

Introductory microbiology

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1

Microbial structure and mode of life

1.1 Introduction

Early biologists found it convenient to classify all living things as either animals or plants. To many people today, this grouping still seems perfectly adequate. However, examination of the life-forms that exist on Earth shows that this classification is unsatisfactory. Although there is a superficial resemblance between green plants and the fungi, these two groups are divided by profound biological differences. Unlike green plants, fungi cannot manufacture their own food from water and carbon dioxide by the process of photosynthesis. Rather, they require a supply of organic matter from which they can derive their energy. Fungal cellular composition is dissimilar from that of green plants, and the structural polymers of their **cell walls** are markedly different. **Fungi** are therefore now accorded their own status as a third kingdom. Furthermore, for many years the classification of microscopic organisms proved to be difficult. Photosynthetic microbes behave very differently from higher plants. It was therefore proposed towards the end of the nineteenth century that microscopic life forms should be classified as a fourth kingdom. This was the kingdom **Protista**, proposed in 1866, at a time when the scientific study of microbiology was in its infancy. This was, however, almost 200 years after Antonie van Leeuwenhoek described ‘**animalcules**’ following his development of the optical microscope.

During the twentieth century there have been many advances in microscopy, including the development of the electron microscope. This has enabled subcellular structures to be studied in great detail and has revealed that the Protista may be divided into two major groups. Primitive microorgan-

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isms such as **bacteria** lack a clearly defined, membrane-bound **nucleus** and are called **prokaryotes**. This word is derived from two Greek words; *pro*, meaning before, and *karyon*, a kernel. Prokaryotes are organisms that evolved before the cell's nucleus, its kernel, was properly developed. More advanced microscopic life-forms, as well as having a variety of subcellular **organelles**, also possess a proper membrane-bound nucleus. These are referred to as **eukaryotes** because they have a true nucleus (Greek: *eu-*, well, true or easy). Biologists now reserve the kingdom Protista for eukaryotic microbial life-forms and separate prokaryotes into their own kingdom, the **Monera**. *Monera* is 'new' Latin for non-nucleated protoplasmic masses. Some microbiologists prefer to refer to this kingdom as the **Prokaryotes**. Thus, life-forms may be classified into five kingdoms: Animals, Plants, Fungi, Protista, and Monera or Prokaryotes. Table 1.1 indicates the characteristic features of members of the different kingdoms.

Microbiology is the study of microscopic life-forms including the microscopic fungi and Protista as well as prokaryotes. It also encompasses a study of **viruses**: subcellular structures comprising nucleic acid inside a protein coat and sometimes covered in a membrane. Viruses cannot replicate outside a host cell, and thus represent the ultimate parasites. Despite their limitations, viruses have managed to parasitise animal, plant and even bacterial cells. In a book of this size, it is not possible to cover the whole of microbiology, and so the text is devoted to a description of the biology of bacteria (prokaryotes), viruses and the microfungi.

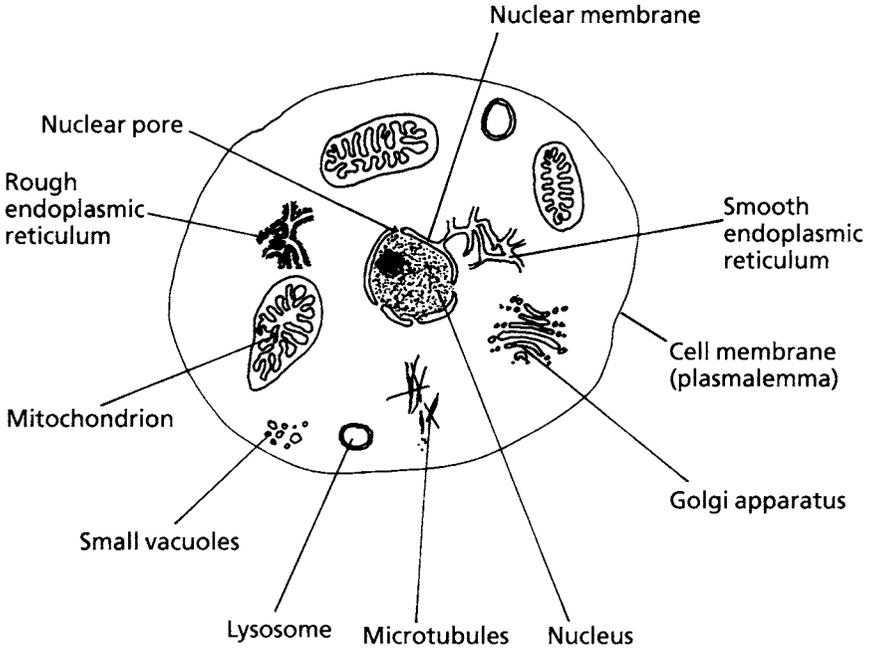
1.2 A comparison of eukaryotic and prokaryotic cells

The cells of eukaryotic organisms have a much more complex structure than do prokaryotic cells. Eukaryotic cells have a highly developed internal membrane and microtubular structure, together with subcellular organelles. Such structures are absent from many prokaryotes, although some prokaryotes do have specialised internal membranes associated with particular metabolic functions. The nucleus of the eukaryotic cell is membrane bound. Associated with the nucleus is the **centriole**, a structure used in the regulation of **mitosis**. This is the process of cell division that ensures that each daughter cell receives a complete set of **chromosomes**. Protein synthesis in eukaryotic cells is carried out on **ribosomes** associated with the **endoplasmic reticulum**, a network of internal membranes. The endoplasmic reticulum is contiguous with the **Golgi apparatus**; a structure associated with the export of proteins (Fig. 1.1).

Table 1.1 *Characteristic features of members of the five kingdoms*

	Animals	Plants	Fungi	Protista	Prokaryotes
Multicellular	Yes	Yes	Yes	No	No
Membrane-bound nucleus	Yes	Yes	Yes	Yes	No
Nuclear material present as naked DNA	No	No	No	No	Yes
Cytoplasmic membrane-bound organelles	Yes	Yes	Yes	Yes	No
Cell walls	No	Yes (mainly cellulose-based)	Yes (mainly chitin-based)	Some	The vast majority. Mostly peptidoglycan-based, but other polymers are found Some
Growth can be achieved using simple chemicals	No	Yes	No	Some	Some
Growth requires a supply of complex organic molecules	Yes	No	Yes	Some	Some
Chloroplasts	No	Yes	No	Some	No
Mitochondria	Yes	Yes	Yes	Yes	No
Ribosomes	Larger cytoplasmic, smaller mitochondrial	Larger cytoplasmic, smaller mitochondrial and chloroplast	Larger cytoplasmic, smaller mitochondrial	Larger cytoplasmic, smaller mitochondrial and chloroplast (if present)	Small

(a)



(b)

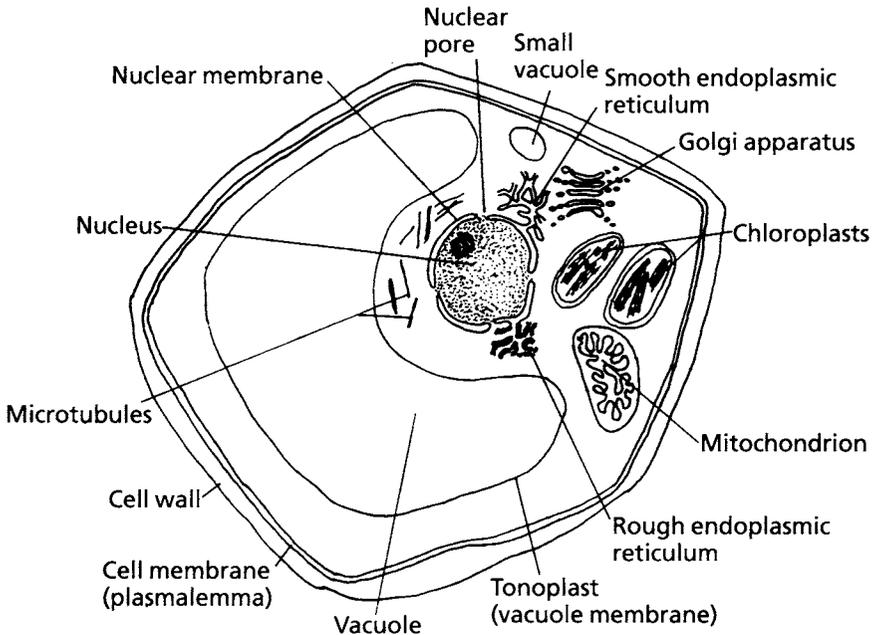


Fig. 1.1. Stylised eukaryotic cells. (a) A stylised animal cell and (b) a stylised plant cell.

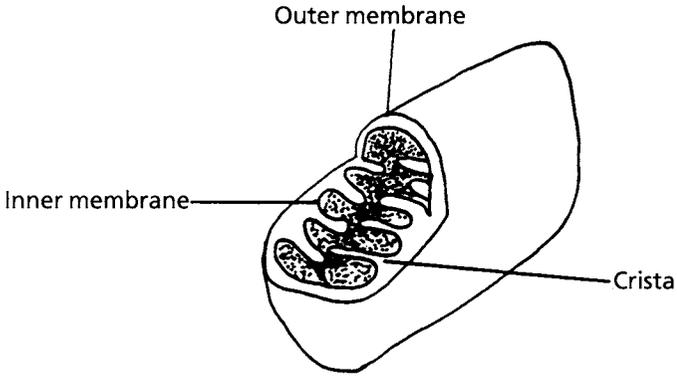
Cellular respiration, generating ATP, is carried out in **mitochondria**. These are organelles delineated by a double membrane. The mitochondrial inner membrane carries the respiratory enzymes or **cytochromes**, and is highly convoluted to form **cristae**. These greatly increase the surface area of the mitochondrial inner membrane, the surface at which ATP generation occurs. Plant cells, in addition, carry **plastids**. These are also organelles bounded by a double membrane, and in green plant tissues plastids become specialised as **chloroplasts**, which are also involved in the generation of ATP. Chloroplasts generate ATP by the process of **photosynthesis** whereas mitochondria generate ATP by aerobic respiration. Chloroplasts have elaborate internal membrane structures called **thylakoids**. Both mitochondria and chloroplasts have their own DNA and are capable of synthesising particular proteins. This process is much more akin to bacterial protein synthesis than to cytoplasmic protein synthesis in eukaryotic cells. Many biologists believe that mitochondria and chloroplasts represent the descendants of obligate intracellular endosymbiotic bacteria that date from the earliest days of eukaryotic cells. The ancestral **endosymbionts** are thought to have evolved to lose most of their bacterial structure. However, mitochondria and chloroplasts retain a genetic apparatus that provides them with the ability to synthesise certain of their own proteins. They also provide metabolic processes that have proved to be of great value to the eukaryotic host (Fig. 1.2).

Both plant and animal cells have membrane-bound **vacuoles**. Plant cell vacuoles are large and bounded by a special membrane called a **tonoplast**. Animal cells contain smaller, more numerous vacuoles. Additionally, animal cells contain **lysosomes**; subcellular sacs filled with lytic enzymes. These may fuse with vacuoles containing foreign bodies to form **phagosomes**. Inside the phagosome, lytic enzymes digest foreign material, and the breakdown products may be absorbed into the cell. Cytoplasmic streaming in eukaryotic cells is the process by which metabolites are distributed through the cell. It is controlled by a system of microtubules. Some eukaryotic cells elaborate (or develop) organelles of motility that extend into the environment. Short structures are referred to as **cilia**; longer organelles are called **flagella**. Eukaryotic cilia and flagella have a characteristic internal structure comprising nine pairs of microtubules encircling a further two pairs of tubules. This is often referred to as a 9 + 2 structure, and is confined to eukaryotic cells.

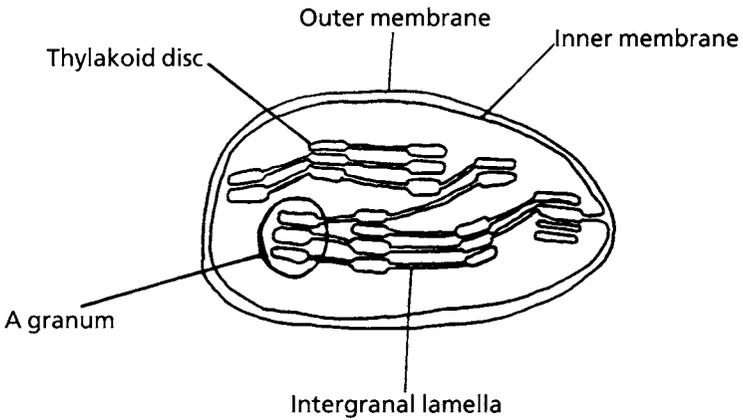
Eukaryotic cells are bounded by a cytoplasmic membrane, the **plasmalemma**. In animal cells, the plasmalemma is the outermost structure; plant cells have a **cellulose**-based cell wall outside the plasmalemma to provide additional protection. Eukaryotic membranes are stabilised by the presence of sterols.

6 Microbial structure and mode of life

(a)



(b)



(c)

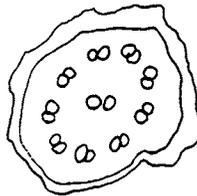


Fig. 1.2. Some subcellular structures found in eukaryotic cells. (a) A cut-away diagram of a mitochondrion, (b) section through a chloroplast, and (c) cross-section through a eukaryotic flagellum, showing the typical '9 + 2' structure.

In contrast to eukaryotic cells, prokaryotes have a very simple internal structure. As their name implies, they do not have a clearly defined nucleus delineated by a nuclear membrane. Rather, the chromosome forms a densely packed area within the cytoplasm known as the **nucleoid**. Extrachromosomal DNA structures, at one time called **episomes** and now more commonly known as **plasmids**, also exist within the cytoplasm. The prokaryotic ribosomes, which are smaller than those found in eukaryotic cells, are not attached to internal membranes. Rather, multiple ribosomes line up along messenger RNA molecules to form structures called **polysomes**.

The size of ribosomes is conventionally expressed as the rate at which they sediment in a density gradient such as a sucrose gradient. The unit of measurement is the Svedberg unit. Eukaryotic cytoplasmic ribosomes are described as 80 S ribosomes, and these are larger than prokaryotic 70 S ribosomes. Ribosomes are made up of two subunits. In eukaryotic cells these are 60 S and 40 S subunits, whereas in prokaryotes there are 50 S and 30 S subunits. The sedimentation rate of a body depends upon its shape as well as its density. Thus the whole ribosome unit has a lower sedimentation rate than that of its component subunits. The ribosomes found in chloroplasts and mitochondria are much closer in size, and behaviour, to the 70 S prokaryotic ribosome than to the 80 S eukaryotic ribosome. This observation lends weight to the endosymbiotic theory for the origin of mitochondria and chloroplasts.

Archaeobacteria represent some of the most ancient of life-forms that persist today. They can be found in some of the most extreme habitats on Earth. For example archaeobacteria have been isolated from coal slag heaps, in an environment with a temperature of about 60 °C, and **pH** of about 1, and they can be found around underwater volcanoes. They may be differentiated from other bacteria, the eubacteria, using a variety of characteristics, especially with respect to ribosomal and membrane structures, but these differences are not sufficient to warrant the separation of archaeobacteria and eubacteria into two separate kingdoms. The prefix *archae-* comes from the Greek word *arkhaio*, meaning ancient. **Eubacteria** are the most familiar and commonly studied of bacteria.

The cytoplasm of prokaryotic cells is bounded by a cell membrane, and many bacteria have a cell wall outside the cell membrane. The principal cell wall polymer of archaeobacteria varies from group to group, whereas in eubacteria the main component is **peptidoglycan**. Various structures that act in cellular adhesion and locomotion are associated with the prokaryotic cell wall. Many prokaryotes have an additional outer membrane beyond the cell wall, and some bacteria have external structures beyond this.

1.3 Morphology of fungi

The study of fungi is referred to as mycology, and is one of the oldest disciplines in microbiology. Fungi are amongst the most generally familiar organisms that are studied in microbiology. Everyone will recognise the furry growths that appear on stale bread and rotting fruits, and since time immemorial, fungi have been exploited for the production of leavened bread and alcoholic beverages. Indeed, so important are fungal products to humans that bread and wine are two of the principal symbols of the Christian Church, representing the body and blood of Christ. The role of fungi in the decay of vegetable and animal matter has also long been recognised. Fungi have been so intimately linked with the decomposition of organic matter that they have become synonymous with mouldiness, putrefaction and decay.

Fungi range dramatically in size from relatively large and compact structures such as puff-balls, mushrooms, toadstools and bracket fungi that can be seen attached to decaying trees, through the diverse network of filaments in soil, frequently associated with plant roots, down to the microscopic unicellular yeasts. However, all fungi are eukaryotic organisms. They possess a membrane-bound nucleus and nucleoli, cellular respiration occurs in mitochondria present in the cytoplasm, and fungal cells have an elaborate arrangement of internal membrane systems.

1.3.1 Moulds and their structures

The fungi display an astonishing variety of size and shape, but can be broadly divided into two groups, the **moulds** and the **yeasts**. The moulds are also referred to as filamentous or mycelial fungi, and they are composed of a network of filaments called **hyphae** (singular: hypha) that are interwoven into a structure called a **mycelium** (plural: mycelia). *Huphe* is the Greek word for a web, and mycelium is derived from *mukes* meaning mushroom. Moulds reproduce asexually or sexually, and the mycelium and **fruiting bodies** of a mould are collectively referred to as the fungal **thallus**, this being the Greek for green shoot. The spores of fungi are of primary importance in the identification of fungi, and are discussed in detail below. Mycelial tissue is also sometimes referred to as an **anastomosis** because it comprises a web of cross-connecting hyphae. The development of mycelial structures is by growth from the tips of the hyphae, with branching of the filaments occurring intermittently. The cytoplasm of young hyphae fills the filament, but further back from the growing tip, the cytoplasm becomes increasingly vacuolated, and the oldest

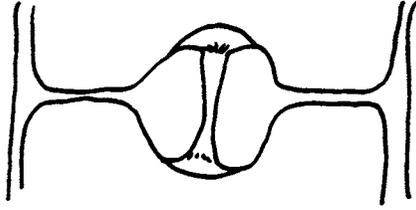
hyphae are empty structures that may become cut off from the rest of the mycelium.

In the majority of moulds, hyphae are divided into sections by the regular occurrence of cross-walls or **septa** (singular: septum). These structures add rigidity to the filament, and help to control the flow of nutrients through the mycelial network. Septa vary in complexity; simple septa have a single central pore, but some septa seen in higher fungi have a **dolipore** structure, in which a narrow central pore is flanked by a cap-like, perforated membranes (parenthesomes) made up of amorphous material (Fig. 1.3). Individual compartments in the septate hypha may contain a single nucleus, and these are said to be uninucleate or they may contain several nuclei, and are thus described as multinucleate. In more primitive fungi such as the Phycomycetes, there are no septa to divide the hyphae into sections, and the aseptate hypha is described as coenocytic (Greek: *kinos*, common; *kutos*, a vessel). Septa do occasionally develop in Phycomycetes, but their function is to separate the reproductive structures from the vegetative body of the fungus, or to cut off the old sections of the thallus. Unlike the septa of other fungi, the structures elaborated by the Phycomycetes are solid plates, and they do not have a central pore (Fig. 1.4).

In certain higher fungi, adjacent hyphae can fuse vegetatively to give a three-dimensional network structure. It is from such structures that reproductive fruiting bodies are formed. Hyphae can also aggregate to give rise to other specialised structures that exhibit a high degree of internal organisation as a result of coordinated growth. **Rhizomorphs**, literally resembling root structures, are rope-like strands that have a highly differentiated structure. These structures appear to develop in response to stress, and in nature they develop in relatively dry environments such as are found in sandy soils. **Sclerotia** (singular: sclerotium) are hardened structures that enable certain moulds to survive in a dormant state. In culture, sclerotia are pigmented, and are sufficiently large to be seen by the naked eye. They are generally rounded, but may display an irregular shape. The cells of the outer wall of a sclerotium possess thick walls, and thus the structure has a thick, protective coat. This encloses a central cortex of hyphae that contains the food reserves necessary for dormancy. Nutrients are typically stored either as oil droplets or as glycogen. The most familiar structures generated by fungi, however, are the **mushrooms** and **toadstools**. These are highly complex reproductive structures that demonstrate an astonishing level of internal differentiation and organisation. A stalk or **stipe** supports the cap or **pileus** under which the **gills** develop. It is from these gills that spores are released. The development of gills is a highly coordinated process that responds to environmental stimuli. In order for

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(a)



(b)

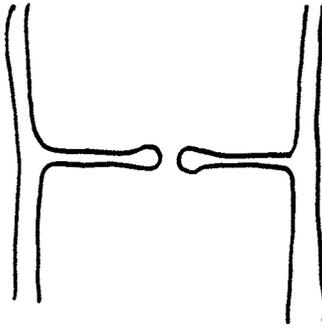


Fig. 1.3. (a) A dolipore and (b) a 'normal' septum.

(a)



(b)



Fig. 1.4. Aseptate (coenocytic) and septate hyphae. (a) An aseptate or coenocytic hypha; the hypha shown in (b) is divided by regular septa.

spores to be released efficiently, gills must develop vertically. This is achieved by **geotropism**, as shown by the observation that if the developing structure is tilted, then the gills will still form to lie vertically.

1.3.2 Yeasts and their structures

In yeasts, the fungal thallus is generally a single cell. Yeasts are predominantly unicellular fungi that are round, oval or elongated in shape. They vary from 2 to about 10 micrometres in size. A limited number of yeasts elaborate extra-cellular capsules. An example is *Cryptococcus neoformans*, a human pathogen that causes a chronic form of meningitis increasingly seen in patients with acquired immune deficiency syndrome (**AIDS**). In this instance, the capsule mucopolysaccharide helps the yeast to evade the body's defence mechanisms, and thus allows it to cause disease.

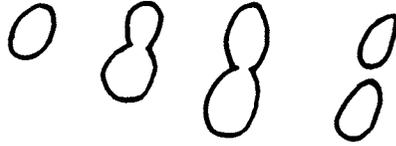
Yeasts generally reproduce by the asexual process of **budding**. The parental cell develops a protruberance that swells and enlarges into a **blastospore** that eventually separates from its parent (Greek: *blastos*, sprout). However, in **fission yeasts**, such as *Schizosaccharomyces pombe*, a parental cell divides into two **progeny** in a manner somewhat similar to the transverse binary fission seen in bacterial reproduction. Yeasts rarely form true multicellular structures. Some yeasts form chains of elongated cells that are called **pseudomycelia** (singular: pseudomycelium) or **pseudohyphae** (singular: pseudohypha). Pseudomycelia are elongated yeast cells that arise from buds adhering together in branching chains. The individual cells within a pseudomycelium are independent of one another and, unlike the units within the septate hyphae of moulds, they are not connected by pores. Yeast cells with a typical unicellular morphology may cluster terminally or along the side of a pseudomycelium. These are called secondary blastospores. Some yeasts can produce septate, true mycelia under certain growth conditions (Fig. 1.5).

1.3.3 Dimorphic fungi

Although it is convenient to divide fungi into two groups, moulds and yeasts, there are fungi that are capable of adapting their structures in response to changes in their environment. They may grow in either a mycelial or a yeast form, depending on the prevalent growth conditions. These are referred to as **dimorphic fungi**. The mycelia formed by dimorphic fungi are true mycelia, unlike the pseudomycelia produced by some yeasts. Many of the fungi that

12 Microbial structure and mode of life

(a)



(b)

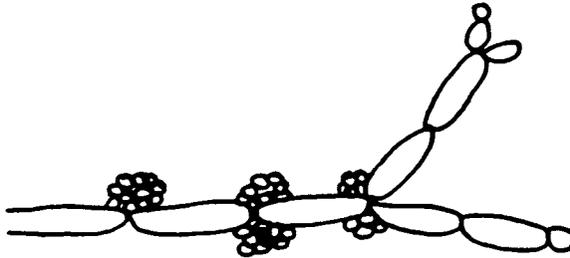


Fig. 1.5. Budding yeast cells (blastospores) and pseudomycelia that can develop in certain yeast species. The budding yeast cells are shown in (a) and the pseudomycelia are represented in (b). Elongated yeast cells are produced in chains, and secondary blastospores arise at cell junctions.

cause diseases in humans and animals are dimorphic, for example *Candida albicans*, the fungus that causes thrush. This is an infection that affects mucous membranes such as are found in the mouth or the genital tract. The visible symptoms of oral thrush are white plaques in the mouth, and vaginal thrush appears as an itchy white vaginal discharge.

1.3.4 The fungal cell wall

Fungi have been described as plant-like, because they are generally non-motile, and because their cells are bounded by a well-defined, multi-layered **cell wall**. However, the cell wall structure of plants and fungi differs considerably, with plant cell walls being made of celluloses and hemi-celluloses, and the fungal cell walls composed mainly of other polysaccharides including **chitin**, a polymer of **N-acetylglucosamine**. As well as being one of the principal components of fungal cell walls, chitin is a structural polymer that is found in the exoskeleton of the arthropod invertebrates.

The major component of the cell walls of both moulds and yeasts is **polysaccharide**, with up to 80% of the cell wall material comprising crystalline microfibrils in an amorphous matrix material. Of the remaining 20% of cell

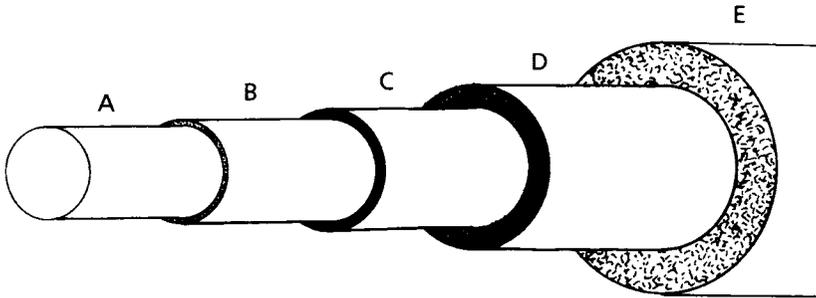


Fig. 1.6. The five-layered fungal cell wall structure. The fundamental layer, A, is the plasmalemma. Beyond this there is a layer, B, of chitin microfibrils in a matrix of proteins, mannans and glucans. This supports protein layer, C, beyond which is a glycoprotein network, D. The thickest, outermost layer, E, comprises amorphous glucans.

wall components, protein and lipid are present in approximately equal proportions. The cell wall polysaccharide component depends upon the type of fungus. In moulds, chitin is the principal fibrillar component and polymers of glucose known as **glucans** form the amorphous matrix material. In contrast to moulds, **mannans**, polymers of mannose, are the predominant structural components of yeast cell walls where they are also found with glucans. In baker's yeast, *Saccharomyces cerevisiae*, the cell wall contains less than 1% chitin, and this polymer is principally associated with bud scars, in which it forms a plug of material. In a special group of fungi (Oomycetes) within the Phycmycetes, cellulose forms the dominant structural component of the cell wall material.

The cell wall of fungi has five layers. It is exemplified by the architecture of the cell wall of the mature hyphae of the mould *Neurospora crassa*. The plasmalemma forms the foundation of the cell wall. Above this lies a layer of chitin microfibrils in an amorphous matrix of proteins, mannans and glucans that is about 20 nanometres thick. Beyond this lies a discrete protein layer of about 10 nanometres in thickness. This supports a **glycoprotein** network embedded in protein. This layer is about 50 nanometres thick. The outer layer of the cell wall is the thickest, and is made up of amorphous glucans. This layer is about 90 nanometres thick in *Neurospora crassa* (Fig. 1.6).

1.4 Reproduction in fungi

Reproduction in fungi may be **asexual** or **sexual** and, in both cases, **spores** are the structures that are responsible for dispersing progeny to colonise new

locations. Some spores are designed to withstand adverse growth conditions or to provide for a period of dormancy. The mycelia of moulds may also become fragmented, and the resulting fragments may each subsequently develop into an individual thallus by the process of **vegetative reproduction**. The term vegetative reproduction is used to refer to asexual reproduction where special reproductive structures other than spores are not formed. The vegetative or asexual state of a fungus is known as the **anamorph** and the sexual state as the **teleomorph** (perfect state).

1.4.1 Asexual reproduction in fungi

The simplest form of asexual reproduction is the production of vegetative spores. There are two principal structures associated with vegetative reproduction. These are **arthroconidia**, and **chlamydoconidia**. *Arthron* is the Greek for joint, and arthroconidia are produced by the hyphae that become disjointed and fragment. These may also be referred to as **thallospores**. Chlamydoconidia are usually larger than arthroconidia, are rounder and are swollen with food reserves. The Greek word for cloak is *chlamus*, and chlamydoconidia derive their name from their thick cell wall. Formation of these structures is usually a response to environmental stresses. Under favourable conditions, both arthroconidia and chlamydoconidia germinate to produce new mycelia (Fig. 1.7(a)).

True asexual spores of fungi differ from vegetative spores in that they are formed on or in specialised structures called **sporophores**. They are also generally produced in large numbers. They arise because cells divide mitotically, and so the genetic composition of the spores is identical with that of the parent. There is an astonishing variety of size, shape, complexity and colour displayed by the asexual spores of fungi. This provides an excellent means of identifying fungi, and also in part forms the basis of mycological classification. Some fungi produce only one type of asexual spore, whereas others may produce different types of spore.

The majority of spores are disseminated by wind, water or insects. Asexual spores provide a means of reproduction for the fungus and, because of the large numbers of spores that are produced, widespread dissemination of the species is possible. Some asexual spores are short-lived, and are sensitive to external stresses such as ultraviolet irradiation and desiccation. However, this is not always the case. Some spores, particularly those that are darkly pigmented or thick-walled, are resistant to such environmental pressures. Resistant spores can be used as a dormant stage in the life-cycle of fungi.

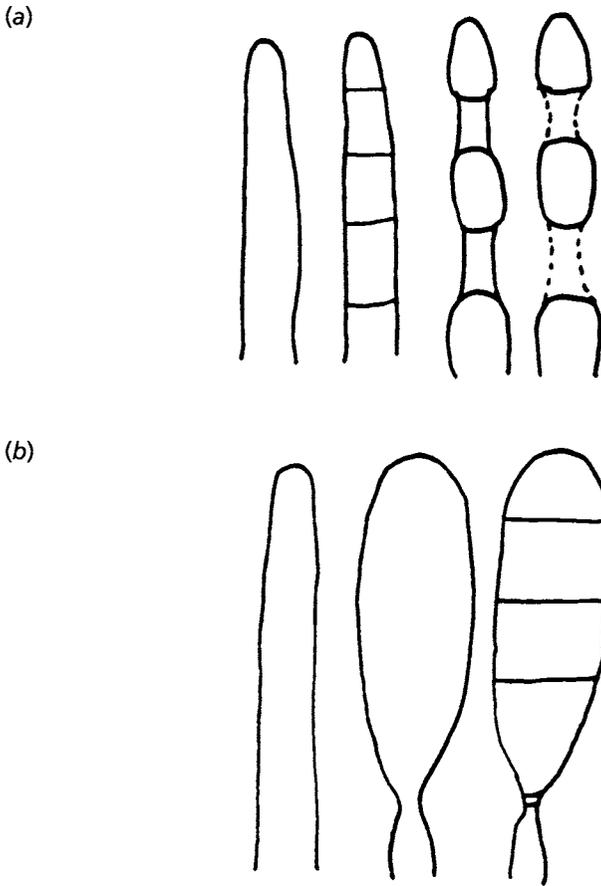


Fig. 1.7. Stylised arthroconidium (arthrospore) and thallospore production. (a) Production of arthroconidia, and (b) thallospore production.

Asexual spores can be produced either **exogenously** on the tips or sides of hyphae, or **endogenously** in specialised sac-like structures called **sporangia** (singular: sporangium). Lower fungi produce spores in sporangia that are typically formed at the tips of ordinary hyphae or on specialised hyphae called **sporangiophores**, although they may be formed along the hyphal structure. Sporangiohores usually contain large numbers of **sporangiospores** that may be motile or non-motile. Some sporangiophores form a **columella**. This is a dome-shaped structure that separates the sporulating and non-sporulating region within the sporangium. A columella of a sporangium that has discharged its spores may take on the appearance of an umbrella. Many terrestrial fungi produce non-motile spores called **aplanospores** (Greek: *a-*, not; *planao*,