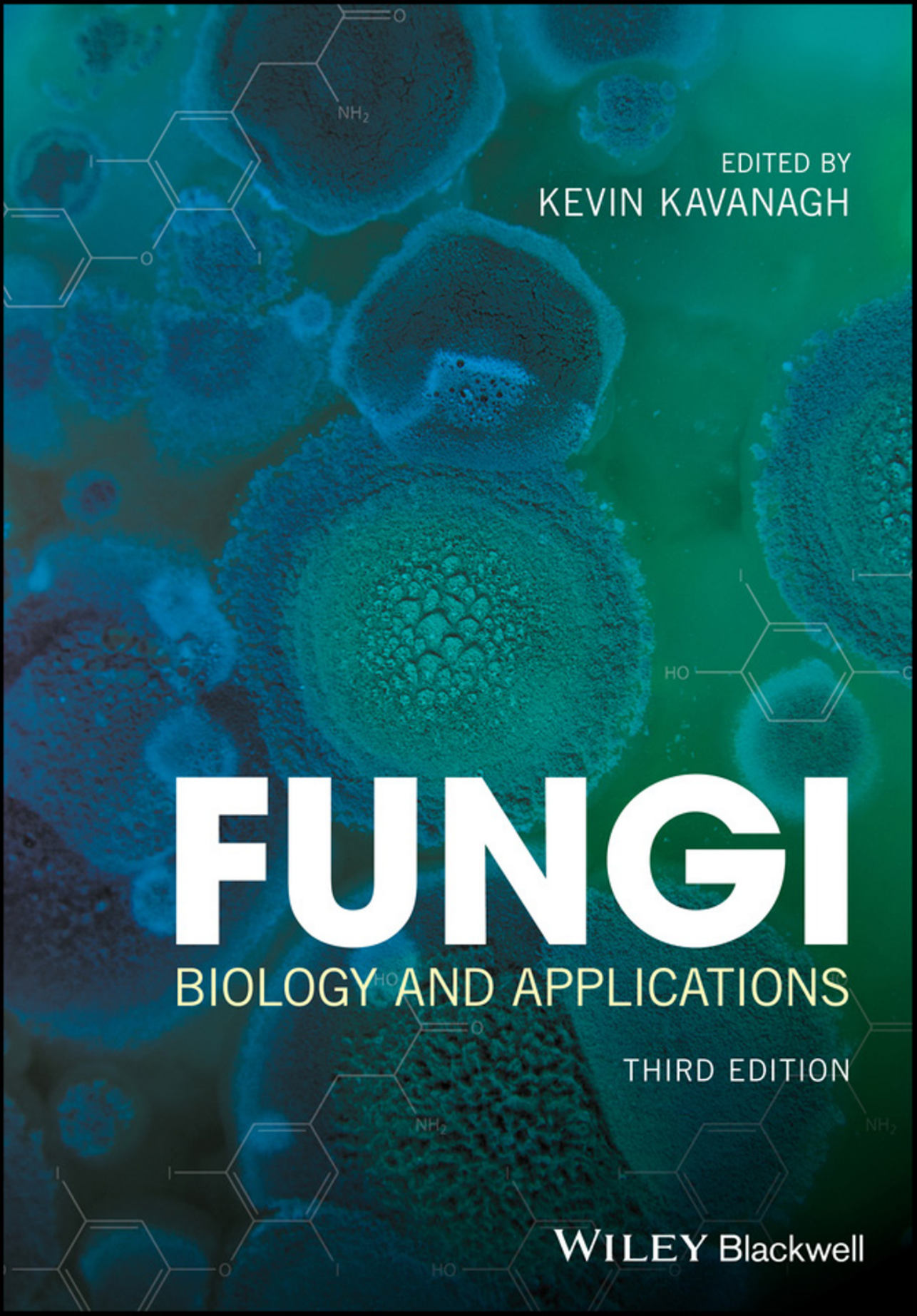
EDITED BY
KEVIN KAVANAGH
FUNGI
BIOLOGY AND APPLICATIONS
THIRD EDITION
WILEY Blackwell

Fungi

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Biology and Applications

Third Edition

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WILEY Blackwell

This edition first published 2018

Registered Office(s)
John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA

Editorial Office
111 River Street, Hoboken, NJ 07030, USA

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Library of Congress Cataloging-in-Publication Data

Names: Kavanagh, Kevin, editor.
Title: Fungi : biology and applications / edited by Kevin Kavanagh.
Other titles: Fungi (Kavanagh)
Description: Third edition. | Hoboken, NJ : Wiley, 2017. | Includes bibliographical references and index. |
Identifiers: LCCN 2017032708 (print) | LCCN 2017033915 (ebook) | ISBN 9781119374169 (ePDF) | ISBN 9781119374275 (ePUB) | ISBN 9781119374329 (paperback)
Subjects: | MESH: Fungi | Biotechnology
Classification: LCC TP248.27.F86 (ebook) | LCC TP248.27.F86 (print) | NLM QW 180 | DDC 579.5–dc23
LC record available at https://lccn.loc.gov/2017032708

Cover design: Wiley
Cover image: (Background) © Anna_Gavrylova/Gettyimages; (Chemical Structures) © chromatos/Shutterstock

Set in 10.5/13pt Sabon by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

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Preface

Fungi make an enormous contribution to our life. The role of yeast in the production of alcohol and bread is well characterized. We consume fungi directly in the form of edible mushrooms and in "blue cheeses" which get their characteristic flavor and aroma from the presence of fungi. Fungi are also used for the production of antibiotics, such as penicillin, and enzymes for use in the food industry. Since the 1990s, fungi have been utilized for the production of recombinant proteins, some of which have great therapeutic potential. Although infrequently recognized as important decomposers of organic detritus, fungi play a significant role in degrading biological matter, such as fallen leaves. On a more negative note, some fungi (for example members of the genus *Candida* and *Aspergillus*) are capable of causing serious life-threatening infections in immunocompromised patients, and other fungi can be serious plant pathogens.

This is the third edition of *Fungi: Biology and Applications* which was first published in 2005. Since that date there have been enormous strides in our understanding of the biology of fungi, and their contribution to our life is becoming increasingly important. The aim of the current edition is to provide a detailed description of the biology, biotechnological applications, and medical significance of fungi. The book commences with an in-depth description of the physiology of fungi in which the structure, metabolism, and growth of fungi are described. This is followed by a chapter dedicated to the genetics of fungi in which the lifecycles of a number of representative fungi are described and the use of fungi for genetic analysis is outlined. The advent of genomics and proteomics has revolutionized our study of the cell. Chapters 3, 4, and 5 describe how genomics, transcriptomics, and proteomics, respectively, have increased our knowledge of fungi and made available new opportunities for exploiting fungi for the good of humanity. Chapter 6 describes the importance of fungi as food and highlights the different techniques for the commercial production of edible fungi. Chapters 7 and 8 describe how fungi can be utilized for producing commercially important antibiotics, enzymes, and a range of chemical

products such as citric acid. Chapter 9 focuses on the exploitation of fungi for the production of heterologous proteins and illustrates how yeast has been used for the production of hepatitis B antigens. Chapter 10 describes the main fungal pathogens of humans and Chapter 11 outlines the human immune response to fungi that restricts infection. Chapter 12 describes the main classes of antifungal drugs and their modes of action. Chapter 13 outlines the role of fungi in the environment where they play a significant role in recycling nutrients. Chapter 14 describes the main fungal pathogens of plants and assesses the impact of such pathogens on the global supply of food.

This book gives a comprehensive introduction to fungi in terms of their biology, genetics, medical significance, and biotechnological potential. Each chapter is written by internationally recognized experts, so the reader is given an up-to-date and detailed account of our knowledge of the biology and various applications of fungi.

Kevin Kavanagh

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 surroundings, including cellular responses to environmental 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 physicochemical environment, or can be exploited as agents of biocontrol or as biofertilizers. Fungal metabolism is also responsible for the detoxification of organic pollutants and for bioremediating heavy metals and other recalcitrant chemicals in the environment (including wastewaters and groundwaters). 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. More negatively, fungi can cause considerable disease, spoilage, and decay of important artefacts, commodities, and materials, buildings, and of course food supplies.

In terms of human health, some yeasts and fungi represent major opportunistic life-threatening pathogens, while others are life-savers as they provide antimicrobial and chemotherapeutic agents. In modern biotechnology, several yeast

Fungi: Biology and Applications, Third Edition. Edited by Kevin Kavanagh.

species are being exploited as hosts for the expression of human therapeutic proteins following recombinant DNA and gene editing technologies (see Chapter 9). Recently, the application of gene editing using CRISPR/Cas is leading to a revolution in fungal genetic engineering (see Chapter 2). Furthermore, an international synthetic biology research consortium, called Sc-2.0, has embarked on the construction of a completely synthetic version of *Saccharomyces cerevisiae*. This would represent the world's first fully synthetic eukaryotic genome! In addition to the direct industrial exploitation of yeasts and fungi, it is important to note that these organisms, most notably the yeast *S. 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, including "synthetic" ones, 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

There are a diversity of yeast and fungal cellular morphologies. Most higher fungi are filamentous, yeasts grow as unicells, and some primitive fungi such as the Chytridomycota grow as individual rounded cells or dichotomous branched chains of cells with root-like rhizoids for attachment to a nutrient resource. Here we 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 varied and often apparent throughout the environment (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 Ascomycota and Basidiomycota and related forms), during a walk through pasture or woodland. Microfungi (the molds) are also diverse and are often observed on decaying foods and detritus, whereas many, including the colored 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 μm 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 (3-D) colony. This radiating, circular pattern is also visible during the growth of fairy ring fungi in grassland and as ringworm infections of the skin (Plate 1.1, parts a and b).

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 are therefore 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.

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 (Plate 1.1, parts l and m).

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 members of the deuteromycete group) fungi that divide asexually by budding or fission and whose individual cell size can vary widely from 2–3 μm to 20–50 μm in length and 1–10 μm in width. *Saccharomyces cerevisiae*, commonly referred to as brewer's or baker's yeast, is generally ellipsoid in shape with a large diameter of 5–10 μm and a small diameter of around 5 μm (Figure 1.1). There is great diversity in cell shapes and modes of cellular reproduction in the yeasts, as summarized in Table 1.1.

The morphology of agar-grown yeasts shows great diversity in terms of color, texture, and geometry (peripheries, contours) of giant colonies. Several yeasts are pigmented and the following colors 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. *Rhodosporidium* 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).

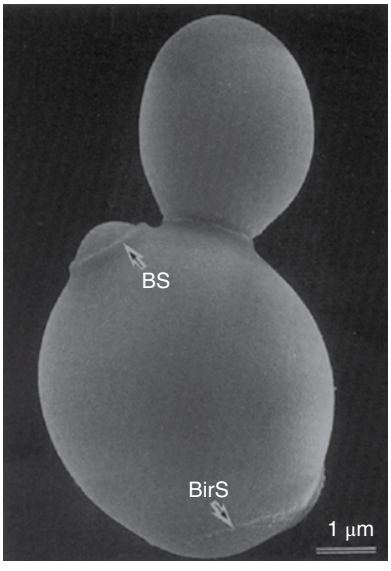

Figure 1.1 Scanning electron micrograph of a typical yeast cell (×10,000). BS, Bud scar; BirS, birth scar. (Reproduced with kind permission of Professor Masako Osumi, Japan Women's University, Tokyo.)

Table 1.1 Diversity of yeast cell shapes.

Cell shape	Description	Examples of yeast genera
Ellipsoid	Ovoid-shaped	*Saccharomyces*
Cylindrical	Elongated cells with hemispherical ends	*Schizosaccharomyces*
Apiculate	Lemon-shaped	*Hanseniaspora*, *Saccharomycodes*
Ogival	Elongated cell, rounded at one end and pointed at other	*Dekkera*, *Brettanomyces*
Flask-shaped	Cells divide by bud-fission	*Pityrosporum*
Miscellaneous shapes	Triangular	*Trigonopsis*
	Curved	*Cryptococcus* (e.g. *C. cereanus*)
	Spherical	*Debaryomyces*
	Stalked	*Sterigmatomyces*
Pseudohyphal	Chains of budding yeast cells which have elongated without detachment	*Candida* (e.g. *C. albicans*)
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	*Candida albicans*
Dimorphic	Yeasts that grow vegetatively in either yeast or filamentous (hyphal or pseudohyphal) form	*Candida albicans*, *Saccharomycopsis fibuligera*, *Kluyveromyces marxianus*, *Malassezia furfur*, *Yarrowia lipolytica*, *Histoplasma capsulatum*

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 (Figure 1.2).

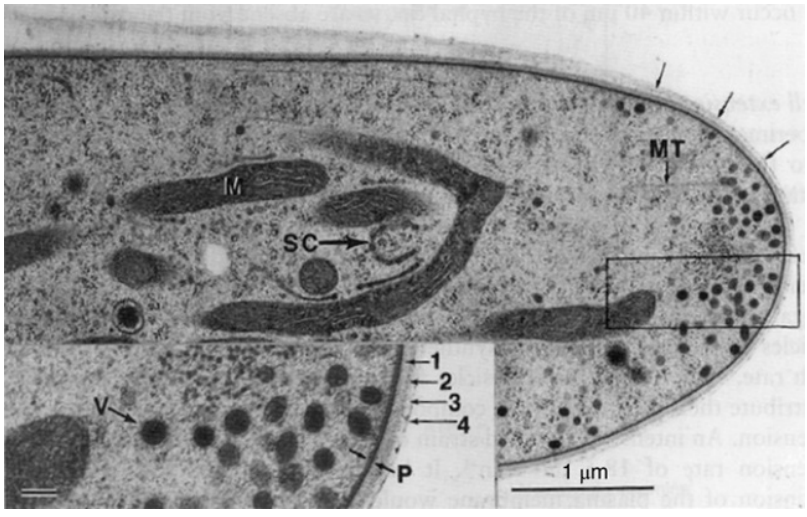

Figure 1.2 Transmission electron microscopy of ultrathin sections of a hyphal tip of *Fusarium* reveals intracellular fine structure. Layers of cell wall are shown in greater detail in lower image. M, Mitochondrion; V, vesicles; P, plasma membrane; MT, microtubules; SC, smooth Golgi cisternae; 1, 2, 3, 4, four layers of the cell wall. The Spitzenkörper appears as a region surrounded by vesicles containing many small particles (rectangle). (From Carlile *et al.* (2001).)

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 μm 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 are often resistant to plasmolysis.

Fungal cell surface topological features can be visualized using scanning electron microscopy (SEM) and nanometre resolution 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.

Table 1.2 Major polymers found in different taxonomic groups of fungi and fungus-like organisms, together with presence of perforate septa in these groups.

Taxonomic grouping	Fibrillar polymers	Matrix polymers	Perforate septa present or absent
Oomycetes (no longer considered to be true fungi)	β(1,3), β(1,6)-Glucan; cellulose	Glucan	Absent
Chytridomycetes	Chitin; glucan	Glucan	Absent
Zygomycetes	Chitin; chitosan	Polyglucuronic acid; glucuronomannoproteins	Absent
Basidiomycetes	Chitin; β(1,3)-β(1,6) glucans	α(1,3)-Glucan; xylomannoproteins	Present (mostly Dolipore)
Ascomycetes/ Deuteromycetes	Chitin; β(1,3)-β(1,6) glucans	α(1,3)-Glucan; galactomannoproteins	Present (mostly simple with large central pore)

Adapted from Deacon (2000); Carlile *et al*. (2001).

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 chitin, glucans, mannoproteins, chitosan, polyglucuronic acid, or cellulose (absent from true fungi), together with smaller quantities of proteins and glycoproteins (Table 1.2). Generally, the semicrystalline 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. The processes of endocytosis and exocytosis occur around apical and subapical regions and serve to shape both hyphal growth and interactions with the environment (Figure 1.2). 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 appressoria (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.

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 provides stability and protection to the cells and its structure comprises polysaccharides (predominantly β-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 1.3 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.

Chitin is also found in yeast cell walls and is a major constituent of bud scars (Figure 1.1). 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. calcoflour 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

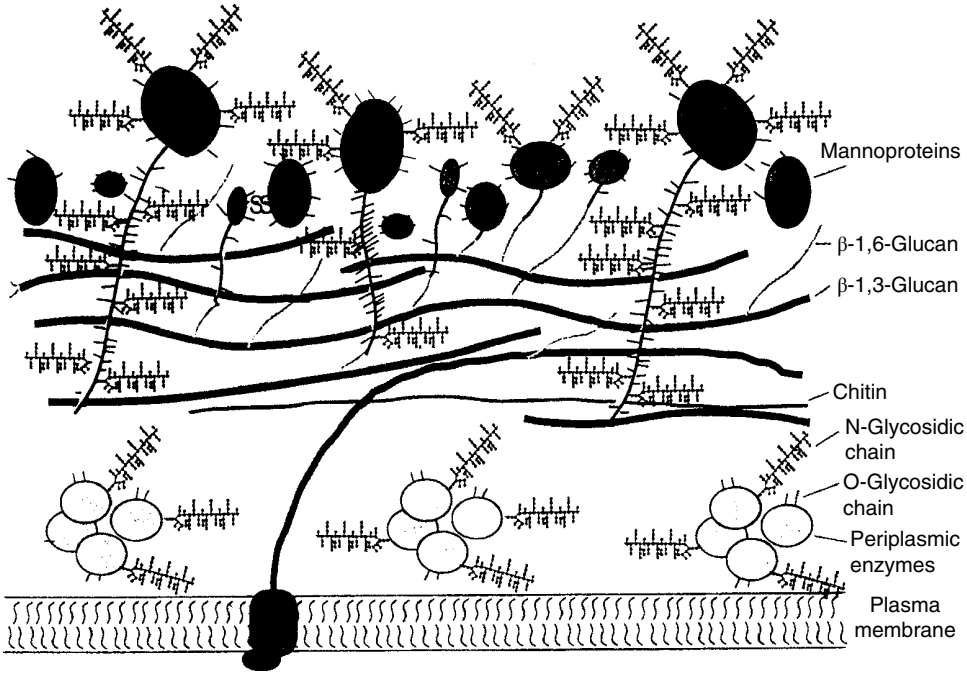

Figure 1.3 Cell envelope structure of the yeast *S. cerevisiae*. (From Walker (1998).)

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 *Aureobasidium pullulans*, and is used in the production of oral hygiene products.

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

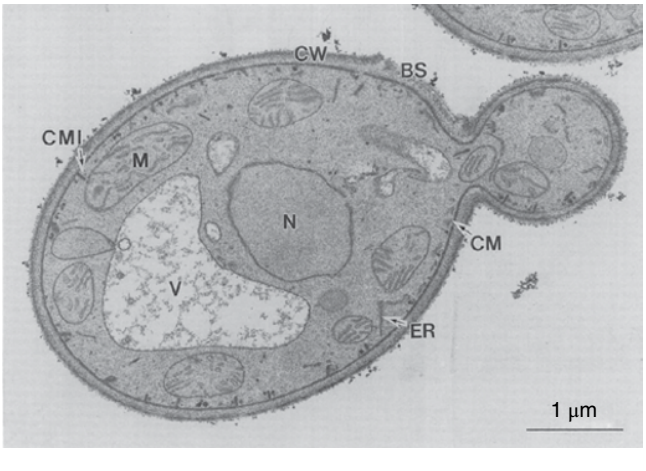

Figure 1.4 Electron micrograph of a typical yeast cell. CW, Cell wall; CM, cell membrane; CMI, cell membrane invagination; BS, bud scar; M, mitochondrion, N, nucleus; V, vacuole; ER, endoplasmic reticulum. (Reproduced with kind permission of Professor Masako Osumi, Japan Women's University, Tokyo.)

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.

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 yeasts and fungi are haploid (singular copies of each chromosome), although some (e.g. *S. cerevisiae*) may alternate between haploidy and diploidy.

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

Many industrial strains of *S. cerevisiae* exhibit aneuploidy (odd numbers of chromosomes) or are polyploid (multiple chromosome copies). 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 and 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 hemotoxylin, 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 (Figure 1.2). 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, comprise trace elements like calcium, copper, iron, manganese, and zinc and 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.

Being chemo-organotrophs, fungi need fixed forms of organic compounds for their carbon and energy supply. Sugars are widely utilized for fungal growth, and can range from simple hexoses like glucose to polysaccharides like starch and cellulose.

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
Oxygen	Air, O_2	Substrate for respiratory and other mixed-function oxidative enzymes. Essential for ergosterol and unsaturated fatty acid synthesis
Nitrogen	NH_4^+ salts, urea, amino acids	Structurally and functionally as organic amino nitrogen in proteins and enzymes
Phosphorus	Phosphates	Energy transduction, nucleic acid, and membrane structure
Potassium	K^+ salts	Ionic balance, enzyme activity
Magnesium	Mg^{2+} salts	Enzyme activity, cell and organelle structure
Sulfur	Sulfates, methionine	Sulfhydryl amino acids and vitamins
Calcium	Ca^{2+} salts	Possible second messenger in signal transduction
Copper	Cupric salts	Redox pigments
Iron	Ferric salts. Fe^{3+} is chelated by siderophores and released as Fe^{2+} within the cell	Heme-proteins, cytochromes
Manganese	Mn^{2+} salts	Enzyme activity
Zinc	Zn^{2+} salts	Enzyme activity
Nickel	Ni^{2+} salts	Urease activity
Molybdenum	Na_2MoO_4	Nitrate metabolism, vitamin B12

Some fungi can occasionally utilize aromatic hydrocarbons (e.g. lignin by the white-rot fungi). Table 1.5 outlines the variety of carbon sources that can be utilized by yeasts and filamentous fungi for growth.

Fungi are nondiazotrophic (cannot fix nitrogen) and need to be supplied with nitrogenous compounds, either in inorganic form such as ammonium salts, or in organic form such as amino acids. Ammonium sulfate is a commonly used nitrogen source in fungal growth media since it also provides a source of utilizable sulfur. Many fungi (but not the yeast *S. cerevisiae*) can also grow on nitrate,

Table 1.5 Diversity of carbon sources for yeast and filamentous fungal growth.

Carbon source	Typical examples	Comments
Hexose sugars	D-glucose, D-galactose,	Glucose metabolized by majority of yeasts and filamentous fungi
	D-fructose, D-mannose	If a yeast does not ferment glucose, it will not ferment other sugars. If a yeast ferments glucose, it will also ferment fructose and mannose, but not necessarily galactose
Pentose sugars	L-arabinose, D-xylose, D-xylulose, L-rhamnose	Some fungi respire pentoses better than glucose. *S. cerevisiae* can utilize xylulose but not xylose
Disaccharides	Maltose, sucrose, lactose, trehalose, melibiose, cellobiose, melezitose	If a yeast ferments maltose, it does not generally ferment lactose and vice versa. Melibiose utilization is used to distinguish ale and lager brewing yeasts. A large number of yeasts utilize disaccharides. Few filamentous fungi (e.g. *Rhizopus nigricans*) cannot utilize sucrose
Trisaccharides	Raffinose, maltotriose	Raffinose only partially used by *S. cerevisiae*, but completely used by other *Saccharomyces* spp. (*S. carlsbergensis*, *S. kluyveri*)
Oligosaccharides	Maltotetraose, maltodextrins	Metabolized by amylolytic yeasts, not by brewing strains
Polysaccharides	Starch, inulin, cellulose, hemicellulose, chitin, pectic substances	Polysaccharide-fermenting yeasts are rare. *Saccharomycopsis* spp. and *S. diastaticus* can utilize soluble starch; *Kluyveromyces* spp. possess inulinase. Many filamentous fungi can utilize these, depending on extracellular enzyme activity
Lower aliphatic alcohols	Methanol, ethanol	Respiratory substrates for many fungi. Several methylotrophic yeasts (e.g. *Pichia pastoris*, *Hansenula polymorpha*) have industrial potential
Sugar alcohols	Glycerol, glucitol	Can be respired by yeasts and a few fungi.
Organic acids	Acetate, citrate, lactate, malate, pyruvate, succinate	Many yeasts can respire organic acids, but few can ferment them
Fatty acids	Oleate, palmitate	Several species of oleaginous yeasts can assimilate fatty acids as carbon and energy sources

(*continued*)

Table 1.5 (*Continued*)

Carbon source	Typical examples	Comments
Hydrocarbons	n-Alkanes	Many yeast and a few filamentous species grow well on C_{12}-C_{18} n-alkanes
Aromatics	Phenol, cresol, quinol, resourcinol, catechol, benzoate	Few yeasts can utilize these compounds. Several n-alkane-utilizing yeasts use phenol as carbon source via the β-ketoadipate pathway
Miscellaneous	Adenine, uric acid, butylamine, pentylamine, putrescine	Some mycelial fungi and yeasts, e.g. *Arxula adeninivorans* and *A. terestre*, can utilize such compounds as sole source of carbon and nitrogen
	Lignin	Can be decayed only by white-rot fungi (basidiomycotina). Little net energy gained directly, but makes available other polysaccharides such as cellulose and hemicellulose
	"Hard" keratin	Keratinophilic fungi

Adapted from Walker (1998).

and if able to do so may also utilize nitrite. Nitrate reductase, followed by nitrite reductase, are the enzymes responsible for converting nitrate to ammonia. Most fungi can assimilate amino acids, amines, and amides as nitrogen sources. Most fungi (but not many yeasts) are also proteolytic and can hydrolyze proteins (via extracellularly secreted proteases) to liberate utilizable amino acids for growth. Urea utilization is common in fungi, and some basidiomycotenous yeasts are classed as urease-positive (able to utilize urea), while several ascomycotenous yeasts are urease-negative.

In terms of oxygen requirements, most fungi are aerobes and are often described as being microaerophilic (preferring an oxygen tension below that of normal atmospheric). Although yeasts like *S. cerevisiae* are sometimes referred to as facultative anerobes, they cannot actually grow in strictly anaerobic conditions unless supplied with certain fatty acids and sterols (which they cannot synthesize without molecular oxygen). In fact, there are thought to be very few yeast species that are obligately anaerobic. Unsaturated fatty acids (e.g. oleic acid) and sterols (e.g. ergosterol) are important constituents of the yeast cell membrane, and oxygen is required for their synthesis and to maintain membrane functional integrity and stress resistance. For aerobically respiring yeasts and fungi, oxygen is required as the terminal electron acceptor, where it is finally reduced to water in the electron transport chain. Different fungal species respond to oxygen availability in diverse ways and Table 1.6 categorizes fungi into different groups on this basis.

Table 1.6 Yeast and fungal metabolism based on responses to oxygen availability.

Mode of energy metabolism	Examples	Comments
Obligate fermentative	Yeasts: *Candida pintolopesii* (*Saccharomyces telluris*)	Naturally occurring respiratory-deficient yeasts. Only ferment, even in presence of oxygen
	Fungi: facultative and obligate anerobes	No oxygen requirement for these fungi. Two categories exist with respect to the effects of air: facultative anerobes (e.g. *Aqualinderella* and *Blastocladia*) and obligate anerobes (e.g. *Neocallimastix*)
Facultatively fermentative		
Crabtree-positive	*Saccharomyces cerevisiae*	Such yeasts predominantly ferment high sugar-containing media in the presence of oxygen
Crabtree-negative	*Candida utilis*	Such yeasts do not form ethanol under aerobic conditions and cannot grow anaerobically
Nonfermentative	Yeasts: *Rhodotorula rubra*	Such yeasts do not produce ethanol, in either the presence or absence of oxygen
	Fungi: *Phycomyces*	Oxygen is essential for such (obligately oxidative) fungi
Obligate aerobes	*Gaemannomyces graminis* (the take-all fungus)	Growth of these is markedly reduced if oxygen partial pressure falls below normal atmospheric

Adapted from Walker (1998), Deacon (2000), and Carlile *et al*. (2001).

Sulfur sources for fungal growth include sulfate, sulfite, thiosulfate, methionine and glutathione, with inorganic sulfate and the sulfur amino acid methionine being effectively utilized. Virtually all yeasts can synthesize sulfur amino acids from sulfate, the most oxidized form of inorganic sulfur.

Phosphorus is essential for biosynthesis of fungal nucleic acids, phospholipids, adenosine triphosphate (ATP), glycophosphates, and polyphosphates. Hence, the phosphate content of fungi is considerable (e.g. in yeast cells, this accounts for around 3–5% of dry weight; the major part of this is in the form of orthophosphate (H_2PO_4–) which acts as a substrate and enzyme effector). The fungal

Table 1.7 Metals required for fungal growth and metabolic functions.

Metal ion	Concentration[1]	Main cellular functions supplied in growth medium
Macroelements		
K	2–4 mM	Osmoregulation, enzyme activity
Mg	2–4 mM	Enzyme activity, cell division
Microelements		
Mn	2–4 μM	Enzyme cofactor
Ca	<1 μM	Second messenger, yeast flocculation
Cu	1.5 μM	Redox pigments
Fe	1–3 μM	Heme-proteins, cytochromes
Zn	4–8 μM	Enzyme activity, protein structure
Ni	~10 μM	Urease activity
Mo	1.5 μM	Nitrate metabolism, vitamin B12
Co	0.1 μM	Cobalamin, coenzymes

[1] Concentration figures relate to yeast (*S. cerevisiae*) growth stimulation, and are dependent on the species/strain and conditions of growth, but they would be generally applicable for fungal growth. Adapted from Walker (2004).

vacuole can serve as a storage site for phosphate in the form of complexed inorganic polyphosphates (also referred to as volutin granules). Both nitrogen and phosphorus availability may be growth limiting in nature. Filamentous fungi have evolved a number of biochemical and morphological strategies allowing capture of often poorly available phosphorus within the natural environment. Plants exploit such efficiency during symbioses between their roots and certain mycorrhizal fungi. The major storage form of phosphorus in plants is phytic acid (myo-inositol hexa-dihydrogenphosphate) which is poorly utilized by monogastrics (e.g. humans, pigs, poultry), and fungal (and yeast) phytases have applications in reducing phytate content of foods and feeds (see Chapter 8).

Concerning requirements for minerals, potassium, magnesium, and several trace elements are necessary for fungal growth. K and Mg are macroelements required in millimolar concentrations primarily as enzyme cofactors, whereas other microelements (trace elements) are generally required in the micromolar range. These include Mn, Ca, Fe, Zn, Cu, Ni, Co, and Mo. Table 1.7 summarizes the main metals required for fungal growth. Toxic minerals (e.g. Ag, As, Ba, Cs, Cd, Hg, Li, Pb) adversely affect fungal growth generally at concentrations greater than 100 μM.

Fungal growth factors are organic compounds occasionally needed in very low concentrations for specific enzymatic or structural roles, but not as energy