ISBN: 978-93-90847-13-6

2. Fungal Ultra Structure, Composition, Septation and Environmental Interaction

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2.1 Information:

Ultrastructure is the architecture of cells and biomaterials that is visible at higher magnifications than found on a standard optical light microscope. Ultrastructure can be viewed with scanning electron microscopy (SEM) and super-resolution microscopy, although transmission electron microscopy (TEM) is a standard histology technique for viewing ultrastructure. Under study of SEM and TEM, fungi is a very complex multicellular mycelia organisms contain a multitude of form in terms of species abundance, genetic diversity and biomass. The fungal ultra structure can be discussed under five main heads - the cell wall, plasma membrane, cytoplasm, nucleus and cellular organelles (Figure-1).

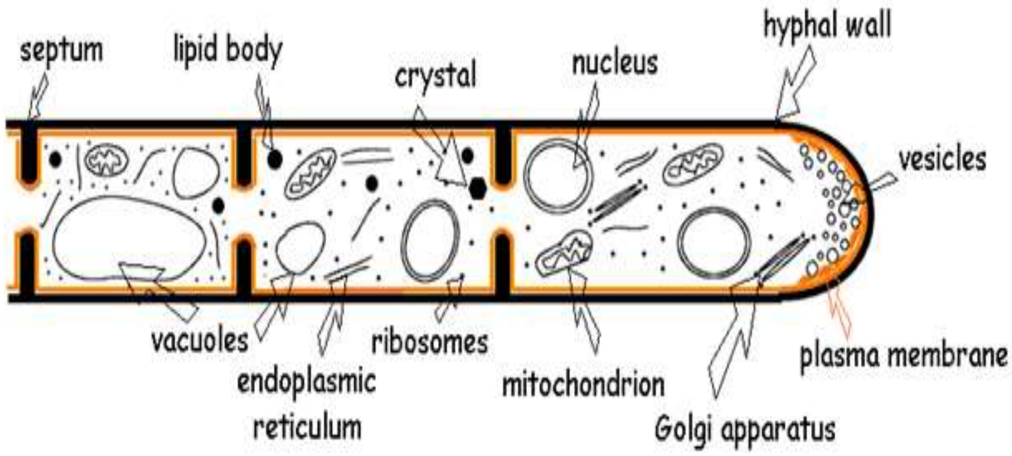

Figure 2.1: Fungal Ultra Structure

2.2 The Cell Wall:

The fungal cell wall is a dynamic structure that protects the cell from any stress in osmotic pressure and other environmental pressures. Except slime molds (Myxomycetes) that is lack of cell wall. The entire eukaryotic fungal cell consists of a rigid cell wall and cell organelles. Chemical analysis of cell wall reveals that it contains 80-90% polysaccharides and 20-10% proteins and lipids. Chitin, cellulose or other glucans are present in cell walls in the form of fibrils. The wall enables reproduction, recognition and reception. Approximately 80% of the cell wall consists of polysaccharides. Most fungi have a fibrillar structure built-up with chitin, chitosan (Zygomycota only), and ß-glucans, and a variety of heteropolysaccharides. Proteins constitute a small fraction of wall bound material, rarely more than 20%, and often as glycoprotein, which function as mating, recognition, wall modification and nutrition. Hydrophobins may constitute up to 10% of total wall protein are expressed constitutively, and become bound to the wall when the hyphae emerge in air. Walls also contain a range of other minor components which include pigments, melanin and salts. Melanin is important for protecting the hyphae and spores from UV stress. Fungal wall made up with polymeric fibrils embedded in an amorphous matrix and this is covered by further layers of matrix material.

At the hyphal tip the wall is thinner and simpler in structure, consisting of only two layers - an inner layer of fibrils (polymeric) embedded in protein and outer layer of mainly protein. Extra layers of wall material are deposited in the lateral walls behind the extending apex - strengthening the wall as the hypha matures. In the oldest parts of the hyphae (and in many fungal spores) lipids and pigments may be deposited in the wall. Lipids are found in walls, usually in very small concentrations that serve as a nutrient reserve and help prevent desiccation of pigments, such as melanin.

Fungal cell wall consists of two cell wall component- polymeric fibrils and amorphous gel-like matrix components (Table 2.1).

2.2.1. Polymeric Fibrils:

Polymeric fibrils made up with chitin in most of the fungal cell wall and cellulose exceptionally in oomycotina. Polymeric fibrils include-

- Chitin [ß (1-4)-linked polymer of N-acetylglucosamine]
- Chitosan [ß (1-4)-linked polymer of glucosamine]
- Glucans [ß (1-3) and ß (1-6)] alkali insoluble
- Cellulose [ß (1-4)-linked polymer of glucose]

The structure of the β (1-4) glycosidic bond allows the formation of microfibrills. The diameter of the microfibrills can be 10-25 nm. The microfibrills form a high strength mesh in the cell wall.

2.2.2. Amorphous Gel-Like Matrix Components:

It is made up with glucans, proteins, lipids and heteropolymers (mixed polymers of mannose, galactose, fucose and xylose). The types and amounts of these components differ amongst different groups of fungi and may even vary during life cycle of a single species.

2.3 Cell Wall Functions:

- Protects the underlying protoplasm.
- Determines and maintains the shape of the fungal cell or hypha; if you remove the wall the resulting protoplast will always assume a spherical shape.
- It acts as an interface between the fungus and its environment.
- It acts as a binding site for some enzymes.
- It possesses antigenic properties which allow interactions with other organisms.
- Compatible glycoproteins are called as agglutinins, in the walls of each complementary hypha fuse to form a complex structure and release hormone-like compounds. Adhesion is also mediated by fibrillar glyco-proteins embedded in a gel-like matrix.
- Hydrophobins may constitute up to 10% of total wall protein. Each molecule consists of a hydrophobic domain and a hydrophilic domain. The protein is attached to the fungal wall by the hydrophilic end. The hydrophobic domain is exposed. Hydrophobins reduces movement of water through the wall of the hypha and providing some protection from desiccation. It may also increase the strength of the wall.
- Some members of the Glomeromycota produce a putative glycoprotein in the walls is called glomalin. Recent work suggests that glomalin is related to Heat Shock Proteins (HSPs) in group 60. However, the structure and function of glomalin is unknown.

Table 2.1: Common wall Constituents Found in Each Division of Fungi (Adapted from Gooday in Gow & Gadd, 1995)

Division	Polymeric Fibrils	Gel-Like Polymeric Metrix
Basidiomycota	Chitin, Glucan	Xylomannoproteins, Glucan
Ascomycota	Chitin, Glucan	Galactomannoproteins, Glucan
Zygomycota	Chitin, Chitosan	Polyglucuronic acid, Glucuronomannoproteins, Polyphosphate
Chytridiomycota	Chitin, Glucan	Glucan

2.3.1 Plasma Membrane:

The plasma membrane, also called the cell membrane, is the membrane found in all cells that separates the interior of the cell from the outside environment. The plasma membrane consists of a lipid bilayer that is semipermeable. The plasma membrane regulates the transport of materials entering and exiting the cell. The fungal cell wall encloses the protoplasm with various membrane proteins. However, there are some specialized organelles in the surface of plasma membrane where the fusion of secretory vesicles of cytoplasm occurs. The plasma membrane invaginates and forms a pouch like structure lomasomes enclosing the granular or vesicular materials. It has been defined as “membranous vesicular material” embedded in the wall external to the line of plasma membrane.

2.3.2 Cytoplasm:

The cytoplasm is typical in all respects of a eukaryotic cell. Cytoplasm is colorless and sap-filled vacuoles are found.

Cytoplasm consists of various inclusions such as lipid droplets and glycogen, carbohydrate trehalose, proteinaceous material and volutin. Several metabolites are secreted by the cytoplasm. In matured cell, lipids and glycogen are abundantly present. The cytoplasmic inclusions are dead, non-functional and unimportant for fungal survival.

Of particular interest is the presence of plasmids in cytoplasm of yeast. As many as one hundred plasmids are found in yeast cells. Plasmids are also found in filamentous fungi, where some are associated with disease virulence. Typical fungal cytoplasmic constituents are multivesicular bodies (MVBs), woronin bodies, filasomes, glycogen storage particles, microbodies, golgibodies, endoplasmic reticulum, microtubules and microfilaments.

2.3.3 Nucleus:

Nuclei are always present in living cells. The fungal cytoplasm contains one or more globose or spherical nuclei of about 1-3μm in diameter. A nucleus consists of a bilayered porous nuclear membrane that encloses the chromosomes and nucleolus. Nuclear membrane persists during division, unlike plants and animals. The process of karyokinesis starts with the formation of 2 sister nuclei by constriction in the middle of the nuclear membrane. No clear metaphase plate was reported, chromosomes randomly dispersed. At anaphase stage two daughter chromatids pull apart along two tracks on spindle fibers of different lengths. Various types of spindle pole bodies appeared to ensure that chromosomes separate correctly during nuclear division. Nuclear associated organelles (NAOs) were associated with the nuclear envelope which functions as microtubule-organizing centers during mitosis and meiosis. Spindle pole bodies (SPBs) that lack flagellated stage in lifecycle in fungi were reported as disc shaped in Ascomycota and Mitosporic fungi and two globular ends connected by a bridge in Basidiomycota.

A fungus contains 3-40 chromosomes which are small and difficult to visualize in stained and squashed preparations. The chromosome consists of DNA and histones proteins. The nuclear pores permit to interchange the materials between the cytoplasm and nucleus. Fungi are reported to be haploid, diploid or polyploidy in nature. Haploid species are varied with their chromosome numbers e.g. *Schizophyllum commune* (6), *Neurospora crassa* (7), *Emericella (Aspergillus nidulans* (8), *Saccharomyces cerevisiae* (16) and *Ustilago maydis* (20*). Candida albicans* exist as naturally diploid species. *Allomyces* sp. and *Phytophthora* sp. exist as polyploid species. Fungi contains up to 13-40 Mb (million base pairs) of DNA for coding 6,000 to 13,000 genes.

2.4 Cell Organelles:

2.4.1 Endoplasmic Reticulum:

Endoplasmic reticulum (ER) is a single interconnected membrane system. It is continuous with the nuclear envelope and intracellulary forms a contiguous system of both non-fenestrated sheets and variously branched tubules (Figure-2). The latter predominates at the cell periphery where the tubules are closely associated with and may possess molecular links to the plasma membrane. ER persists throughout the cell cycle. The type of ER network varies in yeast and filamentous fungi. Prominent peripheral ER network was reported in rust fungus during stages of specific development (tubular vesicular network) mostly rough with polyribosomes. The ER is highly motile. In *Ustilago maydis* cytoplasmic dynein and microtubules were shown to be required for ER motility but not for maintaining basic ER organization. It played essential role in both protein and lipid pathways as the site of biosynthesis of nearly all cellular membrane and trans-membrane proteins regardless of ultimate destination. Luminal proteins destined for Golgi apparatus and Vacoules are co-transnationally delivered into the ER lumen. ER lumen stores calcium ions to be released into cytosol upon induction via appropriate signalling.

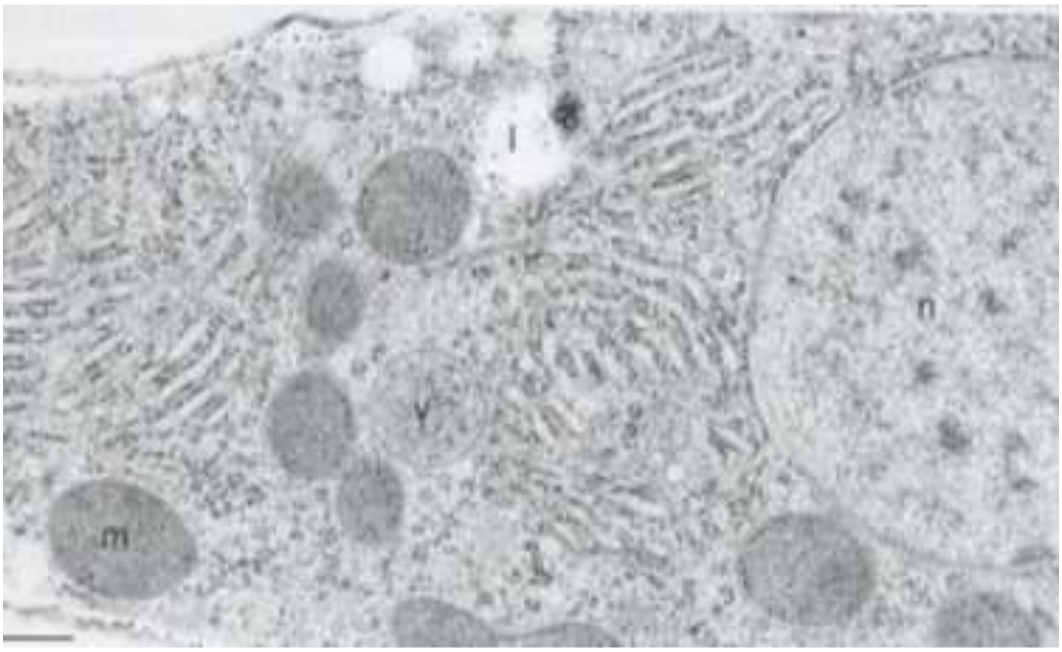

Figure 2.2: Endoplasmic Reticulum (ER) Is A Single Interconnected Membrane System In Fungi.

2.4.2 Golgi apparatus:

In true fungi, Golgi bodies are morphologically very simple and assemblages of tubular cisternae and a lack of cistarnal stacks are the hallmarks of Golgi in filamentous fungi, observed interspersed with vesicle in hyphae of *Aspergillus nidulans*. The width of tubules of a single cisternum is fairly uniform. Golgibody of basidiomycetes are characterized by swollen peripheral terminals.

A multivesicular body is thought to be intermediate compartment between early and late endosome and can be very abundant in hyphae in *Giberella persicaria*. They are inter-spread with microtubules and vesicles that exhibit bidirectional movement. Golgi equivalents are individual organelles, consisting of fenestrated sheets with tubular extensions that are dispersed throughout the hyphae or fenestrated hollow spheres or sheets wrapped closely around mitochondria. The width of cisternal tubules is generally uniform within such a sheet or sphere. Fungal vacuoles are an assemblage of pleiomorphic entities that can exist as elongated tubular structure (Figure 3).

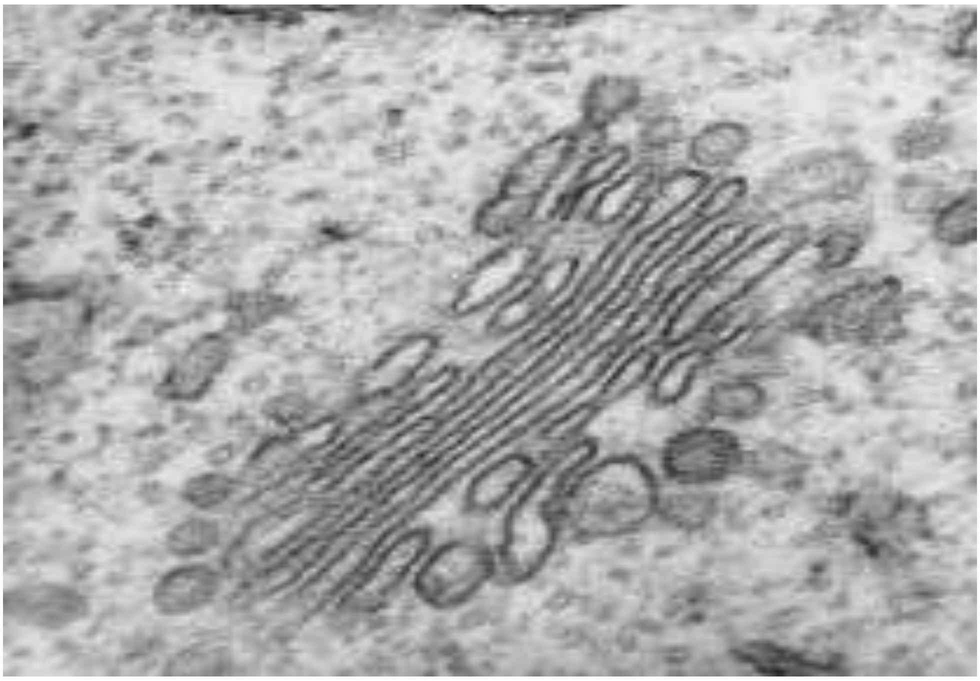

Figure 2.3: Assemblages of Tubular Cisternae and A Lack of Cistarnal Stacks are The Hallmarks of Golgi In Filamentous Fungi, Observed Interspersed With Vesicle In Fungal Hyphae. Golgibody of Basidiomycetes are Characterized by Swollen Peripheral Terminals.

2.4.3 Hydrogenosome:

In some obligately anaerobic organisms, including fungi, an organelle is observed which appears to produce hydrogenase and pyruvate oxidoreductase. The enzymes function in the anaerobic conversion of organic carbon to energy. The organelle is called a hydrogenosome. Hydrogenosomes have been observed in the Chytridiomycota found in the rumen of herbivores; these Chytrids lack mitochondria.

2.4.4 Filasomes:

Relatively little is known about them. Appear as tiny vesicles that are coated with a dense filamentous material. They are numerous in tips of actively growing hyphae (peripheral subapical region) where they are characteristically found in close association with the plasma membrane.

2.4.5 Plasmalemasome and Lomasomes:

Plasmalemmasomes are the various membrane configurations which are external to the plasmalemma, often in a pocket projecting into the cytoplasm, and less obviously embedded in wall material. Lomasome has been defined as membranous vesicular material embedded in the wall external to the line of the plasmalemma. There is a gradation between these two structures.

2.4.6 Mitochondria:

Aside from Nuclei it is the most conspicuous structure. Barely visible in light microscopy as tiny thread or rod like structures, at ultrastructural level appear as electron-dense structures. The mitochondria of fungi are clearly recognizable. They have a double bilayer membrane and contain complex internal membranes. They differ from other eukaryotic organisms in that the mitochondria are commonly elongate, oriented along the hyphal axis. The flattened or plate-like mitochondrial cristae in fungi are similar to that of animals. The membranes are organised as parallel lamellae usually oriented along the long axis. This orientation is particularly common in older regions of the hypha where vacuoles comprise a large proportion of each compartment, and the cytoplasm in between the vacuole and the wall. Giant, branched mitochondria have been observed in yeasts, and intermediate forms occur in cells transforming from yeast to filamentous growth. These are power house of the cell. It has machinery for transcription and translation of organelle specific DNA.

2.5 The Flagellum:

Among the true fungi only chytrids have flagella. The motile zoospores have one posterior flagellum (opisthokont) without tinsel (whiplash). The flagellum has 9+2 microtubules arranged in the typical eukaryotic pattern. The chytrids rumen fungi have posteriorly multiflagellate (up to 16 whiplash flagella) zoospores. The organization of the flagellar apparatus at the base of the flagellum is a characteristic feature of different groups of the chytrids.

2.5.1 Vacuoles:

Vacuoles are essential for cell function in fungi. Fungi are characterised by the presence of spherical to tubular vacuoles. They are found in the old cells of hyphae. The end of hyphal tip of young hyphae lacks vacuole. They are surrounded by membrane called tonoplast. They play an important role in osmoregulation.

2.5.2 Fungal Endomembrane System:

The key feature of endomembrane system is related to hyphal apical growth towards the apex with addition of all the materials needed to create new wall, new membranes and new cytoplasmic components. Most of these materials are exported in vesicles by the endoplasmic reticulum (ER) and Golgi organelles, the vesicles being delivered to the apical vesicle cluster (called the Spitzenkörper) along microtubules powered by motor proteins of the kinesin and dynein families.

The Spitzenkörper organises the final distribution of micro vesicles along actin microfilaments to the plasma membrane at the extending tip. Vesicle fusion with the membrane is enabled by t-SNARE and v-SNARE proteins. Endocytosis at the hyphal tip is dependent upon actin patches where myosin-1 polymerises actin into filaments that take the endocytotic vesicles away from the membrane. The extreme apex of hyphal tips undergoes extensive exocytosis, which is mainly devoted to synthesis of wall polymers outside the membrane and wall construction and maturation (Figure 1.4).

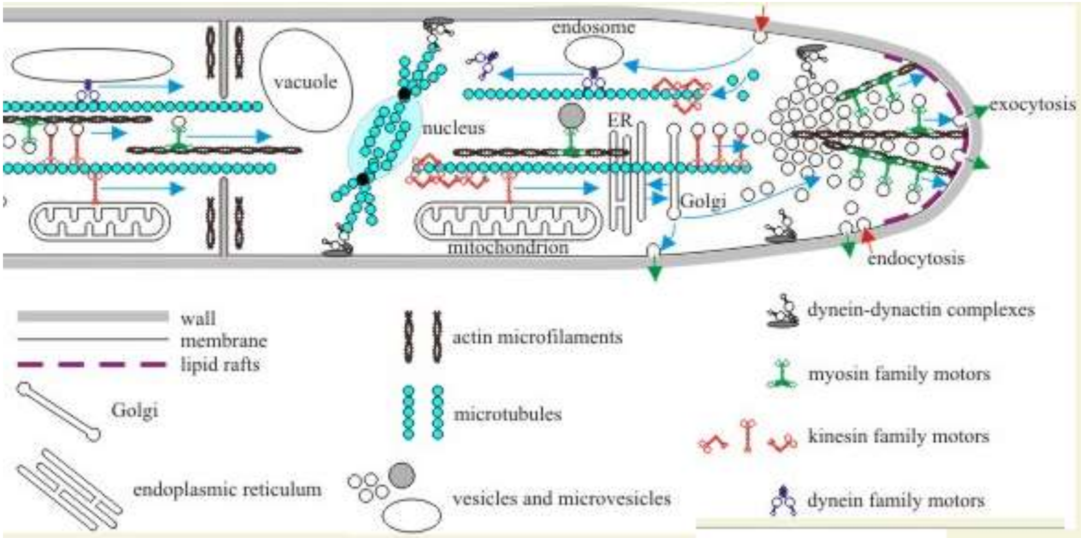

Figure 2.4: Fungal Endomembrane System. New Materials Are Exported In Vesicles By The Endoplasmic Reticulum (ER) And Golgi Organelles, The Vesicles Being Delivered To The Apical Vesicle Cluster (Called The Spitzenkörper) Along Microtubules Powered By Motor Proteins of The Kinesin And Dynein Families.

2.6 Septation and Environmental Interaction:

Septa (cross-walls) can be seen by light microscopy. It looks like a valve between adjacent hyphal cells to prevent the accidental loss of cell organelles between hyphal compartments. Pores within the septa allow the organelles and nutrients to flow freely when required. Depending upon the species, the protoplasm may form a continuous, uninterrupted mass running the length of the branching hyphae with multiple nuclei is called as coecocytic, or the protoplasm may be interrupted at intervals by cross-walls called septa. Septa divide up hyphae into individual discrete cells or interconnected hyphal compartments. There can be various types of septa present in different group of fungi. They are complete septa, perforated septa, dolipore septa, etc (Figure-2.5&2.6). Electron microscopy has revealed that several different types of septa exist among the major taxonomic groups of fungi.

- **Septa in Oomycota and Zygomycota:** In general, the fungi belonging to these groups are not septate (i.e. coenocytic). But septa in the form of complete cross-walls are formed to isolate old or damaged regions of the mycelium or to separate reproductive structures from somatic hyphae.
- **Septa in Ascomycota and Some Mitosporic Fungi:** The Basidiomycota possess perforated septa at regular intervals along their length. The septum consists of a simple plate with a relatively large central pore (50-500 nm diameters) which allows cytoplasmic streaming (i.e. the movement of organelles including nuclei between adjacent hyphal compartments). Cytoplasmic streaming is also enabled to transport nutrients and essential enzymes between adjacent hyphal compartments. Septum of Ascomycota has spherical, membrane-bound organelles called woronin bodies. It was first reported by the Russian mycologist Mikhail Stepanovich Woronin in *Ascobolus pulcherrimus* in 1864. A Woronin body is a peroxisome derived, dense core microbody with a unit membrane found near the septa that plays role in the plugging of the septal pores after hyphal wounding which restricts the loss of cytoplasm to the sites of injury (Figure 2.7).

Not all fungi belonging to the Ascomycota possess Woronin bodies. Those often possess Large Hexagonal Crystal of Proteins (LHCPs) in the cytoplasm that is capable of serving the same function, i.e. they can seal the septal pores of damaged or ageing hyphae.

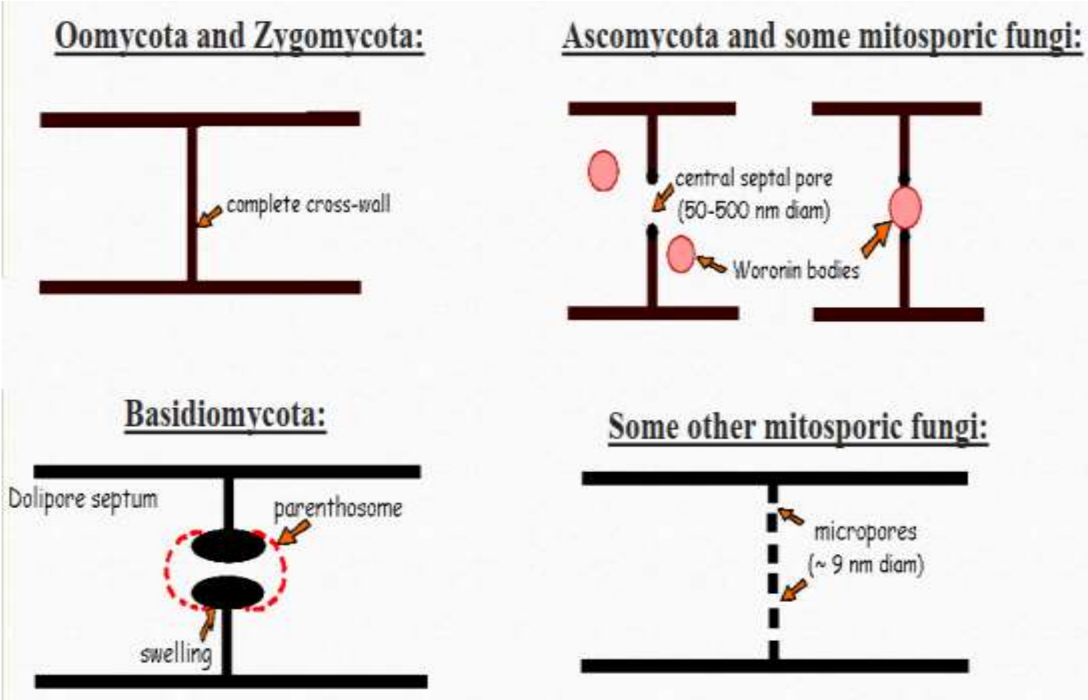

Figure 2.5: Septation in Different Groups of Fungi

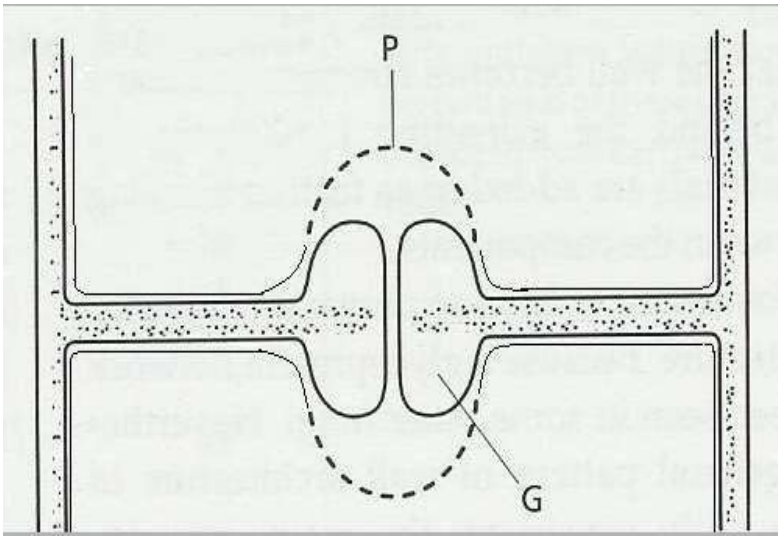

Figure 2.6: The Dolipore Septum of Basidiomycotina. Large Deposits of Glucan (G) Line The Narrow Central Pore, and Specialised Perforated Membranes Termed Parenthosomes (P) Prevent Major Organelles For Passing Through The Septal Pore. [© Jim Deacon]

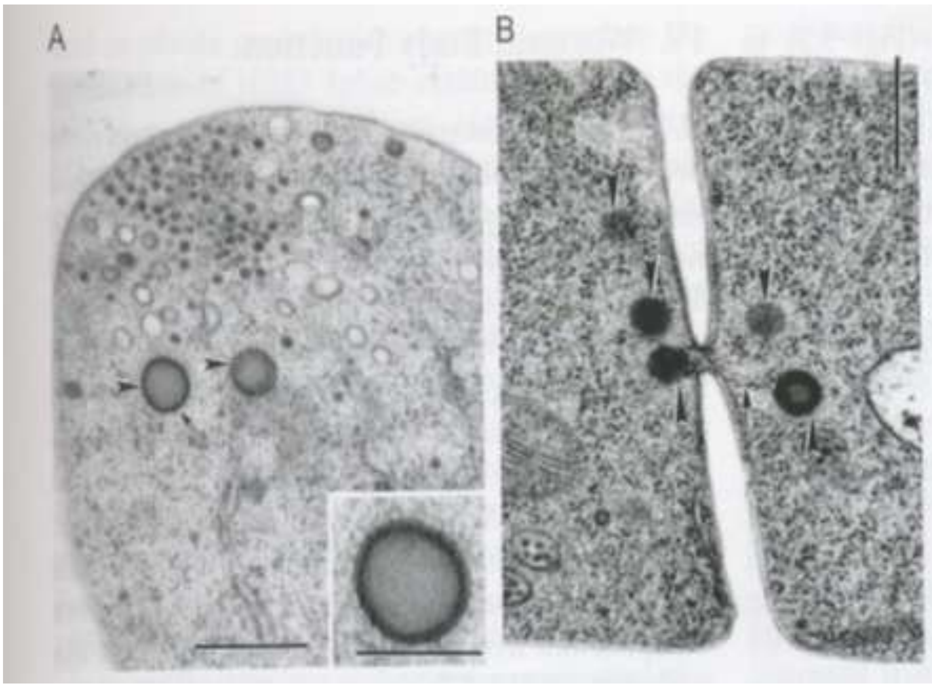

Figure 2.7: A-B: Woronin Body Appearance and Distribution in *Aspergillus Nidulans* and *Neurospora Crassa* by Transmission Electron Microscope (TEM). Woronin Bodies (Arrowheads) Are Observed In The Vicinity of the Spitzenkorper.

Septa in some other mitosporic fungi: Other mitosporic fungi may possess multiperforate septa e.g. the septa of *Geotrichum candidum* possess characteristic micropores (approx.9nm diameters). The number of pores in each septum can vary up to a maximum of 50. These micropores allow cytoplasmic continuity between adjacent hyphal compartments.

Septa in basidiomycota: The most complex type of septum is characterized by a swelling around the central pore (dolipore) and a hemispherical perforated cap i.e.parenthosome on either side of the pore. The perforated parenthosome allows cytoplasmic continuity but prevents the movement of major organelles.

The Dolipore septa are restrictive to the extent that the migration of nuclei is only possible under certain circumstances (mating), when the septum dissolves. The plasma membrane lines both sides of the septum and the dolipore swelling, but the membrane of the parenthosome is derived from endoplasmic reticulum.

2.7 Function of Septa:

- **Structural Supports:** The addition of plate-like cross-walls along long tube-like structure (hypha) gives mechanical support and stability of cell.
- **First Line of Defense:** When part of a hypha is damaged. In a large-pored septa that have Woronin bodies or large proteinaceous crystals associated with them have a mechanism for rapidly sealing the septal pore under conditions of stress (e.g. if the hypha is damaged) thereby protecting the mycelium.

- **Facilitate Differentiation:** Septa can isolate adjacent compartments from one another so that different biochemical and physiological processes can occur within them. These may result in differentiation of the hyphae into specialized structures such as those associated with sporulation.

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