

Physiology and Adaptation

Sarah C. Watkinson

University of Oxford, Oxford, UK

INTRODUCTION

Fungi have evolved to use every kind of carbon source, from dead trees to living tissues. In this chapter we consider the physiological adaptations that fit the huge diversity of species for their different ecological niches. The first section deals with nutrient acquisition: how fungi reach and assimilate their sources of carbon, nitrogen, and other elements. Secondary metabolites, used by fungi in competition and development, include many bioactive compounds, including vital antibiotics, and genetic analysis is proving fruitful in their synthesis and discovery. Cellular sensing, signalling, and response – including development in response to environmental cues – are central to the opportunistic life of fungi. We describe sensing mechanisms for extracellular and intracellular nutrients, and for physical factors including water potential, temperature, and light. We conclude by describing the ways in which fungal activities affect minerals and the soil, including the biotechnical potential of fungi in xenobiotic detoxification.

NUTRIENT ACQUISITION, UPTAKE, AND ASSIMILATION

All fungi require an organic source of carbon and energy, as well as combined nitrogen, phosphorus and sulphur, cations potassium, calcium and magnesium, and many other elements in trace amounts. Most culturable saprotrophic fungi will grow on a synthetic defined medium such as that shown in [Table 5.1](#). The core pathways of primary metabolism are largely the same in fungi as in animals. Energy and carbon skeletons for biosynthesis are supplied from sugars, via the glycolytic, tricarboxylic acid, and pentose phosphate pathways. Under carbon starvation, fungi can assimilate carbon dioxide to supplement the supply of carbon skeletons, but this is an energy-requiring process that cannot sustain growth indefinitely. Oxygen is the normal terminal electron acceptor in respiration, as in plants and animals, but nitrate can be used as an alternative in low oxygen conditions.

TABLE 5.1 Composition of a Synthetic Growth Medium

Mineral base	KH_2PO_4	1 g
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 g
	KCl	0.5 g
	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.01 g
Carbon and energy source ^a	Sucrose	30 g
Nitrogen source ^b	NaNO_3	2 g
Water		1 L
If a solid medium is required	Agar	20 g

^aAn organic form of carbon is always required, as all fungi are heterotrophic. Glucose, sucrose, or starch are commonly used.

^bMost fungi can grow with inorganic sources of nitrogen, usually provided as ammonium or nitrate salts. Some need amino acids.

A detailed account of the pathways of respiratory metabolism is available from specialised texts on fungal physiology (see Further Reading). Although glucose is the primary precursor for these pathways, fungi rarely encounter free glucose in nature. Instead, they must acquire it from widespread and reliable sources, typically insoluble glucan polymers such as cellulose from plant residues, or the tissues of living hosts. The fungal kingdom includes species with enzymes able to break down and feed on most naturally occurring carbon polymers (Table 5.2), and even some manmade ones including plastics.

TABLE 5.2 Some Fungal Enzyme Activities Which Hydrolyse Polymers

Substrate	Enzyme
Arabinans	Arabinofuranosidase
Callose	1 → 3 Glucanases
Cellulose	Endoglucanases, cellobiohydrolases
Chitin	Chitinases
Cutin	Cutinases
DNA	Deoxyribonuclease
Hemicellulose	Hemicellulases
Lignin	Ligninases
Mannans	Mannanase
Pectic substances	Pectin methylesterase, pectate lyase, polygalacturonase
Proteins	Proteinases
RNA	Ribonuclease
Starch	Amylases
Xylans	Xylanase

Carbon and Energy Sources

Sugars Fungi are extremely well equipped for sugar uptake, with both active and passive uptake systems. Transport proteins may be constitutive or induced. Generally, the sugars taken up preferentially from a mixture are those which require the least energy to assimilate and are most widespread in the environment. For example, the soil ascomycete *Chaetomium* takes up glucose as soon as it is supplied in a culture medium, but the relatively rare fructose, given as sole carbon source, is taken it up only after an induction period of several hours. Most culturable fungi will rapidly assimilate common monosaccharides and disaccharides from solution. Genomic analysis (see Chapter 6) reveals the presence in fungi of multiple genes encoding sugar transport proteins, although experiment is needed to establish their functional significance. Almost a hundred different genes of *Saccharomyces cerevisiae* encode transmembrane transporter proteins, mostly from the major facilitator superfamily MFS, with the sugar porter (SP) predominating. Many monosaccharide membrane transporters operate by facilitated diffusion, the passive energy-independent movement of a solute down its concentration gradient. Other monosaccharide transporters, active when sugar is scarce in the environment, are energy requiring and transport the sugar molecule against its concentration gradient, coupled to the simultaneous movement of one or more protons.

In competitive environments, rapid responses confer critical selective advantage. Global transcription factors act as master switches for metabolism, controlling the expression of many suites of pathways simultaneously in response to changing nutrient levels, both in the cell and in its environment. Uptake systems can thus be regulated so as to prefer better value carbon/energy sources from mixtures, and scavenge nutrients efficiently from dilute solutions. Carbon assimilation is under the control of the global transcription regulator CreA, which regulates the expression of whole suites of genes encoding catabolic and anabolic pathways of carbon metabolism, as well as cell membrane transporters.

Polymeric Forms of Carbon

Cellulose is ubiquitous in plants and plant remains and is the most common carbon compound on the planet. Most saprotrophic fungi can utilise it as a carbon source, and fungi are its principal decomposers.

Naturally occurring cellulose as it occurs in the plant cell wall ([Figure 5.1](#)) is insoluble, strong, fibrous, and resistant to hydrolysis because its β 1-4 linked glucan chains, up to 7 μm in length, are held in parallel alignment by hydrogen bonding to form long regular, crystalline bundles ([Figure 5.2](#)). The degree of crystallinity varies along the length of a bundle, with amorphous, less regular regions at recurring intervals. The bundles are themselves aligned to form microfibrils, 4–10 nm in diameter, which are visible in electron micrographs. This closely packed structure helps to protect the glycosidic bonds from hydrolysis, and the fungi that are able to attack crystalline cellulose possess a specialised array of enzyme activities. Physiological studies of cellulolytic fungi in culture have characterised the activities of secreted hydrolytic enzymes as either **endoglucanases** that break the bonds in the middle of the chain, releasing oligosaccharides and opening up the microfibril to further attack, or **exoglucanases** that act on the resulting chain ends. Cellobiohydrolase cleaves off dimers, cellobiose units, which are further hydrolysed to glucose by cellobiase.

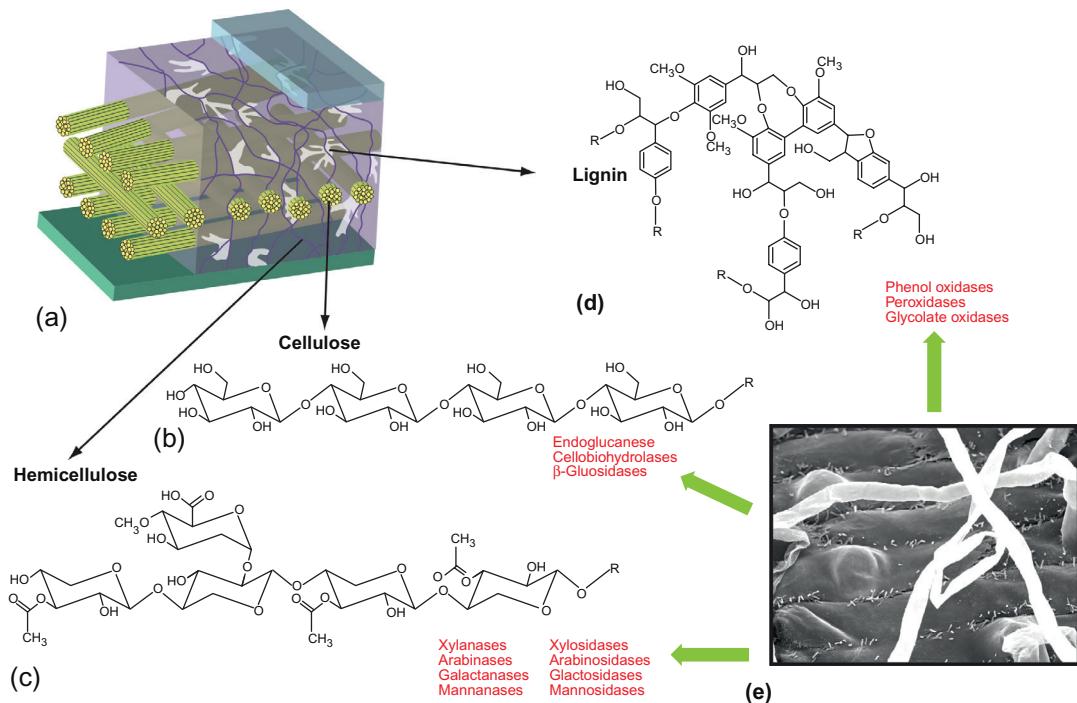


FIGURE 5.1 The plant cell wall (a) and the location and chemical composition of cellulose (b), hemicellulose (c) and lignin (d). Hemicellulose refers to a variety of carbohydrates and shown is O-acetyl-4-O-methyl-d-glucuronoxylan, which is common in angiosperms. Listed beneath each compound are the extracellular enzymes used by soil microorganisms to depolymerize the compounds during the process of plant-litter decay. The process of decomposition is mediated by a community of microorganisms (e), some of which are more or less active as plant detritus enters soil and particular compounds are successively depleted from that material. *Source: Frank Dazzo. From Zak et al., 2006.*

Genomic analysis reveals that fungi which utilise plant remains as carbon resources typically contain multiple genes encoding carbohydrate-active enzymes, in dozens of families. These are catalogued in the CAZy database of carbohydrate-active enzymes, described in Chapter 6. The categories listed, with their activities, are: 'Glycoside Hydrolases (GHs): hydrolysis and/or rearrangement of glycosidic bonds; GlycosylTransferases (GTs): formation of glycosidic bonds; Polysaccharide Lyases (PLs): nonhydrolytic cleavage of glycosidic bonds; Carbohydrate Esterases (CEs): hydrolysis of carbohydrate esters; and Auxiliary Activities (AAs): redox enzymes that act in conjunction with CAZymes'. Enzymes that act on plant polysaccharides often also contain a carbohydrate-binding module (CBM) that helps to attach the enzyme molecule to the glucan chain.

The highly cellulolytic soil ascomycete *Trichoderma reesei* (*Hypocrea jecorina*) is used for the industrial production of enzymes that hydrolyse plant polysaccharides. Interestingly, although its genome contains genes in the CAZy categories GH, GT, PL, CE, and CBM, as expected, these genes are no more numerous in *Trichoderma* than in the genomes of other, less aggressive cellulose decomposing species. *Trichoderma* appears to owe its exceptional

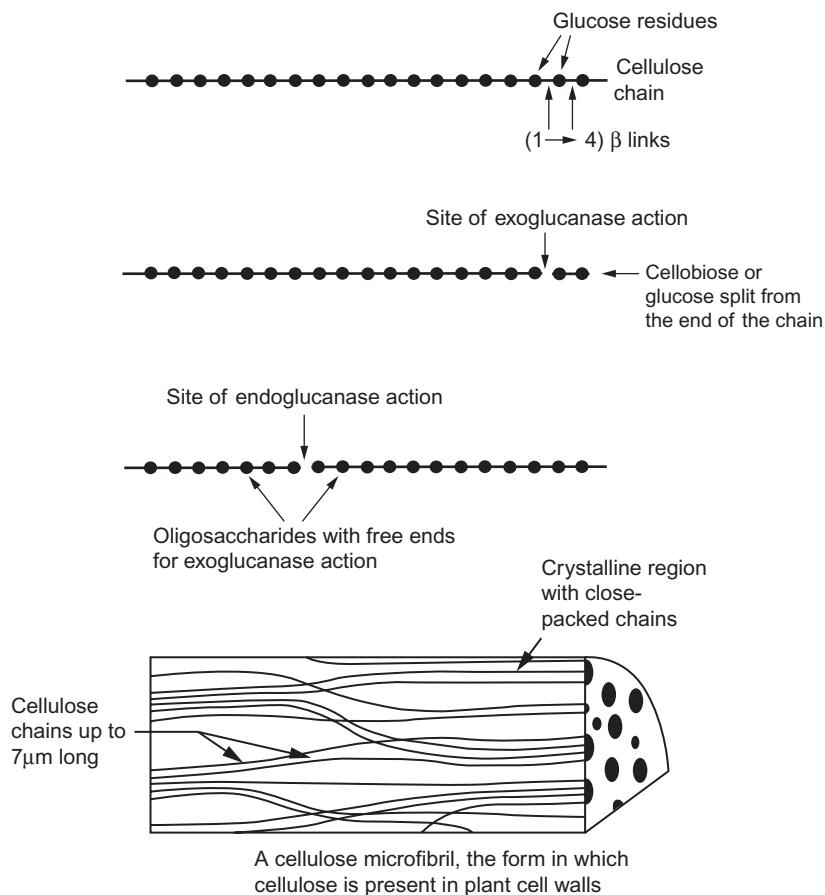


FIGURE 5.2 The structure of cellulose as it occurs in the plant cell wall, and the action upon it of different categories of hydrolytic enzyme. Endoglucanases break the bonds in the middle of the chain, opening up the microfibril to further attack and increasing the numbers of exposed ends of cellulose chains on which exoglucanases can act. Exoglucanases remove monomers or dimers from the ends of the cellulose molecule.

capacity for cellulolysis to the efficient secretion of its enzymes, rather than to an unusually extensive enzyme repertoire.

Genes for carbohydrate active enzymes are clustered in the *Trichoderma* genome, and found close to others for secondary metabolism. This probably facilitates coordinated expression of cellulase and antibiotic genes, helping *Trichoderma* to compete with the many bacteria, fungi, and animals that also utilise fragments of cellulose in the soil. Hundreds of saprotrophic and weakly parasitic microscopic ascomycete soil fungi occupy similar niches and produce secondary metabolites that act as plant toxins and antibiotics as part of their competitive armoury. They are thus a fruitful source of exploitable bioactive compounds and biosynthetic pathway genes (see Secondary Metabolism). Some, such as the genera *Chaetomium*, *Fusarium*, and *Paecilomyces*, cause 'soft rot' of wood by decomposing cellulose, hemicellulose, and pectins on its surface, although they do not penetrate sufficiently to cause structural damage.

Others, including species of *Cochliobolus*, *Fusarium*, and *Gaeumannomyces* are plant pathogens (see Chapter 8) which parasitize plant roots, killing plants with toxins. These are well known for causing losses in cereal crops.

Lignocellulose

The most durable source of cellulose in nature is wood. Comparative phylogenomics and molecular clock data suggest that fungi have been using wood as food since woody plants first evolved 400–500 million years ago (Figure 7.11). Fungal decomposition of wood plays an essential role in ecosystem and global carbon cycling. Wood contains 40–45% cellulose but decomposes only slowly because the cellulose fibres are embedded in lignin, a hydrophobic phenolic polymer which is resistant to microbial attack and cannot be utilised by fungi. Figure 5.3 shows the generalised cellular structure of wood, and the spatial distribution of cellulose, hemicellulose, and lignin. In the wall of woody cells (tracheids and vessels) the cellulose-rich S2 layers are sandwiched within lignified S1 and S3 layers. To feed on wood, hyphae must be able either to dissolve or penetrate lignin.

The so-called **white rot** wood decay fungi destroy lignin to gain access to cellulose and hemicelluloses in lignocellulose. White rot is the ancestral form of fungal wood decay and

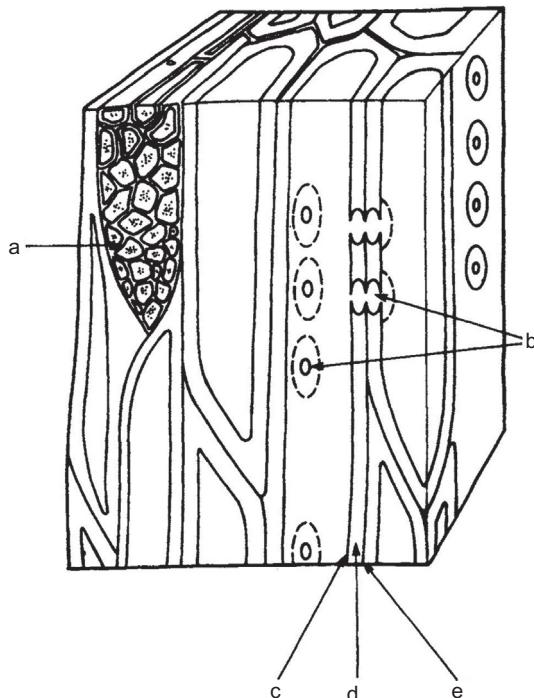


FIGURE 5.3 Diagram of the cellular structure of wood: (a) medullary ray consisting of cells which are alive in living trees, and provide a source of readily available nutrients for fungi invading timber; (b) pits, perforations in the tracheid wall, usually coinciding in position in adjacent tracheids, and providing a pathway for hyphae to grow from one tracheid into another; (c) S3 wall, with abundant lignin added to cellulose framework; (d) S2 wall, mainly cellulosic and relatively less lignified; (e) S1 wall, lignified.

is performed by both basidiomycetes and ascomycetes. Well known examples are the honey fungus, *Armillaria mellea*, and *Phanerochaete chrysosporium* used as an experimental model for white rot wood decomposition. Like many useful categories of biological activities, the distinction between white and brown rot fungi is not sharp in nature, and comparative ecological genomics shows species that do not fit either description precisely and share characteristics of both or neither. While some fungi decompose lignin and cellulose simultaneously, others remove the lignin first to reveal the whitened cellulose in a **selective delignification** (Figure 5.6a). This fungal action of freeing wood cellulose from its protective coat of lignin is important in ecosystem carbon cycling, because the delignified cellulose can be consumed by many different animal phyla (Chapter 9, p.325) including protozoa, nematodes, molluscs, crustacea, insects, and arachnids. This phylum-level diversity led the evolutionary biologist W.D. Hamilton to suggest that fungal decay of trees might have contributed significantly to the early diversification of invertebrates. Vertebrates may also benefit from fungal delignification. Delignified white rotted dead wood (*palo podrida*) is used as cattle forage in the Andes, and fungi are being investigated for use in rendering the woody agricultural wastes of leguminous crops suitable for feeding ruminant farm animals.

The recalcitrance of lignin arises from its polymeric structure as a three-dimensional heteropolymer, formed through random linkage of phenylpropanoid monomers through several types of covalent bond (Figure 5.4). Lignin has no repeating pattern of bonds, and is highly hydrophobic. It cannot, therefore, be broken down by enzymic hydrolysis where an active site of an enzyme binds to a single type of chemical bond. Instead, oxidases and peroxidases act in a non-sterically specific manner to break the molecule into a complex mixture of fragments. Oxidative lignin breakdown is a violent reaction which could not occur inside a living cell, and has been termed 'enzymic combustion'. The reactants are generated extracellularly in contact with the substrate as the hypha lies against the cell walls that form the wood. Peroxidases that use hydrogen peroxide as oxidant, and enzymes that generate hydrogen peroxidase, act together to oxidise and destabilise the lignin molecule, which then breaks down chemically into smaller fragments (Figure 5.5). Lignin peroxidases (LiP), manganese-dependent peroxidases (MnP), and versatile peroxidases (VP) are haem glycoproteins belonging to a monophyletic gene family, termed Class II peroxidases, which has diversified within basidiomycetes. The MnP enzyme forms stable diffusible complexes with organic acids such as oxalate, exuded by many fungi, and these complexes oxidise lignin phenols to phenoxy radicals. MnP is found in all white rot basidiomycetes (unlike LiP, which has arisen only once, in Polyporales) and thus has a key role in the breakdown of lignin in ecosystems as the main enzyme associated with basidiomycete white rot wood decay (Figure 5.5). The LiP enzyme molecule becomes oxidised in the presence of hydrogen peroxide to an active state in which it abstracts an electron from a non-phenolic aromatic compound to generate an unstable aryl cation radical which then undergoes a variety of non-enzymic intramolecular reactions including ring cleavage to produce a mixture of small molecules. Lignin peroxidases were added to the CAZy database in 2013 in recognition of the essential role that they play in the decomposition of lignin-carbohydrate complexes in lignocellulose. They also have their own database, the FOLy database at <http://foly.esi.univ-mrs.fr>.

Accessory enzymes involved in lignin degradation include hydrogen peroxide-generating enzymes such as aryl alcohol oxidase, glyoxal oxidase, and pyranose-2 oxidase. Laccases are blue copper oxidase enzymes which use oxygen to oxidise *p*-diphenols. They are found in

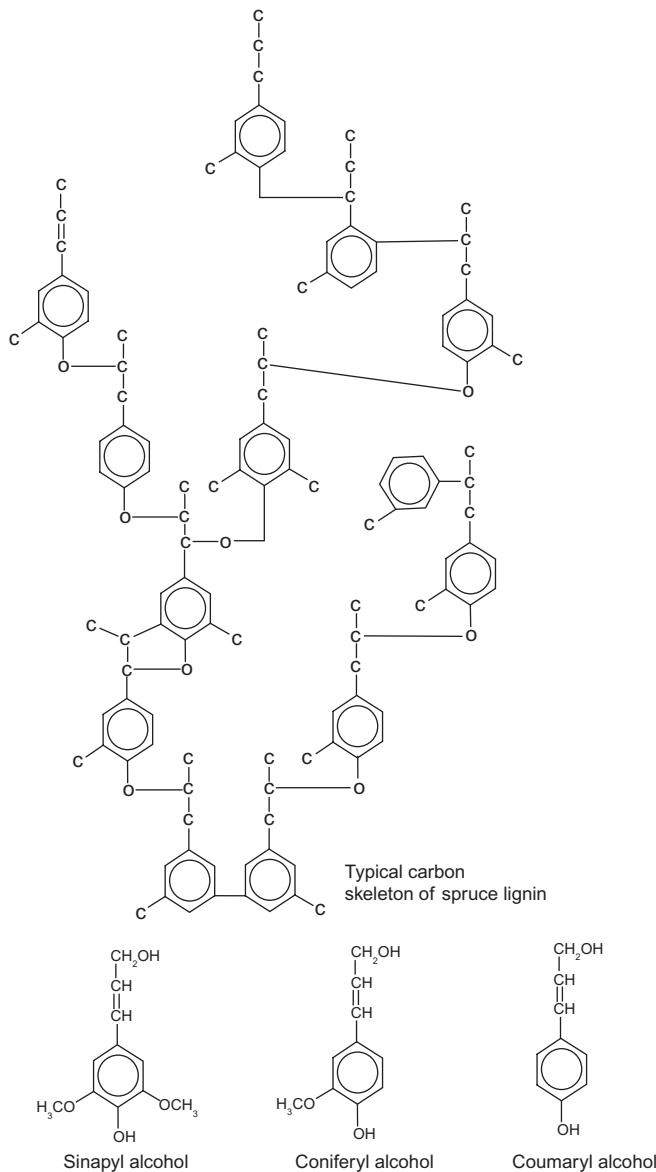


FIGURE 5.4 The molecular structure of lignin, a complex three-dimensional polymer formed from phenylpropanoid subunits. Coumaryl, sinapyl, and coniferyl alcohols are the commonest of the phenylpropanoid subunits, and they are joined randomly in a three-dimensional, asymmetric network by various different linkages, the commonest of which are illustrated. Coniferyl alcohol is the most frequent subunit in the wood of coniferous trees.

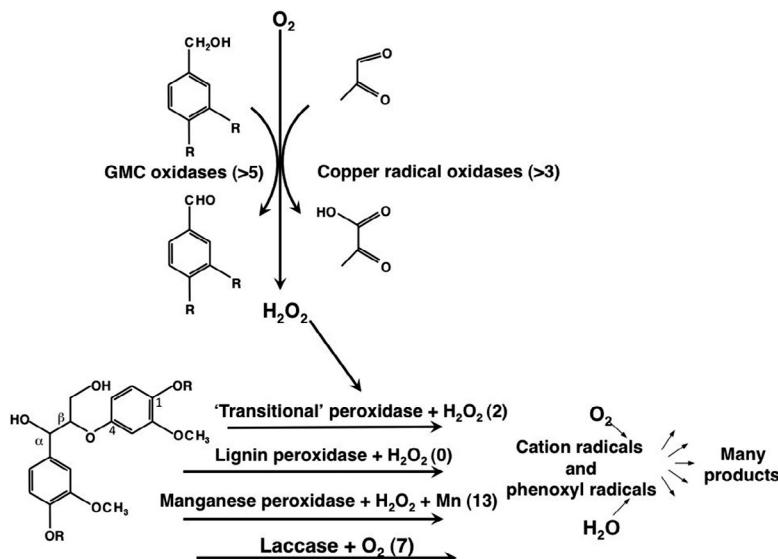


FIGURE 5.5 The role of peroxidases in lignin breakdown. Extracellular processes involved in lignin degradation by a white rot fungus. Hydrogen peroxide generated by reactions catalysed by copper radical oxidases and glucose-methanol-choline (GMC) oxidases acts as substrate for a wide array of oxidations that yield many different products. *Source: Cullen, Wood Decay. pp. 43–62, Section 2, Saprotophrophic Fungi, in Martin (2014).*

most white rot fungi and have been used as indicators of white rot capacity, but cannot attack whole lignin. Lignin fragments cannot provide an energy source for fungi but soluble molecules resulting from oxidative solubilisation can be taken up and degraded intracellularly through the cytochrome P450 system, well represented in fungal genomes and associated with xenobiotic metabolism (see below). The genome of the model white rot basidiomycete *Phanerochaete chrysosporium* has 154 cytochrome P450 genes.

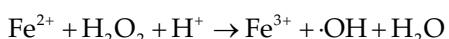
By contrast with white rot, in the **brown rot** mode of wood decomposition, fungi remove cellulose from within the lignocellulose complex, without completely removing the lignin, so that a brown residue of the polymer remains (Figure 5.6b). Most of the mass loss caused by brown rot decay is from the digestion of polysaccharide. However, the lignin is changed, with minor side chain oxidation, demethylation, and some depolymerisation. The capacity for brown rot, a more energy efficient utilisation of wood carbohydrate than white rot, has evolved convergently in at least six different clades of basidiomycetes, including examples in both Polyporales and Boletales (Figure 7.10). Brown rot does not appear to be a feature of ascomycete wood decay. Comparing gene evolution across a range of white and brown rot basidiomycete species indicates that the transition from white to brown rot wood decomposition involved not only the loss of genes for oxidases but also an overall reduction in numbers of some gene families and gene copies of some glycoside hydrolases. However, there has been duplication of genes in other glycoside hydrolase gene lineages, resulting in a reduced and refined collection of enzymes for cellulose depolymerisation.

Brown rot decay of wood is characterised by early reduction in strength, before there has been significant loss in volume or biomass. Timber decay caused by *Serpula lacrymans* in a building can go unnoticed up to the point of collapse. Cellulose fibres provide the tensile



FIGURE 5.6 The appearance of wood decayed by: (a) a white rot fungus. This branch has been selectively delignified, leaving a pale, fibrous, cellulose-rich residue. (b) The remains of a fallen tree decomposed by a brown rot fungus. The decomposed wood breaks apart in a cubical pattern and then crumbles to a brown, lignin-rich residue. Inset figure: close-up of brown rotted wood. Source: (c), *Stuart Skeates*.

strength of wood and the early stages of depolymerisation involve chain breakages in the amorphous regions of crystalline cellulose, thereby weakening the cellulose fibrils. A problem in understanding the role of enzymes in this process is that the micropore diameter within the wood composite structure, at the stage when cellulose depolymerisation is first evident from physical analyses such as X-ray diffraction, appears too small to allow diffusion of hydrolytic enzymes. Microscopy reveals that hyphae are sparsely distributed in decayed wood, and where hyphae can be seen, they are not in close contact with the cell wall, implying that decay is initiated at a distance from the surface of the fungal cell. It appears that the primary attack on the cellulose molecule is by a free radical oxidation rather than a hydrolytic enzyme. Free radical generation outside the hyphae is facilitated by secretion of small molecules including oxalic acid, and phenolates that act as electron donors. Under the low pH conditions generated by these chemicals, the strongly oxidative hydroxyl radical $\cdot\text{OH}$ is generated by Fenton's reaction, in which organic acids and hydrogen peroxide combine in a redox reaction with ferrous iron:



Phenolates, which may be secreted fungal secondary metabolites or derived from lignin decomposition, act both to chelate and also reduce iron, with accompanying oxidation of phenolic groups to quinones.

The hydroxyl radical has a nanosecond half-life; hence it must be produced close to its site of action on the cellulose crystal. The means by which it is targeted is an intriguing question. One answer is suggested by the discovery of compound genes, in the genomes of the white rot fungus *Phanerochaete chrysosporium* and the brown rot species *Serpula lacrymans*, which appear to encode both a CBM, and an iron reductase. This protein, by binding to the cellulose molecule and localising iron reduction for the radical-generating Fenton's reaction close to it, may help to target hydroxyl radical attack to the cellulose molecule. This theory is supported by transcriptional analysis which shows that the compound gene is expressed over a hundred times more when the fungus is grown on wood than when glucose is its sole carbon source.

While the sequenced brown rot species so far investigated have many glucanase enzymes that break bonds in the middle of the glucan chain of cellulose, they all lack the **exoglucanases** which further degrade the oligomeric carbohydrate produced by these reactions. These exoglucanases are 'processive' glucanases that snip glucose or cellobiose units from glucan chain ends. They are present in white rot fungi, but appear to be absent from the genomes of brown rot species that have been sequenced. How exoglucanase action is produced in brown rot fungi is unknown at the time of writing. Brown rot wood decomposer fungi mainly decay softwoods and are the most common cause of wood decay affecting the built environment and timber structures in temperate climates. The dry rot fungus *Serpula lacrymans* (Boletales) is the most common cause of wood decay in buildings in Northern Europe, followed by the cellar fungus *Coniophora puteana*. *Serpula incrassata* and *Postia (Poria) placenta* are responsible for wood decay in buildings in the United States. Molecular clock data (see Chapter 7; Figure 7.11) show that the evolution of white and brown rot fungi coincided with the evolution of angiosperm and gymnosperms, which presumably supplied new nutritional niches for them. In turn, fungi are believed to have played a part in the development of forest soils by generating the humus that conditions these carbon rich, nitrogen-poor soils.

Protein Secretion

Protein secretion is an important feature of fungi that is central to their ecology and is exploited in biotechnology. The release of enzymes is regulated in time and space according to the substrates available and the condition of the fungus. Protein secretion has been investigated intensively in yeasts, because they are experimentally tractable and are used for the heterologous expression of imported genes in biotechnology. Much less is known about secretory processes in filamentous fungi, even though their levels of protein secretion are typically 10-fold higher than those of yeasts. Filamentous fungi are generally better adapted for protein secretion than yeasts. Because their hyphae feed by extracellular digestion of chemically diverse solid substrates, rapid and efficient enzyme secretion confers strong selective advantage. There is, therefore, a practical impetus behind the investigation of protein secretion in filamentous fungi, with huge potential benefit from transforming yeasts to secrete high levels of heterologous proteins.

In the eukaryotic cell, proteins destined for secretion into the external environment are processed for export in the endoplasmic reticulum Chapter 2, p. 45, where they are folded and glycosylated. In a series of interactions, the nascent protein is combined with a binding

protein (BiP), with protein disulphide isomerase (PDI), which catalyses disulphide bond formation and acts like a chaperone, and with peptidyl prolyl isomerase (PPIase), which interacts with other proteins and may accelerate protein folding. Correctly folded proteins are targeted to the Golgi apparatus where they are glycosylated and are then either exported to the exterior via the plasma membrane or targeted to the vacuole. Based on GFP imaging in *Aspergillus niger*, export is believed to be confined to the apical region of the hypha.

Aspergillus niger and other filamentous fungi are of interest for industrial production of a range of heterologous secreted proteins. The secretory pathway of *A. niger* has been successfully modified to secrete proteins that are normally intracellular, to facilitate the harvesting of new heterologous proteins produced by transformants. The intracellular protein for export is targeted into peroxisomes by adding a peroxisome import signal tag. The secretory signal proteins on peroxisomes are also modified so that they fuse with the plasma membrane. The proteins imported into the peroxisomes are found to be released to the external environment through this artificial secretion pathway, termed '**peroxicretion**'.

Identification and purification of secreted proteins makes it possible to carry out imaging to determine the time and place of enzyme release in relation to ecologically relevant substrates. Proteins can be labelled *in situ* serologically, using antibodies conjugated with a visualisable label. However it is now more common to use GFP fusions and image the protein's cellular location by confocal microscopy.

Nitrogen Sources

Fungi share most pathways of amino acid metabolism and protein biosynthesis with other eukaryotes, including animals. However, unlike animals, fungi do not require amino acids from their environment to synthesize protein, but can assimilate inorganic compounds widely available in soil. The ability to make their own amino acids using soil nitrate and ammonia, providing they have a carbon source, enables saprotrophic fungi to convert dead wood and other nitrogen-poor, carbohydrate-rich plant remains into protein. This gives fungi a key role in the trophic webs of terrestrial ecosystems and is also the basis of various processes for converting agricultural waste into animal feed and human foods.

Fungi, unlike many prokaryotes, cannot utilise molecular nitrogen and so must scavenge the element from an unpredictable variety of soluble and insoluble environmental sources, competing with plants and all the other microbes in the habitat. Enzymic, transport, metabolic, and developmental systems for taking up and assimilating nitrogen are expressed according to the ever-changing opportunities for nitrogen acquisition. Fungi respond to nitrogen abundance or starvation conditions with 'global' changes in metabolism, with transcription factors operating to activate whole pathways for assimilation or dissimilation. Conserved regulator genes encode transcription factors that in turn, control and coordinate up- and down-regulation of gene clusters, encoding whole pathways for nitrogen uptake and assimilation. The global transcription factor AreA, conserved across the fungal kingdom, is a positive regulator that switches on pathways of nitrogen catabolism under nitrogen depletion, while nt2 is a negative regulator and represses nitrogen assimilation when nitrogen is available. Both are homologous GATA factors with a shared DNA-binding sequence.

Soil, water, and living tissues provide a wide variety of inorganic and organic nitrogen sources for fungi. In agricultural soils, nitrate derived from bacterial nitrification, fertiliser,

and atmospheric deposition, is the most abundant. By contrast, in natural and semi-natural soils with low disturbance and a large competing microbial population, nitrate and ammonium occur only sporadically and briefly, and fungi in these habitats can assimilate many different organic nitrogen compounds.

Nitrate

Nitrate uptake in fungi occurs via transport proteins encoded by the *NRT1* and *NRT2* gene families. These membrane proteins mediate transport of NO_3^- together with protons (H^+) in a symport driven by the pH gradients across membranes (see Chapter 2, p.42). They are encoded within a coordinately regulated cluster, *fHANT-AC*. This consists of three genes: *nrt2*, which codes for a high-affinity nitrate transporter; *euknr*, which codes for nitrate reductase that keeps cytosolic nitrate low, maintaining a gradient; and *NAD(P)H-nir*, which codes for nitrite reductase. While these individual genes are found in all eukaryotes, the cluster is seen only in fungi and in *Phytophthora*. Phylogenetic analyses suggest that the cluster was first assembled in a lineage leading to oomycetes and subsequently transferred horizontally to an early ancestor of the fungi. The acquisition of *fHANT-AC* may even have been the crucial evolutionary step that enabled higher fungi to colonise land by allowing exploitation of nitrate in aerobic soils. Functional diversification of the nitrate assimilating cluster was demonstrated in a phylogenetic analysis of basidiomycete species with ecological niches representing saprotrophy and biotrophy in three separate lineages. Fungi have two paralogs of *NRT2* encoding a high and a low affinity transporter. Transcription analyses suggest that the high-affinity transporter, which enables fungi to concentrate nitrate from environments in which it is present at less than micromolar concentrations, may be adaptive to environments such as pristine boreal forest soils and water with very low levels of nitrate. In such forest soils, ectomycorrhizal fungi play a crucial role in nitrogen cycling by assimilating nitrogenous compounds from soil and transferring them to tree hosts. Accordingly, genomes of forest mycorrhizal fungi (Chapter 7) are being examined for evidence of nitrogen acquisition mechanisms adapted to the limiting levels of biologically available forms of nitrogen in these soils. Truffles grow as mycorrhizas extending into forest soils from tree roots. Their *nrt2* protein is a very high-affinity transporter ($K_m = 4.7 \mu\text{M}$ nitrate) that is bispecific for nitrate and nitrite. It is expressed in free-living mycelia and in mycorrhiza of the truffle, *Tuber borchii*, where it preferentially accumulates in the plasma membrane of hyphae that contact roots. *Laccaria bicolor*, which is also ectomycorrhizal, but common and widespread compared with truffles, takes up nitrate even when glutamine is present, suggesting that regulation varies according to niche adaptation. The highly efficient nitrate scavenging of fungi is exploited by plants whose own capacity for uptake is inferior. For example, the arbuscular mycorrhizal *Rhizophagus irregularis* (Chapter 7) helps cucumber roots to scavenge nitrate from soil.

In the cell, nitrate is reduced to ammonium in a two-step process via nitrite. Nitrate reduction places an energy demand on the organism, supplied by the coenzyme NADPH produced in the pentose phosphate pathway of glucose metabolism. Many fungi are known to utilise nitrate as an alternative terminal electron acceptor in the respiratory chain when oxygen is limiting, as in temporarily anaerobic soils, where they may therefore contribute to soil denitrification. Nitrate at high concentrations is damaging because it may impose oxidative stress on the cell, triggering responses that include alterations in secondary metabolism and autolysis.

Ammonium

Ammonium ion is taken up by a family of transporter proteins, the ammonium transporter / methylammonium permease family (MEP family), unique to fungi but homologous with the conserved AMTP family found in all eukaryotes. The fungal MEP proteins probably originated as a horizontal gene transfer from a prokaryote early in fungal evolution. They are membrane-spanning proteins composed of 11 highly conserved transmembrane domains that fold into a pore through which ammonia or ammonium pass passively. They are believed to function as modified gas channels, allowing influx of a gas rather than the dissolved ion, conducting ammonia (NH_3). The ammonium proton is lost at the entrance to the pore, ammonia moves through the channel, and is re-protonated at the cytoplasmic side. Ammonia is preferred to nitrate in uptake from mixtures, through repression of nitrate uptake pathways.

Metabolic incorporation of nitrogen occurs by combination of ammonium with precursors from the pathways of respiratory metabolism, forming amino acids. Pyruvate from glycolysis is aminated to alanine, and oxoglutarate from the TCA cycle to glutamate. These serve as intermediates in complex networks of amino acid catabolism and anabolism. Several alternative pathways of ammonium assimilation operate in fungi according to the extracellular concentration of ammonium. Under nitrogen starvation, the so-called GS-GOGAT pathway is activated by expression of genes encoding the component enzymes. The first, glutamine synthase, with a high affinity for ammonium, catalyses its combination with glutamate and the resulting glutamine is used by an amino transferase to aminate 2-oxoglutarate. This reaction is powered by ATP with NADPH as electron donor. When ammonium is more readily available it is assimilated through the energetically cheaper reaction of ammonium with 2-oxoglutarate, which also uses NADPH but does not need ATP.

Amino acids

Amino acids are taken up from the extracellular solution by a variety of constitutive and inducible membrane transport proteins as described in Chapter 2. Core metabolic amino acids such as alanine, glycine, glutamine, aspartate, and glutamate, derived from primary carbon metabolic intermediates by a single amination step, are readily assimilated. Other amino acids, including methionine, are used more slowly and are inferior as sole nitrogen sources. The preferences of fungi for various nutrient sources in culture have been intensively studied by brewing industries, and *Saccharomyces cerevisiae* presented with a cocktail of amino acids will exhaust them one at a time in a clear order of preference.

Not all amino acids taken up by the cell can be metabolised. The non-protein amino acid α -aminoisobutyric acid (AIB) is a product of secondary metabolism in some fungi and bacteria and is combined into peptides with antibiotic function termed **peptaibols**. AIB is preferred even to glutamate by fungal amino acid transporters, but, since it is not metabolised, accumulates unchanged in the cell and is used in biochemical amino acid transport studies. Its inhibitory effect on cell growth has been investigated for tumour suppression. At high concentrations it inhibits mycelial spread in basidiomycetes through competitive inhibition of glutamate uptake. At sub-toxic intracellular concentration it is used a ^{14}C -labelled marker for amino acid translocation in mycelial networks (Figure 5.7), because it is not metabolised and remains unchanged in the free amino acid pool.

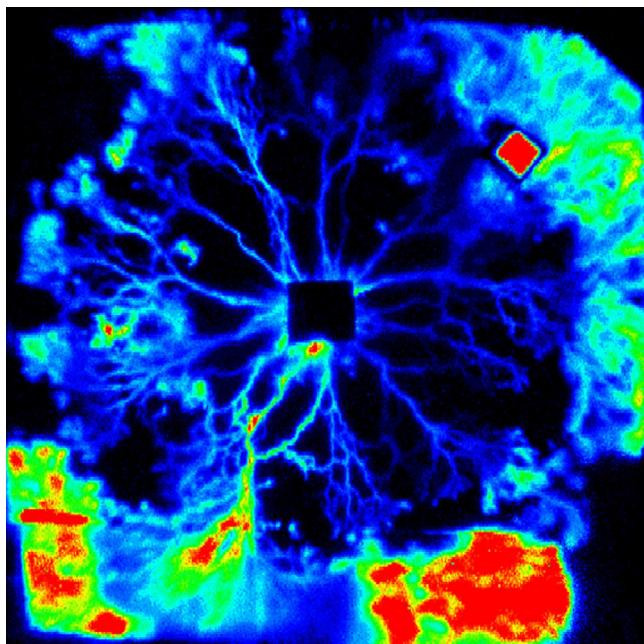


FIGURE 5.7 Photon counting scintillation imaging of ¹⁴C-AIB in the mycelium shown in [Figure 5.18](#), during new wood resource capture (Further Reading in Tlalka et al., 2008, Video S2) Video link, see Further Reading. The mycelium shown in [Figure 5.18](#) was supplied with the non-metabolised tracer amino acid AIB (α -aminoisobutyric acid) labelled with ¹⁴C. After incubation to allow the AIB to become evenly distributed within the mycelium, a fresh wood block was placed near the colony margin, top right, and the system was imaged by PCSI for the ensuing 10 days. The video shows AIB redistribution from the whole mycelium into the site of colonisation of fresh wood. This is consistent with an ability of the mycelium to sense and respond to a localised fresh carbon supply by importing nitrogen, to achieve a balanced internal C/N ratio for biosynthesis. *Source:* Tlalka et al. (2008). <http://dx.doi.org/10.5072/bodleian:d217qq90r>

The mycelium stores free amino acid as a labile expandable pool that responds to internal nitrogen supply and demand, as well as availability in the external environment. Free amino acid levels in the cell can vary 10-fold or more, according to nitrogen availability in the environment. The intracellular pool is composed mainly of core amino acids including glutamate, glutamine, serine, proline, aspartic acid, glycine, and alanine, with smaller amounts of arginine, ornithine, lysine, and histidine. Low levels of non-protein amino acids, including GABA may also be detected. Kinetic studies indicate that separate pools with relatively fast and slow turnover may be present in cytoplasmic and vacuolar locations. Transporters with different affinities may accumulate dibasic arginine, ornithine, and lysine in the vacuole. In addition to its many other functions (Chapter 2), the vacuole can thus act as a short-term mobile nitrogen storage and distribution compartment. Vacuolar movement may be involved in the transfer of nitrogen from mycorrhizal mycelium into the root. Here amino nitrogen, assimilated into arginine in the extramatrical mycelium in soil, is translocated into the root

where the arginine provides a nitrogen source. This process has been described as a spatially separated urea cycle. The plastic and dynamic vacuolar system contributes to nutrient translocation in the hypha. Predictive models show that amino acids for hyphal tip growth may be imported to the tip in this way although such transport is only efficient over distances of a few micrometers.

Other Organic N Compounds

A variety of organic nitrogen compounds are utilised by fungi adapted to habitats where readily assimilable forms of nitrogen are in short supply. Fungi in low-disturbance habitats such as boreal forest soils exist in a state of nitrogen limitation and have evolved strategies to scavenge, store, and recycle nitrogen within their mycelia. Over a thousand organic nitrogen compounds, some of which are highly resistant to biological breakdown, are found in such soils. Up to 50% of soil nitrogen may be present in the form of phenolic nitrogen compounds. Forest fungi mine soil for nitrogen, using oxidising enzyme systems such as the class II peroxidases used in lignin depolymerisation, to break down recalcitrant complexes. The extensive mycelium of woodland basidiomycetes is a repository of nitrogen in the form of chitin, proteins, polyamines, and amino acids.

Protein

Protein is depolymerized by fungi using multiple secreted proteases and peptidases that enable them to hydrolyse most proteins including those in human tissues. Fungal proteinases are of practical interest in the etiology of fungal infections and for industrial applications. The virulence of ringworm fungi that grow on nails, hair, and skin is due to proteinases adapted to keratin hydrolysis.

Seven categories of common protease, and aspartic, serine, metalloproteases, and cysteine protease (characterised by active site and identified biochemically by the effect of standard inhibitors on their activity) are present in fungi. Many others are inferred from biochemical activity or gene sequence but are as yet uncharacterised. Genomes reveal a multiplicity of putatively proteolytic enzymes, far greater than expected from biochemical studies. Proteases destined for the extracellular environment are characterised by the presence of a signal peptide that targets them to the secretory pathway, and an N-terminal propeptide, comprising as much as 50% of the peptide chain, that orchestrates correct protein folding and stability. These are removed by specific proteolysis to activate the secreted enzyme. Enzymes of saprotrophs are typically glycosylated, which contributes to their robustness and durability in soil where proteins are liable to be consumed by other microorganisms.

Fungi parasitic on animals have a wider range of secreted proteinases than saprotrophs, and the particular suite of these found in a species may confer specificity for types of tissue. Proteases conferring virulence on parasitic fungi, but also present in saprotrophs, are categorized as subtilisins and fungalysins. *Aspergillus fumigatus*, cause of aspergillosis in the human lung, secretes fungalysins that attack the interstitial mucopolysaccharides collagen, elastin, and laminin. Dermatophytes, which attack keratin (hair, nails, and skin), secrete not only multiple fungalysins and subtilisins, but also sulphite, which is required to break the disulfide bridges of the insoluble protein to allow access for enzymes. Comparison of the genomes of important fungal pathogens of humans, including *A. fumigatus*, and dermatophytes, has shown the path of gene evolution of proteases. Dermatophytes have many more fungalysins and alkaline

proteases than *A. fumigatus*. The two genes are shared and ancestral to both *Aspergillus* species and dermatophytes, but in dermatophytes there have been multiple expansions in these gene families. Different pathogens express particular suites of protease activities, of which, a subset are virulence factors. From phylogenetic tree topology, gene evolution preceded species divergence, suggesting that the acquisition of new proteolytic capacities opened the way for diversification into new niches. Disease symptoms caused by fungal protease action on animal tissues may be due not solely to proteolytic attack, but also to the allergenic properties of the proteins that induce a localised immune response and cause inflammation.

Other Major Nutrients

Phosphorus

As a component of nucleic acids and cell membranes, and central to energy metabolism, phosphorus is continually required for fungal growth. However, its availability and chemical form in the environment vary in space and time. Fungal systems for phosphorus acquisition are highly adapted for efficient scavenging.

Phosphate ions are assimilated from solution in soil and water by proton-coupled symporters in the cell membrane. The characteristics of phosphate transporters are intensively studied in mycorrhizal fungi because of their key role in plant health. Typically, several transport proteins differing in substrate affinity are encoded in the genome, allowing the fungus to adjust to varying levels of environmental phosphate by differential transcription. The ectomycorrhizal basidiomycete *Hebeloma cylindrosporum*, for example, has two genes, *HcPT1* and *HcPT2*. Under phosphorus limiting conditions, *HcPT1* is up-regulated, suggesting a special role in scavenging scarce phosphate. Internal phosphate levels also regulate the transcription of phosphate transporters, depressing expression when intracellular phosphate increases. The cellular level is also affected by conversion of phosphate to a polymeric form, polyphosphate, consisting of chains of three to 1000 phosphate residues. Polyphosphate is sequestered in the vacuole. The physiology of the fungus thus accommodates to both external supply and internal demand. Phosphate homeostasis within the mycelial network is also regulated by translocation. Intracellular phosphate is shown by ^{32}P -labelling to be transported in mycelial networks to sites of active growth. The rate can be faster than diffusion, but the mechanism and anatomical pathways remain obscure. Phosphorus accumulation and redistribution through fungal networks is central to forest ecosystem nutrient dynamics (Chapter 7).

Uncultivated soil typically contains less than 10 μM levels of free phosphate, and fungi are adapted to utilise other sources of phosphorus in soil, including relatively insoluble minerals like apatite, and organic compounds and complexes. This phosphorus scavenging capacity, together with phosphate translocation, gives mycorrhizal fungi their central role in importing phosphorus from rocks to the biosphere (Table 5.4). Hyphae solubilise phosphate by lowering the pH in their vicinity through proton release and by secreting oxalic, citric, and malonic acids from hyphal tips and along the length of the hypha. Oxalate is produced in large amounts and crystals are common on the surface of aerial hyphae. The dense agglomerations of hyphae formed by some mat-forming ectomycorrhizal species of forest fungi can raise oxalate levels in the surrounding soil to forty times that of uncolonised soil.

In mature natural ecosystems most soil phosphorus is in the form of organic compounds. Simpler organic forms include monoesters (mononucleotides and sugar phosphates) and

diesters (nucleic acids and phospholipids). Monoesterases and diesterases act on these compounds to release assimilable phosphate. Monoesters are more abundant and can comprise up to 50% of the total soil organic phosphorus pool. Inositol phosphates exist in soil in the form of complexes with organic and inorganic material. Phosphate is released from these sources by **phytase** enzymes. Such lysis is an important ecological function of mycorrhizal fungi (Chapter 7) and there is evidence of synergy between the varying phospholytic activities of different ectomycorrhizal taxa, with host phosphorus uptake enhanced when a diversity of fungal symbionts is present. Phytase synthesis is induced under phosphorus-limiting conditions and repressed by intracellular phosphate.

Sulphur

Most fungi are able to utilise sulphate. It is taken up via membrane sulphate transporters, and phosphorylated in the cell via adenosine triphosphate in two steps to give 3'-phosphoednosine-5'-phosphosulphate. This is reduced to sulphite then to sulphide, which is condensed with O-acetyl serine to give cysteine for incorporation into proteins and as an intermediate for the synthesis of methionine. Organic sources of sulphate can also be used, such as choline-O-sulphate, common in plants and fungi, as an internal sulphur store, as well as aromatic sulphate esters. These are transported into the cell via specific transporters, which, along with sulphate transport proteins, are strongly induced in sulphur-limited conditions, and repressed by sulphur-containing amino acids.

Sulphur metabolism is under the control of global transcription factors. Sulphur limitation induces transport systems for methionine or sulphate, and enzymes including aryl sulphatase and choline sulphatase to release sulphur from internal reserves and extracellular organic compounds including protein. All the sulphur acquiring systems are repressed under sulphur sufficiency, which is reached in experimental cultures of yeast and *Aspergillus* at a 5 mM concentration of methionine.

Sulphur is essential not only for protein synthesis but also in the glutathione-based system for combatting stress. Glutathione, a non-protein thiol with a very low redox potential, is present at up to 10 mM in yeasts and filamentous fungi. It is an important antioxidant, reacting non-enzymatically with reactive oxygen species as well as participating in detoxification of xenobiotics. Elemental sulphur, which is cytotoxic, can be reduced extracellularly to hydrogen sulphide by the soil fungus *Fusarium oxysporum*, via the glutathione reductase/glutathione couple. Under experimentally imposed stress, *Saccharomyces cerevisiae* activates a sulphur-sparing system which conserves intracellular sulphur for glutathione synthesis by replacing sulphur-rich proteins with isozymes containing lower levels of sulphur.

Essential Metals

All fungi require substantial amounts of potassium and magnesium, and around 50 mM concentrations of Mg and K salts are used in defined culture media. These cations are present at high concentrations in fungal cells and are structural components of membranes. Iron, copper, calcium, manganese, zinc, and molybdenum are required by all organisms as cofactors for enzymes and other proteins. The amounts needed are relatively low, less than 6–10 µM for iron and molybdenum. Some of the trace elements, including copper and zinc, become toxic for fungi at levels only a few times greater than those required for optimal growth.

In spite of the small quantities of trace metals required, their presence does not always ensure their availability for the fungus. Iron presents a particular problem. Except under strongly acidic conditions, ferrous iron undergoes rapid spontaneous oxidation to the ferric form, followed by precipitation as the highly insoluble ferric hydroxide. Fungi address this problem by synthesising iron chelating agents, **siderophores**, with which iron forms a soluble complex (Figure 5.8). Although the chemical equilibrium strongly favours complex formation, some free metal ions and chelating agent are present, and utilisation of the free ion by the organism results in further liberation of metal ions from the complex. The chelating agents act as metal buffers in a way analogous to pH buffers, maintaining a constant free ion concentration.

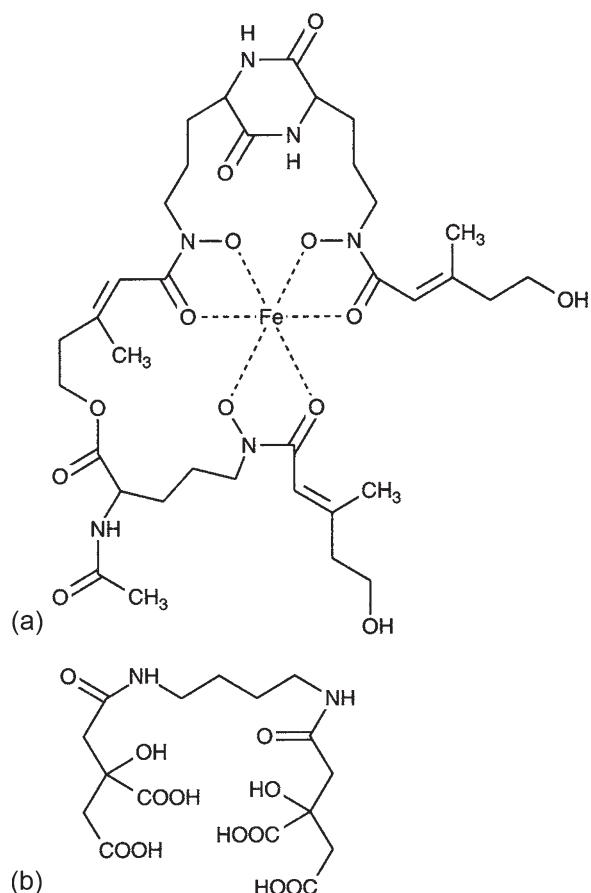


FIGURE 5.8 Fungal siderophores: (a) coprogen, produced by *Neurospora crassa* and some species of *Penicillium*. It is formed by the hydroxylation and acetylation of three L-ornithine molecules, and is an example of a hydroxamate siderophore. These are varied in structure and may originate from 1, 2, or 3 L-ornithine molecules. They are widespread in ascomycetes and basidiomycetes. The figure shows the coprogen-iron complex; (b) Rhizoferrin, produced by Mucorales. It consists of two citric acid molecules linked via amide bonds to putrescine.

Siderophores operate to maintain iron homeostasis. They are released from hyphae when iron availability is limiting growth, and chelate ferric ions in the surrounding solution. Metabolic energy is utilised in transporting the iron–siderophore complex across the plasma membrane into the cell. Siderophores are also involved in the storage of iron in the cell. In some fungi, these siderophores have the same structure as those involved in transport, but in others they differ. Members of the Mucorales (zygomycetes) store iron in their cells by using the iron-binding protein, ferritin, as do plants and animals. Some fungi do not produce siderophores but transport iron into the cell utilizing a ferric reductase at the cell surface. Other fungi release large amounts of the relatively weak chelating agent citric acid into the environment when iron is scarce. Siderophores are essential for virulence of fungal pathogens against animals and plants. Fungi that do not themselves produce siderophores may utilise those produced by other fungi when they are available.

Metals and metalloids can be acquired by fungi from soil minerals and from rock. Secreted organic acids and mechanical action by hyphae combine to solubilise and release cations to make them available for absorption. Metallic cations are strongly adsorbed by fungal cell walls by the physicochemical process of ion exchange. Binding is affected primarily by the valency of the cation, so that trivalent ions displace divalent ions, which in turn displace monovalent cations. Heavy metal pollution can be monitored by analysing fungi or lichens growing in particular environments. Contaminated fungi or lichens can threaten health when used as food by humans.

Some radioactive cations including potassium⁴⁰ and the radioactive element caesium are readily taken up by fungi. For example, the major radionuclide of long-term concern that was released to the atmosphere by the Chernobyl nuclear accident in 1986 is caesium¹³⁷, with a half-life of 30 years. Caesium is accumulated by all living organisms, because of its similarity to potassium. In a survey in 1994–1995 of sites in Russia about 200 km northwest of Chernobyl, levels of ¹³⁷Cs in wild mushrooms were up to a 1000-fold higher than the peak levels in agricultural products. This was reflected in tissue levels of the isotope in the local population, which were 10-fold higher in individuals who ate wild mushrooms regularly compared to those who never did. Even higher levels have been found among the reindeer-herding Samis of northern Scandinavia, where there is a very short food chain from contaminated lichen to reindeer to human. Unexpectedly, analyses in successive years have shown little diminution in isotope levels in lichens, so the problem will be with us for many years to come. Some fungi selectively concentrate particular heavy metals. An example is the common fly agaric, *Amanita muscaria*, which accumulates extraordinarily high levels of vanadium of up to 200 mg per kg.

Growth Factors and Vitamins

Many fungi have rather simple nutrient requirements. Some, as indicated above, need no organic compounds other than a carbon source, and many others have only a few additional needs. It is, however, those organisms which have the simplest needs which are most readily grown in pure culture on defined media. Others have more elaborate requirements, including rarer amino acids and vitamins (substances needed in very small quantities). Many fungi require the vitamins thiamine (e.g. *Phycomyces*) or biotin (e.g. *Neurospora*), water-soluble B vitamins which are important in animal nutrition and were originally isolated from yeast extract. Instances of requirements for most of the other B vitamins are known in the fungi but are less common. Sterols may be necessary, especially under anaerobic conditions. Other requirements may include fatty acids, purines and pyrimidines and inositol.

Autolysis, Autophagy and Apoptosis

These are processes in which cellular protein is broken down to release amino acids for biosynthesis of new proteins. Starved fungal cultures decrease in biomass owing to degradation of cell material by autolysis, and will eventually die if nutrients become exhausted. However, before irreversible autolysis occurs, the cell may show starvation induced changes including the activation of secondary metabolic pathways and the onset of sporulation (see below). In natural environments, starvation may occur in parts of a mycelial system while other parts are well resourced. Foraging systems of basidiomycete mycelial cords can be manipulated in culture to access localised fresh resources, inducing the shut-down of under-resourced parts of the mycelial network. Such nutrient recycling in filamentous fungi can be aided by the process of **autophagy**, a controlled breakdown of protein and organelles that occurs within specialised double membrane-bound vesicles, the **autophagosomes**. These deliver their contents to the vacuole which acts as a lysosomal compartment where proteins are degraded.

Autophagy is conserved across eukaryotes and involves over 30 genes, but only a few of these have been investigated for their function during the growth and development of filamentous fungi. It appears that autophagy serves not only as a pathway for nutrient recycling, but can also be essential for hyphal morphogenesis. The autophagy gene *Atg8* plays a key role when germinating spores of the rice blast pathogen, *Magnaporthe oryzae*, infect leaves (Chapter 8, p.266). Autophagy of the appressorium contents enables cytosolic materials to be relocated from the conidium on the leaf surface into the growing germ tube. The *Atg8* protein has been visualised in fungi using GFP fusions, appearing as dots in the cytoplasm that subsequently move to the vacuole. Caspases, a specialised group of peptidases that operate the process of **apoptosis** or programmed cell death, have recently been found to be involved in antagonistic interactions between different fungal species, and in the development of spores and sporophores.

SECONDARY METABOLISM

Both fungi and plants synthesise innumerable chemically complex substances by means of secondary metabolic pathways that differ from those of primary metabolism in the low substrate specificity of their enzymes. Secondary pathways tend to be branched and interact with one another. One enzyme may catalyse reactions with multiple products, and the products from one reaction may act as substrates for another. This results in the formation of an enormous variety of chemically complex products. Pathways that produce substances with biological activities, such as antibiotics and toxins (Chapter 9, p. 297), appear to have been selected for in habitats where they have conferred some advantage in the interaction of an organism with its neighbours. The genes specifying these pathways have become duplicated with minor changes during species diversification, producing reactions that generate yet more chemical products. Field mycologists identify species according to tiny colour differences – whether, for example, a mushroom is the colour of cinnamon or tobacco – illustrating the taxon-specificity that characterizes many secondary metabolites. Suppressing competitors and attracting dispersing agents are biologically important roles of fungal secondary metabolites. Others that are produced by pathogenic fungi act as virulence factors in disease (Chapter 8). Penicillin and other antibacterial compounds are probably

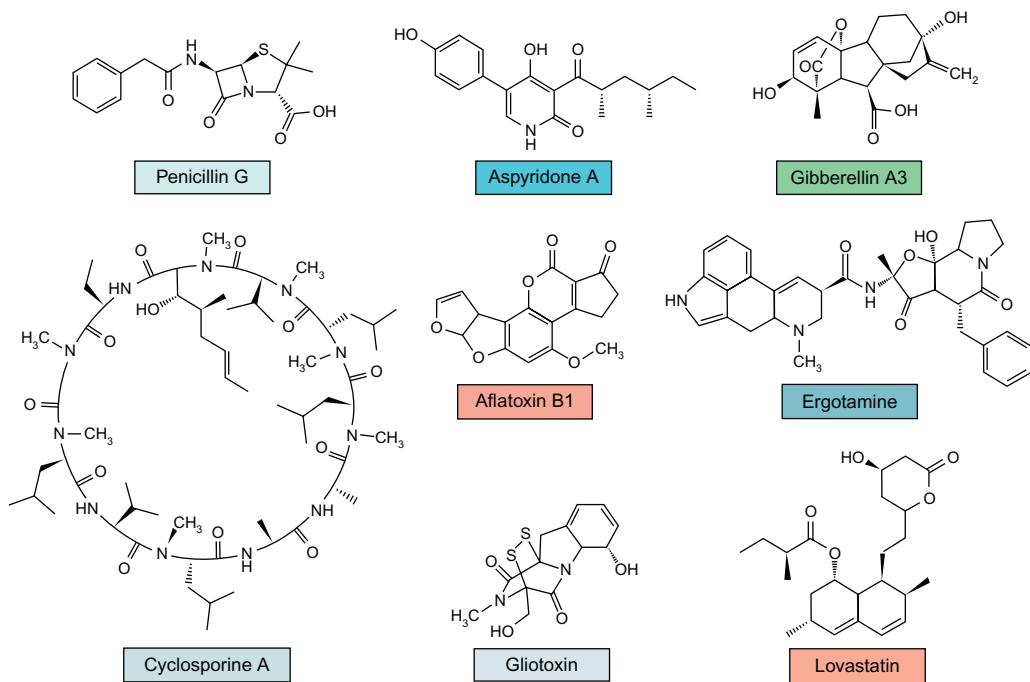
advantageous to soil ascomycetes that produce them, as competitive inhibitors of peptidoglycan synthesis in cell wall synthesis by soil bacteria that compete for carbon substrates. The unique scent and taste of truffles give these ascomycete fruiting bodies a market value of £3000 per kilogram. These fungi have evidently succeeded in attracting mammalian fungivores.

Psilocybin from ‘magic mushrooms’ is a hallucinogen that has long intrigued humans. Recreational use of magic mushrooms is a widespread cultural phenomenon that affects the public perception of mycology. One hundred or so species of *Psilocybe*, including the liberty cap, *Psilocybe semilanceata*, contain an alkaloid called psilocybin whose structure resembles the neurotransmitter serotonin. Psilocybin is converted into psilocin after ingestion, which is thought to be the psychoactive compound. When psilocin binds to a pair of serotonin receptors in the central nervous system, it causes a temporary reduction in blood flow to certain parts of the brain that diminishes neurological activity. This stimulates cross-talk between different regions of the brain resulting in the perception of colours when listening to music. Other common experiences include visions of geometric patterns, altered perception of the passage of time, and stimulation of memory. In a clinical setting, the majority of test subjects who ingested purified psilocybin reported very positive experiences and some ranked their hallucinations among the most elevating experiences of their lives. This finding has encouraged researchers to investigate the potential uses of psilocybin in the alleviation of anxiety and the treatment of clinical depression.

Magic mushrooms have been used in Mesoamerican religious practices. In the Aztec religion, hallucinogenic mushrooms were regarded as the flesh of the gods, or teonanácatl, which fostered communication between the gods and their priests. Descriptions by eighteenth century explorers suggest that the fly agaric mushroom, *Amanita muscaria*, was used as an intoxicant in the Russian Far East. The primary psychoactive compound in this species is muscimol, which has different effects on the central nervous system from psilocybin. Muscimol crosses the blood-brain barrier, binds to the GABA_A receptor and interferes with the transmission of nerve impulses. The resulting elevation of serotonin and dopamine levels induces feelings of weightlessness and changes in size perception. Claims about more widespread ritual uses of hallucinogenic mushrooms, including their worship by Neolithic cultures in Europe, are not supported by critical archaeological and anthropological research.

Psilocybin and muscimol synthesis have both evolved independently in a number of mushroom lineages. Outside the genus *Psilocybe*, which is classified in the family Hymenogastraceae, psilocybin is produced by some species of *Panaeolus* (family Psathyrellaceae) and *Inocybe* (Inocybaceae), and occurs in at least five other families in the Agaricales. Muscimol is similarly widespread among unrelated basidiomycetes. The repeated emergence of psilocybin and muscimol synthesis suggests that these alkaloids have some adaptive significance, but their role in fungal physiology is unknown. The most compelling idea is that they act as antifeedants that protect fruit bodies from insect damage, but this is not bolstered by any experimental data.

Despite their huge variety, most secondary metabolites fall into three broad chemical types: polyketides, non-ribosomal peptides, and terpenes. Some examples of bioactive secondary metabolites are shown in [Figure 5.9](#). The low diversity of synthetic pathways contrasts with the enormous diversity of products. A possible evolutionary explanation for this paradox is that any bioactive product that is only a step away from primary metabolism would be more likely to interfere with metabolism and be eliminated by natural selection. However, once a



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FIGURE 5.9 Examples of secondary metabolites produced by fungi. The clinically used antibiotic penicillin is produced by *Penicillium chrysogenum*. Other clinically used secondary metabolites include immunosuppressants such as the cyclosporines and the cholesterol-reducing compound lovastatin, produced by *Tolypocladium inflatum* and *Aspergillus terreus*, respectively. Many secondary metabolites have adverse toxic activities, such as the aflatoxins produced by *Aspergillus flavus* and gliotoxin, produced by *Aspergillus fumigatus*. The toxicity of gliotoxin has been attributed to a disulphide bridge that is the functional motif of this metabolite. Aspyridones, from *Aspergillus nidulans*, have moderate cytotoxic activity. Gibberellins are plant hormones that are also produced by fungi such as *Fusarium fujikuroi*. Ergot alkaloids are produced by several fungi, including (most prominently) *Claviceps purpurea*, which produces ergotamines. Light grey indicates non-ribosomal peptide derivatives; dark grey represents a non-ribosomal peptide derivative that requires a tryptophan dimethylallyltransferase for synthesis; red (dark grey in the print version) represents polyketide derivatives; blue (dark grey in the print version) represents a mixed polyketide–non-ribosomal peptide compound; green (dark grey in the print version) represents a gibberellin, for which synthesis involves terpene cyclase but no non-ribosomal peptide synthetase or polyketide synthase. Source: Brakhage (2013).

secondary metabolic pathway is established in a lineage of organisms, selection is likely to favour its elaboration, because any bioactive intermediates will then be less likely to interfere with primary metabolism.

Genes encoding secondary metabolic pathways are clustered in the genome, enabling their expression and cellular function to be coordinately regulated. Figure 5.10 shows the steps in synthesis of atromentin, a secondary metabolite characteristic of Boletales, and Figure 5.11 shows the arrangement of the gene cluster governing this biosynthesis. A variety of atromentin-derived molecules are produced by Boletales (Figure 5.12), and endow the sporophores of this group, including those of porcini, *Boletus edulis*, and also the dry rot fungus, *Serpula lacrymans*, with

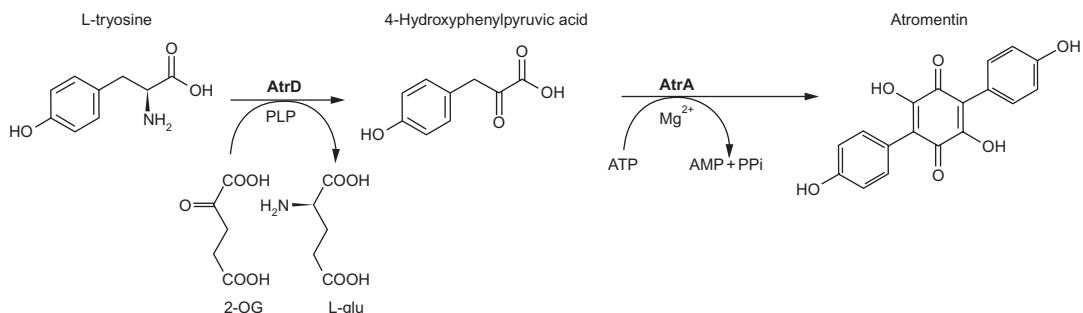


FIGURE 5.10 The pathway of atromentin synthesis in *Tapinella* (Boletales). *Tapinella panuoides* (Boletales) synthesises the secondary metabolite atramentin from L-tyrosine derived from the shikimic acid pathway. Source: Schneider et al. (2008).

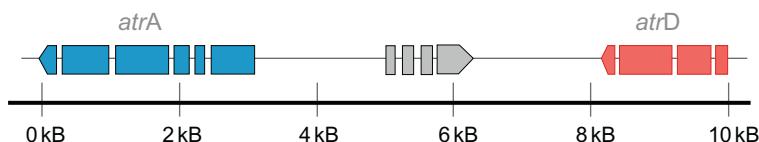


FIGURE 5.11 Genes encoding biosynthetic enzymes for atromentin in *Tapinella*. Genetic map of atromentin biosynthetic genes in *Tapinella panuoides*. The dark grey arrows represent atrA and atrD. The intron positions within the genes are indicated as spaces between arrow segments. The reading frame between atrA and atrD, shown in light grey, codes for a putative alcohol dehydrogenase. Source: Schneider et al. (2008).

their characteristic yellow and brown coloration. The expression of the antibiotic secondary metabolite bikaverin has been investigated in the ascomycete genus *Fusarium*, which includes soil saprotrophs and plant pathogens. Many *Fusarium* species synthesise this red polyketide pigment and have multiple genes encoding its synthesis. In an analysis of the *Fusarium fujikuroi* genome, the bikaverin gene cluster was found to include genes not only for bikaverin synthesis, but also for regulating its biosynthesis in response to nitrogen starvation and acid pH, and for secreting bikaverin via an efflux pump. Fungal endophytes of plants (described in detail in Chapter 7) produce a plethora of bioactive secondary metabolites. In some cases these are also produced by the host plant, the gene cluster encoding their biosynthesis having apparently undergone horizontal gene transfer (see Chapter 4). The gene cluster for biosynthesis of the diterpenoid tumour suppressant taxol is present and expressed both in yew trees, (*Taxus* sp.), and also in their fungal endophyte. The presence of a plastid-targeted signal sequence in one of the enzymes indicates that the pathway for taxol synthesis originated in the plant.

Secondary metabolic pathways are typically expressed in response to environmental cues (Figure 5.13). Developmental processes may be simultaneously regulated, for example when sporophore development is accompanied by the synthesis of new spore wall components, and when fruit bodies acquire pigments, flavours, and toxins. The genetics underlying regulation has been intensively studied for decades in the model fungus *Aspergillus nidulans*, which shows light-induced asexual sporulation, while in the dark it produces both the sexual fruiting bodies and the carcinogenic aflatoxin precursor sterigmatocystin. Development is under the control of the velvet gene family, conserved across all fungi, which encode transcription factors. From genetic and experimental investigation, it emerges that development and secondary

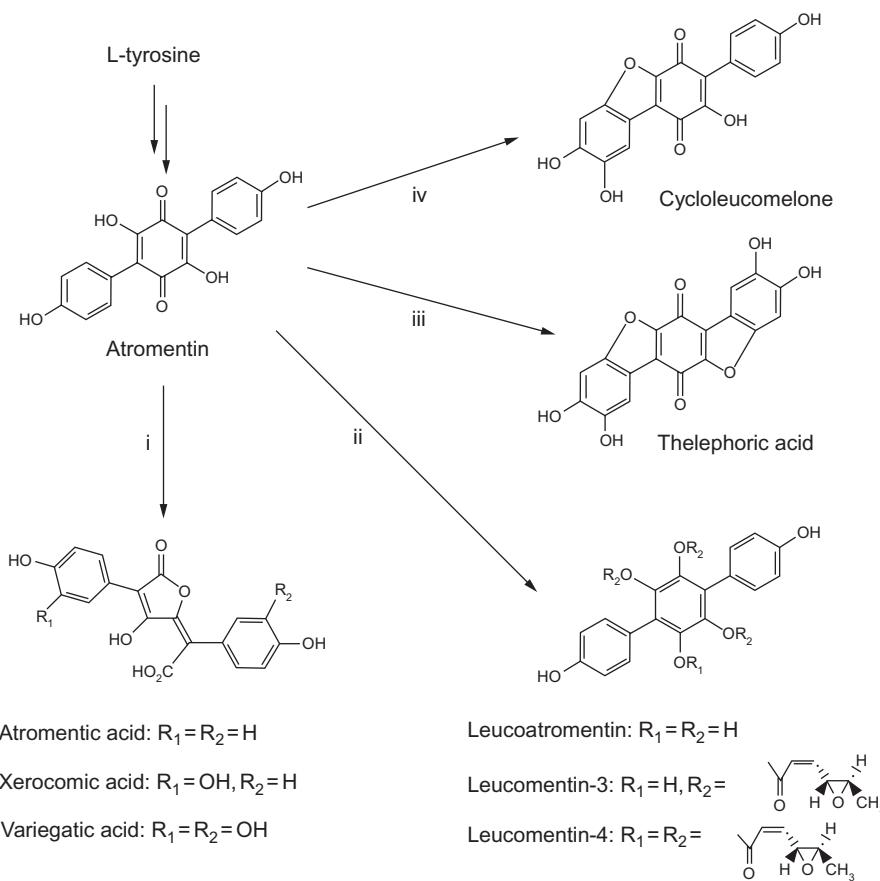
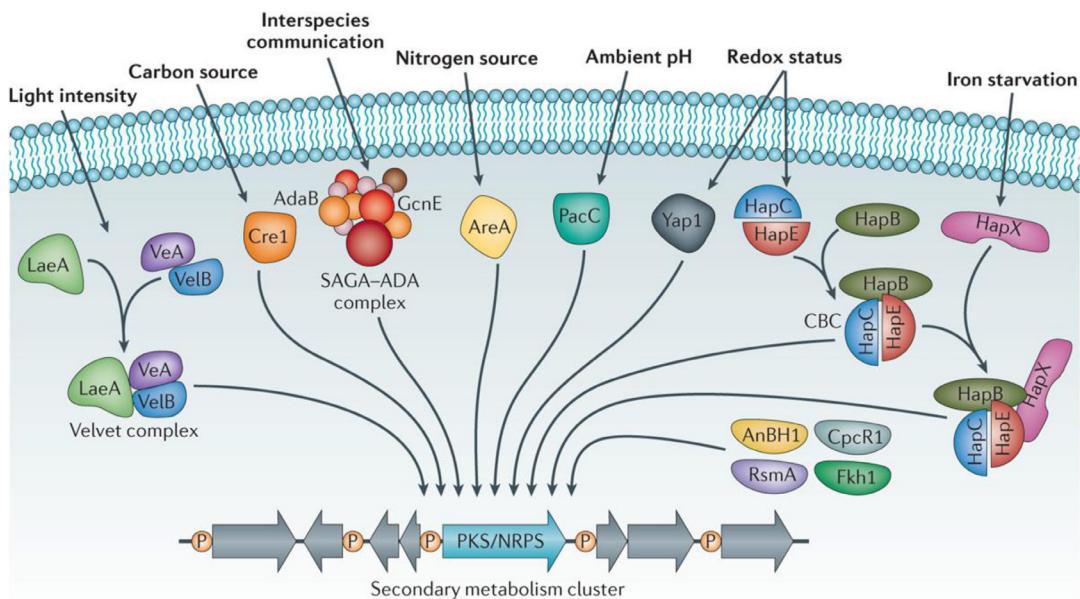


FIGURE 5.12 Synthesis of atromentin derivatives in Boletales. Atromentin and examples of derivatives. Atromentin can undergo modifications such as (i) oxidative ring splitting into atromentic acid, which upon sequential hydroxylation yields xerocomic, and variegetic acid, (ii) reduction and esterification to produce leucoatromentins and leucomentins, (iii) dihydroxylation followed by symmetric heterocyclization for production of thelephoric acid, or (iv) hydroxylation and single heterocyclization to yield cycloleucomelone. Source: Schneider et al. (2008).

metabolism interact through velvet and LaeA proteins, which bind to form a trimeric heteropolymer. In this form, LaeA can cross the nuclear membrane to act directly on DNA.

Mining the full genome sequences of fungi indicates that their potential to produce secondary metabolites is greatly underestimated. Gene clusters related to secondary metabolism are being explored for genes potentially exploitable in biotechnology for the production of bioactive products. Most of the biosynthesis gene clusters are silent under laboratory conditions, leading to a search for the physiological conditions that activate these genes. A direct experimental demonstration of the function of secondary metabolism in an ecological interaction was the finding that silent clusters of biotechnologically interesting PKS genes in the genome of *A. nidulans*, which are not transcribed in sterile conditions, could be induced by contact with soil streptomycetes. Substances induced by this microbial interaction include the polyketide orsellinic acid, the lichen metabolite lecanoric acid, and cathepsin K inhibitors.



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FIGURE 5.13 Regulation of secondary metabolism by environmental factors. Environmental signals can influence the regulation of various secondary metabolism gene clusters through regulatory proteins that respond to these environmental stimuli and, in turn, modulate the expression of the clusters. Shown is a model secondary metabolism gene cluster containing a gene encoding a central non-ribosomal peptide synthetase (NRPS), a polyketide synthase (PKS), or a hybrid PKS-NRPS enzyme. CBC, CCAAT-binding complex; CpcR1, cephalosporin C regulator 1; LaeA, loss of *aflR* expression A; RsmA, restorer of secondary metabolism A; SAGA-ADA, Spt-Ada-Gcn5-acetyltransferase-ADA. Source: *Brakhage* (2013).

SENSING AND RESPONDING TO THE ENVIRONMENT

Fungi sense their environment with cellular systems homologous with animals, and can perceive most of the same stimuli as human cells, including levels of extracellular and intracellular nutrients, pheromones, pH, water and oxidative stress, temperature, light intensity and spectral composition, gravity, and touch.

Nutrient sensing

Nutrient sensing is essential for the opportunistic lives of fungi. In its ever-changing environment the cell and mycelium must be able to respond to the external availability of the different nutrients it requires. Internal homeostasis requires the cell to sense and respond to fluctuating intracellular levels of metabolites, and to physical variables including water potential and pH.

Sensors for extracellular glucose enable hyphae to reduce wasteful enzyme release by repressing the synthesis and secretion of cellulases and other enzymes. Several mechanisms

sense ambient glucose. The membrane protein Gpr1, a G-protein coupled receptor (GPCR), is probably conserved across fungi and acts as a receptor to sense glucose and sucrose. It initiates an intracellular signalling pathway that activates adenylyl cyclase to raise cellular cAMP and activate protein kinase. Fungi also have the hexose transporter gene family *HXT*, which encodes sugar transporters with varied affinities and expression patterns that respond to the amount and type of available sugar. Homologues without transport function, conserved across ascomycetes and basidiomycetes, also act as sugar sensors, or **transceptors**, that regulate the expression of *HXT* genes through the transcription suppressor protein Rgt1. Genes involved in trehalose metabolism also mediate fungal responses to sugars. The Tps1 enzyme protein, trehalose-6-phosphate synthase, is found to act as a central regulator of plant infection in the rice blast fungus, *Magnaporthe oryzae*, (Chapter 8) where it functions as a sugar sensor. Moreover, in this plant pathogen, Tps1 was found to have an additional role in integrating carbon and nitrogen metabolism, via control of the pentose phosphate pathway. This equips the fungus to adapt to nutritional and redox conditions inside the host cell during infection. GPCRs also sense pheromones. In ascomycetes and basidiomycetes, GPCR binding of peptide pheromones initiates signal cascades leading to sexual morphogenesis, taxes, and tropisms to bring mating partners together and orchestrate nuclear fusion (Chapter 4).

Amino acids are sensed with a three gene encoded system, *SPS*, consisting of a transceptor *Ssy1*, which on binding an amino acid activates a protease, *Ssy5*. *Ssy5* activates transcription factors, leading to the expression of amino acid transporters and pathways for amino acid metabolism. In this way, the cellular machinery for uptake is coordinately activated but only when it is required. In the human pathogen *Candida albicans*, this amino acid sensing pathway is required for virulence, presumably because it alerts the fungus to the presence of host tissue. Specific amino acids may act as cues, sensed via a G-protein coupled receptor Gpr1, controlling the switch from yeast to hyphal morphology that allows the fungus to invade tissue. Nitrogen availability is sensed by Mep cell surface ammonium transporters described above. Mep2 is expressed under conditions of low ammonium supply and is believed to act as a transporter-driven sensor which triggers filamentation ([Figures 5.14](#) and [5.15](#)).

Intracellular nutrient levels

Intracellular nutrient levels change continuously as fungi forage in a heterogeneous environment. Changes in cellular nutrient status not only induce coordinated regulation of cellular systems involved in nutrient acquisition and metabolism, but also have developmental effects that include changes in cell shape, cell cycle progression, the initiation of spore development, the formation of sporophores and multicellular tissues, and starvation-induced autolysis and autophagy. Such responses are central to fungal niche adaptation, allowing the mycelium to optimize the spatial and temporal allocation of its available resources to accommodate to its changing environment.

Intracellular carbon nutrients are sensed via the cAMP-PKA pathway, and nitrogen nutrients by the *Tor* (Target of rapamycin) signalling pathway. The central role of the *Tor* protein in cellular responses to nitrogen supply was discovered through studies of the antibiotic rapamycin, which was originally investigated as a tumour suppressant. The *Tor* gene encodes a kinase that controls protein phosphorylation cascades that regulate numerous growth

processes. Fungi have two homologues of *Tor*, *Tor1* and *Tor2*. Each is a multiprotein complex. *Tor*-mediated signalling is activated in the nitrogen replete cell and upregulates multiple cell functions including protein synthesis, ribosome biogenesis, yeast dimorphic transitions, and cell cycle progression. Inactivation of *Tor* signalling by rapamycin or starvation arrests growth and activates the autophagy pathway. Regulation is by control of the entry of transcription factors to the nucleus, mediated by phosphorylation and dephosphorylation reactions. *Tor* signalling is likely to be involved in mediating morphogenetic responses to changes in external nitrogen availability.

Physical Factors

Water

All fungal activities are dependent on the regulation of cellular water content. The hydration and dehydration of fungal colonies is determined by differentials in water availability between the organism and its surroundings. Water potential, ψ , is the recommended term for quantifying water availability, defined as the potential energy of water per unit volume. The benchmark of zero water potential refers to pure water at atmospheric pressure. Water potential is reduced to negative values by dissolved solutes; water potential is increased by hydrostatic pressure. The difference in hydrostatic pressure between a cell and its surroundings is called turgor pressure (also known as pressure potential, ψ_p). The water potential of a cell is determined by its osmotic potential (ψ_π), proportional to the concentration of dissolved solutes, and its turgor pressure according to the following equation:

$$\psi = \psi_\pi + \psi_p$$

All three terms are expressed as pressures with the Pascal (Pa = Nm⁻²) as the SI unit. These simple considerations are often complicated by the use of the term osmotic pressure, which is equal in magnitude to osmotic potential but opposite in sign, and its confusion with turgor: osmotic pressure is not synonymous with turgor. Water potential is also affected by interactions at solid–liquid interfaces such as the surface of colloids. The term matric potential has been used to represent these effects, but, in most cases, the influence on the solid phase contributes to the osmotic potential and turgor of the cell, and is not measured separately. The matric potential of liquid or solid culture media is insignificant, but it becomes an important parameter in soils and can be measured with an instrument called a tensiometer.

Water moves into a hypha by osmosis in response to a differential in water potential. When the osmotic potential of the cytoplasm is lower than the fluid surrounding the hypha, there is a net influx of water and turgor pressure rises until the water potential of the hypha matches its surroundings. As the hypha grows, its turgor pressure tends to fall, but physiological adjustment of the osmotic potential maintains relatively constant turgor. Osmotic adjustment, or regulation, involves solute uptake and excretion as well as the synthesis of compatible solutes including sugar alcohols.

Under most circumstances, the water potential of an active mycelium is closely matched to the water potential of its surroundings. The turgor pressure of the constituent hyphae can be measured directly using a pressure probe (see Chapter 2), or estimated from the difference

between the osmotic pressure of the cytoplasm and the surrounding fluid phase. For fungi growing at atmospheric pressure, the external water potential is equal to osmotic potential because the pressure potential term is zero:

$$\psi_{(\text{outside})} = \psi_{\pi(\text{outside})} = \psi_{(\text{inside})} = \psi_{\pi} + \psi_p$$

and, mycelial turgor pressure = $\psi_{\pi(\text{outside})} - \psi_{\pi(\text{inside})}$

Osmotic potential of fungal samples can be measured very accurately using osmometers. Some of these determine osmotic potential from the depression of the freezing point of a sample of cytoplasm, or other fluid, relative to pure water. However, vapour pressure deficit osmometers are superior for most applications. These measure the vapour pressure of water in equilibrium with the fungal sample, from the lowering of the temperature necessary to reach the dew point.

Research on fungal water relations has been complicated by the use of alternative terminology. Food microbiologists, for example, prefer to use the term water activity (a_w) to quantify water availability. This is the ratio of water vapour pressure between the sample (p_s) and pure water (p_w):

$$a_w = \frac{p_s}{p_w}$$

Water activity varies from zero (no water) to 1.0 (pure water). Water activity is related to water potential using the following logarithmic equation:

$$\text{water potential}(\psi) = k \ln a_w$$

k is a temperature-dependent constant; $k=1.35$ at 20°C and 1.37 at 25°C .

The availability of water in the gas phase is expressed in terms of relative humidity. Relative humidity is equivalent to the water activity represented as a percentage (e.g. $a_w=0.75=75\%$ relative humidity).

WATER POTENTIAL AND THE FUNGAL ENVIRONMENT Fungal activity, whether it is manifested as plant disease, mouldiness of materials, rotting of wood, or the appearance of mushrooms in fields and woodlands, is most obvious in damp conditions. This is consistent with experiments demonstrating that most fungi grow best at high water potentials, above -1 MPa . Commonly used media, including those containing 2% sucrose, have a water potential in this range; 0.4M sucrose, equivalent to a 14% solution, has a water potential of -1 MPa . At lower water potentials, rates of hyphal growth diminish, until values are reached at which growth does not occur (Table 5.3). Most wood-destroying fungi, for example, cannot grow at water potentials below -4 MPa . The inhibition of fungal growth at low water potentials is the basis for such traditional methods of food preservation as drying and the addition of salt or sugar. A few fungi, however, are adapted for growth at very low water potentials. These are usually termed **osmophiles** or **xerophiles**, although most are really osmotolerant, growing best at relatively high water potentials. Many yeasts and species of *Aspergillus* (teleomorph *Eurotium*) and *Penicillium* (*Talaromyces*) are osmotolerant and are important agents of biodegradation. Metagenomic analysis of very dry habitats in the high Andes revealed a group of fungi nested within the Spizellomycetales, an order of chytrids. This was particularly

TABLE 5.3 Water Availability in Different Environments and Approximate Lower Limits for Growth of Some Fungi

Water activity	Water potential (MPa)	Examples
1.0	0	Pure water
0.996	-0.5	<i>Phytophthora cactorum</i> , lower limit
0.995	-0.7	Typical mycological media
0.98	-2.8	Sea water
0.97	-4	Most wood-destroying fungi, lower limit
0.95	-7	Bread. Leaf-litter Basidiomycetes, lower limit
0.90	-14	Ham. <i>Neurospora crassa</i> , lower limit
0.85	-22	Salami. <i>Saccharomyces rouxii</i> in NaCl solution, lower limit
0.80	-30	<i>Aspergillus nidulans</i> and <i>Penicillium martensii</i> , lower limits
0.75	-40	Saturated NaCl solution, <i>Aspergillus candidus</i> , lower limit
0.65	-60	22 molal glycerol
0.60	-69	Limit for cell growth – <i>Zygosaccharomyces rouxii</i> in sugar solutions and the mould <i>Monascus (Xeromyces) bisporus</i>
0.58	-75	Spores of some <i>Eurotium</i> , <i>Aspergillus</i> , and <i>Penicillium</i> species are able to survive for several years
0.55	-80	Saturated glucose solution. DNA denatured
0.48	-90	Antarctic dry valleys

Data from various sources. Molality (molecular weight in grams per 1000 g solute) and not molarity (MW in g per final volume of 1000 ml) is used in dealing with osmotic potentials. The lower limits of growth are those obtained at optimal temperature and nutrition; when these factors are sub-optimal, the limits are not so low. As indicated with *Saccharomyces rouxii*, organisms are usually more tolerant of high sugar than high salt concentrations.

unexpected because most Chytridiomycota are freshwater organisms. It is tempting to speculate that this discovery of apparently xerotolerant chytrids came from resistant spores deposited in herbivore dung. The most osmotolerant fungi known can grow at a water potential of -69 MPa. The dry rocky valleys of Antarctica, with a soil water potential of about -90 MPa, are sterile, except for transient microbial growth following snowfall and in relatively humid microenvironments such as cracks in rocks. The spores of some fungi are able to escape desiccation through development of impermeable walls, and can survive bone dry conditions until moisture allows growth to resume.

ADAPTATION TO CHANGES IN EXTERNAL WATER POTENTIALS The water potential of a growing fungus is a little lower than the water potential of its surroundings, driving water influx and permitting cell expansion. The external water potential may fall, however, through evaporation, increasing the concentration of solutes dissolved in the ambient medium. If this reverses the gradient in water potential, water will leave the cell and growth will cease. The cell membrane may detach from the inner surface of the cell wall, an effect

known as plasmolysis, and desiccation and cell death may occur. If dew or rain increases the external water potential, the cell will absorb water and its hydrostatic pressure, or turgor, will rise. If this occurs swiftly and the cell is poorly adapted to low external water potential, the increase in turgor pressure may rupture the cell wall. Fungi that live in some habitats are exposed to wide fluctuations in water potential and must adjust their cytoplasmic osmotic potential to cope with these environmental circumstances. Lichens are especially well adapted for life in habitats that experience extreme changes in environmental water potential. Desert lichens, for example, are active photosynthetically for a brief time at dawn, when their thalli containing the photobiont cells are damp with dew, but become desiccated in the morning and remain inactive until dampened again the next day.

One way in which the osmotic potential of a fungal cell can be lowered is by the uptake of dissolved solutes from the environment. *Thraustochytrium aureum*, a chytrid that lives in brackish water and in the sea, can adjust its internal osmotic potential by the uptake of inorganic ions. At high concentrations, however, inorganic ions and many other solutes can change the configuration and catalytic activity of enzyme molecules. Hence, where very low cytoplasmic osmotic potentials are required, many fungi synthesise polyhydric alcohols (polyols), which are 'compatible solutes' that have little effect on enzyme activity even at high concentrations. These polyols may be produced from sugars taken up from the growth medium or from the breakdown of polymeric reserves. The polyol concerned with osmotic adjustment in the moderately osmotolerant yeast *Saccharomyces cerevisiae* as well as the highly osmotolerant species *Zygosaccharomyces rouxii* is glycerol. Other polyols important in osmotic adaptation in fungi are mannitol and arabitol. When an increase in the internal osmotic potential of a fungus is needed, this can be brought about by the loss or export of solute to the environment, or by conversion of the solute to an insoluble reserve material.

Molecular genetic responses of fungi to hyper- and hypoosmotic stress (drying and wetting, respectively) were first investigated in *Saccharomyces cerevisiae*. Yeast cells respond to hyperosmotic stress by temporary cessation of growth, with disassembly of the actin cytoskeleton and loss of cell polarity, decrease in cell wall porosity and membrane permeability to glycerol, and accumulation of glycerol. The chitin content of the cell wall increases too. Yeast cells detect and respond to high and low extracellular osmolarity by activating two different MAPK signalling pathways. High osmolarity activates the HOG (high osmolarity glycerol) pathway. This pathway exhibits multiple redundancies, starting at the sensor level, where two independent branches activate the MAPK signalling cascade: a putative transmembrane osmosensor, and a protein phosphorylation relay system which is inhibited by hyperosmotic stress. In both cases, the result is activation of the MAPK cascade, which in turn activates the *HOG1* gene. The *HOG1* protein in turn induces transcription of several genes, including *GDAP1*, the structural gene for glycerol 3-phosphate dehydrogenase involved in glycerol synthesis. The cell then accumulates glycerol which lowers its osmotic potential to restore water influx. Accompanying responses include a decrease in membrane permeability to glycerol. Hypoosmotic stress activates the protein kinase C (PKC) signal transduction pathway. The PKC pathway is activated by a range of other stimuli such as nutrient stress, and it is thought that a major role for this molecular response is the maintenance of cell integrity by controlling the assembly of the cell wall and plasma membrane synthesis.

It has already been emphasised that the cell wall is a dynamic structure that must be maintained to resist mechanical and chemical stress. The modifications required for this entail

activation of enzymes that synthesise cell wall polymers, and the vectorial transport of vesicles that carry wall and membrane components. The stretching of the cell membrane caused by hypoosmotic stress is thought to be detected by a mechanosensor, which activates a small GTP-binding protein, Rho1, which controls the activity of the Pkc1 protein and a $\beta(1 \rightarrow 3)$ glucan synthase. Pkc1 activates the MAPK cascade, and a result is the activation of chitin synthase genes for cell wall biosynthesis, proteins involved in mannosylation and a GPI-anchored membrane protein. Activities of these proteins produce a stronger wall with higher chitin content. Chitin synthases from a range of fungi are also activated quickly following hypoosmotic stress, probably from preexisting inactive forms, giving a rapid increase in wall strength. Genomic analysis has allowed comparison of HOG pathway component genes across a number of fungal species. This reveals subtle differences between the proteins comprising the pathway, many of which are likely to have adaptive significance. Apart from raising glycerol content, responses mediated via the HOG pathway in filamentous fungi include the onset of conidiation, the ability to infect and invade plant cells, and secondary metabolite production, including the induction of aflatoxin synthesis in *Aspergillus*.

Fungal cells control their water potential not only as a stress response but also as an essential part of 'normal' growth and development. In addition to regulating their levels of compatible solutes, fungi can also control their water content via changes in the permeability of the cell membrane and cell wall. Gene expression associated with developmental water partitioning between cells has been analysed in the plant pathogen *Magnaporthe oryzae*. Development accompanying leaf infection involves a series of controlled changes in the water potential of adjacent cells. First the appressorium inflates and drives the infection peg through the plant cell wall, and then it deflates as the cytoplasm moves into the mycelium that develops in the plant (Chapter 7). **Aquaporins**, membrane proteins that channel water and small solutes, have been investigated in *Saccharomyces* and the mycorrhizal fungus *Laccaria bicolor* (see Chapter 7). *L. bicolor* has seven aquaporins that differ in substrate specificity and regulation, and are likely to be important in integrating host and symbiont physiology.

Light

Light is a vital environmental cue for development in all organisms. In the fungal kingdom, light responses are particularly important to ensure that spores for dispersal are produced in the open air and not buried in the substratum. Fungal physiology is highly responsive to light, with light-induced effects including change in asexual conidiation, the circadian clock, sexual development, and secondary metabolism. Light is sensed over the whole spectrum, from ultraviolet to far-red, and over a range of intensity from starlight to bright sunshine. Detailed investigation at molecular level has revealed three light sensing systems in fungi, based respectively on flavin-based photoreceptors for blue light, phytochrome for red light, and opsins related to the rhodopsin of animals and some archaea.

Blue-light responses are found in all organisms including prokaryotes and eukaryotes. The fungal blue-light response has been investigated in detail in *Neurospora crassa*, in which effects include carotenoid synthesis, induction and phototropism of protoperithecia, induction of hyphal growth, asexual sporulation, and the entrainment of the circadian clock. The best-described blue-light receptor in the fungal kingdom is the protein White Collar 1 (WC-1). The WC (white collar) genes WC-1 and WC-2 were discovered in from *Neurospora* mutants unable to produce mycelial carotenoids in the light, resulting in a 'white collar' round the colony

margin. WC-like proteins are conserved at sequence and functional level across basidiomycetes, ascomycetes, and zygomycetes. They incorporate a flavin-based photoreceptor which absorbs blue light causing a reaction from the protein. From sequence analysis, WC-1 and WC-2 proteins are GATA-type transcription factors, and share further domains that interact during the response to light. WC genes are essential components of the *Neurospora* circadian system which regulates cellular processes so that they follow a 24-h cycle. The **circadian** system is a biological clock that enables organisms to measure time, anticipate diurnal changes in environmental factors, and regulate growth, sporulation, and associated patterns of gene expression. Biological rhythms are termed circadian when the periodicity is set endogenously and is not affected by changes in nutrient availability or temperature variations. The rhythm can be entrained by manipulating light or temperature conditions to set the clock. WC proteins interact with a very large number of clock control genes including a central timekeeping oscillator in the form of rhythmically expressed *frq* genes. This system has been intensively investigated in the *Neurospora* clock, producing a detailed model of the circadian system of intracellular feedback loops between environmental cues and cellular processes.

Red-light responses mediated by phytochrome are widespread in plants, which use phytochromes to respond to the balance between red and far-red light. Phytochromes are photoreceptor proteins with a linear tetrapyrrole as the chromophore. Similar red light sensing has been found in fungi. *Aspergillus nidulans*, as described above, forms abundant asexual conidia in light at wavelength 680 nm, but at 740 nm, or in the dark, produces sexual spores and toxic secondary metabolites. Phytochromes have been identified in the genomes of several fungal species, and have been analysed in detail in *Neurospora* and *Aspergillus*. *Aspergillus nidulans* has a single phytochrome gene, *fphA*, which appears to be involved in suppressing sexual development, because deletion results in a mutant phenotype which produces sexual spores in the light.

Fungi also have light-absorbing pigments, **opsins**, related to rhodopsin, the transmembrane-light-absorbing proteins used for light sensing in the animal retina and for energy transduction in archaeal prokaryotes. Opsins are conserved across ascomycetes and basidiomycetes and are believed to have originated from prokaryotes by horizontal gene transfer. Their role in fungi is not well understood. The opsin NOP-1 from *Neurospora crassa* absorbs green light, but deletion of *NOP-1* has no known phenotypic effect.

Apart from responding to light as an environmental cue, fungi have mechanisms for protecting the cell from its damaging effects. Fungi are exposed to potentially damaging ultraviolet light when sporulating on exposed surfaces and when spores travel long distances through air. Spore walls are protected by light-absorbing secondary metabolites including melanin, carotene and sporopollenin. Melanin is also produced as a response to ionising radiation.

Temperature

Fungi are exposed to a wide range of temperatures in natural environments that affect water availability. Many species cannot grow at temperatures above 30–40 °C. Fungi that can grow near freezing point or even a little below are termed psychrotolerant (from the Greek, *psychros*, cold), and if incapable of growth above 20 °C, psychrophilic. A group of fungi called snow moulds grow on vegetation such as grass or unharvested crops when these are buried by snow. These can be problematic when they kill grass or produce mycotoxins. Many cold-tolerant yeasts are known and some of them, including some basidiomycete

yeasts, are psychrophilic. Yeasts are among the few microorganisms found in the cold, dry valleys of Antarctica. Temperatures well above 40°C are often encountered in accumulations of decomposing vegetation and compost. *Mucor pusillus*, *Chaetomium thermophile*, and *Thermoascus aurantiacus* are examples of species that play an important role in the succession of organisms involved in successful composting. Fungi are described as thermotolerant if they are capable of growth at 50°C or higher temperatures, and thermophilic if incapable of growth below 20°C. The highest temperature at which fungal growth has been recorded is 60°C. The vast majority of fungi that are neither psychrophilic nor thermophilic, favour intermediate temperatures and are termed mesophiles. Mesophiles can be psychrotolerant or thermotolerant.

Phylogenetic analysis shows that thermophily is restricted to a few groups of fungi including ascomycetes in the Sordariales, Onygenales, and Eurotiales, and zygomycetes classified in the Mucorales. Culture-independent analyses of fungal communities in hot natural habitats may well discover others.

Carbon Dioxide

Carbon dioxide is involved in signalling as well as in respiration. Its concentration increases around mycelia respiring in confined spaces, and it can act as a cue for morphogenesis. The morphogenetic effect of CO₂, particularly on sporulation and sporophore development, is well known, but the cellular and molecular mechanisms have not been investigated until recently. In the human pathogens *Candida albicans* and *Cryptococcus neoformans*, carbon dioxide sensing is found to be essential for virulence. In host tissues the carbon dioxide level can vary a hundredfold. Carbon dioxide enters the cell passively, by diffusion. CO₂ / HCO₃⁻ homeostasis involves carbonic anhydrase, a zinc metalloenzyme which catalyses conversion of CO₂ to the bicarbonate used as a substrate in biosynthetic carboxylation reactions. High levels also mediate signalling pathways that induce *C. albicans* to form hyphae and *C. neoformans* to produce capsules resistant to phagocytosis. Bicarbonate stimulates adenylyl cyclase to produce cAMP, which then activates a protein kinase to induce further steps mediating development.

pH

While the pH of the surrounding environment may vary widely, cellular function demands a constant internal pH. Fungal cells operate a pH-homeostatic system. This system also senses ambient pH and adjusts gene expression so that secreted enzymes and bioactive metabolites are released only at pH levels that permit function. The genes and cellular processes involved have been characterised in *Aspergillus nidulans*, *Saccharomyces cerevisiae*, and *Candida albicans*. Ambient pH is sensed by a complex of membrane proteins and a further endosomal complex mediates signal transduction. A transcriptional regulator (*PacC* in *A. nidulans*, *Rim101p* in *S. cerevisiae*) activates genes expressed under acidic conditions and represses them under alkaline conditions. The pH regulatory system is crucial to fungal pathogenicity in animals and plants. In *C. albicans*, genome-wide transcriptional profiling identified 514 pH-responsive genes, including those expressed in iron acquisition and invasive hyphal growth.

Fungi can affect environmental pH by secreting protons and organic acids. Brown rot basidiomycetes that secrete oxalic acid can acidify a culture medium to pH as low as 2.5. It has

been suggested that this ability aids wood decay by tipping the ionic equilibrium towards a preponderance of ferrous iron that participates in cellulose breakdown through a Fenton's reaction, as described above. Fungal acidification of the environment can cause mobilisation and leaching of soil cations, and mineral transformations in rock are mediated through pH changes as described below.

DEVELOPMENTAL ADAPTATION FOR NUTRIENT ACQUISITION

Fungi feed by growing into fresh food. Alterations in form are necessary to adapt to the resources available. Nutrient sensing and developmental responses are critical in niche adaptation. We illustrate this concept with two well-studied examples, at opposite ends of the fungal size range: dimorphism in a pathogenic yeast, and the development of vast mycelial networks by basidiomycetes inhabiting the forest floor.

Dimorphic yeasts

Dimorphic yeasts are able to switch from unicells to filamentous hyphae in order to penetrate solid materials by means of tip growth. The ascomycete yeast *Candida albicans* causes thrush, affecting the mucous membranes (Chapter 9, p.302). It can grow either as unicellular budding yeast, or in the filamentous form (Figure 5.14) in which it invades epithelial

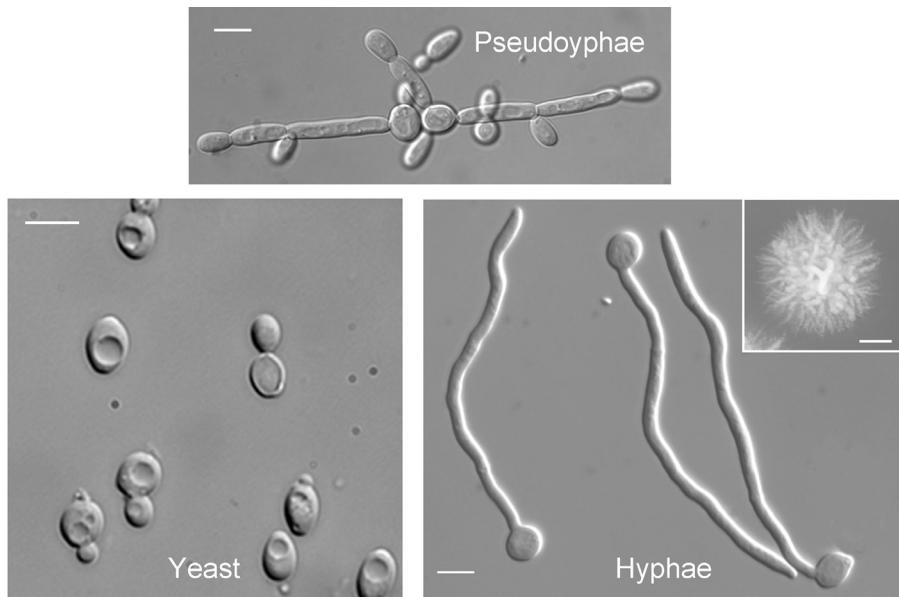


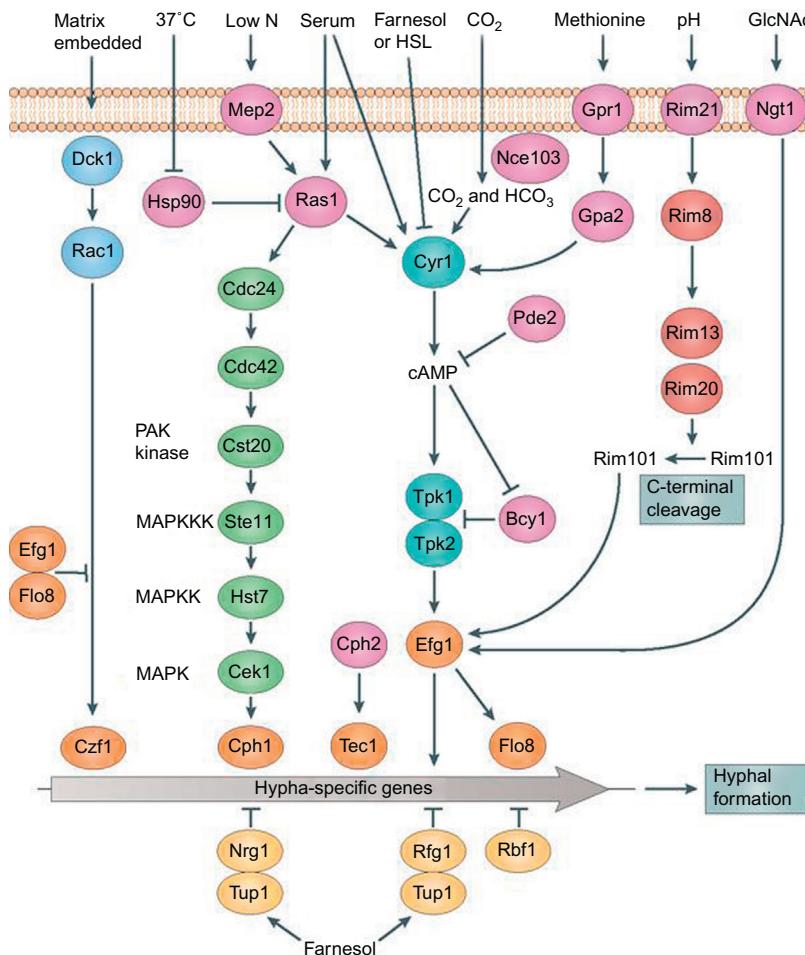
FIGURE 5.14 Yeast, pseudohyphal, and hyphal form of *Candida albicans*. Source: Sudbery (2011).

cells of the mucosa. It also uses the hyphal growth form to escape from macrophages that engulf the yeast phase. The switch between growth forms can be readily manipulated in liquid and solid culture, which makes it possible to analyse the process at the cellular and molecular level. A range of chemical and physical cues induce the switch from the yeast to hyphal form. These include starvation, growth in solid medium, and presence of *N*-acetylglucosamine. Cues encountered in mammalian tissue include the presence of serum and amino acids, microaerophilic conditions, neutral pH, a temperature around 37 °C, and carbon dioxide (at levels found in the bloodstream). Hyphal development is inhibited in cells growing close together, when farnesol acts as a cue for quorum sensing. Farnesol is a sesquiterpenoid secondary metabolite secreted by the cells. Molecular genetic methods have uncovered an integrated set of sensors and signal transducing pathways that act via positive or negative transcription factors on a gene, *Hgc1*, to regulate the expression of the cellular machinery for polarised hyphal growth ([Figure 5.15](#)). In addition, *N*-acetylglucosamine can induce hyphal growth directly, independently of *Hgc1*. Polarised hyphal growth, described in detail in Chapter 2, requires the assembly of a spitzenkörper and its accompanying apparatus of oriented cytoskeleton and secretory vesicle exocytosis, as well as control of nuclear division and septation as the cell elongates.

Cord-forming basidiomycetes

Cord-forming basidiomycetes, in contrast to yeasts, include the biggest and longest lived fungal individuals known. Honey fungus belonging to the genus *Armillaria* is well known to gardeners and foresters as it kills plants by colonising and killing woody roots. Its mycelium can spread over many metres by growing through soil to invade and destroy adjacent plants. Householders are familiar with the dry rot fungus, *Serpula lacrymans*, which infects damp softwood in buildings, and like *Armillaria* can grow across intervening non-nutrient spaces to colonise and decay successive timbers. Both fungi provide examples of a common mode of growth among woodland basidiomycetes that utilise living roots or fallen trees as massive carbon sources. Sugar translocation from the food base fuels the extension of the mycelium of these fungi and they scavenge mineral nutrients as they grow. When the hyphae that compose the advancing margin of the mycelium encounter and colonise fresh food, the intervening connecting mycelium aggregates and differentiates to form a nutrient-translocating pipe called a mycelial cord. As the fungus extends across the forest floor, more and more connections are made until the mycelium takes the form of a network of mycelial cords. Each cord is composed of tens to hundreds of aligned hyphae. The area occupied by a single clone (though not its connectedness) can be established by genetic analysis of samples from the forest floor, and may reach many metres.

When mycelia are grown in cultures designed as microcosms of the forest floor, they show a remarkable degree of overall coordination across the entire network. As they develop, older, exhausted parts of the mycelium regress by autolysis, and growth is directed preferentially in the directions where fresh resources have been reached. Species vary in the topology of their networks according to their preferred resources. Leaf litter decomposers like *Hypholoma fasciculare* forage over short ranges with many short cords, while species such as *Megacollybia platyphylla* that utilise large logs may produce cords that extend long distances from log to log with far fewer branches and connections ([Figure 5.16](#)).



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FIGURE 5.15 Signal transduction pathways leading to expression of hypha-specific genes in *Candida albicans*. Multiple sensing and signalling mechanisms that regulate the developmental response of the pathogenic yeast *Candida albicans* to its environment. Environmental cues feed through multiple upstream pathways to activate a panel of transcription factors. The cyclic AMP-dependent pathway that targets the transcription factor enhanced filamentous growth protein 1 (Efg1) is thought to have a major role. In this pathway, adenylyl cyclase integrates multiple signals in both Ras-dependent and Ras-independent ways. Negative regulation is exerted through the general transcriptional corepressor Tup1, which is targeted to the promoters of hypha-specific genes by DNA-binding proteins such as Nrg1 and Rox1p-like regulator of filamentous growth (Rfg1). Protein factors are colour-coded as follows: mitogen-activated protein kinase (MAPK) pathway (green, dark grey in the print version), cAMP pathway (turquoise, dark grey in the print version), transcription factors (orange, grey in the print version), negative regulators (yellow, light grey in the print version), matrix-embedded sensing pathway (light blue, grey in the print version), pH sensing pathway (brown, dark grey in the print version), other factors involved in signal transduction (mauve, dark grey in the print version), C-terminal, carboxy-terminal; Cdc, cell division control; GlcNAc, N-acetyl-D-glucosamine; Gpa2, guanine nucleotide-binding protein α -2 subunit; Gpr1, G-protein-coupled receptor 1; HSL, 3-oxo-homoserine lactone; Hsp90, heat shock protein 90; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; PAK, p21-activated kinase; Rbf1, repressor-activator protein 1. Source: Sudbery (2011).

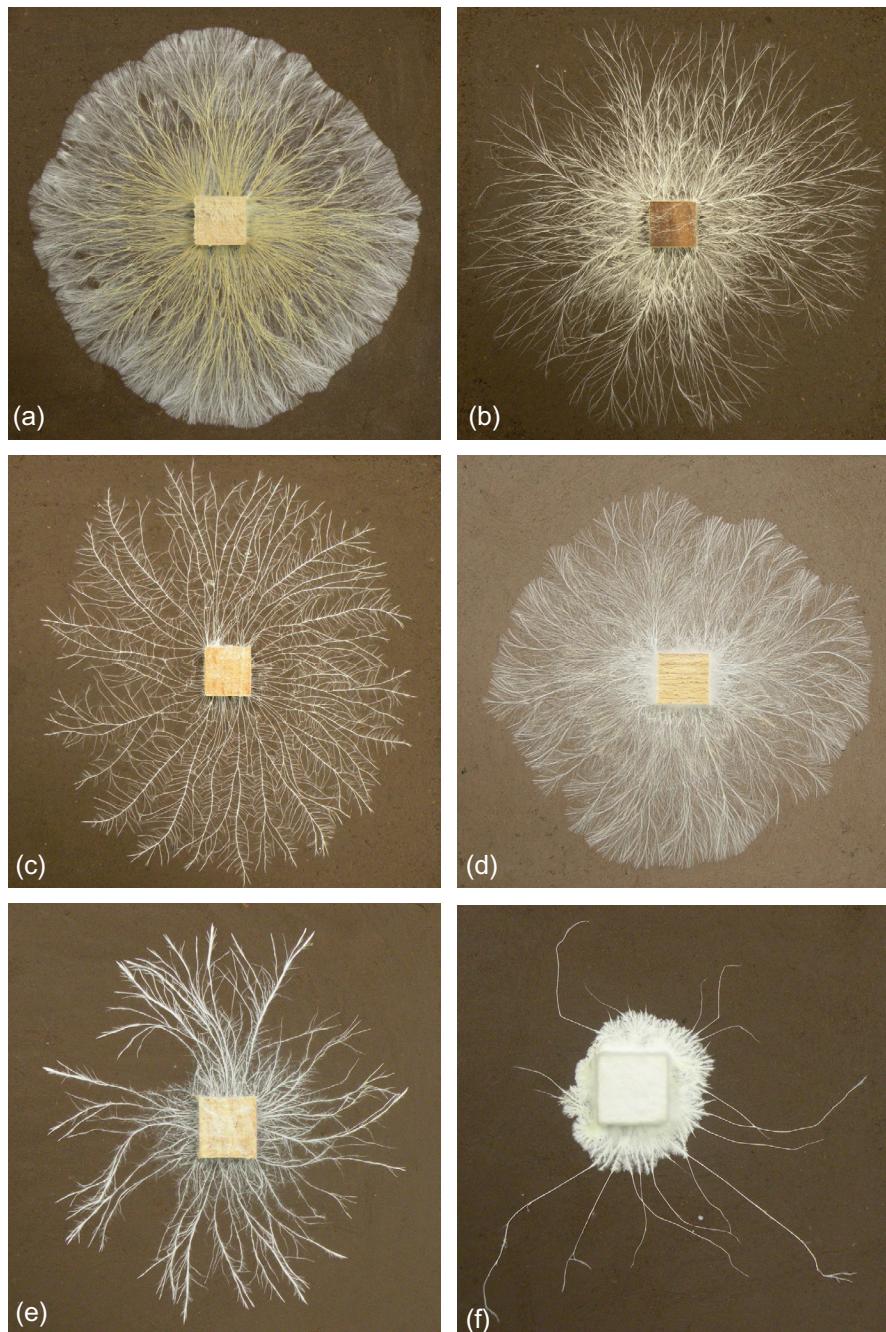


FIGURE 5.16 Mycelial cord-forming networks of six woodland basidiomycetes with varying network form related to foraging strategy. (a) *Hypholoma fasciculare*, (b) *Coprinopsis picacea*, (c) *Phallus impudicus*, (d) *Phanerochaete velutina*, (e) *Resinicium bicolor*, (f) *Megacollybia platyphylla*. Mycelium is shown growing from colonised wood blocks across soil, in microcosms designed to mimic natural conditions. Source: Photo: A.'Bear.

The corded systems of saprotrophic and mycorrhizal woodland fungi scavenge and accumulate mineral nutrients, as described in Chapter 7. They may retain most of the available nitrogen and phosphate in the ecosystem. Nitrogen equivalent to an annual agricultural fertiliser input may be held in fungal biomass. Network topology responds to nitrogen and phosphorus availability in the soil, with the mycelium showing diffuse growth of hyphae in patches where nutrients are abundant, and extending faster and more thinly in locations where nutrients are limited. Cords are differentiated and consist of three distinguishable hyphal types (Figure 5.17). Wide 'vessel' hyphae, apparently empty of contents, run in the centre, surrounded and interspersed with hyphae showing living cell contents. Fibre hyphae, with wall thickening that almost occludes the lumen, run longitudinally in a band around the periphery of the cord. By using radioactive tracers C^{14} , P^{32} , and K^{40} nutrient translocation has been shown to occur in both directions along a cord and to respond to supply and demand in different sites within the network. Figure 5.18 shows a mycelium of the cord forming fungus *Serpula lacrymans* extending over sand from a colonised block of wood. The whole mycelium is allowed to take up sub-toxic amounts of a radiolabelled, non-metabolisable amino acid, 2-aminoisobutyric acid, detected by photon counting scintillation imaging and recorded in a video trace. The labelled amino acid tracks the flow of

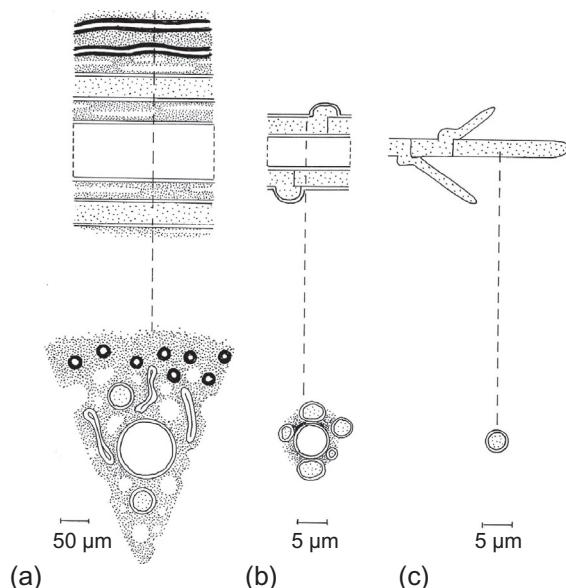


FIGURE 5.17 Cartoon showing the pattern of hyphal differentiation during cord formation and the relationship between different hyphal types in mature cords. (a) Structure of mature cord. Vessel hyphae apparently empty of contents and tendril hyphae with visible cytoplasm are run longitudinally in an extracellular matrix which is permeated by longitudinal spaces. Some hyphae appear to have collapsed. Thick-walled "fiber" hyphae are run longitudinally in the outer layers of the cord. (b) A cord starting to develop approximately 50 mm behind the mycelial margin. Wider and relatively empty "vessel" hyphae become surrounded by thigmotropic "tendril" hyphae with denser cytoplasm, which grow both acropetally and basipetally over the vessel hypha surface. Extracellular matrix material binds aggregations of vessel and tendril hyphae into cords. (c) Assimilating, extending hyphal tips forming a diffuse mycelium of separate hyphae at the advancing mycelial margin. (Source: Drawing courtesy of Rosemary Wise.)

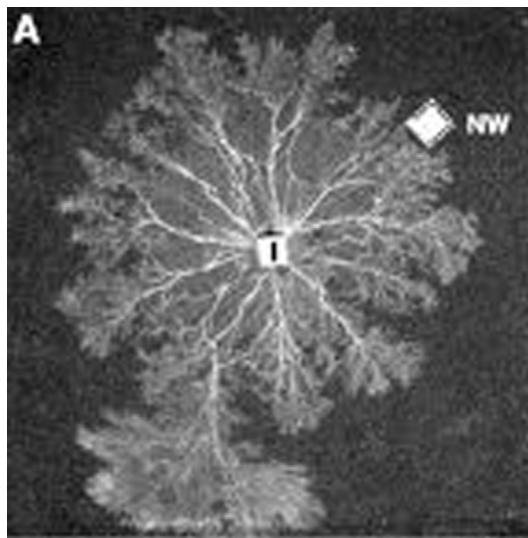


FIGURE 5.18 Mycelium of the cord forming fungus *Serpula lacrymans* growing out over sand from a colonised block of wood. See also [Figure 5.7](#) which shows these mycelial cords functioning as channels for the rapid import of mycelial amino acid into the freshly-colonised wood, visualised by photon-counting scintillation imaging. I, the original inoculum block and food source for the mycelium, NW, a new wood block that the mycelium will meet and colonise. The video in [Figure 5.7](#) shows the reallocation of amino acid within the mycelium following its capture of this new resource, and the rapid flow along mycelial cords.

the amino acid pool within the mycelium in real time. When a fresh wood block is presented to the edge of the colony it becomes colonised, and the majority of the organism's amino acids are rapidly translocated to the new source of food ([Figure 5.18](#)). The speed of translocation suggests that mass flow is significant, although the location of the mass flow pathway and the mechanism of loading and unloading remain unclear. Fungal accumulation and redistribution of plant nutrients through mycelial networks can affect plant growth, particularly of plants connected via a common mycorrhizal network (Chapter 7). Fungal translocation can facilitate the decomposition of nitrogen-poor plant remains, by importing nitrogen scavenged by hyphae in contact with soil ([Figure 5.7](#)). Sugar import can also provide the energy to enable hyphae to assimilate nitrogen. These observations show that the mycelium can act as a responsive resource-supply network with a key role in carbon and nitrogen dynamics in forests.

GEOMYCOLOGY

Geomycology is the study of fungal processes important in geology. Fungi are essential in global element cycling and soil fertility ([Table 5.4](#)). The effect of fungi on minerals has been studied mainly in aerobic terrestrial environments. Growing hyphae acidify their surroundings, as we have seen earlier. They release protons and exude carboxylic acids, and respiratory carbon dioxide produces carbonic acid in water. Together with the invasive abilities of hyphae, this makes some saprotrophic fungi capable of pioneering growth on rock. Many are

TABLE 5.4 Summary of Important Roles and Activities of Fungi in Geomycological Processes

Fungal attribute or activity	Geomycological consequences
GROWTH	
Growth and mycelium development; fruiting body development; hyphal differentiation; melanization	<p>Stabilisation of soil structure</p> <p>Penetration of rocks and minerals</p> <p>Biomechanical disruption of solid substrates, building stone, cement, plaster, concrete, etc.</p> <p>Plant, animal and microbial colonisation, symbiosis and/or infection; mycorrhizas, lichens, pathogens</p> <p>Nutrient and water translocation</p> <p>Surfaces for bacterial growth, transport, and migration</p> <p>Mycelium acting as a reservoir of N and/or other elements</p>
METABOLISM	
Carbon and energy metabolism	<p>Organic matter decomposition and cycling of component elements, e.g. C, H, O, N, P, S, metals, metalloids, radionuclides</p> <p>Altered geochemistry of local environment, e.g. changes in redox, O₂, pH</p> <p>Production of inorganic and organic metabolites, e.g. H⁺, respiratory CO₂, organic acids, siderophores</p> <p>Exopolymer production</p> <p>Organometal formation and/or degradation</p> <p>Degradation of xenobiotics and other complex compounds</p>
Inorganic nutrition	<p>Altered distribution and cycling of inorganic nutrient elements (e.g. N, S, P, essential and inessential metals, metalloids, organometals and radionuclides)</p> <p>Transport, accumulation, incorporation of elements into macromolecules</p> <p>Redox transformations of metal(lloid)s and radionuclides</p> <p>Translocation of water, N, P, Ca, Mg, K, etc., through mycelium and/or to plant hosts</p> <p>Fe(III) capture by siderophores</p> <p>MnO₂ reduction</p> <p>Element mobilisation or immobilisation including metals, metalloids, radionuclides, C, P, S, etc.</p>
Mineral dissolution	<p>Mineral and rock bioweathering</p> <p>Leaching/solubilisation of metals and other components (e.g. phosphate)</p> <p>Element redistributions including transfer from terrestrial to aquatic systems</p> <p>Altered bioavailability of (e.g. metals, P, S, Si, and Al)</p> <p>Altered plant and microbial nutrition or toxicity</p> <p>Mineral formation (e.g. carbonates, oxalates, clays)</p> <p>Altered metal and nutrient distribution, toxicity, and bioavailability</p> <p>Mineral soil formation</p> <p>Biodeterioration of building stone, cement, plaster, concrete, etc.</p>
Mineral formation	<p>Element immobilisation including metals and radionuclides, C, P, and S</p> <p>Mycogenic carbonate formation</p> <p>Limestone calcrite cementation</p> <p>Mycogenic metal oxalate formation</p> <p>Metal detoxification</p> <p>Contribution to patinas on rocks (e.g. 'desert varnish')</p>
PHYSICO-CHEMICAL PROPERTIES	
Sorption of soluble and particulate metal species, soil colloids, clay minerals, etc.	<p>Altered metal distribution and bioavailability</p> <p>Metal detoxification</p> <p>Metal-loaded food source for invertebrates</p> <p>Prelude to secondary mineral nucleation and formation</p>

TABLE 5.4 Summary of Important Roles and Activities of Fungi in Geomycological Processes—cont'd

Fungal attribute or activity	Geomycological consequences
Exopolymer production	Complexation of cations Provision of hydrated matrix for mineral formation Enhanced adherence to substrate Clay mineral binding Stabilisation of soil aggregates Matrix for bacterial growth Chemical interactions of exopolymers with mineral substrates
SYMBIOTIC ASSOCIATIONS	
Mycorrhizas	Altered mobility and bioavailability of nutrient and inessential metals, N, P, S, etc. Altered C flow and transfer between plant, fungus, and rhizosphere organisms Altered plant productivity Mineral dissolution and metal and nutrient release from bound and mineral sources Altered biogeochemistry in soil-plant root region Altered microbial activity in plant root region Altered metal distributions between plant and fungus Water transport to and from the plant
Lichens	Pioneer colonisers of rocks and minerals, and other surfaces Bioweathering Mineral dissolution and/or formation Metal accumulation by dry or wet deposition, particulate entrapment, metal sorption, transport, etc. Enrichment of C, N, P, etc. in thallus and alteration of elemental concentrations and distribution in local microenvironment Early stages of mineral soil formation Development and stimulation of geochemically-active microbial populations Mineral dissolution by metabolites including 'lichen acids' Biomechanical disruption of substrate
Insects and invertebrates	Fungal populations in gut aid degradation of plant material Invertebrates mechanically render plant residues more amenable for decomposition Cultivation of fungal gardens by certain insects (organic matter decomposition and recycling) Transfer of fungi between plant hosts by insect vectors (aiding infection and disease)
PATHOGENIC EFFECTS	
Plant and animal pathogenicity	Plant infection and colonisation Animal predation (e.g. nematodes) and infection (e.g. insects, etc.) Redistribution of elements and nutrients Increased supply of organic material for decomposition Stimulation of other geochemically-active microbial populations

These processes may take place in aquatic and terrestrial ecosystems, as well as in artificial and man-made systems, their relative importance depending on the species and active biomass present and physico-chemical factors. The terrestrial environment is the main site of fungal-mediated biogeochemical changes, especially in mineral soils and the plant root zone, decomposing vegetation, and on exposed rocks and mineral surfaces. There is rather a limited amount of knowledge on fungal geobiology in freshwater and marine systems, sediments, and the deep subsurface. In this Table, fungal roles have been arbitrarily split into categories based on growth, organic and inorganic metabolism, physico-chemical attributes, and symbiotic relationships. It should be noted that many if not all of these are linked, and almost all directly or indirectly depend on the mode of fungal growth (including symbiotic relationships) and accompanying chemoorganotrophic metabolism, in turn dependent on a utilisable C source for biosynthesis and energy, and other essential elements, such as N, O, P, S and many metals, for structural and cellular components. Mineral dissolution and formation are detailed separately although these processes clearly depend on metabolic activity and growth form. (Gadd, 2011)

microcolonial, frequently melanized forms, capable of growing both as filaments and yeasts. They can grow only slowly, as they depend on scarce organic nutrient sources from the atmosphere and from other microbes. Their hyphae respond thigmotropically to microscopic pores and fissures, penetrating into the mineral structure and progressively eroding it with mechanical pressure and acid exudates. Fungal deterioration and bioweathering can affect rocks and minerals including carbonates, silicates, phosphates, and sulphides. These processes are part of the early stages of mineral soil formation, but also cause deterioration of building stone, cement, plaster, and concrete.

In mycorrhizas and lichens (Chapter 7), where symbiosis with a photobiont provides a continuous and reliable source of energy, hyphae can act continuously over long periods to dissolve nutrients from soil mineral particles and from rock. Nutrients obtained from minerals include anions, including phosphate and sulphate, and cations of essential metals, including potassium, calcium, and magnesium. In this way, ectomycorrhizal forest fungi can mine the underlying rock and minerals to supply phosphate to their host plants, in exchange for sugars produced by photosynthesis. This exchange is facilitated by the extensive translocating systems of many basidiomycetes, as described above, which enable them to access deep soil horizons and bedrock. The fungal partner in lichens growing on bare rock can similarly acquire mineral nutrients through solubilisation of nutrients, fuelled by the photobiont's photosynthesis.

Fungi can alter the spatial distribution of inorganic substances in soil. Acidification releases metals, including aluminium and iron, from complexes with clay particles in soil, allowing cations to be leached through soil horizons by water. Subsequent immobilisation at lower levels produces the layered horizons of acid **podsols** characteristic of forest soils over acid rock in regions of high rainfall. Fungal activity can also immobilise metal cations in exopolysaccharides deposited on the cell surface and lead to the formation of new minerals. Whewellite and weddellite have both been produced by fungi in culture and are considered to originate from fungal activity in nature. Fungal mobilisation and immobilisation of minerals is exploited in some approaches to removing toxic **xenobiotic** metals, metalloids and organometallic compounds from soil and water.

Xenobiotics

Water and soil are contaminated with increasing amounts of **xenobiotic** substances resulting from human activities. Organic xenobiotics include polycyclic aromatic hydrocarbons, halogenated solvents, endocrine-disrupting agents and drugs, explosives, and agricultural chemicals. Inorganic xenobiotics are metals, metalloids including arsenic and selenium, organometallic compounds, and radionuclides. Metals are released by mining, smelting and the disposal of metal waste. Many xenobiotic substances are toxic. Recently there has been concern about endocrine-disrupting agents in wastewater, which are bioactive at low concentrations and are not retained by water treatment plants. Microbes are promising agents for bioremediation of xenobiotic pollution, but bacteria have been the main focus of research. Strains of bacteria have been engineered with suites of enzymes to degrade toluene and other organic pollutants in soil. The potential uses of fungi deserve more research. Exploratory hyphae that can penetrate soil micropores and bridge air gaps, seem ideally suited for scavenging pollutants from contaminated soil. Unlike bacteria, fungi can translocate

carbon energy sources to sites of activity from distant points of uptake, enabling them to colonise the spatially heterogeneous environment of soil. Moreover, natural selection has favoured the evolution of fungi that secrete powerful oxidising enzymes of low substrate specificity to scavenge nutrients from recalcitrant compounds. This reduces the need to develop substrate-specific strains. Applications that have been explored include the use of white rot fungi to degrade polycyclic aromatic hydrocarbons in soil and to decolorize wastewater from the Kraft process for paper manufacture. However, many other fungi can degrade toxic organic compounds in the environment and have not been tested as agents for xenobiotic bioremediation. Bioprospecting in the environment is likely to uncover many fungi with a capacity for dismantling resistant manmade molecules. Metals cannot be broken down but may be separated from susceptible biota or complexed into forms that are unlikely to be processed in natural food webs (see *Essential Metals*). Plants inoculated with selected strains of mycorrhiza can be used to reduce concentrations of heavy metal pollutants such as cadmium from soil.

Fungal enzymes that might be suitable for future applications in xenobiotic detoxification include those with intracellular activities: multiple mixed-function cytochrome P₄₅₀ mono-oxygenases, phenol 2-mono-oxygenases, nitro reductases, quinone reductase, reductive dehalogenases, and miscellaneous transferases. Even though intracellular, these enzymes show wide substrate specificity. For example, fungal mixed-function cytochrome P₄₅₀ oxidases can catalyse epoxidation and hydroxylation of numerous pollutants, including dioxins and polycyclic aromatic hydrocarbons. They can also catalyse the degradation of anti-inflammatory drugs, lipid regulators, anti-epileptic and analgesic pharmaceuticals, suggesting the possibility of new environmental applications for the superlative scavenging activity of hyphae.

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Links

Assembling the Fungal Tree of Life: <http://aftol.org/>
MycoCosm: a fungal genomics portal (<http://jgi.doe.gov/fungi>), developed by the US Department of Energy Joint Genome Institute to support integration, analysis and dissemination of fungal genome sequences and other ‘omics’ data by providing interactive web-based tools. Grigoriev, I.V., Nikitin, R., Haridas, S., Kuo, A., Ohm, R., Otiillar, R., Riley, R., Salamov, A., Zhao, X., Korzeniewski, F. 2014. MycoCosm portal: gearing up for 1000 fungal genomes. *Nucl. Acids Res.* 42, D699–D704.

The Carbohydrate-Active Enzyme (CAZy) database: <http://www.cazy.org/> describes the families of structurally-related catalytic and carbohydrate-binding modules (or functional domains) of enzymes that degrade, modify, or create glycosidic bonds.

FOLy: An integrated database for the classification and functional annotation of fungal oxidoreductases potentially involved in the degradation of lignin and related aromatic compounds <http://www.sciencedirect.com/science/article/pii/S1087184508000066>

Figure 5.7. Photon Counting Scintillation Imaging of ¹⁴C-AIB in a fungal colony during capture of a new wood source. Tlalka, M., Fricker, M.D., Watkinson, S.C., 2008. Imaging of long-distance [alpha]-aminoisobutyric acid translocation dynamics during resource capture by *Serpula lacrymans*, *Appl. Environ. Microbiol.* 74, 2700–2708. <http://dx.doi.org/10.5072/bodleian:d217qq90r>