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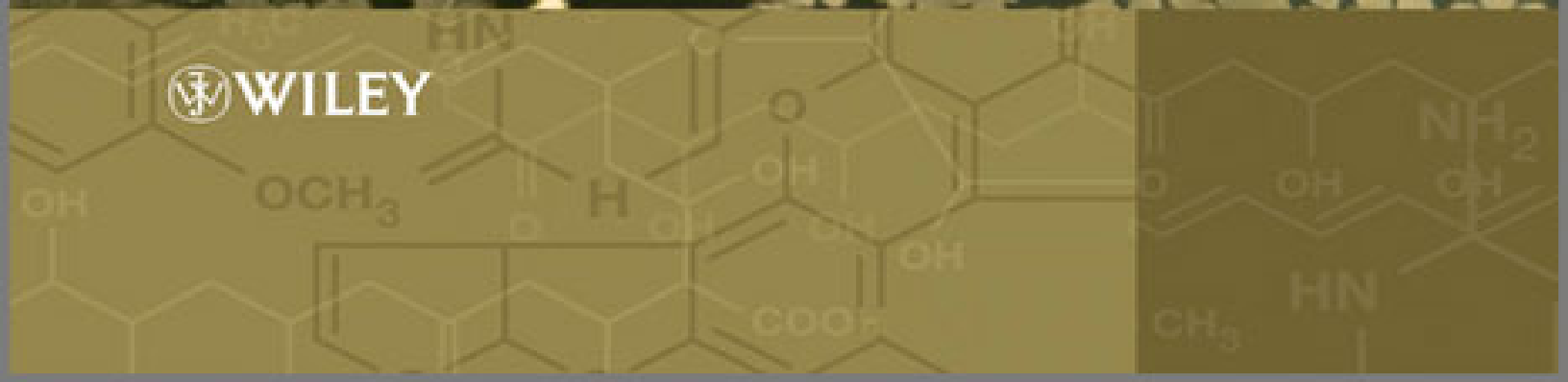
Kevin Kavanagh

# Fungi

Biology and Applications

Second Edition

 WILEY

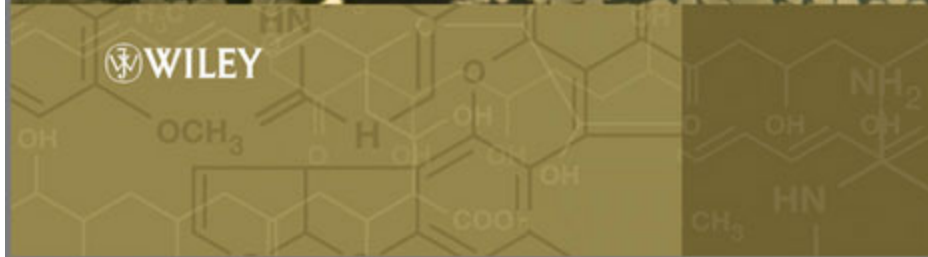


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Kevin Kavanagh

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# Fungi

Biology and Applications

Second Edition

Editor

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# ***1***

## ***Introduction to Fungal Physiology***

**Graeme M. Walker and Nia A. White**

### **1.1 Introduction**

Fungal physiology refers to the nutrition, metabolism, growth, reproduction and death of fungal cells. It also generally relates to interaction of fungi with their biotic and abiotic environment, including cellular responses to stress. The physiology of fungal cells impacts significantly on the environment, industrial processes and human health. In relation to ecological aspects, the biogeochemical cycling of carbon in nature would not be possible without the participation of fungi acting as primary decomposers of organic material. Furthermore, in agricultural operations, fungi play important roles as mutualistic symbionts, pathogens and saprophytes, where they mobilize nutrients and affect the physico-chemical environment. Fungal metabolism is also responsible for the detoxification of organic pollutants and for bioremediating heavy metals in the environment. The production of many economically important industrial commodities relies on the exploitation of yeast and fungal metabolism, and these include such diverse products as whole foods, food additives, fermented beverages, antibiotics, probiotics, pigments, pharmaceuticals, biofuels, enzymes, vitamins, organic and fatty acids and sterols. In terms of human health, some

yeasts and fungi represent major opportunistic life-threatening pathogens, whilst others are life-savers, as they provide antimicrobial and chemotherapeutic agents. In modern biotechnology, several yeast species are being exploited as ideal hosts for the expression of human therapeutic proteins following recombinant DNA technology. In addition to the direct industrial exploitation of yeasts and fungi, it is important to note that these organisms, most notably the yeast *Saccharomyces cerevisiae*, play increasingly significant roles as model eukaryotic cells in furthering our fundamental knowledge of biological and biomedical science. This is especially the case now that numerous fungal genomes have been completely sequenced, and the information gleaned from fungal genomics and proteomics is providing valuable insight into human genetics and heritable disorders. However, knowledge of cell physiology is essential if the functions of many of the currently unknown fungal genes are to be fully elucidated.

It is apparent, therefore, that fungi are important organisms for human society, health and well-being and that studies of fungal physiology are very pertinent to our understanding, control and exploitation of this group of microorganisms. This chapter describes some basic aspects of fungal cell physiology, focusing primarily on nutrition, growth and metabolism in unicellular yeasts and filamentous fungi.

## **1.2 Morphology of Yeasts and Fungi**

Most fungi are filamentous, many grow as unicellular yeasts and some primitive fungi, such as the chytridomycetes, grow as individual rounded cells or dichotomous branched



chains of cells with root-like rhizoids for attachment to a nutrient resource. Here, we will consider the most common growth forms: the filamentous fungi and unicellular yeasts.

## **1.2.1 Filamentous Fungi**

The gross morphologies of macrofungi and microfungi are very diverse (see [Plate 1.1](#)). For example, we can easily recognize a variety of mushrooms and toadstools, the sexual fruiting bodies of certain macrofungi (the higher fungi Ascomycotina and Basidiomycotina and related forms), during a walk through pasture or woodland. Microfungi (the moulds) are also diverse and are often observed on decaying foods and detritus, whereas many, including the coloured rusts, smuts and mildews, are common plant pathogens. Closer inspection of these visible structures, however, reveals that all are composed of aggregated long, branching threads termed hyphae (singular: hypha), organized to support spores for reproduction and dissemination. The hyphae of these aerial structures extend and branch within the supporting substratum as a network, termed a mycelium, from which the apically growing hyphae seek out, exploit and translocate available nutrients. Apically growing hyphae usually have a relatively constant diameter ranging from 1 to 30  $\mu\text{m}$  or more, depending on fungal species and growth conditions. Filamentous fungi may be cultivated within the laboratory on a variety of different liquid or solid media. On agar, the radially expanding colonial growth form of the fungal mycelium is most evident, extending from an inoculum, on, within and sometimes above the substrate, forming a near spherical three-dimensional colony. This radiating, circular pattern is also visible during the growth of fairy ring fungi in grassland and as ringworm infections of the skin.

The hyphae of individual fungi may (theoretically) extend endlessly via apical growth, provided they are supported

with appropriate nutrients and other environmental conditions. Eucarpic fungi, therefore, are spatially and temporally indeterminate organisms and, unlike animal, plant and other microbial individuals, have no predetermined maximum size or age. The mycelium is not, however, simply a homogeneously extending entity, but displays considerable developmental plasticity. Different interconnected regions of the fungal mycelium may grow, branch, anastomose (fuse), age, die, sporulate and display varying physiological and biochemical activities at different times or even simultaneously, depending on local micro-environmental conditions. Thus, colonies growing on relatively homogeneous media may be pigmented, exhibit different morphological sectors, produce aerial structures, grow as fast-effuse or slow-dense forms and even exhibit rhythmic growth ([Plate 1.1](#)). As well as reproductive structures and substrate mycelium, certain higher fungi, most notably the basidiomycetes, when growing within an environment where nutrients are distributed heterogeneously, can differentiate into long string-like structures called rhizomorphs or cords. These linear organs have evolved to rapidly explore for, connect and translocate water and nutrients between patches of resource (e.g. pieces of fallen timber on the forest floor or from tree root to tree root). Accordingly, many, particularly mature, rhizomorphs contain internal vessel hyphae which possess a wide diameter, forming a channel running along the organ. The peripheral hyphae are often closely packed and melanized for insulation.

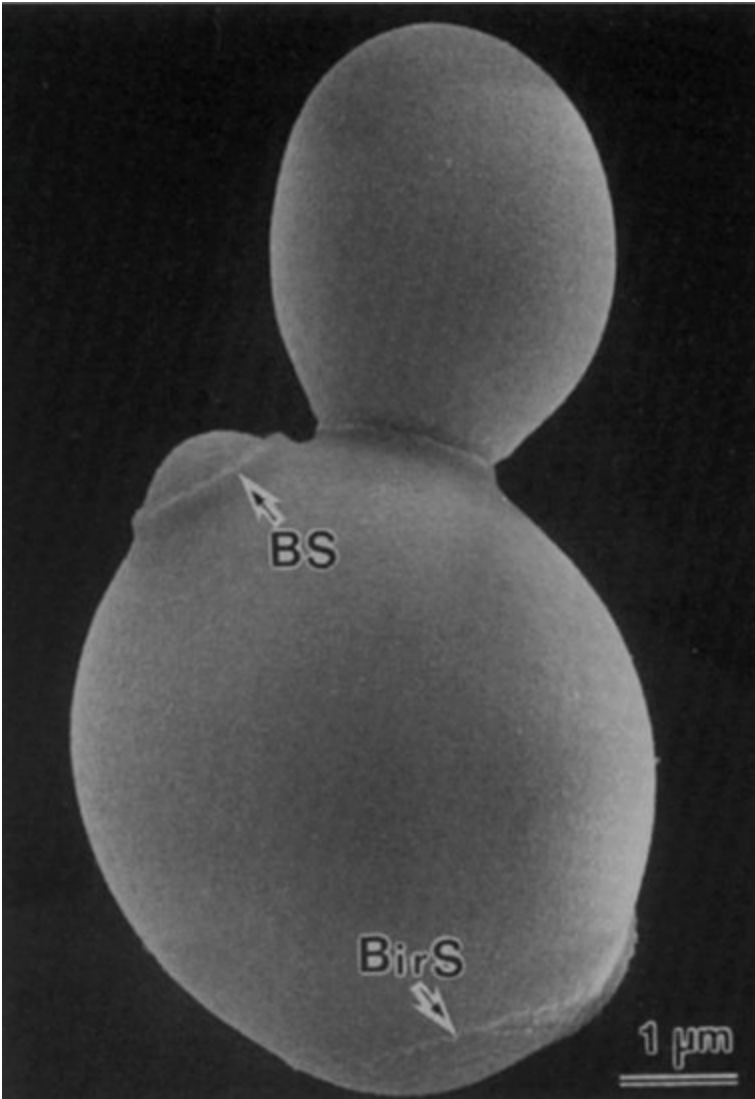
Filamentous fungi and yeasts are simply different styles of fungal growth suitable for occupation of different habitats and produced by differing cell growth polarities. Many species termed dimorphic fungi can adopt either the hyphal or unicellular yeast forms according to environmental circumstances. For example, certain important human and

animal pathogens exist as yeast forms mobilized in body fluids but are able to form hyphae or pseudohyphae for tissue invasion.

## 1.2.2 Yeasts

Yeasts are unicellular (mostly Ascomycete, Basidiomycete or Deuteromycete) fungi that divide asexually by budding or fission and whose individual cell size can vary widely from 2 to 3  $\mu\text{m}$  to 20–50  $\mu\text{m}$  in length and 1–10  $\mu\text{m}$  in width. *S. cerevisiae* (commonly referred to as brewer's or baker's yeast), is generally ellipsoid in shape with a large diameter of 5–10  $\mu\text{m}$  and a small diameter of 1–7  $\mu\text{m}$  ([Figure 1.1](#)).

**[Figure 1.1](#)** Scanning electron micrograph of a typical yeast cell. ( $\times 10\,000$ ). BS, bud scar; BirS, birth scar. (Reproduced with kind permission of Professor Masako Osumi, Japan Women's University, Tokyo.)



The morphology of agar-grown yeasts shows great diversity in terms of colour, texture and geometry (peripheries, contours) of giant colonies. Several yeasts are pigmented, and the following colours may be visualized in surface-grown colonies: cream (e.g. *S. cerevisiae*); white (e.g. *Geotrichum candidum*); black (e.g. *Aureobasidium pullulans*); pink (e.g. *Phaffia rhodozyma*); red (e.g. *Rhodotorula rubra*); orange (e.g. *Rhodospiridium* spp.); and yellow (e.g. *Cryptococcus laurentii*). The pigments of some yeasts have biotechnological uses, including astaxanthin from *P. rhodozyma* in aquacultural feed supplements for

farmed salmon (that are unable to synthesize these natural pink compounds) ([Table 1.1](#)).

**Table 1.1** Diversity of yeast cell shapes.

Cell shape	Description	Examples of yeast genera
Ellipsoid	Ovoid shaped cells	<i>Saccharomyces</i>
Cylindrical	Elongated cells with hemispherical ends	<i>Schizosaccharomyces</i>
Apiculate	Lemon shaped	<i>Hanseniaspora</i> , <i>Saccharomycodes</i>
Ogival	Elongated cell rounded at one end and pointed at other	<i>Dekkera</i> , <i>Brettanomyces</i>
Flask-shaped	Cells dividing by bud-fission	<i>Pityrosporum</i>
Miscellaneous shapes	Triangular	<i>Trigonopsis</i>
	Curved	<i>Cryptococcus</i> (e.g. <i>Cryptococcus cereanus</i> )
	Spherical	<i>Debaryomyces</i>
	Stalked	<i>Sterigmatomyces</i>
Pseudohyphal	Chains of budding yeast cells which have elongated without detachment	<i>Candida</i> (e.g. <i>Candida albicans</i> )
Hyphal	Branched or unbranched filamentous cells which form from germ tubes. Septa may be laid down by the continuously extending hyphal tip. Hyphae may give rise to blastospores	<i>Candida albicans</i>
Dimorphic	Yeasts that grow vegetatively in either yeast or filamentous (hyphal or pseudohyphal) form	<i>Candida albicans</i> <i>Saccharomycopsis fibuligera</i> <i>Kluyveromyces marxianus</i> <i>Malassezia furfur</i> <i>Yarrowia lipolytica</i> <i>Histoplasma capsulatum</i>

## 1.3 Ultrastructure and Function of Fungal Cells

### 1.3.1 The Fungal Cell Surface

The cell envelope in yeasts and fungi is the peripheral structure that encases the cytoplasm and comprises the plasma membrane, the periplasm, the cell wall and additional extracellular structural components (such as fimbriae and capsules). The cell wall represents a dynamically forming exoskeleton that protects the fungal protoplast from the external environment and defines directional growth, cellular strength, shape and interactive properties. In filamentous fungi, cell-wall formation and organization is intimately bound to the process of apical growth. Thus, for example in *Neurospora crassa*, the wall is thin (approximately 50 nm) at the apex but becomes thicker (approximately 125 nm) at 250  $\mu\text{m}$  behind the tip. The plasma membrane component of the fungal cell envelope is a phospholipid bilayer interspersed with globular proteins that dictates entry of nutrients and exit of metabolites and represents a selective barrier for their translocation. Ergosterol is the major sterol found in the membranes of fungi, in contrast to the cholesterol found in the membranes of animals and phytosterols in plants. This distinction is exploited during the use of certain antifungal agents used to treat some fungal infections, and can be used as an assay tool to quantify fungal growth. The periplasm, or periplasmic space, is the region external to the plasma membrane and internal to the cell wall. In yeast cells, it comprises secreted proteins (mannoproteins) and enzymes (such as invertase and acid phosphatase) that are unable to traverse the cell wall. In filamentous fungi, the cell membrane and wall may be intimately bound as hyphae and are often resistant to plasmolysis.

Fungal cell surface topological features can be visualized using scanning electron microscopy (SEM), and nanometre resolution is achieved using atomic force microscopy (AFM). The latter is beneficial, as it can be employed with unfixed, living cells and avoids potentially misleading artefacts that

may arise when preparing cells for electron microscopy. [Figure 1.1](#) shows SEM micrographs of a typical unicellular yeast cell envelope.

Ultrastructural analysis of fungal cell walls reveals a thick, complex fibrillar network. The cell walls of filamentous fungi are mainly composed of different polysaccharides according to taxonomic group. For example, they may contain either chitin, glucans, mannoproteins, chitosan, polyglucuronic acid or cellulose, together with smaller quantities of proteins and glycoproteins ([Table 1.2](#)). Generally, the semi-crystalline microfibrillar components are organized in a network mainly in the central cell wall region and are embedded within an amorphous matrix. Bonding occurs between certain components behind the extending hyphal tip, thereby strengthening the entire wall structure. There is evidence to suggest that the cell wall is a dynamic structure where considerable quantitative and qualitative differences occur not only between different fungal species, but also between different morphological forms of the same species and even in response to environmental stress. For example, a class of hydrophobic proteins called hydrophobins are localized within the aerial growth or appresoria (terminal swellings involved in infection) of certain fungi, whereas pigmented melanins are often found within some fungal cell walls to insulate against biotic and abiotic stresses.

**[Table 1.2](#)** The major polymers found in different taxonomical groups of fungi, together with the presence of perforate septa in these groups

*(adapted from Deacon (2005) and Carlile et al. (2001)).*

Taxonomic grouping	Fibrillar polymers	Matrix polymers	Perforate septa present or absent
Oomycetes	$\beta(1,3)$ -, $\beta(1,6)$ -Glucan Cellulose	Glucan	Absent
Chytridomycetes	Chitin; glucan	Glucan	Absent
Zygomycetes	Chitin; chitosan	Polyglucuronic acid; glucuronomannoproteins	Absent
Basidiomycetes	Chitin; $\beta(1,3)$ -, $\beta(1,6)$ -glucans	$\alpha(1,3)$ -Glucan; xylomannoproteins	Present (mostly Dolipore)
Ascomycetes/ Deuteromycetes	Chitin; $\beta(1,3)$ -, $\beta(1,6)$ -glucans	$\alpha(1,3)$ -Glucan; galactomannoproteins	Present (mostly simple with large central pore)

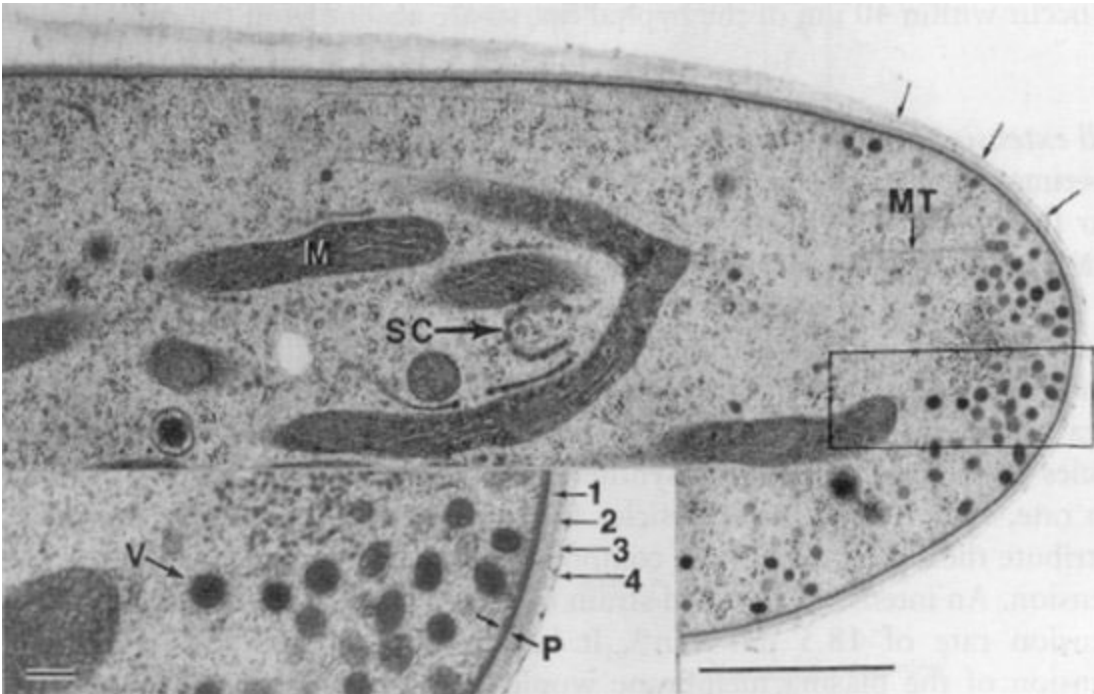
The hyphae of higher fungi extend via tip growth followed by cross-wall formation or septation, whereas the lower fungi remain aseptate (except when segregating spores or in damaged colony regions). Septa may offer some structural support to hyphae. Significantly, septa serve to compartmentalize hyphae but are typically perforated, thereby permitting passage and communication of cytoplasm or even protoplasm between compartments. However, septal pores can become blocked by Woronin bodies or other materials. This aids morphological and biochemical differentiation and serves to seal off stressed or damaged hyphae from undamaged colony regions. Again, different pore types are representative of different taxonomic groups and species ([Table 1.2](#)).

In yeasts, the cell-wall structure comprises polysaccharides (predominantly  $\beta$ -glucans for rigidity), proteins (mainly mannoproteins on the outermost layer for determining porosity), together with some lipid, chitin (e.g. in bud scar tissue) and inorganic phosphate material. [Figure 1.2](#) shows the composition and structure of the *S. cerevisiae* cell wall. Hyphal cell walls generally contain fewer mannans than yeast cell forms, and such changes in composition are



even observed during the transition from unicellular to mycelial growth of dimorphic fungi.

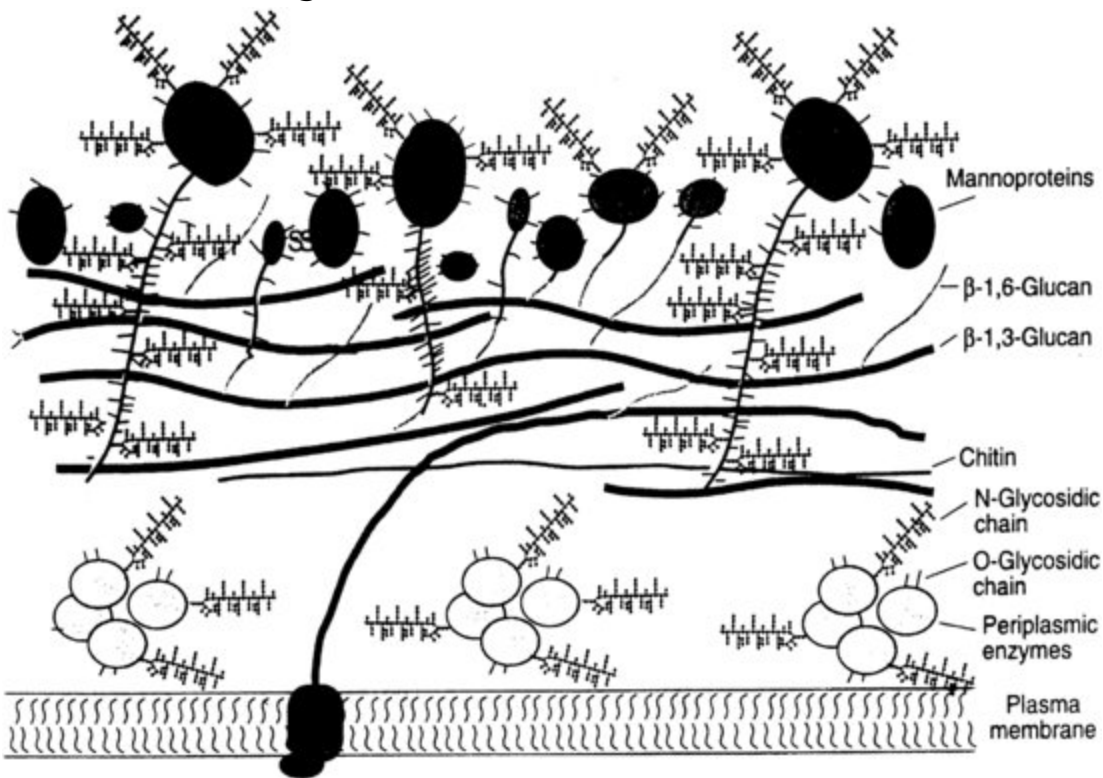
**Figure 1.2** Cell envelope structure of the yeast *S. cerevisiae* (from Walker (1998). Permission obtained for First Edition).



Chitin is also found in yeast cell walls and is a major constituent of bud scars ([Figure 1.3](#)). These are remnants of previous budding events found on the surface of mother cells following birth of daughter cells (buds). The chitin-rich bud scars of yeast cells can be stained with fluorescent dyes (e.g. calcofluor white), and this can provide useful information regarding cellular age, since the number of scars represents the number of completed cell division cycles. Outside the cell wall in fungi, several extramural layers may exist, including fimbriae and capsules. Fungal fimbriae are long, protein-containing protrusions appearing from the cell wall of certain basidiomycetous and ascomycetous fungi that are involved in cell-cell conjugation. Capsules are extracellular polysaccharide-containing structures found in basidiomycetous fungi that

are involved in stress protection. In *Cryptococcus neoformans* (the pathogenic yeast state of *Filobasidiella neoformans*) the capsule may determine virulence properties and evasion from macrophages. One extrahyphal substance, the polymer pullulan, is produced commercially from *A. pullulans*.

**Figure 1.3** Transmission electron microscopy of ultrathin sections of fungal cells reveals intracellular fine structure.

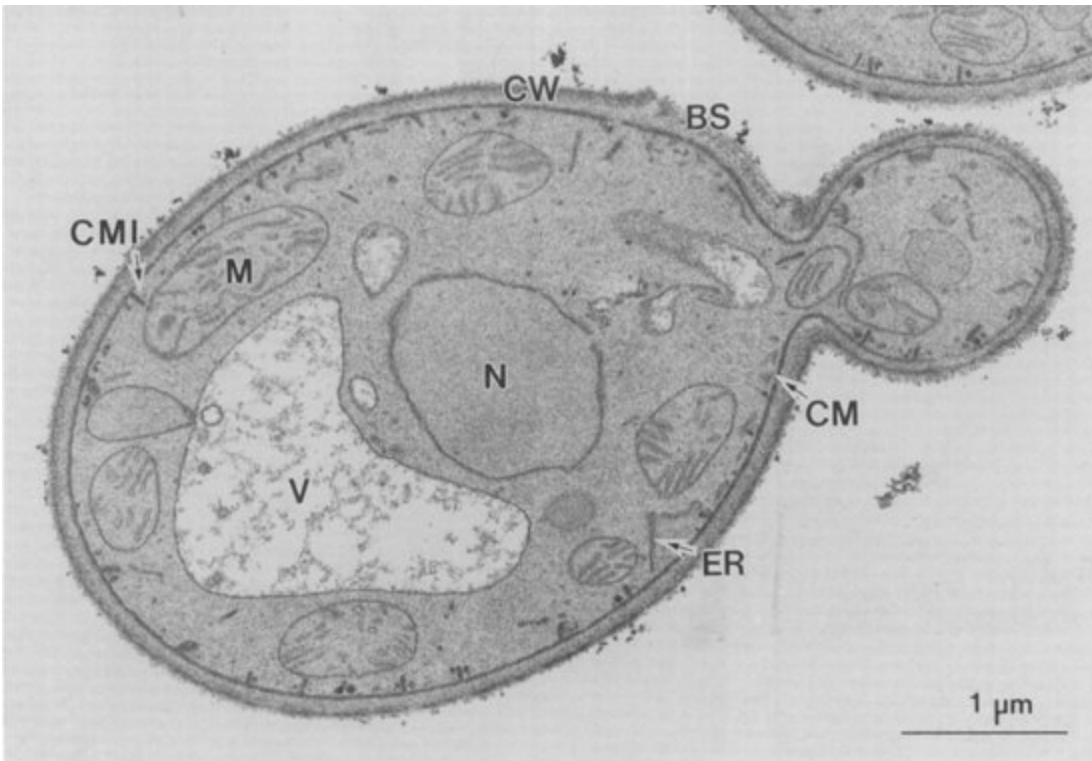


## 1.3.2 Subcellular Architecture and Organelle Function

Transmission electron microscopy of ultrathin sections of fungal cells reveals intracellular fine structure ([Figures 1.2](#) and [1.4](#)). Subcellular compartments (organelles) are bathed in an aqueous cytoplasm containing soluble proteins and other macromolecules, together with low-molecular weight metabolites. However, the hyphae of central (and therefore older) colony regions of filamentous fungi may become

devoid of protoplasm and organelles, as protoplasmic components are driven forward or are recycled, to support the growth of actively growing hyphal tips. Cytoplasmic components additionally comprise microbodies, ribosomes, proteasomes, lipid particles and a cytoskeletal network. The latter confers structural stability to the fungal cytoplasm and consists of microtubules and microfilaments. The following membrane-bound organelles may be found in a typical fungal cell: nucleus: endoplasmic reticulum (ER), mitochondria, Golgi apparatus, secretory vesicles and vacuoles. Several of these organelles form extended membranous systems. For example, the ER is contiguous with the nuclear membrane and secretion of fungal proteins involves intermembrane trafficking in which the ER, Golgi apparatus, plasma membrane and vesicles all participate. The physiological function of the various fungal cell organelles is summarized in [Table 1.3](#).

**Figure 1.4** Electron micrograph of a typical yeast cell. (CW, cell wall; CM, cell membrane; CMI, cell membrane invagination; BS, bud scar; M, mitochondrion, N, nucleus; V, vacuole; ER, endoplasmic reticulum. (Reproduced with kind permission of Professor Masako Osumi, Japan Women's University, Tokyo.)



**Table 1.3** Functional components of an idealized fungal cell.

Organelle or cellular structure	Function
Cell envelope	Comprising: the plasma membrane, which acts as a selectively permeable barrier for transport of hydrophilic molecules in and out of fungal cells; the periplasm, containing proteins and enzymes unable to permeate the cell wall; the cell wall, which provides protection, shape and is involved in cell-cell interactions, signal reception and specialized enzyme activities; fimbriae involved in sexual conjugation; capsules to protect cells from dehydration and immune cell attack.
Nucleus	Relatively small. Containing chromosomes (DNA-protein complexes) that pass genetic information to daughter cells at cell division and the nucleolus, which is the site of ribosomal RNA transcription and processing.
Mitochondria	Site of respiratory metabolism under aerobic conditions and, under anaerobic conditions, for fatty acid, sterol and amino acid metabolism.
Endoplasmic reticulum	Ribosomes on the rough ER are the sites of protein biosynthesis.
Proteasome	Multi-subunit protease complexes involved in regulating protein turnover.
Golgi apparatus and vesicles	Secretory system for import (endocytosis) and export (exocytosis) of proteins.
Vacuole	Intracellular reservoir (amino acids, polyphosphate, metal ions); proteolysis; protein trafficking; control of cellular pH. In filamentous fungi, tubular vacuoles transport materials bidirectionally along hyphae.

Organelle or cellular structure	Function
Peroxisome	Oxidative utilization of specific carbon and nitrogen sources (contain catalase, oxidases). Glyoxysomes contain enzymes of the glyoxylate cycle.

The nucleus is the structure that defines the eukaryotic nature of fungal cells. It is bound by a double membrane and encases the chromosomes in a nucleoplasm. Most yeast and fungi are haploid, although some (e.g. *S. cerevisiae*) may alternate between haploidy and diploidy. Chromosomes comprise DNA-protein structures that replicate and segregate to newly divided cells or hyphal compartments at mitosis. This, of course, ensures that genetic material is passed onto daughter cells or septated compartments at cell division. Yeasts usually contain a single nucleus per cell. However, the hyphal compartments of filamentous fungi may contain one or more nuclei. Monokaryotic basidiomycetes possess one nucleus per compartment, whereas dikaryons or heterokaryons possess two or more genetically distinct haploid nuclei. The maintenance of multiple nuclei within individual hyphal compartments allows fungi to take advantage of both haploid and diploid lifestyles. This is discussed further in Chapter 2.

In filamentous fungi, a phase-dark near-spherical region, which also stains with iron haematoxylin, is evident by light microscopy at the apex during hyphal tip growth. The region is termed the Spitzenkörper, the apical vesicle cluster or centre or apical body, and it consists of masses of small membrane-bound vesicles around a vesicle-free core with emergent microfilaments and microtubules. The Spitzenkörper contains differently sized vesicles derived from Golgi bodies, either large vesicles or microvesicles (chitosomes), with varying composition. It orientates to the side as the direction of tip growth changes, and disappears when growth ceases. This vesicle supply centre is involved in wall extension and, hence, tip growth, branching, clamp

connection formation (in Basidiomycetes) and germ tube formation.

## 1.4 Fungal Nutrition and Cellular Biosyntheses

### 1.4.1 Chemical Requirements for Growth

Yeasts and fungi have relatively simple nutritional needs and most species would be able to survive quite well in aerobic conditions if supplied with glucose, ammonium salts, inorganic ions and a few growth factors. Exceptions to this would include, for example, obligate symbionts such as the vesicular-arbuscular mycorrhizal (VAM) fungi which require growth of a plant partner for cultivation. Macronutrients, supplied at millimolar concentrations, comprise sources of carbon, nitrogen, oxygen, sulfur, phosphorus, potassium and magnesium; and micronutrients, supplied at micromolar concentrations, comprising trace elements like calcium, copper, iron, manganese and zinc, would be required for fungal cell growth ([Table 1.4](#)). Some fungi are oligotrophic, apparently growing with very limited nutrient supply, surviving by scavenging minute quantities of volatile organic compounds from the atmosphere.

**Table 1.4** Elemental requirements of fungal cells.

Element	Common sources	Cellular functions
Carbon	Sugars	Structural element of fungal cells in combination with hydrogen, oxygen and nitrogen. Energy source
Hydrogen	Protons from acidic environments	Transmembrane proton motive force vital for fungal nutrition. Intracellular acidic pH (around 5-6) necessary for fungal metabolism