

Fungal Cell Biology and Development

Nicholas P. Money

Miami University, Oxford, OH, USA

ORGANELLES, CELLS, ORGANS

Fungi are eukaryotes and much of their cell biology is shared with animals, plants, and protists. Fungal cells are built from the same kinds of organelles as other eukaryotes. They have a plasma membrane, nuclei, and complicated endomembrane system. Most species have mitochondria. A few organelles are not found in other kingdoms. These include a dense assembly of secretory vesicles called the Spitzenkörper that is located in hyphal tips, and the Woronin body of Ascomycota that serves as an intracellular plugging device that stops cytoplasmic leakage. The chitinous cell wall is another distinctive feature of fungal cells. This chapter will provide an overview of the structure of fungal cells, discuss how they grow and multiply to form yeast colonies or branching mycelia, and describe the developmental processes that result in the formation of complex, multicellular organs including cords, sclerotia, and mushrooms (Figure 2.1).

CELL STRUCTURE

The Cell Wall

The cytoplasm of growing yeasts and mycelia contains a higher concentration of salts and sugars than the surrounding fluid. This osmotic differential drives the net influx of water through the plasma membrane and this causes cell expansion. Unlike many protists, fungi do not limit expansion by exporting water using contractile vacuoles, but do so by constructing a cell wall on the surface of the plasma membrane. As water enters the cell, the plasma membrane is pressed against the inner surface of the wall, resulting in the development of hydrostatic pressure or **turgor**. The increase in internal pressure allows the cell to approach a condition of homeostasis in which water influx matches the increase in cell volume that occurs during growth.

The wall is a highly dynamic structure, resisting expansion over much of its surface, but extending in specific regions including hyphal tips and yeast buds. The adaptive significance of the wall is controversial. It is important to avoid the chicken-and-egg trap of

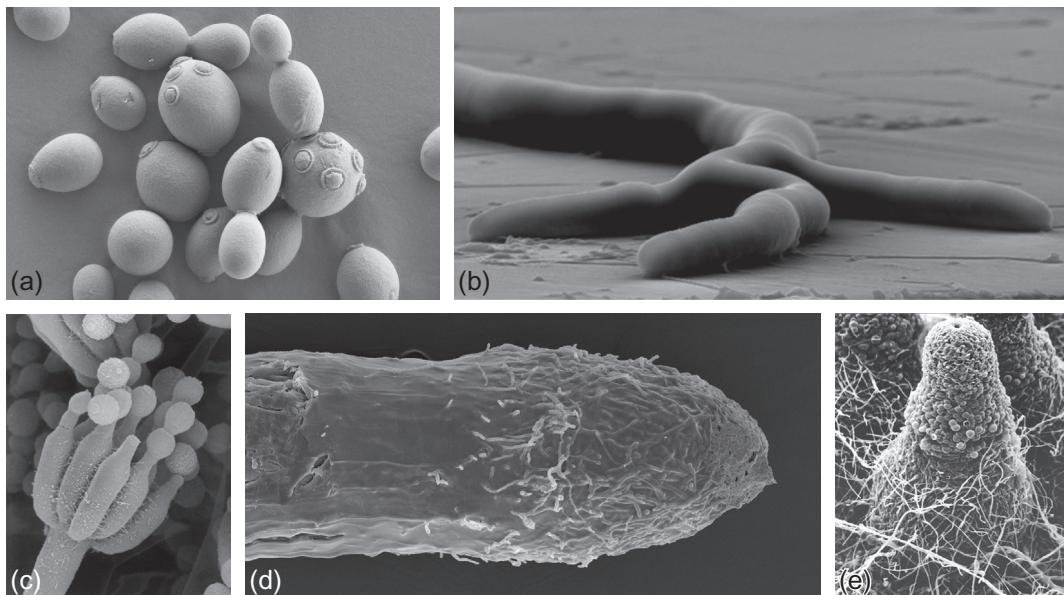


FIGURE 2.1 Fungal cells and multicellular structures imaged using scanning electron microscopy. (a) Yeast cells of *Saccharomyces cerevisiae*. The older cells have faint birth scars, stretched since separation from their mother cells, and bud scars produced by the formation of daughter cells. (b) Hypha of *Aspergillus niger*. (c) Conidiophore of a *Penicillium* species. (d) Rhizomorph of *Armillaria gallica*. (e) Peritheciun of *Sordaria humana*. Source: (a) Kathryn Cross, Institute of Food Research, Norwich. (b) Geoffrey Gadd, University of Dundee. (c) Richard Edelmann, Miami University, Ohio. (d) Levi Yafetto, University of Cape Coast, Ghana. (e) Nick Read, University of Manchester.

suggesting that the cell wall functions to resist the explosive effects of turgor, because the cell would not be pressurised without the resistive behaviour of its wall. The cell wall allows the cell to generate turgor pressure, so perhaps it is more fruitful to think about why turgor might be useful. We will come back to this issue later in the chapter.

The fungal cell wall is a porous macromolecular composite assembled at the surface of the plasma membrane (Figure 2.2). It contains stress-bearing microfibrils of **chitin**, linear polymers of glucose, or **glucans**, and a variety of **cell wall proteins** (CWP). The chitin polymer is built from β -1 \rightarrow 4-linked monomers of the amino sugar, *N*-acetyl- α -D-glucosamine (Figure 2.3). Adjacent chitin chains assemble into hydrogen-bonded antiparallel arrays, producing microfibrils that can reach lengths of more than 1 μm . Chitin microfibrils have tremendous tensile strength; when chitin is disrupted, the cell loses its osmotic stability and may rupture. **Chitosan**, or β -1 \rightarrow 4-glucosamine, is a polymer of the deacetylated sugar that is produced by many fungi in addition to chitin. β -1 \rightarrow 3-glucan is often the most abundant wall polymer (Figure 2.4). The β -1 \rightarrow 3-glycosidic linkage in glucans twists the polymer and three glucan chains form a triple helix that is held together by hydrogen bonds. β -1 \rightarrow 3-glucans are connected with β -1 \rightarrow 6-glucans in the mature wall structure to produce a highly branched elastic network of polymers. The structural proteins in the cell wall are **glycoproteins** with *N*- and *O*-linked carbohydrates. These include **mannoproteins**, which are glycosylated with mannose-rich chains, and other glycoproteins with both mannose and

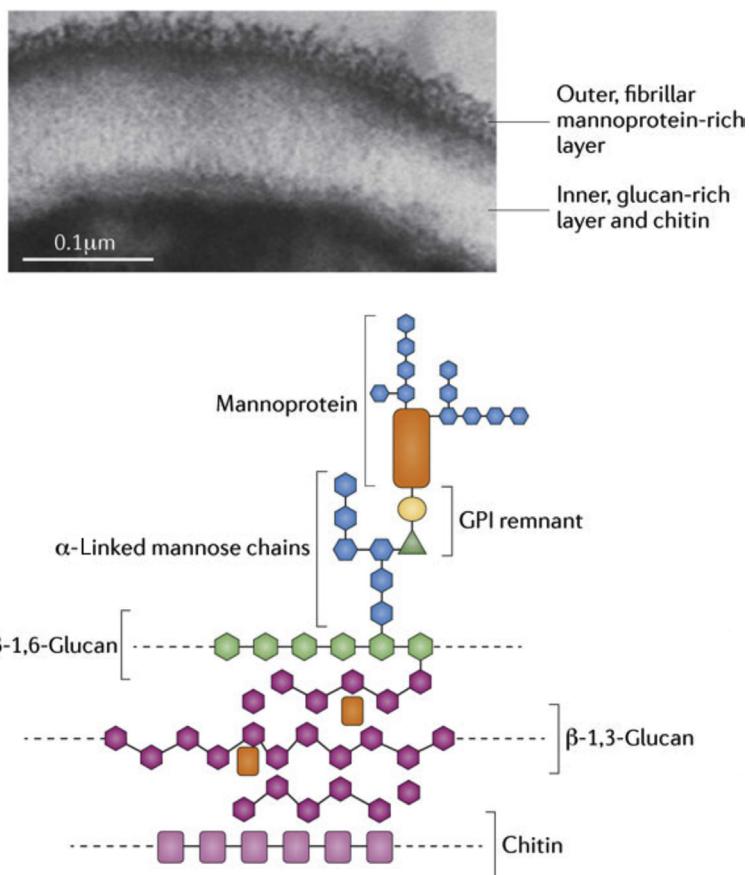


FIGURE 2.2 Structure of the fungal cell wall. Transmission electron micrograph of *Candida albicans* cell wall with diagram showing chemical composition. Source: Cassone, A., 2013. Development of vaccines for *Candida albicans*: fighting a skilled transformer. *Nature Rev. Microbiol.* 11, 884–891.

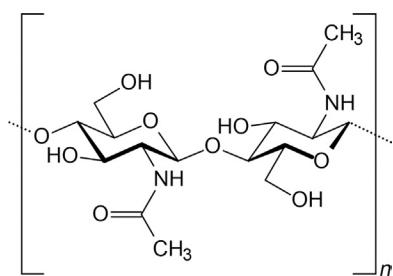


FIGURE 2.3 Chemical structure of the cell wall polymer chitin.

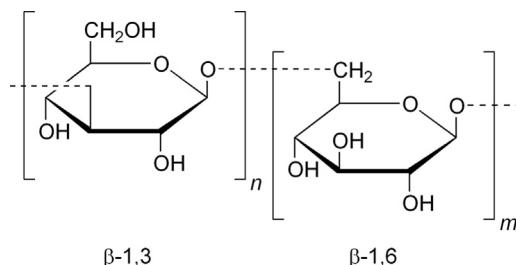


FIGURE 2.4 Chemical structure of the cell wall glucan.

galactose residues. Cell wall glycoproteins are connected to the plasma membrane by a glycoprophosphatidylinositol (GPI) anchor and cross-linked to the chitin microfibrils and glucans.

Although chitin is a stress-bearing component within the extracellular matrix of most fungi, the maxim that ‘fungi have chitinous walls’ can be misleading. β-1→3-glucan is often the dominant wall polymer and up to half the wall of some fungi is proteinaceous. The relative proportions of chitin, glucans, and glycoproteins vary according to fungal species. Chitin is a minor constituent of the cell wall of the yeast, *Saccharomyces cerevisiae*, and is synthesised when the mother cell produces a bud. The bulk of the yeast cell wall is a scaffold of β-1→3-glucans capped by a layer of mannoproteins. The β-1→3-glucans are cross-linked to β-1→6-glucans and to a variety of CWP. The cell wall of fission yeast, *Shizosaccharomyces pombe*, is very different, containing glucans with varied glycosidic linkages (α-1→3-, β-1→3-, and α-1→3-glucan) but completely lacking chitin. The same polymers are found in mycelial fungi, but chitin is more important in these species, accounting for 10% or more of the dry weight of the wall. In *Neurospora crassa*, for example, β-1→3-glucans and chitin form an inner layer, which is covered by a protein–polysaccharide complex. There is no β-1→6-glucan in the cell wall of this ascomycete.

Chitin synthesis is catalysed by **chitin synthase** which is an integral membrane protein. Chitin synthase extrudes chitin chains through the plasma membrane and the new polymers hydrogen bond with one another and crystallise into microfibrils. *Saccharomyces cerevisiae* has three chitin synthases (Chs1p, Chs2p, and Chs3p); the filamentous ascomycete *Aspergillus fumigatus* has seven chitin synthases, and four have been identified in *Neurospora crassa*. Glucan synthases operate in a similar fashion to the chitin synthases. They are integral membrane proteins and form long chains of glucans by the sequential addition of glucose residues. Crosslinks between adjacent branches within glucan molecules and linkages between the glucans, chitin, and glycoproteins are vital for maintaining wall strength. Glucan synthesis is a potential target for antifungal agents. A family of drugs called **echinocandins** that bind to β-1→3-glucan synthase show promise in the treatment of aspergillosis and candidiasis (Chapter 9).

Most of the wall proteins are glycosylated during transit through the secretory pathway. Delivered to the plasma membrane by exocytosis and tethered by GPI anchors, their sugar residues form covalent links with other wall polymers and help to maintain cell shape. Glycoproteins also serve signalling and transport functions, participate in fusion with other cells (e.g. agglutinins involved in cell–cell recognition during mating reactions), and function in adhesion to surfaces, biofilm formation, and pathogenesis. They also mediate the

absorption of compounds from the surrounding environment and protect the cell from noxious substances. The wall is also rich in enzymes. Some of these enzymes are involved in the synthesis of other wall components and are critical for the continuous remodelling of the wall during growth. The function of many cell wall enzymes is unknown.

The electron microscope reveals that the walls of some fungi are organised into discrete layers, but this does not mean that the wall has a plywood-like structure that separates each of the different kinds of molecule. The wall is more like a fibre reinforced polymer (e.g. fibreglass), with multiple interwoven components within each layer. Another important feature of the wall is its dynamic nature. Even the thick cell wall of a long-dormant spore is reconfigured and rendered more fluid as the cell germinates. Although there are many features of cell wall structure and function that are unique to the fungi, there are numerous similarities between this structure and the extracellular matrix of other eukaryotes. The plant cell wall contains stress-bearing microfibrils of cellulose (β -1 \rightarrow 4-glucan), but it also contains glucans with β -1 \rightarrow 3 linkages. The extracellular matrix of animal cells is constructed from collagens intermeshed with proteoglycans, including glycoproteins. Fungal cell walls are particular types of extracellular matrix.

Plasma Membrane

The sterol molecule, **ergosterol** is a distinguishing component of the fungal plasma membrane (Figure 2.5a). Ergosterol performs the same function as cholesterol in animal membranes, namely, modulating membrane fluidity and permeability through its interactions with phospholipids and other membrane constituents. The absence of ergosterol in animals allows treatment of a broad range of fungal infections (mycoses) using antifungal agents that inhibit its synthesis (Chapter 9). **Terbinafine hydrochloride** (Lamisil), which is used to treat athlete's foot and other superficial infections caused by dermatophytes, inhibits an

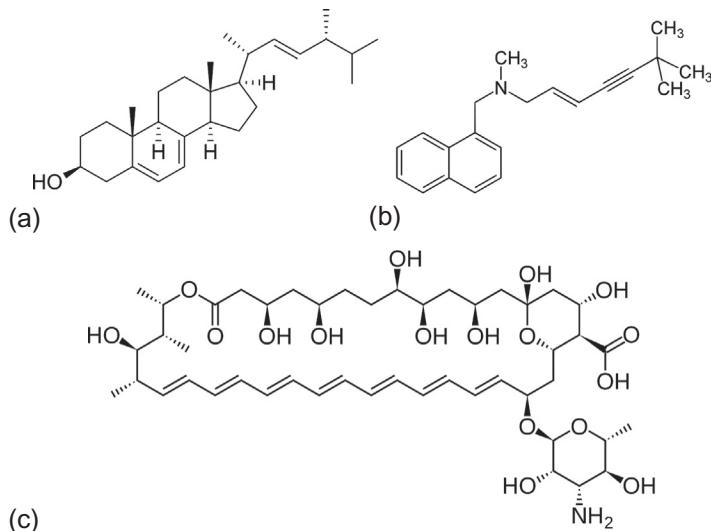


FIGURE 2.5 Chemical structures of (a) ergosterol, (b) terbinafine hydrochloride, and (c) amphotericin B. (Source: Creative Commons).

enzyme involved in ergosterol synthesis called squalene epoxidase ([Figure 2.5b](#)). Disruption of sterol biosynthesis causes lysis of the target cells. **Amphotericin B** is an antifungal agent that is used to treat more serious infections, including cryptococcal meningitis ([Figure 2.5c](#)). Amphotericin B binds to ergosterol and, like terbinafine hydrochloride, is thought to interfere with membrane integrity.

The lipid bilayer of the fungal plasma membrane contains proteins that function in solute transport, signal transduction, cell wall synthesis, and as anchors for the underlying cytoskeleton. **Lipid-anchored proteins**, including G proteins, are linked covalently to the lipid bilayer via lipidated amino acid residues (or by the GPI anchor described in the previous section). Peripheral membrane proteins are associated with the membrane by electrostatic forces and other kinds of non-covalent interactions. **Integral membrane proteins** are permanent residents of the phospholipid bilayer. These include **transmembrane proteins**, or integral polytopic proteins, that function in transporting ions and molecules through the plasma membrane. **Integral monotopic proteins** are embedded in only one side of the membrane (rather than threading through the entire membrane).

Ion transport functions catalysed by transmembrane proteins are critical for understanding fungal physiology ([Figure 2.6](#)). The plasma membrane acts as a semipermeable barrier to the diffusion of many ions and small molecules. For example, protons cannot diffuse freely through the membrane. They are extruded from the cytoplasm by an enzyme, or **ion pump**, called the proton (H^+)-ATPase. Proton extrusion is an example of **primary active transport**. This creates an electrochemical gradient with a reduction in pH at the surface of the fungal cell and a negative voltage inside the cell (as much as -250mV). This voltage, or membrane potential, can be measured with a microelectrode inserted through the cell wall and underlying membrane ([Figure 2.7](#)).

The electrochemical gradient established by proton ATPase activity is vital to the absorptive feeding mechanism characteristic of the fungi, because it powers the import of small molecules including sugars and amino acids. This cellular physiological mechanism is very elegant. The proton ATPase moves protons against their concentration gradient, so that protons will flow into the cytoplasm as soon as a diffusion path is opened. As a hypha digests protein-rich food, a localised pool of amino acids accumulates around the cell. Because biomolecules, including amino acids, are at far higher concentration inside the cytoplasm than

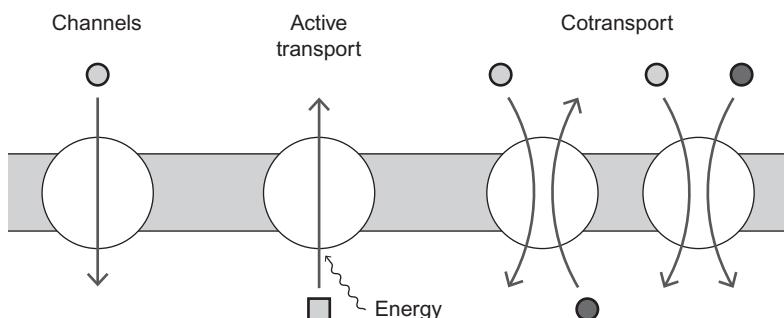


FIGURE 2.6 Transmembrane proteins that transport ions and molecules between fungal cells and the surrounding environment. Source: Mark Fischer, Mount St. Joseph University, Cincinnati.

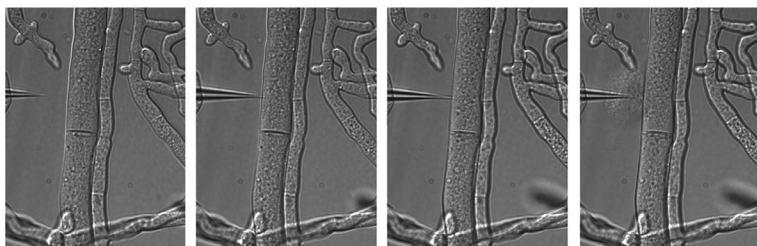


FIGURE 2.7 Microelectrode piercing hyphal cell wall and plasma membrane during a recording of the membrane potential. Source: Roger Lew, York University, Ontario.

the surrounding environment, their import will not occur passively by diffusion, even if physical pathways through the membrane are available. The concentration gradient is in the wrong direction. Transmembrane proteins called **carrier proteins** solve this problem by harnessing the influx of protons to the movement of amino acids. Carrier proteins function by undergoing a specific conformational change in response to the flux of each proton that shuffles an amino acid molecule from the exterior of the cell into its cytoplasm. The amino acid is captured and then released by the carrier protein. This is an example of **secondary active transport**.

The carrier protein that conducts amino acid import is an example of a **symporter**. Other symport proteins couple proton influx to the import of potassium ions and sugars. Antiporters couple the passive influx of a proton, or another ion, to the export of a different ion or a molecule. Na^+/H^+ antiporters have been characterised in yeast and filamentous fungi. These proteins export one sodium ion for each proton that passes into the cell and are important in maintaining ionic homeostasis and determine salt tolerance in saline environments. Carrier proteins can also provide aqueous pathways for the **facilitated diffusion** of ions and molecules that are more diluted within the cell, but cannot diffuse through the hydrophobic interior of the plasma membrane. **Ion channels** are another subset of integral membrane proteins that control ion import and export. Some ion channels act as gates that open and close in response to changes in membrane potential or to mechanical signals including stretching of the membrane. Calcium channels regulate the concentration of calcium ions in the cytoplasm. Calcium is maintained at very low levels in the cell, but bursts in concentration caused by channel opening may serve as important signals regulating cellular development. The concerted action of all these transport proteins determines the chemical composition of the cytoplasm, the food supply for the mitochondria, the export of waste metabolic materials, and exclusion of environmental toxins.

In the 1980s, it was thought that the electrical activity of cells caused by all of these ion movements reflected important developmental cues. The unequal distribution of ion pumps along fungal hyphae, for example, produces a halo of ionic current with the net influx of positive charge at the growing hyphal tip. More recent studies suggest that these patterns reflect the feeding activity of the hypha and have no deeper meaning for developmental biologists searching for signals that determine how fast these cells extend and where branches appear.

Endomembrane System

The cytoplasm of hyphae and yeast cells is packed with the membrane-bound compartments of the **endomembrane system**. These organelles function in the secretory (export) and endocytotic (import) pathways that sustain growth and development. The endomembrane system includes the **endoplasmic reticulum**, **Golgi apparatus**, **vacuoles**, and **vesicles** (Figure 2.8). The system is very dynamic, both in terms of the movement of the different components within the cytoplasm and the biochemical activities within them. Analysis of the endomembrane system has been revolutionised by the use of **fluorescent protein tagging** methods to pinpoint the distribution of specific molecules within the cell (Figure 2.9). Researchers can track the locations and movement of these fluorescent probes using laser scanning **confocal microscopy**. Major advances in fungal cell biology have been made using these techniques in **live cell imaging** in conjunction with modern methods of molecular genetics and **comparative genomics**. The computer-based tools of comparative genomics have highlighted genes in the fungus that share sequences with genes with proven activity in the endomembrane system of humans. The actual function of these genes in the fungus can then be studied by comparing the growth and development of a mutant strain in which the genes have been deleted, with wild-type strains expressing the functional genes. Live cell imaging is used to track the location of proteins in the wild-type

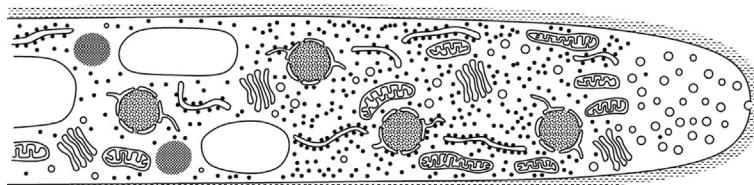


FIGURE 2.8 Diagram of the endomembrane system within a fungal hypha. Source: www.cronodon.com

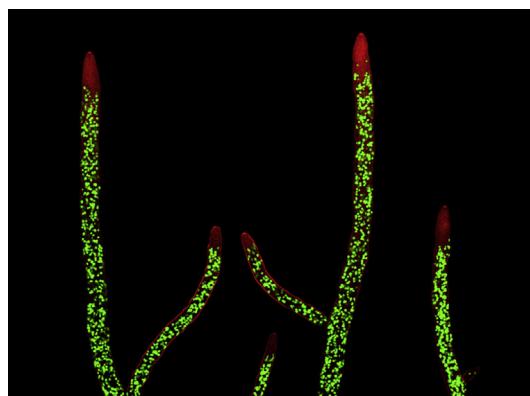


FIGURE 2.9 Confocal image showing tip-growing and branched, multinucleate hyphae of *Neurospora crassa*. The nuclei are shown in green (nuclear-targeted GFP) and membranes, especially the plasma membrane and secretory vesicles, are shown in red (stained with FM4-64). Source: www.fungalcell.org

strains and protein–protein interactions can be visualised when two or more fluorescent probes are introduced into the same cells.

The **endoplasmic reticulum** is an interconnected system of membranes from which most other membranes originate. In *Saccharomyces cerevisiae*, the endoplasmic reticulum is organised as an array of contiguous sheets and tubules that link to the plasma membrane. In filamentous fungi, the endoplasmic reticulum is organised as stacks of membrane-bound sacs or **cisternae**. Most of these cisternae are studded with groups of ribosomes called polyribosomes, constituting **rough endoplasmic reticulum**. Other portions of the endoplasmic reticulum, called **smooth endoplasmic reticulum**, are not decorated with ribosomes. The whole assembly contains mixtures of rough and smooth endoplasmic reticulum and transitional regions from which the cisternae of the **Golgi apparatus** are generated. Studies with fluorescent dyes show that the endoplasmic reticulum is a mobile organelle rather than a static platform. The Golgi in fungi is a very different structure from the organelles in other eukaryotes. The stacks of cisternae, called dictyosomes, found in animals and plants are replaced by single cisternae perforated with holes and tubular extensions that are dispersed throughout the fungal cytoplasm. The term **Golgi equivalent** is often used to describe this fungal organelle, but Golgi apparatus has the virtue of being less cumbersome. The Golgi develops by the coalescence of vesicles that bud from the endoplasmic reticulum and it functions in the modification of proteins for delivery to the plasma membrane by exocytosis. In preparation for these functions, the proteins are cleaved, folded into their tertiary structures, glycosylated and phosphorylated in the Golgi. Molecular labels or **signal sequences** are also added to proteins that specify their destination after release from the Golgi. These proteins include the integral membrane proteins, CWP_s, and secreted enzymes that catalyse the breakdown of polymers and fuel the fungus with absorbable nutrients. Cell wall polysaccharides are also generated in the Golgi and reach the cell surface by exocytosis.

Vacuoles form the largest endomembrane compartments in fungal hyphae ([Figure 2.10](#)). These are highly mobile organelles, capable of extension, retraction, and peristaltic shape changes. These structures also occur in yeast where their function in the endocytotic pathway for sorting and recycling proteins has been studied in most detail. In filamentous fungi, the vacuoles form an interconnected system of elongated tubules behind the tip and rounded vacuoles in older portions of the cell. Dissolved substances are free to diffuse throughout these compartments, which is consistent with a passive transport function for these organelles. In addition to their endocytotic function, vacuoles may also serve as a repository for waste products from metabolism and for heavy metals and other toxins absorbed from the environment.

Cytoskeleton

The shape of yeast cells and filamentous hyphae is determined by physical interactions between the cell wall, whose composition and mechanical properties vary at different points on the cell surface, the pressurised cytoplasm, which tends to inflate the cell, and the cytoskeleton. The importance of the cytoskeleton in this shaping process has been demonstrated by live cell imaging of its behaviour, experiments with inhibitors that disrupt specific cytoskeletal components, and work with mutants with various defects in cytoskeletal function ([Figure 2.11](#)). The fungal cytoskeleton comprises three polymers: actin microfilaments

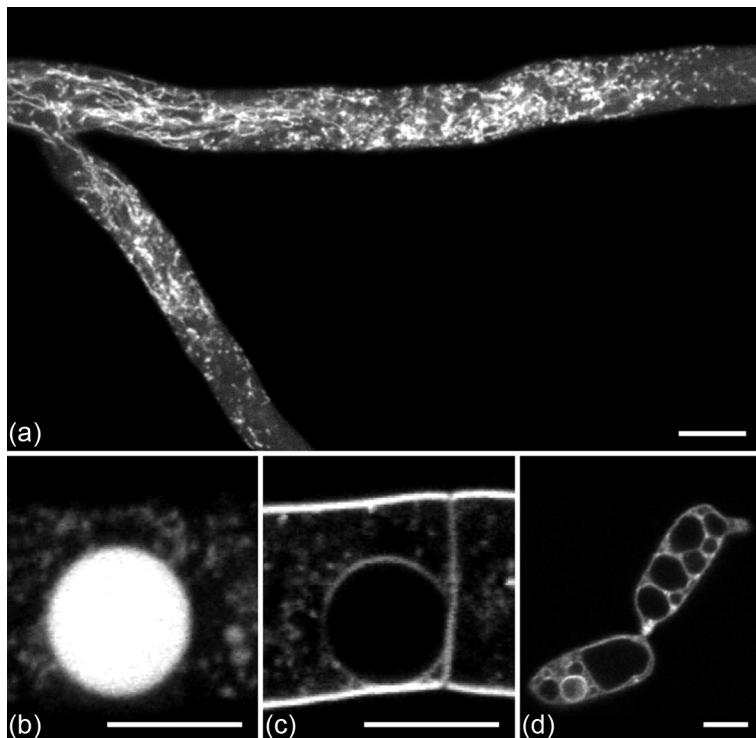


FIGURE 2.10 Vacuoles in (a–c) *Neurospora crassa* and (d) *Colletotrichum lindemuthianum*. (a) Tubular vacuolar network in apical hyphal compartment and branch stained with carboxy-DFFDA. (b) Large and small spherical vacuoles in subapical hyphal compartment stained with carboxy-DFFDA. (c) Vacuole membrane of large spherical vacuole in subapical hyphal compartment stained with FM4-64. (d) Vacuole membranes in conidia stained with MDY-64. Note that the two conidia are fusing via conidial anastomosis tubes. All scale bars = 10 µm. Source: www.fungalcell.org

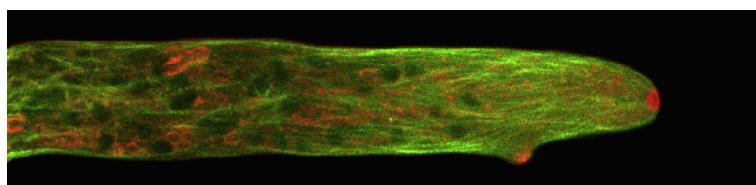


FIGURE 2.11 Confocal image of a growing hypha expressing β -tubulin-GFP, localised in microtubules (green), and co-labelled with FM4-64 to show distribution of membranes (red). Microtubules extend into the negatively stained Spitzenkörper at the tip. A subapical swelling that will become a hyphal branch is highlighted with a concentration of vesicles that will become a separate Spitzenkörper. Source: Patrick Hickey.

(F-actin), microtubules, and septins. These form an intracellular scaffold that probably plays a role in maintaining cell shape as well as directing the traffic of organelles. The structural polymers regulate cell shape by serving as directional pathways for the movement of vesicles. The trafficking functions are dependent upon molecular motors, including myosin, dynein, and kinesin, which glide along the actin filaments and microtubules. Interactions between

F-actin and septins are important in controlling the direction of trafficking, but septins do not interact directly with the motor proteins.

Actin microfilaments are assembled from globular actin monomers (G-actin) that polymerize as pairs of intertwined helices. Each filament is polarised, with the addition of new G-actin monomers to one end (called the plus, or barbed, end) and depolymerization at the other end (the minus or pointed end). The plus ends of the microfilaments tend to be oriented toward sites of growth and relative rates of polymerization and depolymerization determine the length of the filaments. Interactions between F-actin and actin binding proteins organise the microfilaments into cables, patches, and rings. Actin cables function in exocytosis, forming pathways for the movement of vesicles powered by myosin motors. Patch structures are involved in endocytosis and actin rings function in cell division and the formation of septa (see next section).

As their name suggests, microtubules have a tubular form. They are assembled from 'protofilaments' of paired α - and β -tubulin monomers (forming heterodimers), whose parallel arrangement creates a tube with an outer diameter of 25 nm. Like the microfilaments, the microtubules have a plus and a minus end and this polarity dictates the direction of organelle movement along the filament surface: organelles equipped with kinesin motors move toward the plus end of the microtubules, and dynein sends them in the reverse direction. In common with the role of microtubules in other eukaryotes, fungal microtubules also position nuclei, form the mitotic spindle, and drive chromosome separation.

Less is known about the third type of cytoskeletal polymer, called septins. Septins are types of GTPases that can be configured in a variety of ways to produce rods, longer filaments, and sheets. GTPases hydrolyse the purine nucleotide guanosine triphosphate (GTP) into guanosine diphosphate and inorganic phosphate, and perform critical functions in signal transduction, protein synthesis, cell differentiation, and vesicle transport. Septins become concentrated at the periphery of fungal cells and play important roles in tip growth and the control of cell shape.

GROWTH AND CELL DIVISION

The quest for an integrated model of tip growth in filamentous fungi is a continuing endeavour for experimental mycologists. Rather more is known about the mechanism of bud formation in yeast and we will look at this first.

Growth and Cell Division in Budding Yeast and Fission Yeast

Bud formation in *Saccharomyces cerevisiae* is regulated throughout the cell cycle, producing one daughter cell at each mitotic division (Figure 2.12). The position of the bud on the cell surface is determined during the G1 phase of the cell cycle. In haploid cells, the new bud will develop next to the scar left by the separation of the preceding daughter cell. Buds form at opposite poles of a diploid mother cell, alternating from one end of the ovoid cell to the other from division to division. The new bud site is designated by specific marker proteins in the cytoplasm during the previous cell cycle. These are recognised during G1 by a GTPase and associated proteins constituting the Rsr1p GTPase module; these proteins interact with another protein complex, the polarity establishment Cdc42p GTPase module, which directs

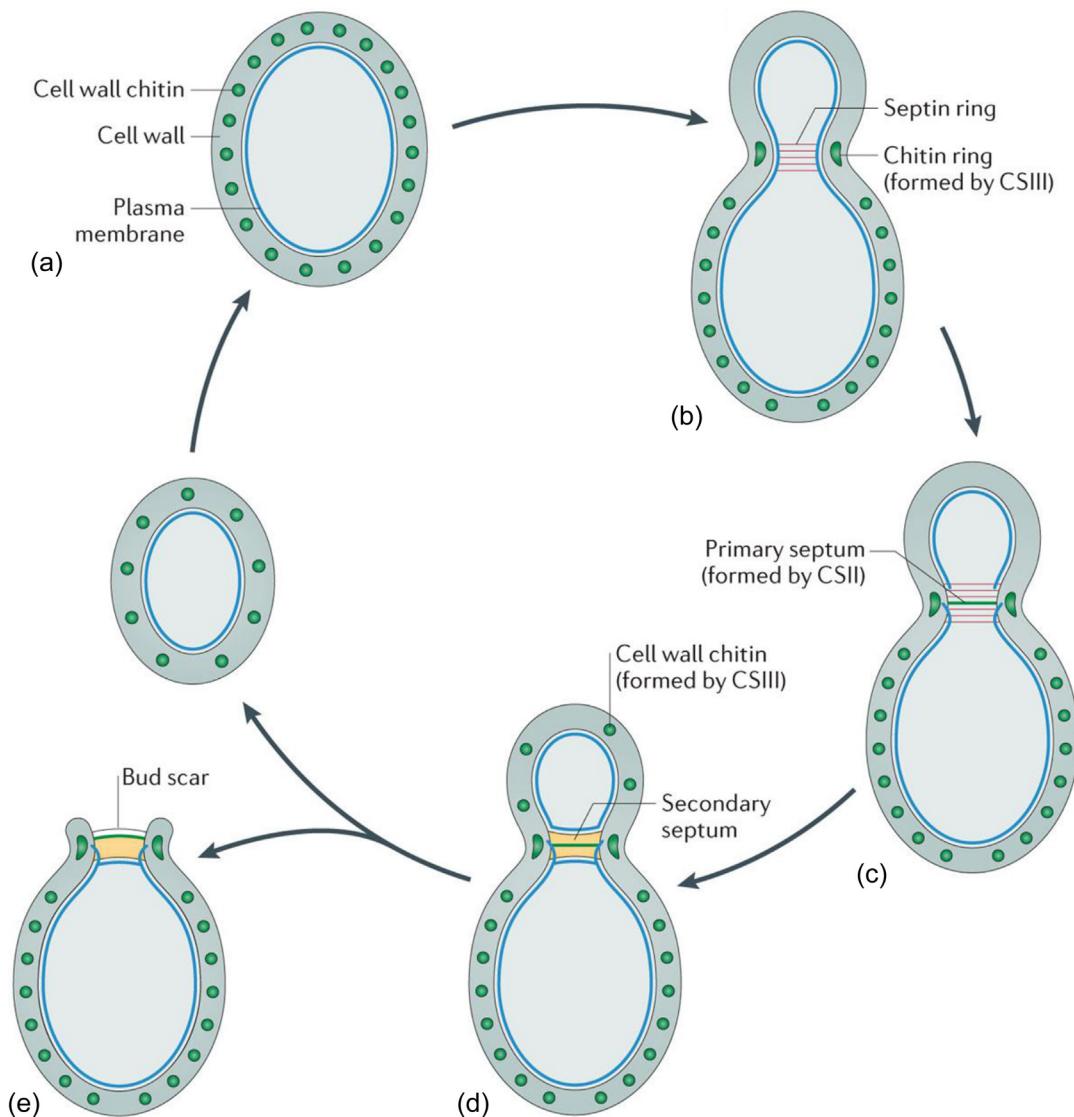


FIGURE 2.12 Cell division in *Saccharomyces cerevisiae*. Source: Cabib, E., Arroyo, J., 2013. How carbohydrates sculpt cells: chemical control of morphogenesis in the yeast cell wall. *Nat. Rev. Microbiol.* 11, 648–665.

the construction of the new cell. Toward the end of G1, and throughout S and S/G2, septins, myosin, actin, and associated molecules are organised at the neck of the bud. The septins form a ring at the neck that seems to function as a template, or scaffold, upon which a second ring of actomyosin is constructed as mitosis takes place. The septin ring is also thought to be important in positioning the mitotic spindle along the mother–daughter axis. Exocytosis provides new membrane for the growing bud and chitin synthesis is localised to the bud neck,

directed by the septin ring. This produces a septum that divides the mother and daughter cell at the completion of mitosis. The contractile actomyosin ring guides membrane formation during septum formation. Unlike the rest of the yeast cell wall, the septum is rich in chitin. Chitin synthases are localised at the neck of the bud in anaphase and create the septum when mitosis is completed. The coordination between the intricate processes of bud formation and the cell cycle is an active area of research. A signalling cascade called the mitotic exit network, or MEN, plays an important role in this biochemical control.

Fission yeast, *Schizosaccharomyces pombe*, produces cylindrical cells that divide by the formation of septa. The division site is determined by the position of nucleus that is, in turn, fixed in the centre of the cell by microtubules. The position of the nucleus is communicated by the export of a protein, Mid1p, that marks the cell cortex. Proteins related to Mid1p are involved in cell division in *Drosophila* and humans, suggesting conservation of the mechanism of division site selection in multicellular organisms. Cell division in fission yeast shows a number of similarities to the process in budding yeast, including the assembly of an actomyosin ring that directs the synthesis of new plasma membrane and cell wall at the division site.

Nuclear Division

The nuclear envelope of animal cells disassembles in prometaphase and is absorbed by the endoplasmic reticulum. The envelope of paired membranes reassembles around each daughter nucleus at the end of anaphase. This type of nuclear division is described as an 'open mitosis'. 'Closed mitosis', in which the intact nuclear envelope encloses the spindle and chromosomes, is characteristic of most fungi. This has been studied in greatest detail in *Saccharomyces cerevisiae*, and a similar process takes place in *Neurospora crassa*. There are significant variations in the mitotic process among other ascomycetes, including the partial breakdown of the nuclear pore complex in *Aspergillus nidulans*. Open mitosis may be widespread among the Basidiomycota. Unlike the fragmentation of the nuclear envelope that occurs in animal cells, the entire envelope is stripped away from the dividing nucleus in the basidiomycete *Ustilago maydis* and recycled in telophase. The structures that organise the microtubules of the mitotic spindle in fungi are called **spindle pole bodies**. These **microtubule organising centres** perform the same function as the centrosomes of animal cells, but they are given a distinct name because they do not contain centrioles. The spindle pole body is attached to the nuclear envelope and is duplicated through a series of discrete steps that proceed throughout interphase. The behaviour of the nuclear envelope during mitosis and the structure of the spindle pole body suggest that the mitotic mechanism in the fungi may have had an independent evolutionary origin from the process in other eukaryotes.

Tip Growth in Filamentous Fungi

Tip growth in hyphae is a continuous process of extension that is not coupled to nuclear division in the same manner that bud formation and mitosis are connected in yeast. The colony, or mycelium, of the ascomycete *Neurospora crassa* can expand radially at a rate of a few millimetres per hour with the repetitive formation of lateral branches. As each hypha extends, its population of nuclei increases through asynchronous mitosis, but the formation of new cells, or hyphal compartments, does not seem tightly coordinated to nuclear division. Mitosis is

described as 'parasynchronous' in *Aspergillus nidulans*, and other ascomycetes, because waves of nuclear division progress along the hyphae so that adjacent nuclei are engaged in mitosis at the same time. As the hyphae elongate, septa form across (at a right angle to) the long axis of the mitotic spindles of dividing nuclei. But because most nuclei divide without directing septum formation, each compartment of a hypha can contain hundreds of nuclei. Hyphal structure is different in other phyla. Mycelia produced by most basidiomycetes have uninucleate (homokaryotic) hyphal compartments before mating, and binucleate (heterokaryotic) compartments following the fusion of sexually compatible colonies. Nuclear division is coupled to cell division via the formation of septa in the basidiomycetes. Zygomycetes form non-septate multinucleate hyphae.

The apex of a growing hypha is a site of concentrated vesicle traffic. Vesicles arriving at the tip create new membrane surface through the process of exocytosis and deliver new cell wall materials. A dense nugget of vesicles in the hyphal tip called the **Spitzenkörper** plays an important role in the growth process (Figure 2.13). It contains macrovesicles, also known as apical vesicles, and microvesicles, plus ribosomes and cytoskeletal components. In the ascomycete *Neurospora crassa*, the microvesicles are packed into the core of the Spitzenkörper, which is surrounded by macrovesicles. The microvesicles are called **chitosomes**, referring to their function in chitin biosynthesis. A protein involved in glucan synthesis has been identified in the same region of the cell as the macrovesicles, suggesting that these larger vesicles are involved in the synthesis of other cell wall polymers. The organisation of these components varies between species, but the structure, biochemistry, and behaviour of the Spitzenkörper offer compelling evidence for its role as an exocytic apparatus (or vesicle supply centre) that controls cell wall synthesis at the hyphal tip. The activity of the Spitzenkörper is regulated by a pair of protein complexes called the **exocyst** that forms a bridge between the vesicles and the cell membrane,

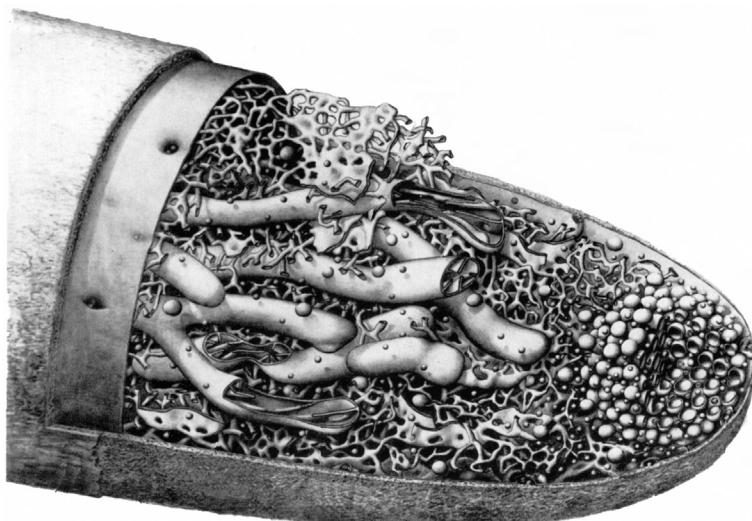


FIGURE 2.13 Diagram showing the inside of a hyphal tip packed with vesicles that form the Spitzenkörper whose position directs the direction of growth. Source: Girbardt, M., 1969. *Die Ultrastruktur der Apikal region von Pilzhyphen*. *Protoplasma* 67, 413–441.

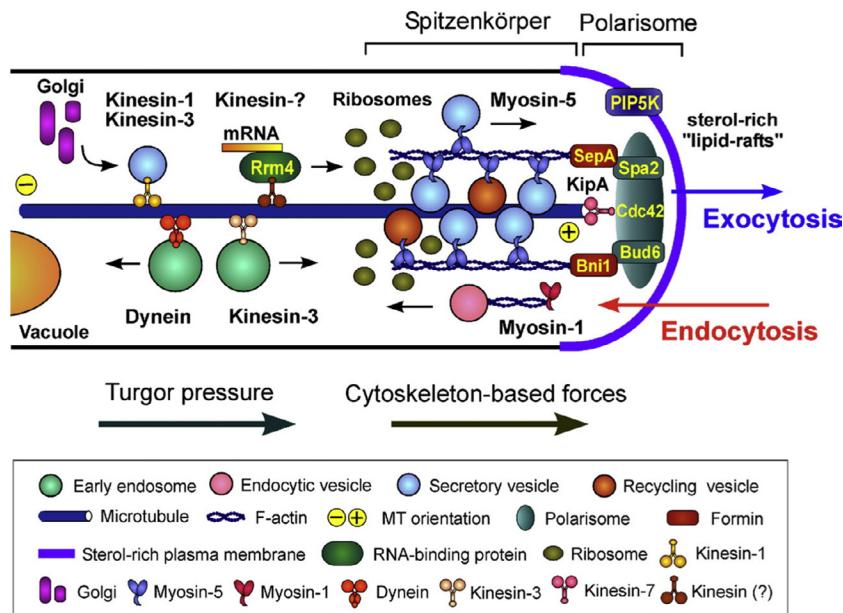


FIGURE 2.14 Model of tip growth showing some of the molecular components in the extending hypha. Source: Steinberg, G., 2007. Hyphal growth: a tale of motors, lipids, and the Spitzenkörper. *Euk. Cell* 6, 351–360.

and the **polarisome** that controls the position of the Spitzenkörper (Figure 2.14). The activity and position of the Spitzenkörper are absolutely critical for hyphal growth, branching, and mycelial development. Shifts in the position of the organelle result in changes in the direction of hyphal extension. If the cluster of vesicles remains in the centre of the tip, the hypha will grow straight; if it shifts to one side, the hypha will reorient its growth axis in this direction. Small Spitzenkörpers develop at sites along hyphae where new branches emerge and occupy the tips of new growth axes. Computer models have been effective in ‘growing’ virtual hyphae, generating hyphal tips of different shapes, and forming branches by moving and duplicating a vesicle supply centre whose behaviour mimics the Spitzenkörper.

The Spitzenkörper functions in the export of materials packaged in vesicles, but the process of membrane (vesicle) import, or endocytosis, may be equally important in fungal growth. Much of this is hypothetical. Endocytosis is thought to occur behind the growing hyphal tip where patches of actin are concentrated in a subapical collar. As the tip extends, the enzymes that control cell wall synthesis, and molecular complexes that maintain the position of the Spitzenkörper, are swept back. Endocytosis in the subapical region of the hypha would serve to recycle these cell components and enable their repositioning at the tip. According to this **apical recycling model**, endocytosis assumes a pivotal role in controlling cell shape. Put simply, the balance between exocytosis and endocytosis will determine the increase in surface area of the hyphal tip and this may control a range of developmental processes including the formation of spores at the tips of aerial hyphae (Chapter 3). Researchers have developed a detailed picture of the molecular mechanisms of endocytosis in yeast, and there is an intensive effort to understand these processes in filamentous fungi.

Tip growth depends upon water influx to support the increase in cell volume, and exocytosis to provide the new plasma membrane and cell wall materials of the expanding surface. Osmosis causes water influx when the cytoplasm contains a higher concentration of dissolved ions and molecules than the surrounding fluid. Net water influx occurs until the chemical potential of water (water potential) of the cell and its surroundings equilibrate (Chapter 5). This is achieved by an increase in hydrostatic pressure, or turgor, in the cytoplasm as the plasma membrane is pressed against the inner surface of the cell wall. In most instances, growing hyphae are pressurised to a few atmospheres of hydrostatic pressure. (The pascal, symbol Pa, is the SI unit for pressure; 1 atmosphere is equal to 100 kPa or 0.1 MPa.)

Turgor pressure is an important feature of growing hyphae, in the sense that the cell is always at risk of rupturing if its surface is damaged (see Woronin bodies below), and plasma membrane and cell wall synthesis must be regulated carefully to allow controlled expansion. Our understanding of the mechanisms that permit cell wall polymers to slip past one another as new materials are incorporated into the wall is very patchy. The study of these biomechanical processes is a potentially fruitful research topic for new investigators. We have learned a great deal about the different components in the fungal cell wall, but know very little about their interactions during hyphal growth. Turgor pressure acts to smooth the cell surface and, presumably, pushes the cell wall polymers apart as the tip expands. But turgor does not operate as any kind of special 'driving force', any more than water influx drives growth. The walled cells of plants are also pressurised, but the cells of animals and many protists maintain ionic homeostasis with their surroundings or export water using contractile vacuoles and do not generate any significant turgor pressure. In other words, if many eukaryotes can dispense with turgor pressure, why is this intracellular pressure essential for hyphae? The answer may lie in the special feeding mechanism of filamentous fungi.

Hyphae are microscopic mining devices that envelop and permeate dead plant tissues and other sources of nutrients. Many of the foods used by fungi have low concentrations of soluble sugars and other readily digested molecules and are rich in polymers, including complex polysaccharides, proteins, and lipids. Hyphae are superbly adapted for solubilizing these materials. Rather than digesting solids from the outside and working inward, colonies of branched hyphae penetrate their food and digestion is achieved over a large area of cell surface. This is called **invasive growth**. The mechanism involves the interlinked processes of enzymatic digestion and pressure-driven penetration. Hyphae secrete enzymes from their growing tips. These enzymes hydrolyse polymers, releasing low molecular weight molecules that are absorbed by the cell (Chapter 5). This digestive mechanism renders the food more fluid, or, at least, less of a physical obstacle to growth. By loosening the cell wall polymers at the hyphal tip, the cell allows a proportion of its internal turgor pressure to press upon the surrounding material. These invasive pressures have been measured using miniature strain gauges and range from a few tenths of one atmosphere to two atmospheres (up to 200 kPa). The combination of enzymatic digestion and pressure-driven penetration allows fungi to penetrate an astonishing range of solid substances.

Enzymes secreted from hyphae may diffuse into the environment and catalyse the digestion of macromolecules some distance from the cell surface. Some enzymes seem to be less mobile, remaining in the cell wall or close to its surface. If these act as nutritional enzymes, molecules will be solubilized much closer to the cell, with the invasive hypha mining food from the immediate vicinity of its cell wall. Fungal nutrition will be considered in greater detail in Chapter 5.

Aerial Hyphae and Conidiophores

Fungal colonies cultured on agar medium often cover themselves with a forest of aerial hyphae. This tangle of filaments can fill the airspace above the agar and squash itself by extending against the lid. Extension of these aerial hyphae is driven by the same mechanisms described in the previous section, but the cells encounter a different set of environmental challenges. Hyphae-penetrating agar must exert substantial force to push through the gel matrix and do so by loosening the cross-links between polymers in the apical cell wall, thereby applying a proportion of their internal turgor pressure against their surroundings. The requirement for force generation is greatly reduced for a cell extending into the air, but internal pressure is necessary to support the vertical orientation of the cell. This is obvious, based on the observed collapse of aerial hyphae when they are exposed to dry air. Before hyphae can reach the air above the colony, they must overcome the surface tension of the air–water interface. This problem seems to be addressed by a combination of the continued exertion of turgor-derived force and the secretion of hydrophobic proteins called **hydrophobins** that reduce the surface tension at the interface.

Hydrophobins are small, cysteine-rich, water-repellant proteins that are secreted on the surface of aerial hyphae and fruit bodies. They have been studied in the mushroom-forming basidiomycete, *Schizophyllum commune*. Targeted deletion of one of the hydrophobin genes in this fungus, SC3, results in the resulting mutant's inability to produce aerial hyphae. The protein, SC3, operates as a surfactant, self-assembling as a monolayer at an air–water interface and reducing the surface tension of the water. The reduction in surface tension may allow hyphae of the fungus to escape a fluid environment and grow into the air. Secretion of the protein continues with the extension of the aerial hyphae, coating them with a hydrophobic layer. Hydrophobin secretion is also important in facilitating the attachment of hyphae to hydrophobic surfaces. In many species, aerial hyphae differentiate into conidiophores (spore stalks) that produce asexual spores, or conidia. We will return to the process of conidium formation, or conidiogenesis, in the next chapter. The secretion of hydrophobins onto the surface of developing mushrooms (basidiomata) is important in cementing hyphae together, waterproofing the surface of the reproductive organ, and supporting gas exchange by preventing tissues from becoming saturated with water.

Septa, Woronin Bodies, and the Septal Pore Complex

Septal structure is quite different in the Ascomycota and Basidiomycota. In filamentous ascomycetes, septa are perforated by a single, centrally located pore. Open pores allow the transmission of organelles, including nuclei, between compartments. The movement of organelles is clearly visible in active hyphae viewed with a conventional light microscope. Organelles move toward the growing tip, and in the opposite direction toward older compartments. Some organelles move along relatively straight lines: these structures are carried along actin microfilaments, or microtubules, powered by motor proteins. More obvious bulk flow of cytoplasm is also a feature of active hyphae, with pulses of tip-directed flow, interrupted by retrograde motion. These movements are probably driven by tiny differences in turgor pressure along the hypha. The septate hyphae of the subphylum Pezizomycotina (Ascomycota) contain organelles called Woronin bodies

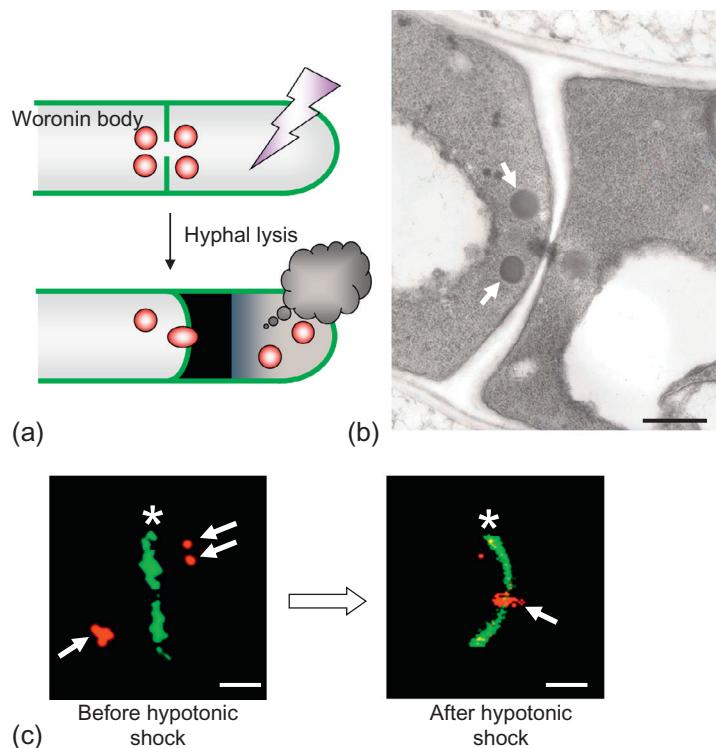


FIGURE 2.15 Morphology and function of the Woronin body. (a) Schematic of Woronin body function. (b) Transmission electron micrograph showing Woronin bodies (arrows) in *Aspergillus oryzae*. Scale bar: 500 nm. (c) Confocal images of Woronin bodies (red arrows) and septa (green asterisks) stained with fluorescent labels before (left) and after (right) hypotonic tip bursting. Scale bar = 2 µm. Source: Maruyama, J., Kitamoto, K., 2013. Expanding functional repertoires of fungal peroxisomes: contribution to growth and survival processes. *Front. Physiol.* 4, 177.

that protect the mycelium from catastrophic injury following damage to one or more cell compartments (Figure 2.15). Rupture of the cell wall of a hypha leads to loss of the pressurised cytoplasm and leakage would continue unabated in the absence of some type of sealing mechanism. Woronin bodies isolate the damaged portion of a colony by plugging septal pores on either side of a wound, allowing the rest of the colony to continue growth. Woronin bodies range in size from 100 nm to more than 1 µm and can be seen with a light microscope in some species. The organelle is a type of **peroxisome**. The membrane-bound structure contains a dense core that develops as a self-assembling hexagonal crystal of a single protein called HEX-1. *hex-1* mutants are prone to 'bleeding', and show many developmental defects.

Basidiomycetes produce **dolipore septa** (Figure 2.16). The central canal (pore) of this structure is surrounded by a barrel-shaped swelling of the septum cell wall. Nuclei cannot migrate through unmodified dolipore septa and their distribution within the hyphae that develop after the fusion of sexually compatible colonies involves the formation of **clamp connections**

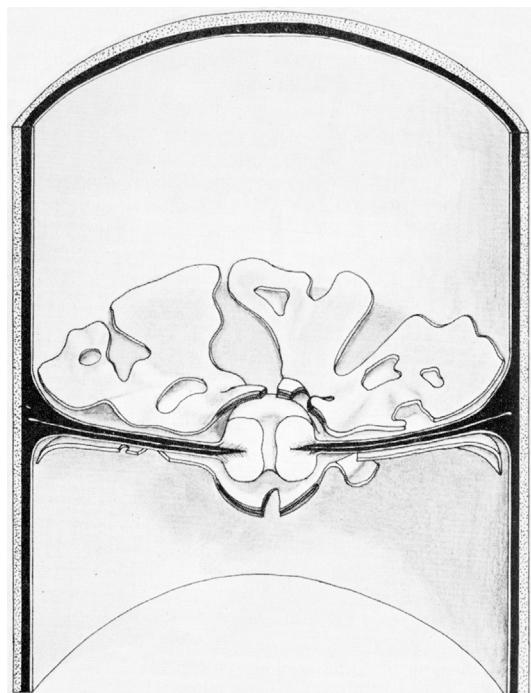


FIGURE 2.16 Dolipore septum of *Rhizoctonia solani*. Source: Bracker, C.E., Butler, E.E., 1963. The ultrastructure and development of septa in hyphae of *Rhizoctonia solani*. *Mycologia* 55, 35–58.

(Chapter 1). Both open ends of the dolipore swelling are surrounded by membranes of the **septal pore cap** derived from the endoplasmic reticulum. When the hypha is injured, these membranes collapse and seal the open ends of the septal swelling, performing the same function as the Woronin bodies.

The evolution of complex multicellular structures, from elaborately branched and interconnected colonies to mushrooms and other kinds of fruit bodies, is associated with the development of mechanisms for protecting hyphae from uncontrolled leakage of cytoplasm. This makes sense from an economic point of view. A mushroom constructed from millions, or hundreds of millions of hyphae represents a tremendous investment on the part of the fungal colony. The probability of injury from abrasion or from invertebrates during fruit body expansion seems high. Without a mechanism for isolating damaged hyphal compartments, the operation of the entire organ would be compromised. This process also allows differentiation of the mycelium into compartments that assume specialised functions including survival and reproduction. A simple illustration of this is seen in zygomycetes where the formation of a septum at the base of a sporangium precedes the development of spores; the rest of the colony is non-septate. It seems possible that the sealing mechanisms in the Ascomycota and the Basidiomycota evolved independently, allowing species in both phyla to elaborate complex kinds of feeding colonies and multicellular organs.

THE MYCELIUM

Hyphal extension in non-septate fungi, including *Mucor* and its relatives (the zygomycetes), produces tubes that can extend for many millimetres. Three-dimensional colonies of these fungi develop by lateral branching, creating continuous networks of cytoplasm. These colonies are multinucleate, but not multicellular. Colonies of septate fungi also proliferate through tip growth and branching. Development is complicated in basidiomycetes and ascomycetes by the formation of septa, and clamp connections in the basidiomycetes (Chapter 1), and the number of nuclei in each hyphal compartment varies between different taxonomic groups.

Germination of single spores by the emergence of a young hypha, or germ tube, followed by continuous elongation and repeated branching produces circular colonies, or mycelia, whose superficial form may be likened to the pattern of spokes radiating from the hub of a bicycle wheel ([Figure 2.17](#)). There are, however, developmental features of the mycelium that limit the usefulness of this analogy. As the hyphae extend they diverge from one another and primary branches form secondary branches, and so on, so that the entire area becomes occupied by the fungus. Any gaps between hyphae become occupied by new branches. This infilling makes sense when we consider that every hyphal apex is a feeding device and that any substantial gaps in the colony may contain unabsorbed nutrients. Hyphal activity on exposed surfaces is only part of the picture. Invasive growth drives hyphae into its food producing a mature colony with a three-dimensional form. In basidiomycetes and ascomycetes, hyphal branches fuse with one another to create highly interconnected networks or webs. Connections between hyphae, or **anastomoses**, provide pathways for the bulk flow of cytoplasm and regulated movement of organelles over the cytoskeleton ([Figure 2.18](#)). Anastomoses can form between

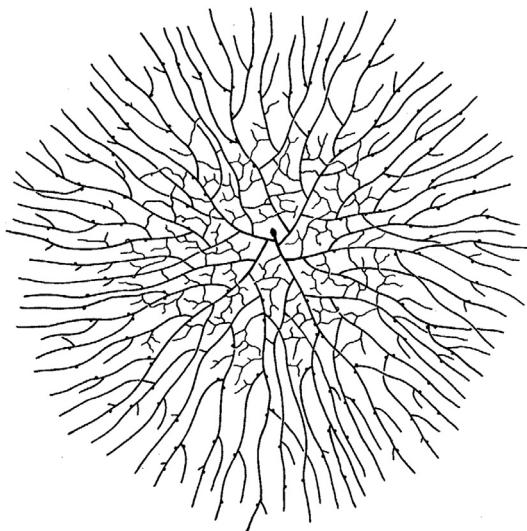


FIGURE 2.17 Young colony or mycelium of a fungus that has grown outwards from a single spore at the center of the drawing. Source: Buller, A.H.R., 1931. *Researches on Fungi*, vol. 4. Longmans, Green, and Co., London.

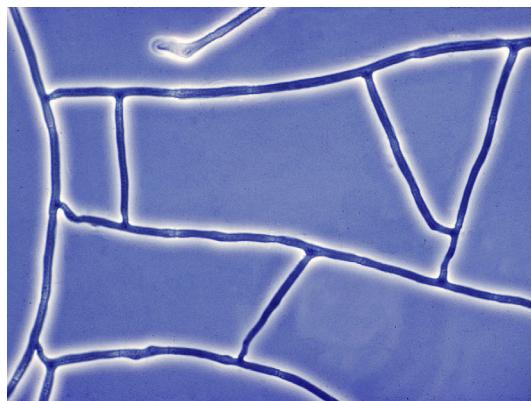


FIGURE 2.18 Anastomoses between hyphae of *Sordaria*. Source: George Barron, University of Guelph.

germlings directly after spore germination in some ascomycetes, as well as later in the development of the mycelium. Viewed under the light microscope, the growing colony is revealed as a complex network of tubes supporting the continuous motion of fluid. The interconnected architecture of the colony is important because it allows resources to be shuttled from one location to another tangentially as well as radially. The significance of this resource distribution is apparent when we consider the behaviour of basidiomycetes that digest wood.

Living trees and decaying wood in forests are connected by populations of mycelia formed by mycorrhizal fungi and saprotrophs. Mycelia can span large territories in these ecosystems, disbursing nutrients from portions of the colony embedded in a rotting log, for example, to other parts of the colony spanning out in search of fresh nutrient sources. The architecture of mycelia varies according to species and to environmental conditions. Young colonies can be very dense, forming a thick weft of filaments close to the point of origin. Others remain more diffuse, extending rapidly rather than concentrating in a single area. When one part of the colony encounters a promising resource, the form of the entire colony can change. It is worth underscoring that the exploratory part of the mycelium is pursuing genetically determined algorithms of colonial behaviour rather than expressing any intent. This probably seems obvious, but it can be easy to convey impressions of fungal intelligence when we think sloppily about the complex signalling that controls development. The redistribution of resources between distant parts of the mycelium has been examined in beautiful experiments on cultured wood-decay basidiomycetes. The formation of multicellular ‘organs’, called cords, is a very important part of this process, and we will examine their structure in the next section.

Hyphal size and shape show considerable variation among the fungi. Hyphae range in diameter from a few micrometres in many ascomycetes and basidiomycetes to much larger cells, with a diameter of more than 20 µm in zygomycete fungi. Tip shapes range from perfect hemispheres to more pointed forms and some variations are seen within individual cultures. Differentiation of hyphae occurs within mycelia producing hyphal swellings and cells with thickened walls. The function of these features is often obscure. More complex morphological changes are common, too, and these can be matched to particular functions. **Appressoria** are inflated cells produced by plant pathogens on the leaf surfaces of their hosts (Figure 2.19). These are initiated as hooks and swellings on the host and expand into domed cells that adhere tightly to the plant cuticle.

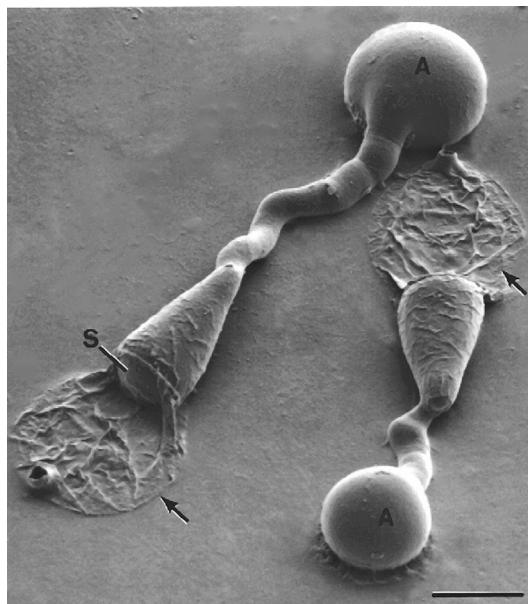


FIGURE 2.19 Young appressoria of the rice blast fungus, *Magnaporthe grisea*, connected by germ tubes to their conidia. The conidia deflate (arrows) as their contents are transferred into the appressoria (A). A septum (S) within one of the conidia is evident between the collapsed portion of the spore and the adjacent cell that has not emptied its cytoplasm. Source: Money, N.P., Howard, R.J., 1996. Confirmation of a link between fungal pigmentation, turgor pressure, and pathogenicity using a new method of turgor measurement. *Fungal Genet. Biol.* 20, 217–227.

Slender hyphae, or infection pegs, grow from the base of the appressoria and penetrate the leaf. Appressoria of some species form over stomata and penetration occurs when the stomata are open. In other diseases, penetration occurs directly through the intact cuticle and underlying cell wall and is a mechanical process that seems to be dependent upon the exertion of force derived from cytoplasmic turgor pressure (Chapter 8). Following penetration of the host, hyphae of biotrophic pathogens produce **haustoria** that absorb nutrients from the plant without destroying the infected cell. Arbuscular mycorrhizal fungi classified as Glomeromycota produce analogous structures called **arbuscules** that form the interface between the fungus and its host. Arbuscules are highly branched cells that control nutrient transfer from plant to fungus and vice versa. Nematophagous or nematode-trapping fungi produce a variety of hyphal structures ranging from adhesive knobs to constricting rings to snare their prey. Distinctive hyphal shapes are often seen in cultures, but the functions of these modifications are not understood.

MULTICELLULAR ORGANS

Cords and Rhizomorphs

The use of the term ‘organ’ to describe the multicellular structures of fungi is subjective. Animal organs are differentiated structures that perform specific functions. A mycelium may be viewed as an organ by this definition, but the degree of differentiation among its hyphae

is very limited. A higher degree of differentiation is achieved through the formation of cords by mycelia of wood-decay and ectomycorrhizal fungi and it seems justifiable to describe the more complex of these structures as organs. We will return to this issue of terminology when we consider the structure and function of fruit bodies later in the chapter.

Strands, cords, and rhizomorphs vary in complexity from bundles of hyphae, whose cell walls adhere to one another to produce slender cylinders of unpigmented cells on the surface of a culture dish, to fat pipes with a diameter of a few centimetres that can extend for hundreds of metres. The larger pipes are formed from hundreds of thousands of hyphae, develop a complex internal anatomy, and are sometimes tipped with a rounded, mucilaginous cap. Variations in the internal structure of these organs make it difficult to discriminate between strands, cords, and rhizomorphs. The term rhizomorph may be useful to designate the larger of these invasive organs that have an identifiable tip that pushes through the soil. Cord is the preferred term for other linear organs without an organised tip.

Rhizomorphs facilitate the spread of *Armillaria* species between host plants and rotting wood, and support the coverage of vast territories by colonies of these mushroom-forming fungi. The surface of the rhizomorph is covered with a peripheral layer of thin hyphae that encloses a cortex of radially oriented hyphae (Figure 2.20). Hyphae of various sizes run along the length of the organ beneath the cortex. These longitudinally oriented cells constitute the medulla. The largest of these medullary hyphae, toward the centre of the organ, are dead and devoid of cytoplasm. A gas-filled lumen occupies the centre of the rhizomorph. Within the medulla, it seems likely that the smallest hyphae (just beneath the cortex) are the youngest and most active cells. As the rhizomorph elongates, these cells are pushed toward the centre of the organ by new hyphae growing directly beneath the cortex. The displaced medullary cells enlarge, eventually becoming inactive and line the empty lumen. Physiological experiments suggest that some of the medulla cells act as conduits for fluid transport and the term 'vessel hypha' has been applied to the largest hyphae that have this presumed function. Beautiful experiments have been performed on fluid translocation within cords and rhizomorphs using radioactive tracers, but there are many unresolved questions about the physiology of rhizomorphs. The gas-filled lumen aids oxygenation of rhizomorphs that bury themselves within soil and rotting wood.

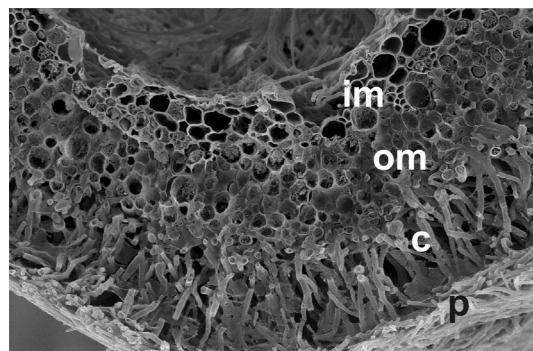


FIGURE 2.20 Anatomy of the rhizomorph of *Armillaria gallica* revealed by fracturing the frozen specimen and viewing in a scanning electron microscope. (p) peripheral layer of hyphae, (c) cortex, (om) outer medulla, and (im) inner medulla. The inner medulla surrounds an open central cavity. Source: Yafetto, L., Davis, D.J., Money, N.P., 2009. Biomechanics of invasive growth by *Armillaria* rhizomorphs. *Fungal Genet. Biol.* 46, 688–694.

Experiments on rhizomorphs of *Armillaria gallica* reveal that the organs extend at faster rates than individual hyphae growing in their normal unbundled form. This accelerated extension is driven by a combination of apical growth of cells at the tip of the rhizomorph and intercalary growth of hyphae behind the apex. Like hyphae growing individually, the cells of the rhizomorph exert a pressure of up to one atmosphere (100 kPa), which provides the organ with mechanical force to overcome physical obstacles in its path.

Cords vary from loose bundles of hyphae to more complex structures with interior vessels enclosed in a thick outer rind. Rather than forming an organised tip like a rhizomorph, hyphae aggregate into cylindrical cords behind tip-growing hyphae that spread into a fan. Cords often develop between separate woody resources colonised by a single mycelium.

Cords and rhizomorphs allow fungi to mobilise food resources and water from one location and transport them over long distances to support other parts of an extended mycelium. They greatly extend the area that may be explored by a single mycelium. These organs allow dry-rot fungi that specialise in the destruction of timber in buildings to bridge nutrient deserts of brick and concrete and colonise dry wood by translocating nutrients and water from wetter locations. Dry-rot fungi include *Serpula lacrymans*, which causes tremendous damage to buildings in Europe, and an equally destructive basidiomycete, *Meruliporia incrassata*, which destroys homes in North America.

Sclerotia

Sclerotia are hardened masses of hyphae that serve as survival structures for ascomycetes and basidiomycetes (Figure 2.21). Sclerotia can be rounded, flattened, or elongated. Their sizes range from the 0.1-mm-diameter microsclerotia of the plant pathogen *Macrophomina phaseolina*, to the 30 cm sclerotia of the edible Australian fungus *Laccocephalum mylittae* that weigh several kilograms. Sclerotial development sometimes occurs when nutrients are running out, but many are formed in active cultures showing that there are other stimuli for the growth of these structures. Their development involves the repeated branching of hyphae and formation of closely spaced septa. Differentiation of hyphae within the sclerotium produces a central medulla of thin-walled hyphae rich in lipid and glycogen reserves. These cells are surrounded by a cortex of hyphae with thicker walls and an outer layer, or rind, of

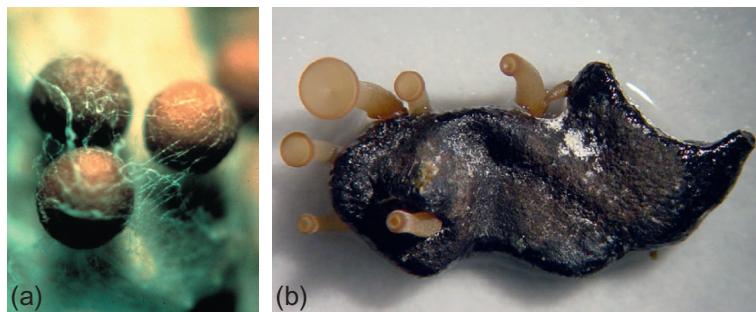


FIGURE 2.21 Sclerotia of *Sclerotinia sclerotiorum*. (a) Dormant and (b) germinating to produce apothecia. Source: www.sclerotia.org

cells that are highly melanized in some species. Some fungi form a feeding mycelium when their sclerotia germinate; others produce fruit bodies. Elongated sclerotia known as ergots are produced by species of *Claviceps*, including *Claviceps purpurea* that causes ergot of rye. Ergots develop in autumn, survive the winter, and germinate in the spring to produce a stalked fruit body from which infectious ascospores are discharged into the air. Ergots of *Claviceps purpurea* contain toxic alkaloids, including ergotamine that causes vasoconstriction. Ingestion of this toxin in the form of bread baked from contaminated flour has caused outbreaks of gangrene, loss of limbs, and resulted in many deaths. *Sclerotium sclerotiorum*, that causes white mould of flowers and vegetables, produces lumpy sclerotia as over-wintering structures. Germination of these sclerotia produces stalked fruit bodies tipped with cup-shaped apothecia. Sclerotium production by plant pathogens has been studied in the greatest detail, but these structures are also formed by ectomycorrhizal and ericoid mycorrhizal fungi, saprotrophs, and fungi that have adopted a variety of other lifestyles. The distribution of these species in diverse taxonomic groups shows that sclerotial development is an example of evolutionary convergence.

Pseudosclerotia and Pseudosclerotial Plates

Pseudosclerotial development promotes the survival of a fungal colony by incorporating the material on which it is growing. Species of *Ophiocordyceps* mummify the bodies of the invertebrates that they have killed within pseudosclerotia. Stalked fruit bodies bearing perithecia develop from the pseudosclerotia of these ascomycetes. Pathogenic species of *Monilia* (*Sclerotinia*) produce similar structures around the infected fruits of their host plants. The polypore *Laccocephalum basilapiloides* is the Australian 'stone-making fungus', whose 8 cm-diameter pseudosclerotium binds sand grains, root fragments and other debris, and supports a single fruit body.

Pseudosclerotial plates are thin sheets of mycelium that incorporate wood and other organic matter. The hyphae of these structures are highly branched and pigmented with melanin, forming a barrier that resists penetration by water and the hyphae of other fungi. Pseudosclerotial plates are often visible as black zone lines in rotting wood. *Armillaria* species confine 'decay columns' of wet wood rot within pseudosclerotial plates. Conversely, the ascomycete *Xylaria hypoxylon* maintains the wood beneath its pseudosclerotial plates in a dry condition.

Fruit Bodies: Ascomata and Basidiomata

The formation of multicellular fruit bodies is the most complex and least understood developmental process in the fungi. Fungi are regarded traditionally as microorganisms because many species (e.g. zoosporic fungi, yeasts) are visible only using a microscope, and others exist in microscopic form for much of their life cycles. This distinction can seem irrational, however, when we consider macroscopic fruit bodies (mushrooms) including the ascomata of morels (Ascomycota), or basidiomata of the Agaricomycotina (Basidiomycota). Indeed, the fruit bodies of many of the basidiomycetes can be difficult to ignore. The caps of *Termitomyces tianicus* mushrooms that grow from abandoned termite nests in West Africa can expand to 1 m in diameter. Even larger fruit bodies are produced by wood-decay fungi. A metre-long crust produced by a white rot fungus called *Phellinus ellipsoideus* on the underside of an oak

log was reported in 2010 on Hainan Island in China. It weighed 500 kg and shed an estimated one trillion spores per day. In this respect, fungi are unlike other microorganisms, including bacteria and many protists, which are invisible to the unaided eye. Nevertheless, the fungi are considered part of the purview of microbiologists.

Limited differentiation of tissues is an important feature of fruit body development. Many kinds of cells are visible when thin sections of a young plant stem are studied with a microscope: guard cells form stomata in the epidermis, cortical cells lie beneath the epidermis, and phloem, cambium, and xylem cells are organised in vascular bundles. The same exercise performed on the stem (or stipe) of a mushroom reveals a considerably simpler anatomy. Mushroom stems and caps are built from thin-walled hyphae that differ only in diameter, the spacing of septa, and frequency of branching. This anatomical plainness is a little disconcerting. At least 16,000 different species of mushroom-forming basidiomycete have been described and each has a distinctive basidiome. Besides the conventional umbrella-shaped mushroom, these fungi form brackets, coral-shaped fruit bodies, little spindles, discs, ruffled crusts, puffballs, and phallic mushrooms tipped with stinking slime. All of these beautiful organs are formed by filamentous hyphae that grow from the feeding mycelium. Different types of hyphae are recognised in some fruit bodies, including thin-walled generative hyphae, thick-walled skeletal hyphae, and elaborately branched binding hyphae. This limited differentiation affects the texture of the fruit body, with a high proportion of skeletal and binding hyphae, for example, producing the harder and less flexible basidiomata of certain bracket mushrooms.

Sections of fruit bodies viewed with the transmission electron microscope show tightly packed cells that resemble the parenchyma of plants ([Figure 2.22](#)). This structure is also apparent when the surfaces of a perithecium or other types of ascocarps are viewed with a scanning electron microscope ([Figure 2.1e](#)). Investigators looking at the anatomy of rhizomorphs in the

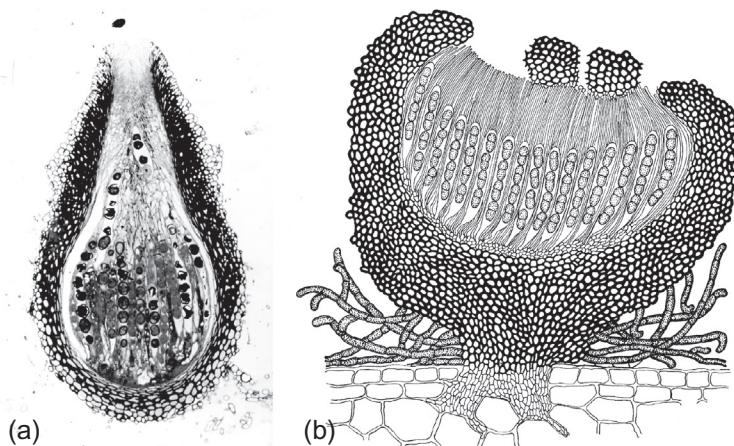


FIGURE 2.22 Pseudoparenchyma tissue formed by branched and interwoven hyphae in the walls of ascocarps. (a) Transmission electron micrograph of perithecium of *Sordaria humana*. (b) Ascoma (pseudothecium) of *Pseudoparodia pseudopeziza*. Source: (a) Read, N.D., Beckett, A., 1985. *The anatomy of the mature perithecium in Sordaria humana and its significance for fungal multicellular development*. Can. J. Bot. 63, 281–296 and (b) Müller E., von Arx, J.A., 1962. *Beit. Kryptogamen. Schweiz* 11 (2), 922 p.

1970s believed that they had identified a group of tiny isodiametric cells in the tip of the organ that might function as a meristem. These observations produced a confusing picture of fungal development, until careful microscopic analysis demonstrated that fruit bodies are formed by the intermingling and branching of hyphae and frequent formation of septa to produce short hyphal compartments. The resulting tissue is called **pseudoparenchyma**, for its superficial resemblance to the parenchyma tissue of plants. Recognition of the hyphal nature of every fungal structure is critical for understanding fungal development because it highlights the importance of 'invisible' differentiation at the molecular level in sculpting mushrooms and other fungal organs. The complex form of ascocarps and basidiomata derives from intricately choreographed interactions between hyphae that reflect their positions within the fruit body.

Environmental and genetic factors that control fruit body development are treated later in this book. The focus here is on the developmental processes that take place once fruiting is initiated. The tissue that forms ascocarps is composed of hyphae that contain haploid nuclei. Fusion of sexually compatible hyphae occurs within the fruit body and produces the cells from which the asci develop. This delayed mating distinguishes the ascomycetes from the basidiomycetes, as described in Chapter 1. Basidiomata are produced by heterokaryotic hyphae that contain nuclei of two compatible mating types. Information on the genetic control of the development of ascocarps is limited. Genetic studies have concentrated on a handful of 'model' ascomycetes including *Aspergillus nidulans*, *Neurospora crassa*, and *Podospora anserina*. Several genes involved in primary metabolism affect sexual development. Sexual development is disrupted when these genes are mutated, but vegetative growth of hyphae is unaltered. This indicates, as one would anticipate, that fruit body development involves distinctive molecular processes that are not activated as long as the fungal colony is feeding.

The transition between the two phases in the life cycle is driven by a number of environmental factors, but nutrient availability is the primary stimulus. The production of fruit bodies represents a tremendous investment in resources for the colony and demands a high level of metabolic activity. This is the reason that mutants defective in mitochondrial activity can be sterile (unable to form fruit bodies). Increased respiration during sexual differentiation produces more reactive oxygen species (ROS) and oxidative damage to DNA and proteins is deleterious for developing ascocarps. Not surprisingly, genes encoding protective superoxide dismutases, oxidases, and peroxidases that control the production and degradation of ROS are also crucial for fruit body formation. Signal transduction pathways (cascades) involved in fruiting have also been examined and a catalogue of G proteins, G protein receptors, cAMP-dependent protein kinases, GTP-binding proteins, and other signalling molecules and transcription factors have been characterised in the model ascomycetes. Once the sexual cycle has been initiated, genes that control the positioning of nuclei and the cell cycle are essential for fruit body differentiation, and tubulin and other components of the cytoskeleton are implicated in these mechanisms. Genes involved in the synthesis of cell wall components are also critical, but researchers are a long way from understanding ascocarp development at the level of molecular genetics. This is another of those topics in fungal biology with the potential for huge advances by future researchers.

Research on basidiome development in basidiomycetes is similarly limited. Formation of a mushroom requires the transfer of cytoplasm from a large volume of supporting mycelium. Experiments on this process on Petri dish cultures of *Coprinopsis lagopus* indicate that 50% of the biomass in the mycelium may be shuttled into developing basidiomata in response to the

appropriate environmental cues. Fruiting begins (or becomes visible) with the formation of aggregates of branched hyphae called knots. These knots expand to form the embryonic fruit body, or **primordium**. Differentiation of cells occurs in the centre of the primordium demarcating regions that will become the stipe, cap, and gills of the mushroom. Because mushroom development has evolved multiple times, it is not surprising that it occurs in different ways. In **gymnocarpic** development, the cap enlarges from the top of the elongating stipe and the hymenium is exposed for most of the expansion process. Gymnocarpic development is characteristic of *Boletus*, *Clitocybe*, *Lactarius*, and *Russula*. **Angiocarpic** development refers to protection of the immature hymenium. In some mushrooms, the hymenium differentiates within an internal cavity in the primordium and is exposed when the fruit body expands. In others, tissues derived from the surface of the primordium surround the hymenium. A mantle of hyphae, called the **universal veil**, wraps around the entire primordium of the paddy straw mushroom, *Volvariella volvacea*, and species of *Amanita* (Figure 2.23). This is torn apart when the mushroom expands and remains as a basal cup, or **volva**, and as scales on the surface of the cap. Another sector of tissue within some primordia is stretched into a sheet that covers the underside of the gills. This **partial veil** is pulled away from the outer rim of the expanding cap and remains as a ring, or **annulus**, around the stipe in species of *Amanita* and as a thin, cobweb-like drape called the **cortina** in *Cortinarius*. Volva and ring structures are important features for identifying many mushrooms. The **pseudorhiza** is another diagnostic structure associated with some basidiomycetes. This is a root-like extension of the stipe that connects the fruit body to its buried source of nutrients. *Xerula radicata* and *Collybia fusipes* that decompose woody roots are examples of fungi that form pseudorhizas. Colonies of *Termitomyces* are cultivated by termites and produce long pseudorhizas to raise their fruit bodies above the termite mounds.

The mechanism of expansion differs among the basidiomycetes. In some species, expansion is due to the inflation of preexisting hyphae with very limited formation of new hyphal branches. In other cases, stipe elongation involves a new programme of branching and separation. Some mushrooms expand through a combination of inflation and the development of new hyphal compartments, with the balance between these processes differing according to

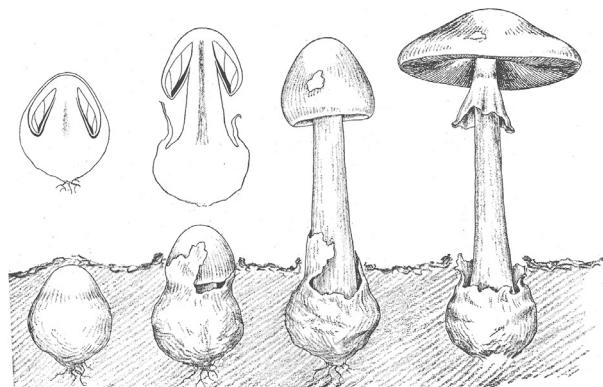


FIGURE 2.23 Mushroom expansion in *Amanita phalloides*, the death cap. Source: Longyear, B.O., 1915. *Some Colorado Mushrooms*. Agricultural Experiment Station of the Agricultural College of Colorado, Fort Collins, CO.

location within the fruit body. Intercalary extension of hyphae is another important process in mushroom expansion with evidence of the synthesis of new cell wall components along the length of the hyphae. The remarkable feature of this phase of development is its speed: mushrooms can show a 1000-fold increase in volume in a few hours! The mechanism is hydraulic, meaning that the inflation is driven by the uptake of water from the environment. This water absorption allows the expanding tissue to exert a hydrostatic pressure of one atmosphere or more against any surrounding obstacles, which allows mushrooms to emerge from rotting wood, hard-packed soil and even to burst through asphalt paving.

The sectors of tissue within the primordium that prefigure the different regions of the mature basidiome differ in the density of the hyphae (how tightly they are packed together), the hyphal diameter, and the frequency of septation. Surprisingly, small pieces of tissue from anywhere within the primordium will generate a feeding colony if they are transplanted onto nutrient agar. This simple experiment illustrates the totipotency of fruit body tissues. Irrespective of their position within the primordium, hyphae are not committed, irreversibly, to a particular developmental fate. This observation marks a sharp contrast to the cells in animal embryos in which differentiation of the cells into various tissues is an irreversible process. Animal stem cells are the important exception to this feature of animal development. Part of the reason for the flexibility in the developmental fate of cells in the basidiome is that most of them perform a purely mechanical function by supporting the spore-producing tissues. Totipotency is lost in the hyphal compartments at the tips of the cells that emerge on the gill surface that become basidia. The basidia are the specialised sites for nuclear fusion, meiosis, and spore formation and these cells show irreversible commitment to these processes.

Knowledge that differentiation of tissues within the basidiome is limited does not help us to understand how the distinctive shapes and sizes and colours of mushrooms are formed. Analysis of fungal genomes has failed to find genes that are homologous to key genes that are universal players in animal development with names like *Hedgehog* and *Notch*. Computer simulations are effective at generating virtual mushrooms from groups of filaments whose behaviour is governed by a handful of rules. These rules include the degree to which neighbouring filaments attract or repel one another as they extend, the frequency of branching, the angles at which those branches grow, and the gravitational response of the growing tips. The power of these models lies in the fact that so few rules can specify a mushroom. The computer simulations also hint at the reason that the kinds of developmental genes ubiquitous among animals are absent in the fungi. According to the models, a developmental clock dictating the expression of successive waves of cell attraction and repulsion might be sufficient to shape everything from a mushroom with delicate gills to a fat bracket sticking out of a dying tree. Having advanced this possibility, however, investigators still need to identify the cell biological mechanisms that enable hyphae to sense the position of their neighbours, control branching, and perceive gravity.

Most of the ongoing molecular studies on fruit body development are directed at an ink cap, *Coprinopsis cinerea*, and the split gill mushroom, *Schizophyllum commune* (Figure 2.24). Experiments on these fungi have singled out a few of essential regulatory genes. These include the *THN* gene in *Schizophyllum* that is involved in the formation of aerial hyphae and fruit bodies. This is interesting given the shared challenges for hyphae and multicellular fruit bodies in ‘escaping’ the submerged mycelium. Genes that encode hydrophobins and are regulated by mating type-genes are essential participants in the fruiting process. Other



FIGURE 2.24 Cultured fruit bodies of (a) *Coprinopsis cinerea* and (b) *Schizophyllum commune*. Source: (a) Hajime Muraguchi, Akita Prefectural University, Japan and (b) www.mycology.adelaide.edu.au

participants include genes encoding lectins (carbohydrate-binding proteins), and, in common with the process of ascocarps development, oxidative enzymes and enzymes involved in carbohydrate metabolism. Despite significant advances in research on fungal multicellularity, however, it is important to recognize that researchers are nowhere close to pinpointing the genes that distinguish the fruit bodies of the 16,000 species of mushroom from one another.

Further Reading

- Berepiki, A., Lichius, A., Read, N.D., 2011. Actin organization and dynamics in filamentous fungi. *Nat. Rev. Microbiol.* 9, 876–887.
- Howard, R.J., Gow, N.A.R. (Eds.), 2007. *The Mycota, Volume 8, Biology of the Fungal Cell*. second ed. Springer Verlag, New York.
- Jedd, G., 2011. Fungal evo-devo: organelles and multicellular complexity. *Trends Cell Biol.* 21, 12–19.
- Lew, R.R., 2011. How does a hypha grow? The biophysics of pressurized growth in fungi. *Nat. Rev. Microbiol.* 9, 509–518.
- Read, N.D., Goryachev, A.B., Lichius, A., 2012. The mechanistic basis of self-fusion between conidial anastomosis tubes during fungal colony initiation. *Fungal Biol. Rev.* 26, 1–11.
- Richards, A., Veses, V., Gow, N.A.R., 2010. Vacuole dynamics in fungi. *Fungal Biol. Rev.* 24, 93–105.
- Steinberg, G., 2007. Hyphal growth: a tale of motors, lipids, and the Spitzenkörper. *Euk. Cell* 6, 351–360.
- Steinberg, G., Martin, S., 2011. The dynamic fungal cell. *Fungal Biol. Rev.* 25, 14–37.
- Taylor, J.W., Ellison, C.E., 2010. Mushrooms: morphological complexity in the fungi. *Proc. Natl. Acad. Sci. USA* 107, 11655–11656.
- Voisey, C.R., 2010. Intercalary growth in hyphae of filamentous fungi. *Fungal Biol. Rev.* 24, 123–131.

Weblink

<http://www.gerosteinberg.com/introduction.php>