The Hyphal Mode of Life

prepared by

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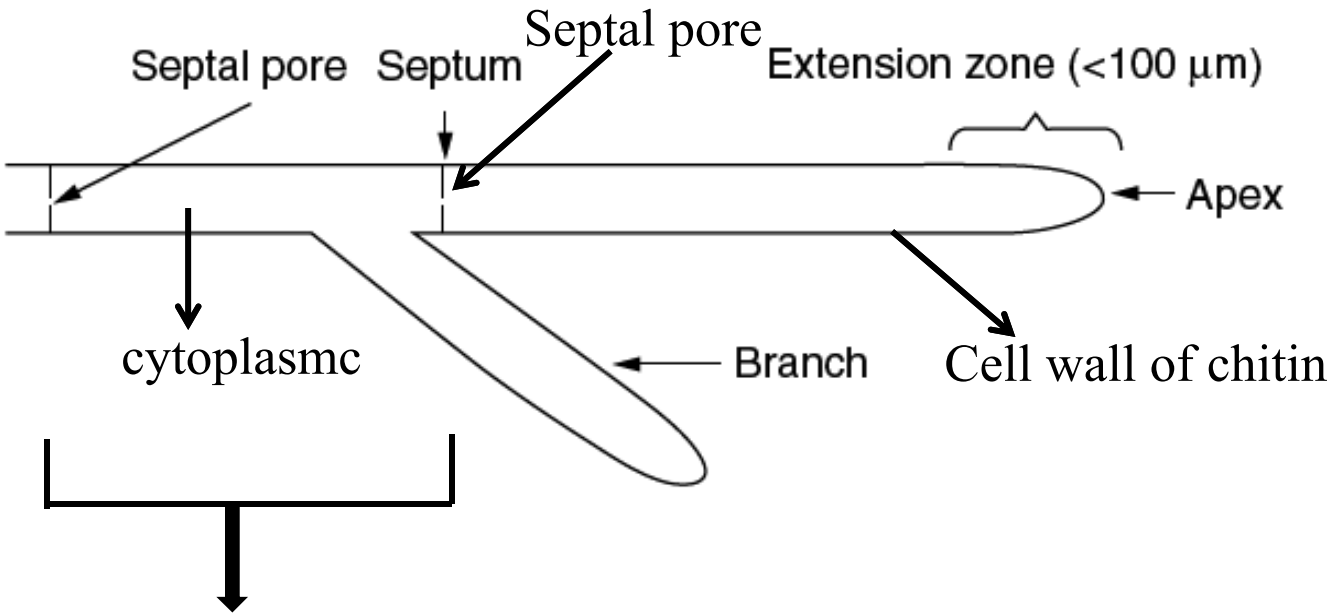
1. Introduction

- Fungi are composed of microscopic, tube-like structures called *hyphae* (singular hypha).
- The hypha
  - shape of a cylindrical tube of even diameter (2-30 μm, mostly 5-10 μm)
  - with a tapering tip meaning grow from its tip.
  - branches subapically, with each branch having a tip of its own.



Behind growing tip the hypha ages progressively

In oldest regions hyphae may break down by

Autolysis (self –enzymes)

Heterolysis (other organisms enzymes)

Radiating system of hyphae = **Mycelium**

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So the hyphae interconnected in away mycelium brought into protoplasmic continuity.

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Mycelium spreads over and penetrate into subtratum

↓

Secreting digestive enzymes

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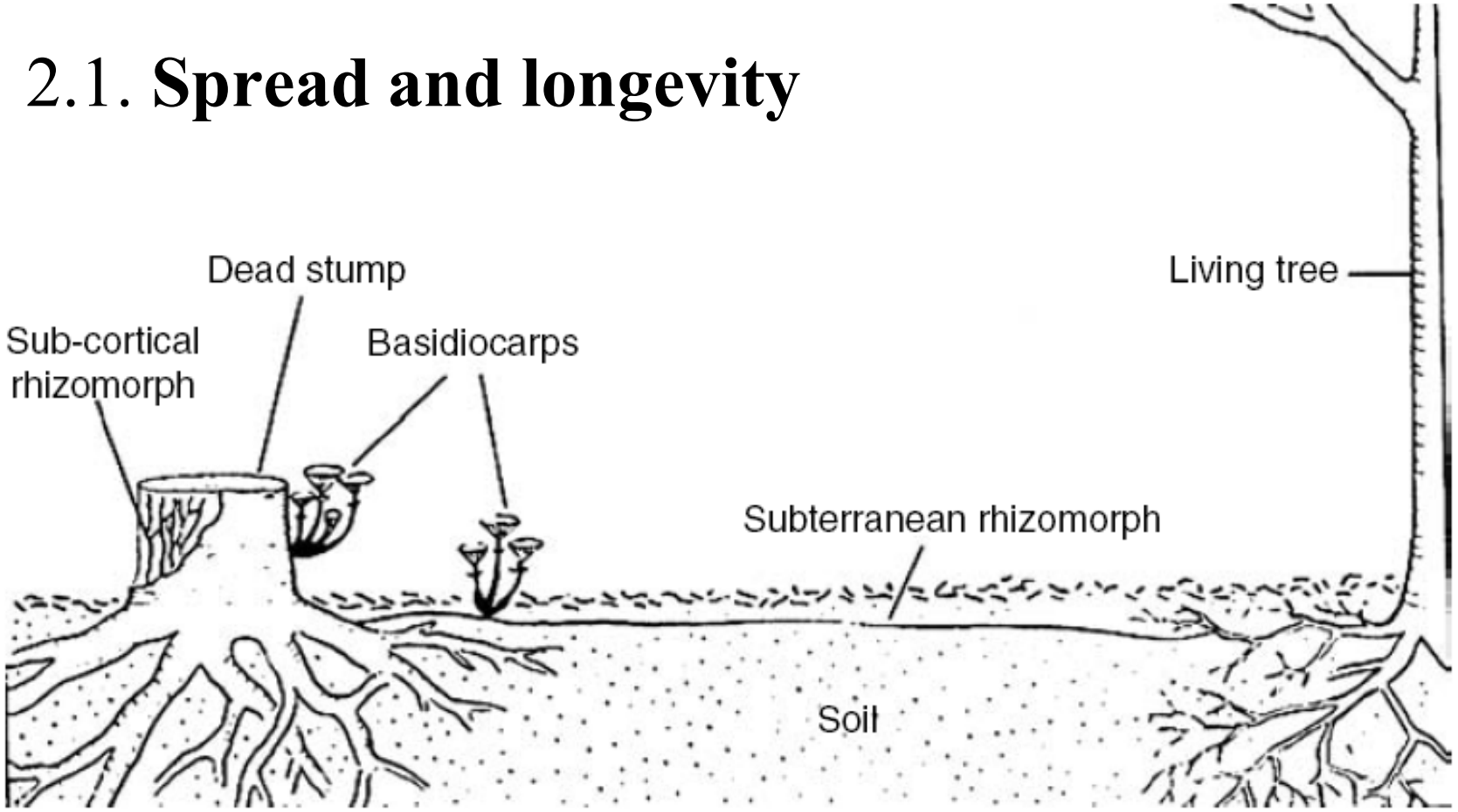
Decompose polymeric constitue of substratum

bsorbing solubilized carbon, P, N, K, S.

2. Features of hyphae

2.1. **Spread and longevity**



2. 2. **indeterminate growth**

-hyphae of individual fungus (theoritically) extend endlessly via apical growth, provided they are supported with nutrients and environmental conditions.

- to investigate genetic relatedness of mycelial isolates by **DNA fingerprinting methods**

DNA fingerprinting methods

Restriction Fragment Length Polymorphism (RFLP)

- Principle: organisms can be differentiated from one another by analysis patterns derived from cleavage of their DNA.
- Distance variation between 2 organisms particular restriction endonuclease cleavage sites.
- DNA resulted fragments differ in their size.
- Separated by electrophoresis according to size difference.
- Different DNA bands patterns due to motilities difference indicate different individuals.

Random Amplification of Polymorphic DNA (RAPD)

- Principle: Arbitrary designed 10 –base pair sequence is annealed to DNA isolated from strains
- Polymerase chain reaction carried out by DNA polymerase.
- DNA resulted products separated by electrophoresis according to size difference.
- Different DNA fragments patterns compared.
- different patterns indicate different individual.

- Different fungal isolates from an extensive area DNA fingerprinting results showed identicallity.
- Example : isolates from an area around 15 hectars belonged to same fungus.
- Therefore, hyphae are capable of potentially unlimited growth.
- Fungi regarded as Immortal organisms.
- Eucarpic fungi are spatially and temporally indeterminate organisms.

- However, different regions of fungal mycelium displays considerable developmental plasticity in:
  - Growth.
  - Branching.
  - Anastomosis (fusion).
  - Age.
  - Death.
  - Sporulation.
  - Varying physiological and biochemical activities at different times and even simultaneously… depending on local micro-environment conditions.

2. 3. **Extension and Synchronized growth:**

- During growth protoplasm moves continuously from hypal old regions towards the tip.

↓

hyphae extends at one end & ages at one end

- Measurements over a period between hyphal tip and the first septum showed that while the former increased the latter remained constant.

↓

Hyphal growth is confined to the tip

- Experiment: C14-labelled N-acetylglucosamine (radioactive precursor of chitin) ⟶ fed to growing mycelium ⟶ autoradiographic imaging detection of incorporation site in hyphae ⟶ terminal regions =100µm ⟶ zone of elongation

2.4. **Large surface area**

- Hyphae are generally 5 to 10 μm in diameter.
- Hypha has a large surface area in relation to the total mass of protoplasm.
- **Advantages:**

1. Maximizes its contact with environment for uptake of raw materials for biosynthesis, for gas exchange and for release of metabolism products.
2. Sugars and metabolizing enzymes are stored in the space between the plasma membrane and the cell wall (intramural space), which the hypha can apparently use as the energy and carbon source during exploration of the surrounding area for nutrients. This strategy is manifest in the high respiratory rate of mycelium in the absence of exogenously supplied respiratory substrate (endogenous respiration)—a feature that undoubtedly contributes to tolerance to adverse conditions for extended periods.

2.4. Large surface area

- Disadvantages:

1. Because of the single-cell thickness of the hypha, the environment has a direct effect on it, rendering the thin walled hypha vulnerable to desiccation.

2. Fungi, therefore, must grow either in aqueous media or in a very humid atmosphere. In adverse conditions, the tip perceives a signal and apparently produces a conidiation-inducing factor that diffuses behind it, inducing formation of double septa along the length of hypha. The cells disarticulate and function as propagules called *Arthroconidia* (Arthroconidia are the simplest type of spores formed by hypha.)

3. **Fungal Wall**

- **Definition**: peripheral structure encases cytoplasm and internal organelles.

- **Components:**
  1. Fungal wall.
  2. Periplasm
  3. Plasma membrane.
  4. Additional extracellular structural components.

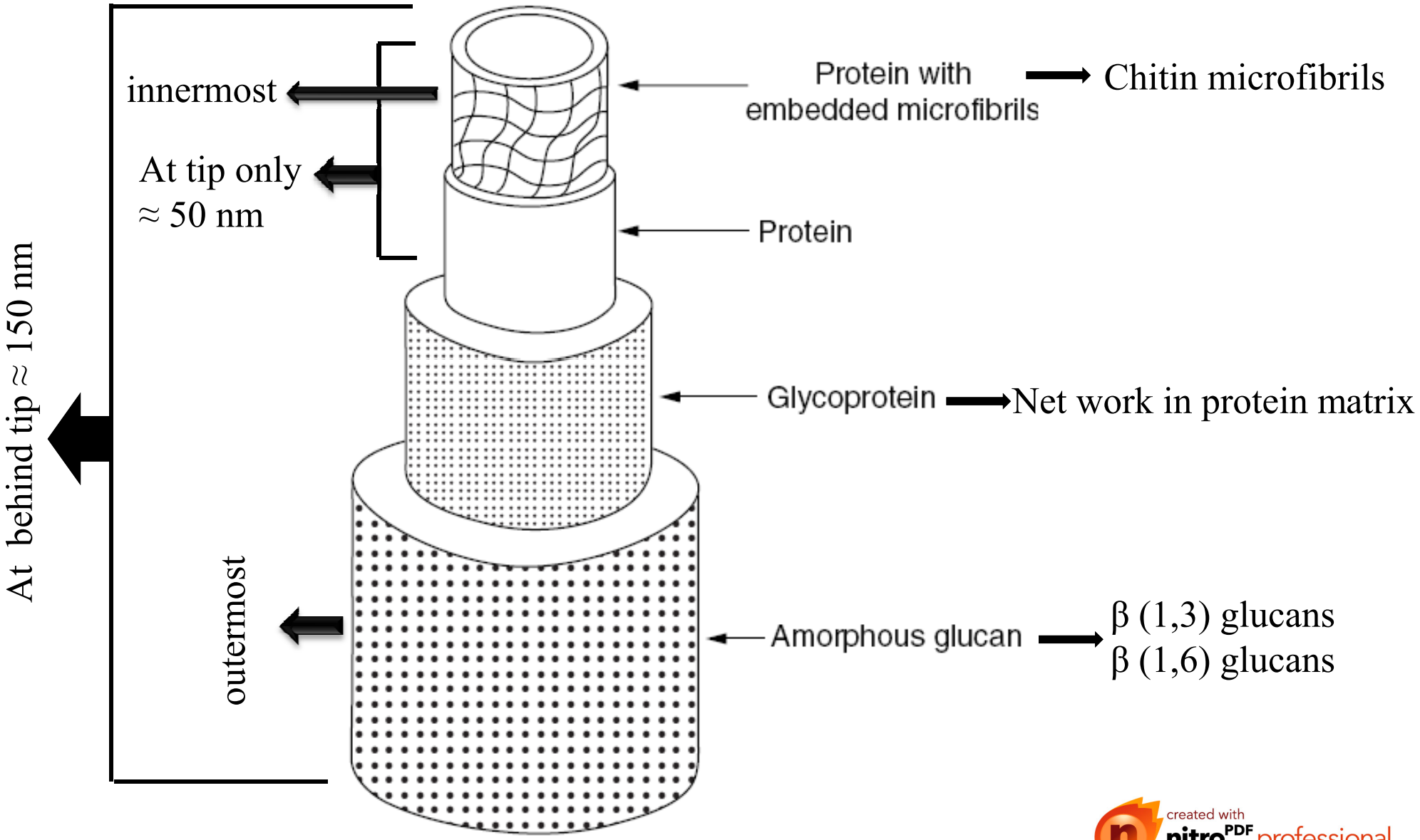
- **Functions:**
  1. Structural barrier (interface between fungus & environment)
  2. Protection against osmotic lysis.
  3. Act as molecular sieve regulatory of passage of large molecules through the wall pore space.
  4. If contain melanin it can protect cell against U.V.
  5. Having binding sites for enzymes as many disaccharides (sucrose, cellulose) and small peptides need to be degraded to monomers before pass through cell membrane → **Wall-bound enzymes.**
  6. Have surface compounds mediate interactions of fungi with other organisms e.g. plants, animals.
  7. Tubular form of hyphae is an ideal structure for
     1. Forcing entry into living tissues.
     2. Extending through soil.
     3. Growing erect to produce propagules and disseminate them into air.

- **3.1. Fungal wall**
  - **Composition:**

1. Structural (fibrillar) polymers → predominantly of straight-chain molecules → structural rigidity.

2. Matrix components → cross-link the fibrils and that coat and embed the structural polymers.

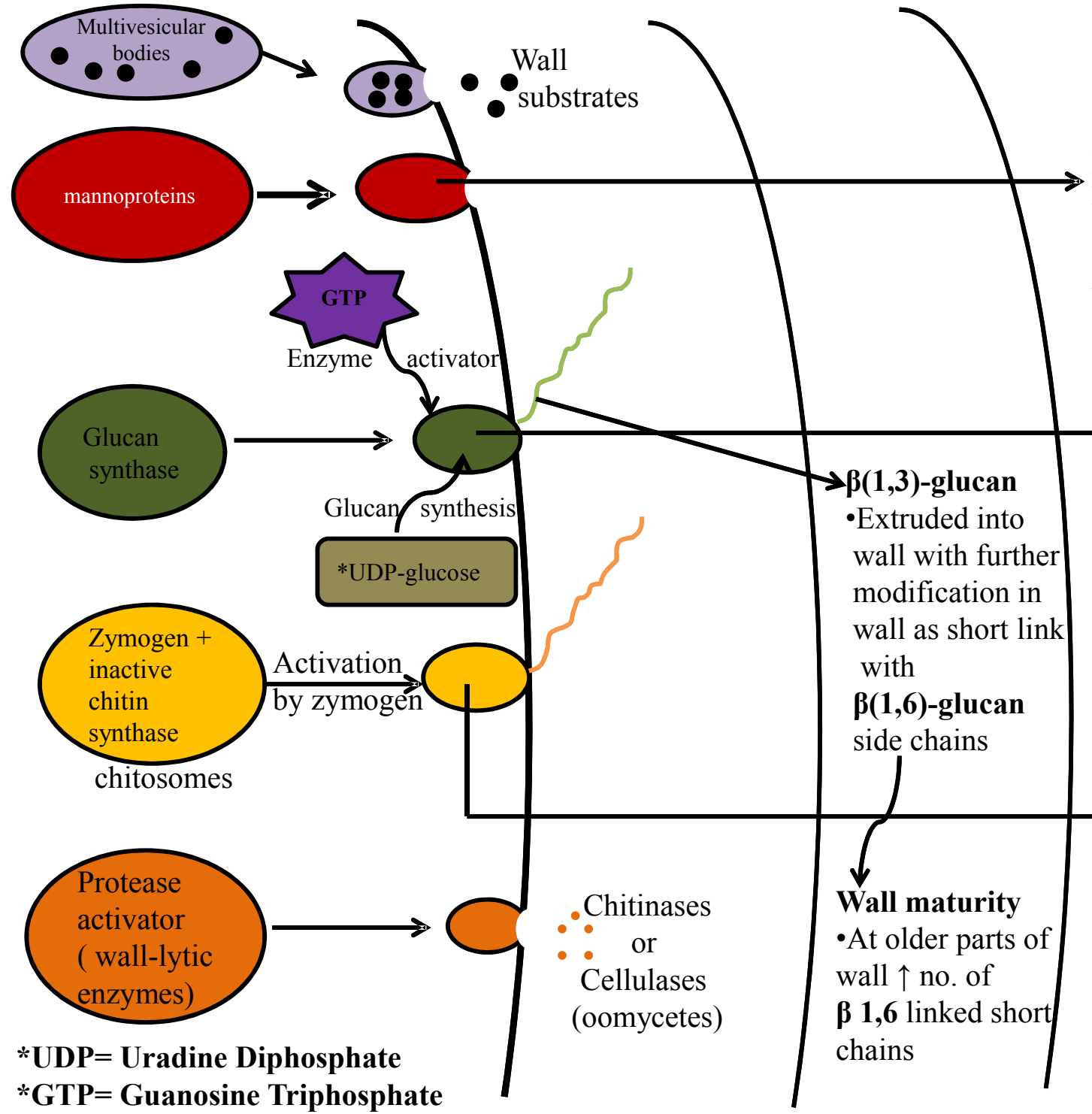
- Wall architecture:



- **Different fungal wall composition in different fungal taxonomic groups**

Taxonomic group	Fibrillar polymers	Matrix polymer	Septa
Straminipila	β (1,3) glucans β (1,6) glucans cellulose	Glucan	No
Chytridiomycotina	Chitin Glucan	Glucan	No
Zygomycota	Chitin Chitosan Glucan	Polyglucuronic acid	No
Basidiomycota	β (1,3) glucans β (1,6) glucans Chitin	α(1,3) glucan xylomannoprotein	Dolipore
Ascomycota/ Deuteromycota	β (1,3) glucans β (1,6) glucans Chitin	α(1,3) glucan galactomannoproteins	With large central pore

• Assembly of the wall at hyphal apex



Mannoproteins
Form relatively small proteins of wall.
Glycosylated proteins are few wall components preformed in E.R. & G. complex then derived in vesicles.

Glucan synthetase
- Become as integral protein.
- Major enz. In wall growth.
- Its substrate is **UDP-glucose**
- **β(1,3)-glucan** synthesis (bulk of fungal wall)
- the enzyme of 2 subunits
  - **Catalytic site** (on outer membrane surface)
  - ***GTP binding protein site** (on inner membrane surface)

β(1,3)-glucan
- Extruded into wall with further modification in wall as short link with **β(1,6)-glucan** side chains

Chitin synthase
- Become as integral protein.
- Catalyses chitin chains synthesis.
- Chitin formed at apex as the substrate **N-acetylglucoamine** which is delivered as nucleotide (UDP)
- At inner face of chitin synthase chitin synth
- Extru
- Contr[illegible] prevent spill-over

Wall maturity
- At older parts of wall ↑ no. of **β 1,6** linked short chains

***UDP= Uradine Diphosphate**
***GTP= Guanosine Triphosphate**

- **Cross-linking and maturation of hyphal wall:**
  - **Chitin-glucan:**
    - Linked by covalent bond which is formed between major wall polymers after been inserted in wall.
    - Occur progressively back from hyphal tip.
  - **Chitin-chitin:**
    - Are associated with one another by H-bonds to form microfibrils.
  - **Glucan-glucan:**
    - are associated with one another also.

Therefore, these additional bondings behind the growing apex could serve to convert initially plastic wall into progressively more cross-linked and rigidified structure.

- It has been suggested that existing wall must be softened in order for new wall components to be inserted.
- Wall growth involves balance **wall lysis** and **wall synthesis**.

3.2. Periplasm

- Periplasmic space is the region external to the plasma membrane and internal to the cell wall.
  - in yeast cells: comprises secreted proteins = **mannoproteins** and **enzymes** = invertase and acid phosphotase (unable to traverse wall)
  - In filamentous fungi, cell membrane and wall may be intimately bound. As the hyphae are often resistant to plasmolysis.

3.3. Plasma membrane:

- Phospholipid bilayer.
- Interspersed with globular proteins (detect entry of nutrients and metabolites)
- Ergosterol, unique sterol found in fungal plasma membrane.
  - (animals have cholestrol & plants have phytosterols)
  - This distinction is exploited during
    - The use of certain antifungal agents to treat some fungal infections.
      - » Antifungal drugs.
      - » Fungicides (controlling plant pathogenic fungi by targeting ergosterol
    - Used as an assay tool to quantify fungal growth

Functions:

1. directly or indirectly evolved in nutrient uptake.

2. wall-synthesis enzymes as can anchor some enzymes e.g. Chitin synthase and glucan synthase.

which become as integral plasma membrane proteins → producing polysaccharides e.g. chitin and glucan from the outer membrane face.

3. relaying signals from external environment to cell interior → signal transduction

3.4. Extrahyphal matrix:

Extrahypal substance is **Pullulan**

Capsule

- Some yeasts can have discrete polysaccharide capsule.
- Function:
  1. Stress protection.
  2. Virulence determination in some pathogenic yeasts as the capsule masks the antigenic components of wall so the fungas proliferate in tissues.

Fungal Fimbriae

- Long protein containing protrusions appearing from fungal wall of certain basidiomycetes and ascomycetes involved in

  Cell-cell conjucation