification schemes which are now known to be unnatural. Several examples of unnatural groups may be found by comparing the present edition with the previous edition of this textbook (Webster, 1980). Examples of traditional taxonomic features include the presence or absence of septa in hyphae, fine details of the type, formation and release mechanisms of spores (e.g. Kendrick, 1971), or aspects of the biology and ecology of fungi. Useful ultrastructural details, provided by transmission electron microscopy, concern the appearance of mitochondria, properties of the septal pore, details of the cell wall during spore formation or germination, or the arrangement of secretory vesicles in the apex of growing hyphae (Fig. 1.4). Biochemical methods have also made valuable contributions, especially in characterizing higher taxonomic levels. Examples include the chemical composition of

the cell wall (Table 1.1), alternative pathways of lysine biosynthesis (see p. 67), the occurrence of pigments (Gill & Steglich, 1987) and the types and amounts of sugars or polyols (Pfyffer *et al*., 1986; Rast & Pfyffer, 1989).

Microscopic features are still important today for recognizing fungi and making an initial identification which can then, if necessary, be backed up by molecular methods. Indeed, the comparison of DNA sequences obtained from fungi is meaningful only if these fungi have previously been characterized and named by conventional methods. It is therefore just as necessary today as it ever was to teach mycology students the art of examining and identifying fungi.

1.5.2 Molecular methods of fungal taxonomy

A detailed description of modern taxonomic methods is beyond the scope of this book, and the reader is referred to several in-depth reviews of the topic (e.g. Kohn, 1992; Clutterbuck, 1995). A particularly readable introduction to this subject has been written by Berbee and Taylor (1999). Only the most important molecular methods are outlined here. They are based either directly on the DNA sequences or on the properties of their protein products, especially enzymes.

Proteins extracted from the cultures of fungi can be separated by their differential migration in the electric field of an electrophoresis gel. The speed of migration is based on the charge and size of each molecule, resulting in a characteristic banding pattern. Numerous bands will be obtained if the electrophoresis gel is stained with a general protein dye such as Coomassie Blue. More selective information can be obtained by **isozyme analysis**, in which the gel is incubated in a solution containing a particular substrate which is converted into a coloured insoluble product by the appropriate enzyme, or in which an insoluble substrate such as starch is digested. In this way, the number and electrophoretic migration patterns of isoenzymes can be compared between different fungal isolates. Protein analysis is useful mainly for distinguishing different strains of the same species or members of the same genus (Brasier, 1991a).

Gel electrophoresis can also be used for the separation of DNA fragments generated by various methods. One such method is called **RFLP** (restriction fragment length polymorphisms) and involves the digestion of a total DNA extract or a previously amplified target sequence with one or more restriction endonucleases, i.e. enzymes which cut DNA only at a particular target site defined by a specific oligonucleotide sequence. Fragments from this digest can be blotted from the gel onto a membrane; fragments belonging to a known gene can be visualized by hybridizing with a fluorescent or radioactively labelled DNA probe of the same gene. In this way, a banding pattern is obtained and can be compared with that of other fungal isolates prepared under identical experimental conditions.

A similar method, **RAPD** (random amplified polymorphic DNA), produces DNA bands not by digestion, but by the amplification of DNA sequences. For this purpose, a DNA extract is incubated with a DNA polymerase, deoxynucleoside triphosphates and one or more short oligonucleotides which act as primers for the polymerase by binding to complementary DNA sequences which should be scattered throughout the genome. Amplification is achieved by means of the **PCR** (polymerase chain reaction), in which the mixture is subjected to repeated cycles of different temperatures suitable for annealing of DNA and primer, polymerization, and dissociation of double-stranded DNA. The largest possible size of the amplification product depends on the polymerization time; bands visible on a gel will be produced only if two primer binding sites happen to be in close proximity to each other, so that the intervening stretch of DNA sequence can be amplified from both ends within the chosen polymerization time. The number and size of RAPD bands on electrophoresis gels can be compared between different fungi, provided that all samples have been produced under identical conditions.

Isozyme, RFLP and RAPD analyses all generate data which are useful mainly for comparing

closely related isolates. Since the results strongly depend on the experimental conditions employed, there are no universal databases for these types of analysis. Further, they are unsuitable for comparisons of distantly related or unrelated organisms. A breakthrough in the taxonomy of fungi as well as other organisms was achieved when primers were developed which guided the PCR amplification of specific stretches of DNA universally present and fulfilling a homologous function in all life forms. Once amplified, the sequence of bases can be determined easily. Such methods were first applied to bacterial systematics with spectacular results (Woese, 1987). In eukaryotes, the most widely used target sequences are those encoding the 18S or 28S ribosomal RNA (rRNA) molecules, which fulfil a structural role in the small or large ribosomal subunits (respectively), or the non-coding DNA stretches (**ITS**, internal transcribed spacers), which physically separate these genes from each other and from the 5.8S rRNA sequence in the nuclear genome (Fig. 1.24; White *et al*., 1990). The structural role which rRNA molecules play in the assembly of ribosomes requires them to take up a particular configuration which is stable because of intramolecular base-pairing. Since certain regions of each rRNA molecule hybridize with complementary regions within the same molecule or with other rRNA molecules, mutations in the DNA encoding these regions are rare because they would impair hybridization and thus the functioning of the rRNA molecule unless accompanied by a mutation at the complementary binding site. The non-pairing loop regions of the rRNA gene and the ITS sequences are not subjected to such a strong selective pressure and thus tend to show a higher rate of mutation. Nucleotide sequences therefore permit the comparison of closely related species or even strains of the same species (ITS sequences), as well as that of distantly related taxa or even members of different kingdoms (18S or 28S rRNA). Further, because extensive databases are now available, the sequence analysis of a single fungus can provide meaningful taxonomic information when compared with existing sequences. In addition to ribosomal DNA sequences, genes encoding cytochrome oxidase (*COX*), tubulins or other proteins with conserved functions are now used extensively for phylogenetic purposes.

Once comparative data have been obtained either by banding patterns or gene sequencing, they need to be evaluated. This is usually done by converting the data into a matrix, e.g. by scoring the absence or presence of a particular band. With comparisons of aligned DNA sequences, only informative positions are selected for the matrix, i.e. where variations in the nucleotides between different fungi under investigation are observed. When the matrix has been completed, it can be subjected to statistical treatments, and phylogenetic trees are drawn by a range of algorithms. In some, the degree of relatedness of taxa is indicated by the length of the branch separating them (see Figs. 1.25, 1.26). Such information is thought to be of evolutionary significance; the greater the number of differences between two organisms, the earlier the separation of their evolutionary lines should have occurred.

1.5.3 How old are fungi?

Several lines of evidence indicate that fungi are a very ancient group of organisms. Berbee and Taylor (2001) have attempted to add a timescale to phylogenetic trees by applying the concept of a 'molecular clock', i.e. the assumption that the rate of mutations leading to phylogenetic diversity is constant over time and in various groups of organisms. By calibrating their molecular clock against fossil evidence, Berbee and Taylor (2001) estimated that fungi may have separated from animals some 900 million years ago, i.e. long before the evolution of terrestrial organisms. This estimate is consistent with the discovery of fossilized anastomozing hypha-like structures in sediments about 1 billion years old (Butterfield, 2005). Fungi recognizable as Chytridiomycota, Zygomycota and Ascomycota have been discovered among fossils of early terrestrial plants from the Lower Devonian Rhynie chert, formed some 400 million years ago (Taylor *et al*., 1992, 1999, 2005). It is apparent that these early terrestrial plants already entertained mycorrhizal symbiotic associations

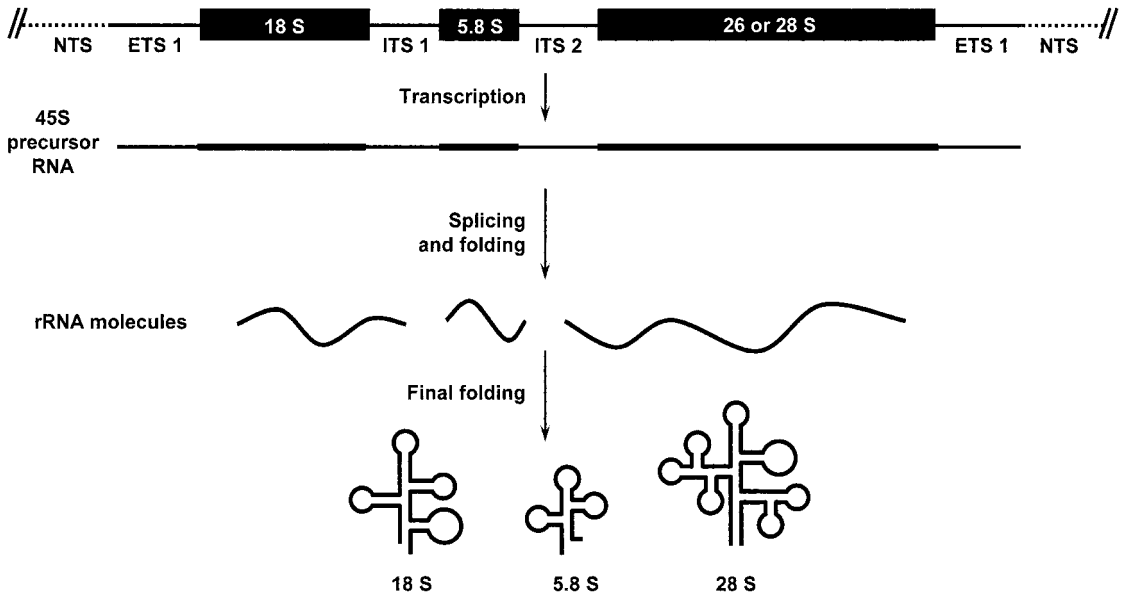

Fig 1.24 The spatial arrangement of a nuclear rRNA gene repeat unit. Each haploid fungal genome contains about 50–250 copies of this repeat, depending on the species (Vilgalys & Gonzalez, 1990). The three structural rRNA genes encoded by one repeat unit, i.e. 18S, 5.8S and 28S, are separated by internal and external transcribed spacers (ITS and ETS, respectively). Adjacent copies of the repeat unit are separated by a short non-transcribed spacer (NTS). The whole unit is transcribed into a 45S precursor RNA in one piece, followed by excision of the three structural RNA molecules from the spacers which are not used. The 5S rRNA gene is encoded at a separate locus. The 18S rRNA molecule is part of the small ribosomal subunit, whereas the other three contribute to the large subunit.

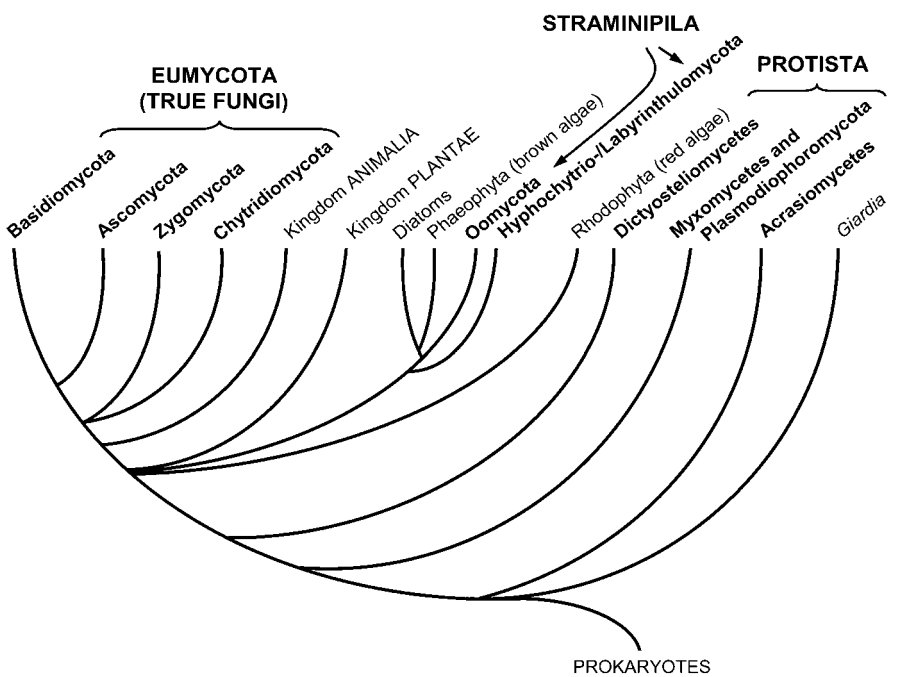

Fig 1.25 The phylogenetic relationships of Fungi and fungus-like organisms studied by mycologists (printed in bold), with other groups of Eukaryota. The analysis is based on comparisons of 18S rDNA sequences. Modified and redrawn from Bruns *et al*. (1991) and Berbee and Taylor (1999).

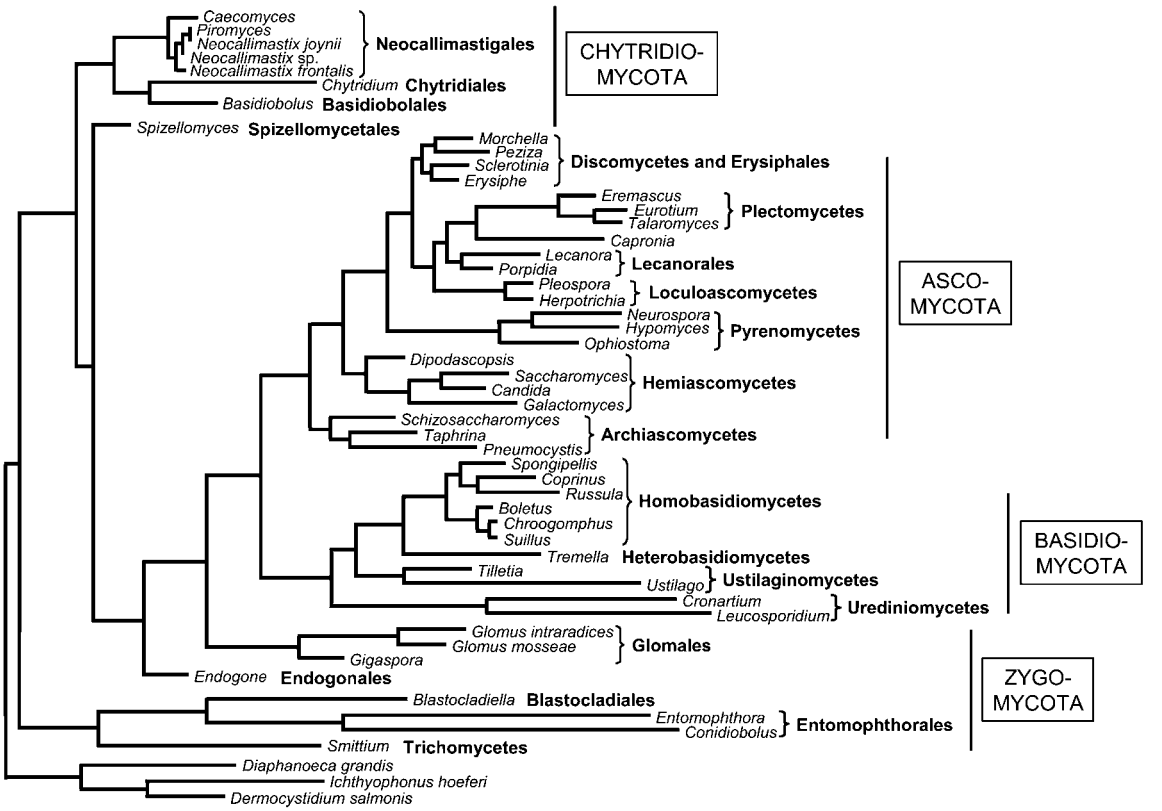

Fig 1.26 Phylogenetic relationships within the Eumycota, based on 18S rDNA comparisons. This tree illustrates the analytical power of molecular phylogenetic analyses; all four phyla of Eumycota are resolved. However, it also highlights problems in that *Basidiobolus* groups with the Chytridiomycota, although sharing essential biological features with the Zygomycota, and that conversely *Blastocladiella* groups with the Zygomycota instead of the Chytridiomycota. Modified and redrawn from Berbee and Taylor (2001), with kind permission of Springer Science and Business media.

with glomalean members of the Zygomycota (see p. 218).

1.5.4 The taxonomic system adopted in this book

The discipline of fungal taxonomy is evolving at an unprecedented speed at present due mainly to the contributions of molecular phylogeny. Numerous taxonomic systems exist, but this is not the place to discuss their relative merits (see Whittaker, 1969; Margulis *et al*., 1990; Alexopoulos *et al*., 1996; Cavalier-Smith, 2001; Kirk *et al*., 2001). In this book we have tried to follow the classification proposed in *The Mycota* Volumes VIIA and VIIB (McLaughlin *et al*., 2001), but even in these volumes the authors of different chapters have used their own favoured systems of classification rather than adopting an imposed one. In cases of doubt, we have attempted to let clarity prevail over pedantry.

Fungi in the widest sense, as organisms traditionally studied by mycologists, currently fall into three kingdoms of Eukaryota, i.e. the Eumycota which contain only fungi, and the Protozoa and Chromista (= Straminipila), both of which contain mainly organisms not studied by mycologists and were formerly lumped together under the name Protoctista (Beakes, 1998; Kirk *et al*., 2001). The Protozoa are notoriously difficult to resolve by phylogenetic means, and the only firm statement which can be made at present is that they are a diverse and ancient group somewhere between the higher Eukaryota ('crown eukaryotes') and the

Table 1.2. The classification scheme adopted in this book, showing mainly those groups treated in some detail.

KINGDOM PROTOZOA
Myxomycota (Chapter 2)
- Acrasiomycetes
- Dictyosteliomycetes
- Protosteliomycetes
- Myxomycetes

Plasmodiophoromycota (Chapter 3)
- Plasmodiophorales
- Haptoglossales (Oomycota?)

KINGDOM STRAMINIPILA
Hyphochytriomycota (Chapter 4)
Labyrinthulomycota (Chapter 4)
- Labyrinthulomycetes
- Thraustochytriomycetes

Oomycota (Chapter 5)
- Saprolegniales
- Pythiales
- Peronosporales

KINGDOM FUNGI (EUMYCOTA)
Chytridiomycota (Chapter 6)
- Chytridiomycetes

Zygomycota (Chapter 7)
- Zygomycetes
- Trichomycetes

Ascomycota (Chapter 8)
- Archiascomycetes (Chapter 9)
- Hemiascomycetes (Chapter 10)
- Plectomycetes (Chapter 11)
- Hymenoascomycetes
  - Pyrenomycetes (Chapter 12)
  - Erysiphales (Chapter 13)
  - Pezizales (Chapter 14)
  - Helotiales (Chapter 15)
  - Lecanorales/lichens (Chapter 16)
- Loculoascomycetes (Chapter 17)

Basidiomycota (Chapter 18)
- Homobasidiomycetes (Chapter 19)
- Homobasidiomycetes: gasteromycetes (Chapter 20)
- Heterobasidiomycetes (Chapter 21)
- Urediniomycetes (Chapter 22)
- Ustilaginomycetes (Chapter 23)

prokaryotes (Kumar & Rzhetsky, 1996). An overview of eukaryotic organisms, in which those groups treated in this book are highlighted, is given in Fig. 1.25. Among the Protozoa, the Plasmodiophoromycota are given extensive treatment because of their role as pathogens of plants (Chapter 3), whereas the various forms of slime moulds are considered only briefly (Chapter 2). Similarly brief overviews will be given of most groups of Straminipila studied by mycologists (Chapter 4), except for the Oomycota which, despite their separate evolutionary origin, represent a major area of mycology (Chapter 5). All remaining chapters deal with members of the Eumycota (= Kingdom Fungi). The scheme is summarized in Table 1.2 and illustrated in Fig. 1.26. An overview of the nomenclature used for describing taxa within the Eumycota is given in Table 1.3.

In the past, fungi which solely or mainly reproduce asexually (Fungi Imperfecti, Deuteromycota, mitosporic fungi, anamorphic fungi) were considered separately from their sexually reproducing relatives the teleomorphs, and separate anamorph and teleomorph genera were erected. However, information from pure-culture studies and molecular phylogenetic approaches has linked many anamorphs with their teleomorphs. For instance, the conidial (imperfect) state of the common brown-rot fungus of apples and other fruits is called *Monilia fructigena*, whereas the sexual (perfect)

Table 1.3. Example of the hierarchy of taxonomic terms. The wheat stem rust fungus, *Puccinia graminis*, is used as an example.

Kingdom Fungi
Subkingdom Eumycota
Phylum Basidio**mycota**
Class Uredinio**mycetes**
Order Uredin**ales**
Family Puccini**aceae**
Genus *Puccinia*
Species *Puccinia graminis*
Race *Puccinia graminis*
f. sp. *tritici*

state is apothecial, being called *Sclerotinia* (*Monilinia*) *fructigena*. As far as is possible, we shall consider anamorphic states of fungi in the context of their known sexual state. Thus, an account of the brown-rot of fruits, although encountered predominantly as the conidial state, will be given in the chapter dealing with apothecial fungi (Helotiales, Chapter 15). Where practical, we have given the teleomorph name priority over the anamorph. As a long-term future goal, Seifert and Samuels (2000) and Seifert and Gams (2001) have outlined a unified taxonomy which might ultimately lead to the abolition of the names of anamorphic genera.

However, with certain ecological groups such as the Ingoldian aquatic fungi (Section 25.2) and nematophagous fungi (Section 25.1), which have diverse relationships, we have deliberately chosen to consider them in their ecological context rather than along with their varied taxonomic relatives.