MOLECULAR AND CELL BIOLOGY IIA: SCIENTIFIC PRACTICE (MCBG2036)

Dr Angela Botes

23 August 2018

Recap

- Cytokinesis?
- 6 fungal phyla?
- Fungal cell wall structure?

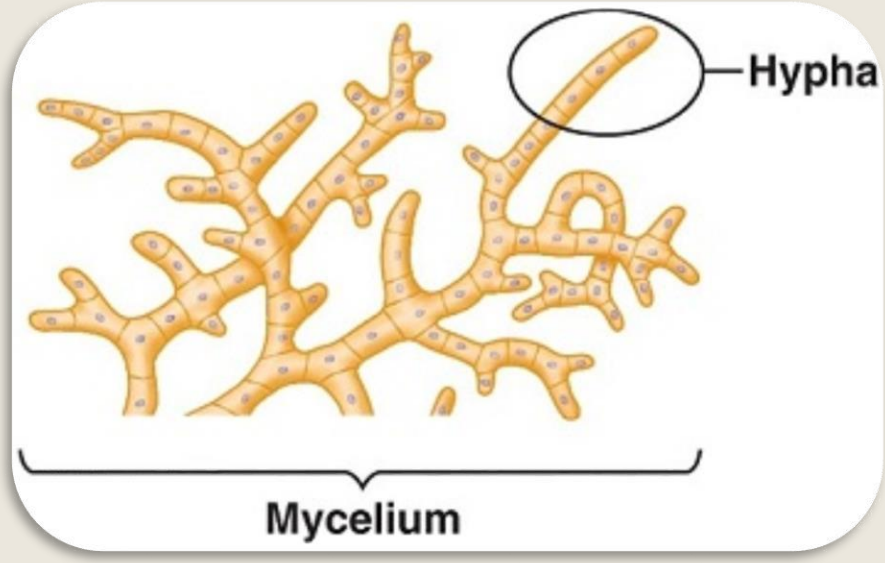
Intended learning outcomes (ILOs)

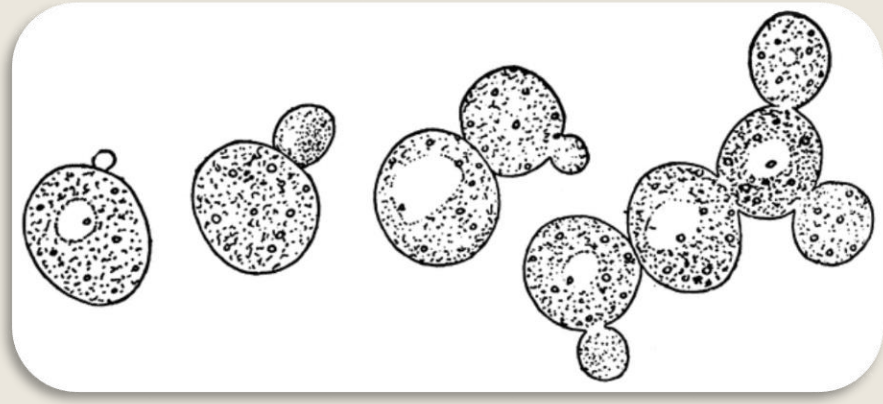
- Define the term / Hlalosa lentsoe
  - *Dimorphic*
  - *Bipolar mating system*
  - *Meiosis*
- Illustrate and explain / Etsa mohlala le ho hlalosa
  - *The basidiomycete life cycle*
  - *The importance of clamp connections*
- List and broadly disucss / Thathamisa le ho buisana haholo
  - *Mechanisms of spore dispersal*
- Discuss and compare / Buisanang le ho bapisa
  - *The different models of hyphal extension*
- Distinguish between / Khetholla pakeng tsa
  - *Mitosis and meiosis (self study)*
- Explain / Hlalosa
  - *Hyphal extension*
  - *The life cycle of* Cryptococcus neoformans
  - Hyphal branching

FUNGAL GROWTH

Fungal Cell Structure

- Filamentous or unicellular






©TIMELAPSE VISION INC.

Hyphal Extension

- Tip growth
  - *Continuous process*
  - *Not linked to nuclear division*

- 3 proposed models
  - *Steady-state model (Wessels., 1986)*
  - *Vesicle supply centre model (Bartnicki-Garcia et al., 1989)*
  - *Amoeboid model (Reinhardt., 1892; Heath et al., 1999)*

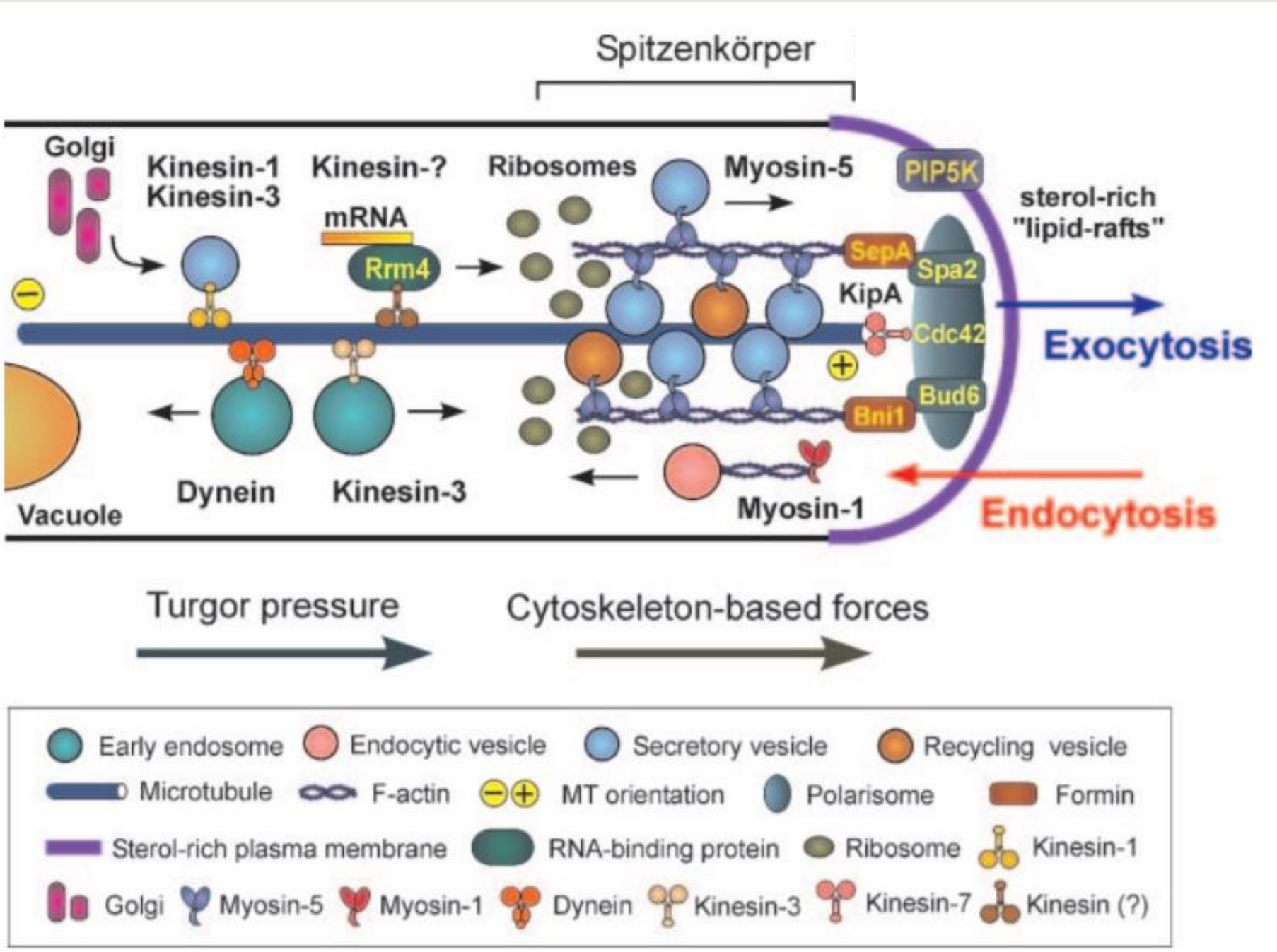
Hyphal Extension

TABLE 1. Models for hyphal tip growth

Model	Basic concept	Major supportive observations/results	Reference
Vesicle supply center	Post-Golgi vesicles are gathered in an apical vesicle supply center. This center regulates growth by generating a gradient of exocytosis of enzymes, such as lysins. Its active tip-ward movement, in combination with turgor pressure, determines hyphal shape and elongation rate	Growing hyphae contain a Spitzenkörper, and its displacement alters hyphal growth and direction and initiates new growth sites. Hyphal growth can be mathematically simulated	9
Steady state	Polar exocytosis delivers wall-forming enzymes (e.g., chitin synthases and glucanases). Newly added wall material is noncrystalline and plastic, and thus can be expanded by turgor pressure. The wall solidifies as it progresses towards the subapex, thereby resisting turgor pressure and shaping the hypha	Chitin is noncrystalline at the tip, but in older and subapical regions of the wall, chitin forms more rigid microfibrills that become covalently cross-linked to β-1,3-glucans	138
Amoeboid[a]	A membrane cytoskeleton supports integrity of the tip and regulates tip extensibility. The hyphal tip expands by the force produced by the cytoskeleton. The cell wall is considered to be an extracellular matrix that confers shape to the hypha	The fungal cytoplasm contracts and is able to form pseudopodia	50

[a] The basic concept of a fungal cell being an amoeba in a tube was first introduced by Reinhardt in the late 19th century (99). Note that hyphal growth is most likely based on all three models.

Steinberg., 2007



Hyphal Extension - Spitzenkörper

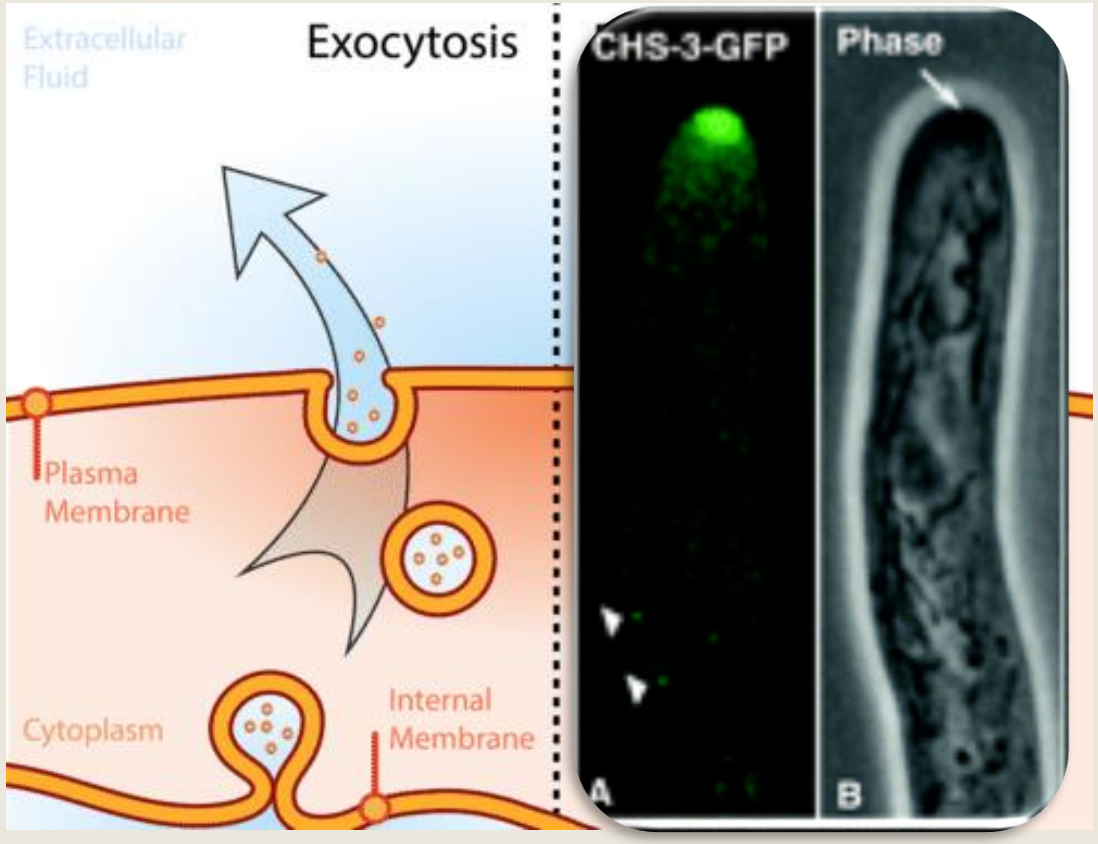
- Located at the hyphal apex
- Core region anchored with F-actin to the cytoskeleton
- Required for rapid rates of hyphal extension
- Not required for hyphal polarization

- Vesicle supply centre (VSC)
  - Exocytic / endocytic vesicles transit through the *Spitzenkörper*
  - *Exocytic vesicles*
    - Ultimate site where these vesicles fuse to the target cell membrane

Riquelme et al., 2007; https://www.ck12.org/biology/exocytosis-and-endocytosis/lesson/Exocytosis-and-Endocytosis-BIO/

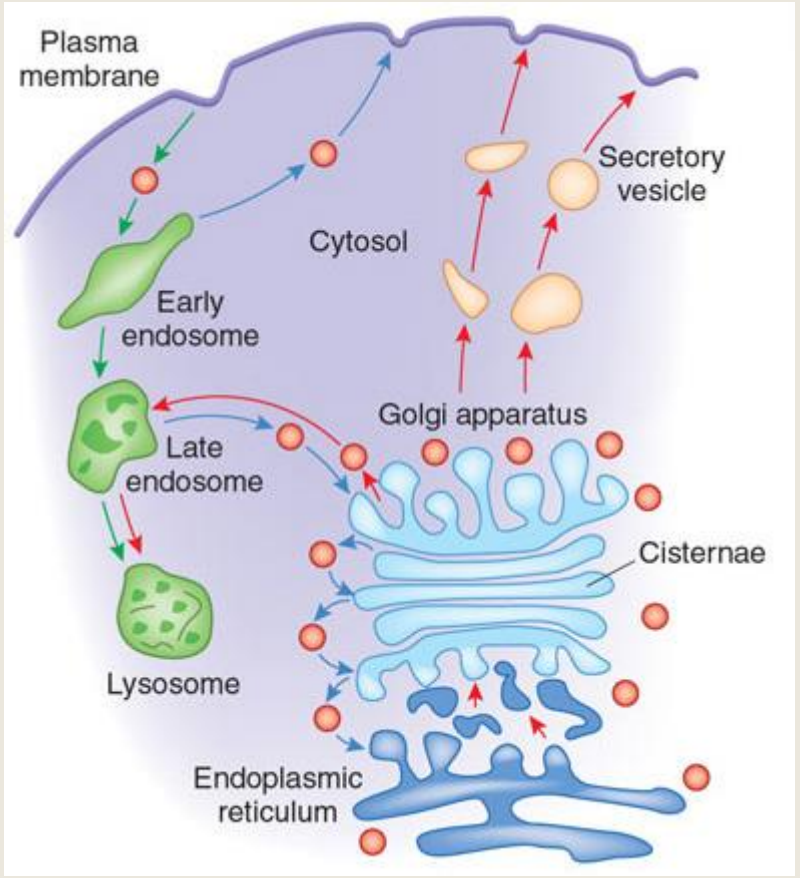
Hyphal Extension - Spitzenkörper

- Exocytosis
  - *Receives vesicles from the Golgi*
  - *Released in a controlled manner*
    - Exocytosis gradient
  - *Creates new cell membranes*
  - *Traffics necessary proteins*
    - Chitin synthase
- Endocytosis
  - *Supports recycling*
    - Enzymes that are secreted are swept back has the hyphae extends
    - Reabsorbed further along the hyphae

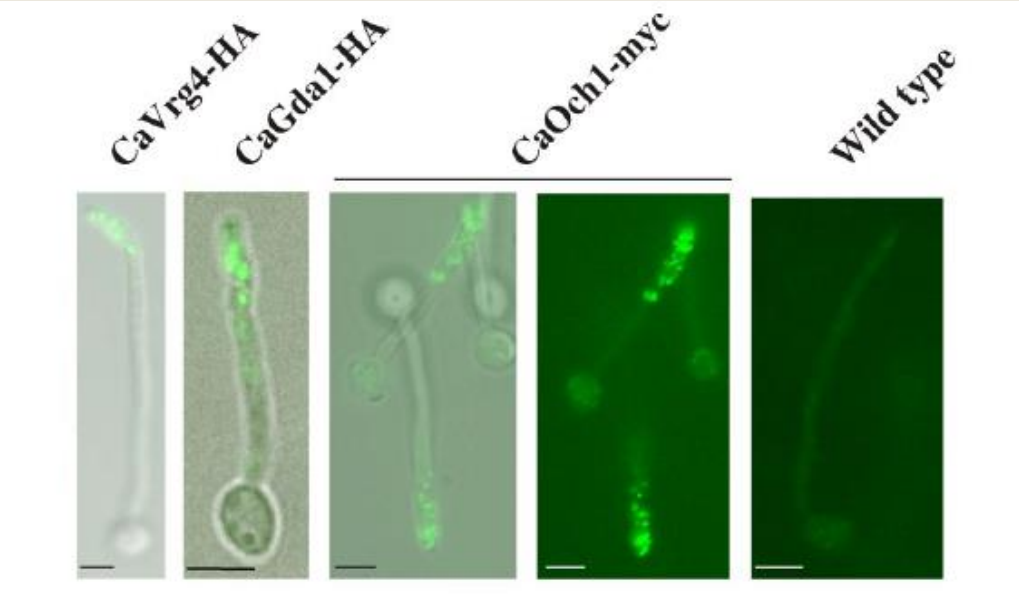


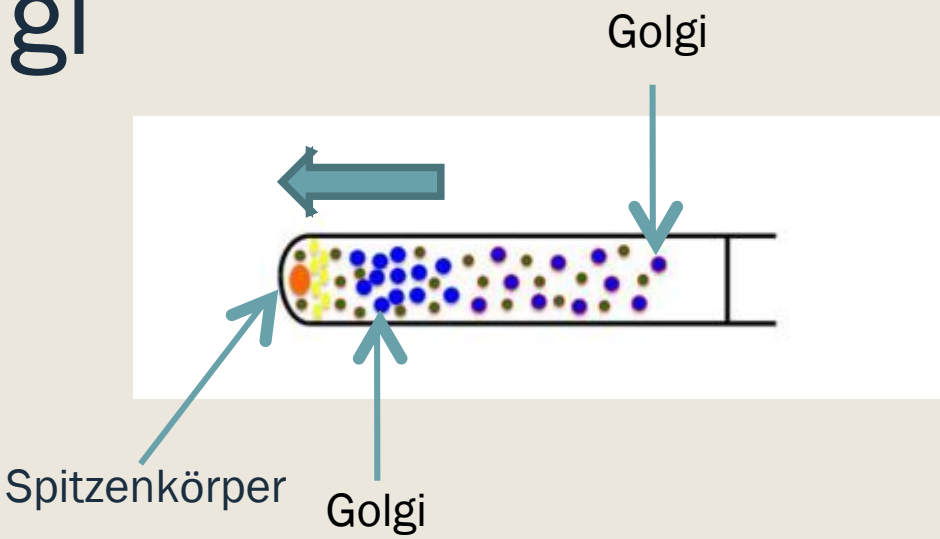
Hyphal Extension – Golgi

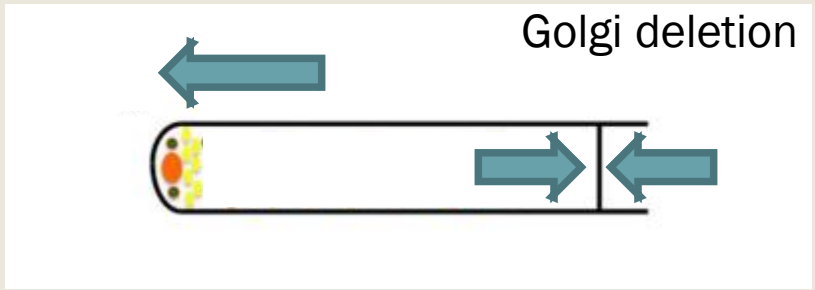
- Golgi
  - *Pivotal role in vesicle trafficking*
    - Source of exocyitic vesicles
    - End journey for some endosomes
- Localize to the hyphal apex
  - *Actin/cytoskeleton dependent process*
- Essential for hyphal polarization
- Essential for extension

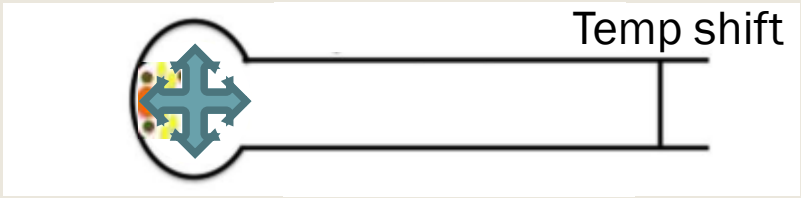


Hyphal Extension – Golgi









- Pinar et al., 2013
  - *Deleted specific Golgi proteins*
    - Golgi dysfunctional
  - *Hyphae grew in various directions*
  - *Temp shift – hyphal tip started to swell*

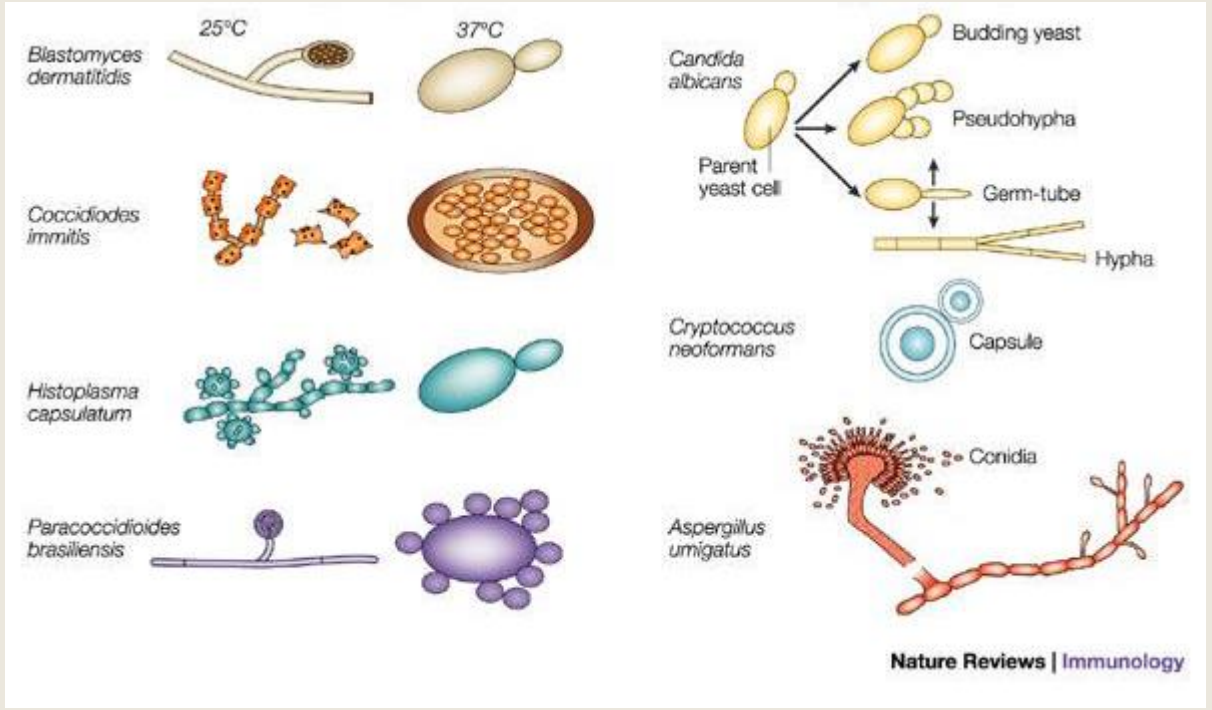
Hyphal Extension – Tugor Pressure

- Turgor pressure
  - *Overcome physical barriers*
    - Pressure driven penetration
    - Nutrient acquisition
      - *Digest substrate inside and out*
    - Pivotal role in pathogenesis of dimorphic fungi
      - *Immune system avoidance*

http://www.medical-labs.net/forms-of-fungi-261/

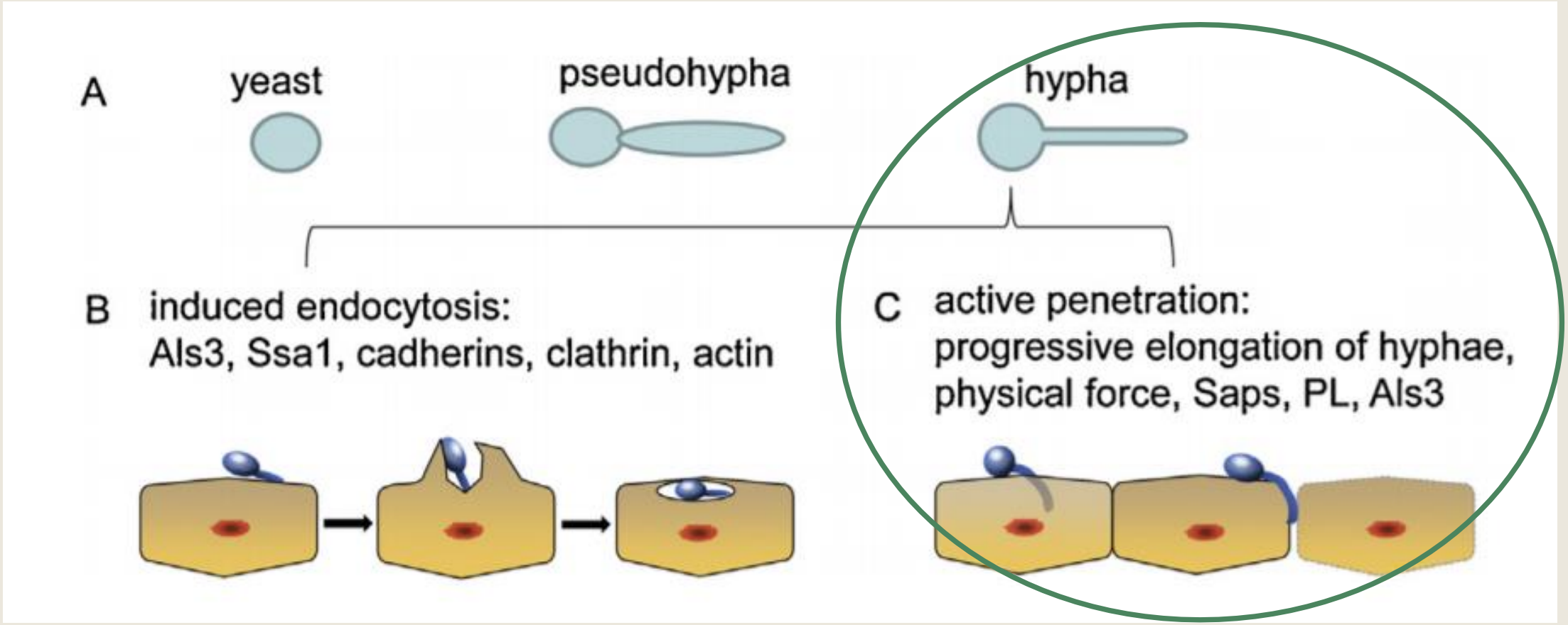
Dimorphic Fungi

- Can switch morphologies
  - *Unicellular ⇨ Multicellular ⇨ Unicellular*
- Usually temperature induced
- Also pH, nutrients, hypoxia, CO_2, N-acetylglucosamine (GlcNAc)



Yang et al., 2014

Hyphal Penetration – *Candida albicans*

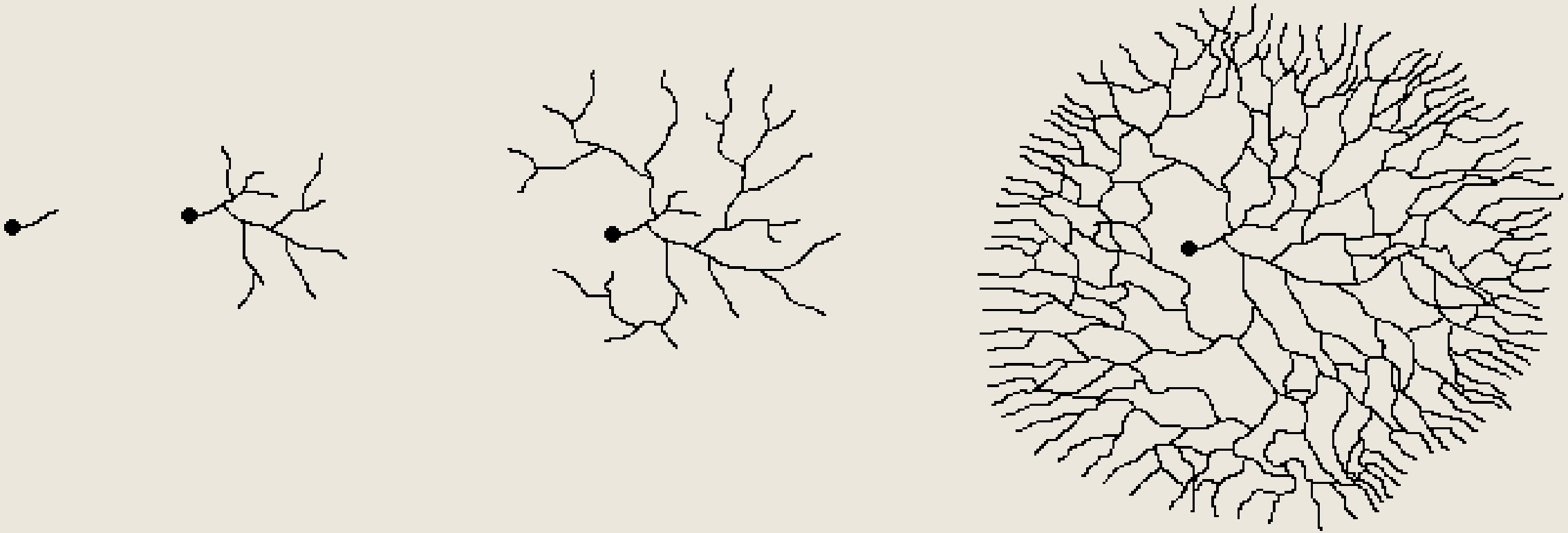


Hyphal Penetration – *Candida albicans*

- Hyphal initiation
  - *Requires asymmetry*
    - Polarization of one side
  - *Ccn1 (G1 cyclin)*
    - Essential for transition between yeast and filamentous growth
    - Starts to accumulate and persist longer
    - Polarizes one side of the cell
- Hyphal maintenance
  - *Increased activation of Cdc42*
    - Rho GTPases are central to dynamic actin cytoskeletal assembly and rearrangement
  - Decreased expression of RAM proteins
    - Cell separation

https://www.anbg.gov.au/fungi/mycelium.html

Hyphal Branching



Hyphal Branching

- Distal to the apex
  - *Apex exerts dominance preventing branching*
  - *Associated with the de novo formation of a Spitzenkorper*
- 2 modes
  - *Branching associated with septa*
    - Septa provide a spatial cue
  - *Random branching*
- Branching in mycorrhizae strongly influenced by external factors
  - *Plant nutrients, hormones etc*

AFTER THE BREAK…

…Fungal Sexual Reproduction

FUNGAL SEXUAL REPRODUCTION

Fungal Sexual Reproduction

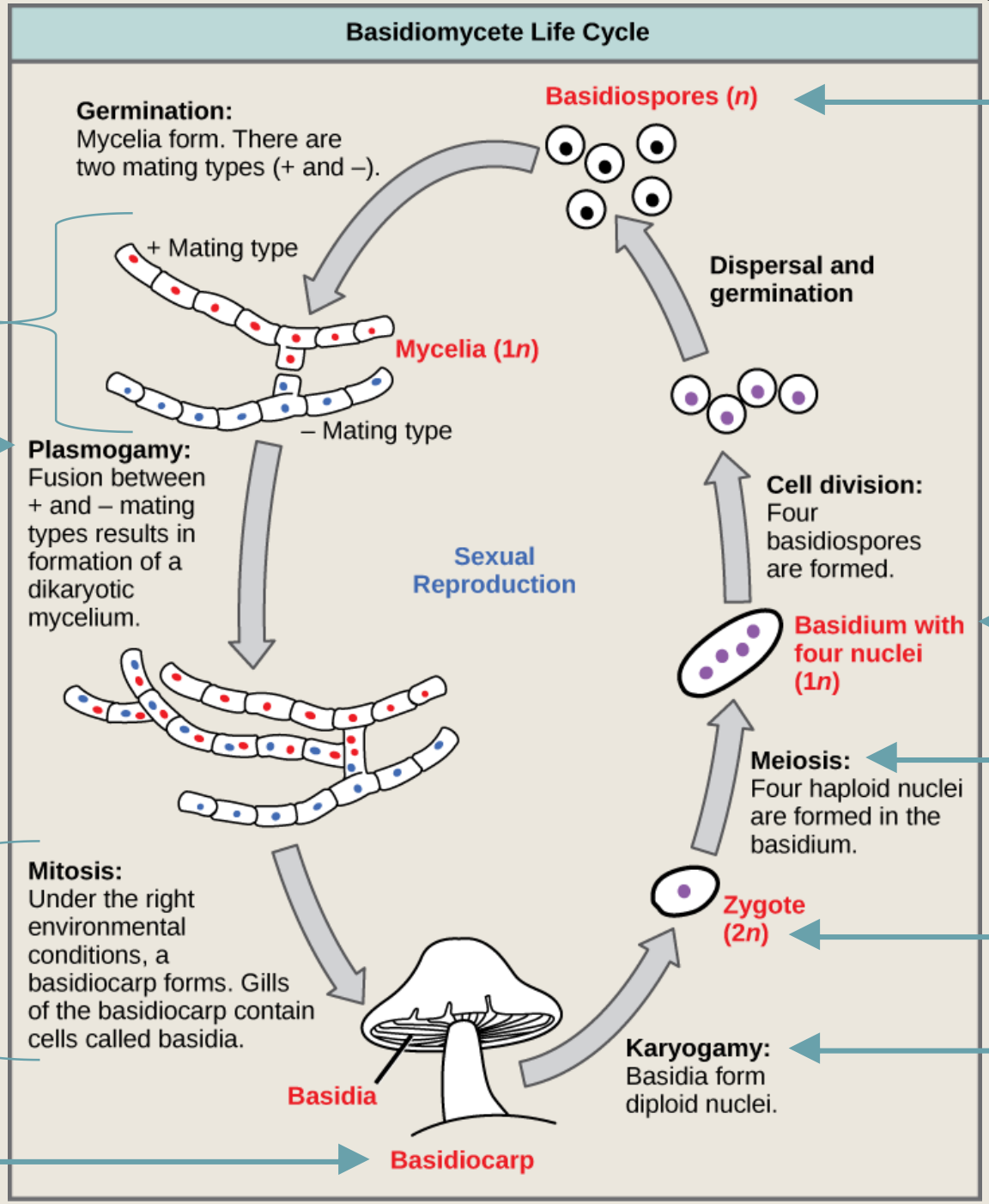
- Meiosis
  - *From the Greek – lessening*
  - *Specialized cell division that reduces the chromosome number by half*
- Conserved core features
- 3 types of sexual spores
  - *Ascospores (Ascomycota)*
  - *Basidiospores (Basidiomycota)*
  - *Zygospores (Zygomycetes)*

Sexual Reproduction - Basidiomycota

- 3 major lineages
  - *Mushrooms*
  - *Rusts*
  - *Smuts*
- Sexual reproduction varies for each

- All produce basidiospores

https://courses.lumenlearning.com/wm-biology2/chapter/basidiomycota/



Bipolar mating system

Fusion of the cytoplasm

Dikaryotic
2 nuclei

Nuclei do NOT fuse

Can take years!

Fruiting body

Haploid (n)

Specialized structure bearing haploid basidiospores

Reduces chromosome number by half

Diploid (2n)

Nuclei do fuse

Bipolar Mating System

- 2 mating types

- Genes are encoded on a section of the genome termed the mating type loci
  - *Pheromone receptor*
  - *Pheromone precursor*
  - *Homeodomain protein HD1 and HD2*
    - Targeted transcriptional regulators (STE12a and STE12α)

Wallen et al., 2018

Bipolar Mating System

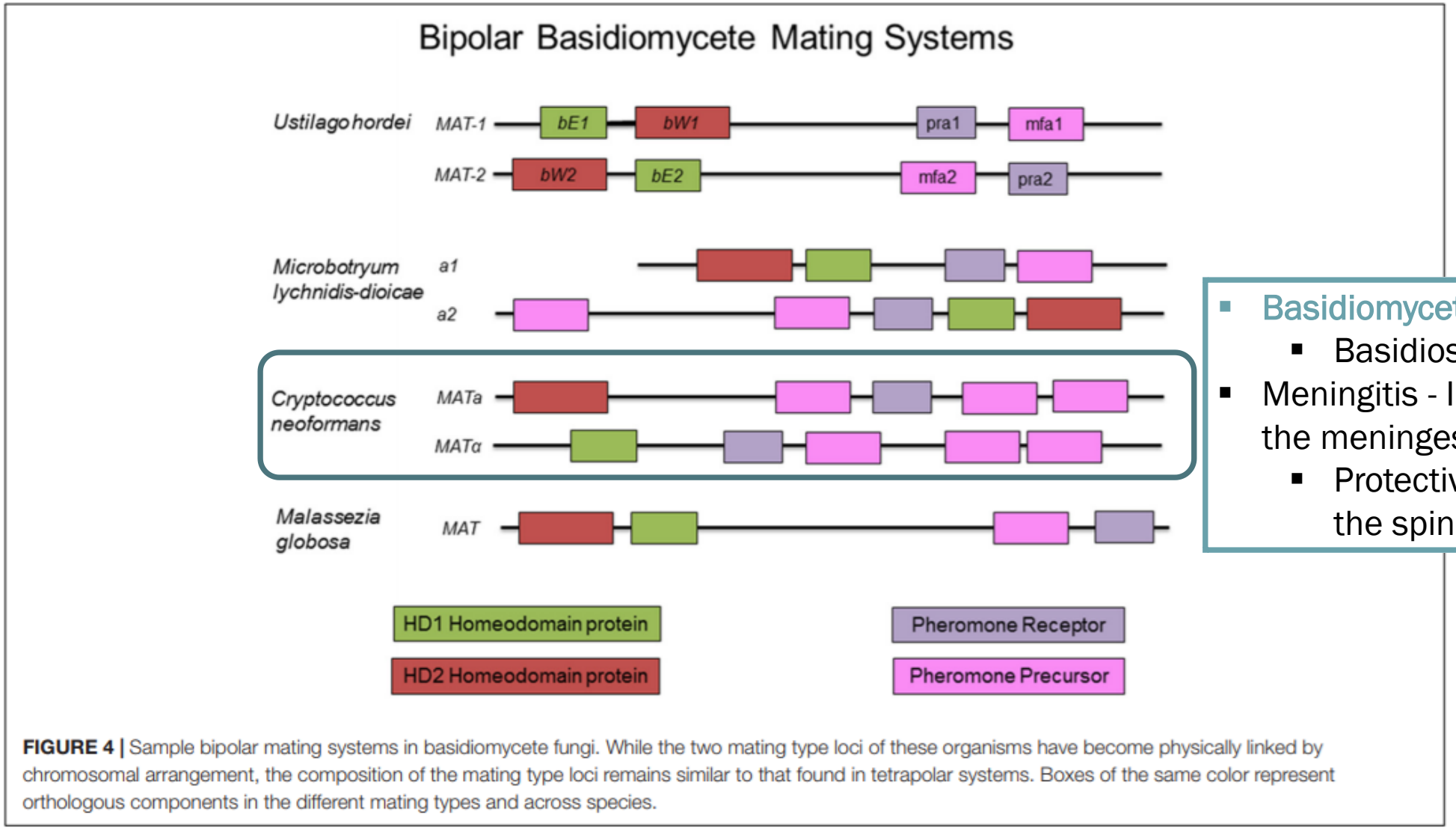
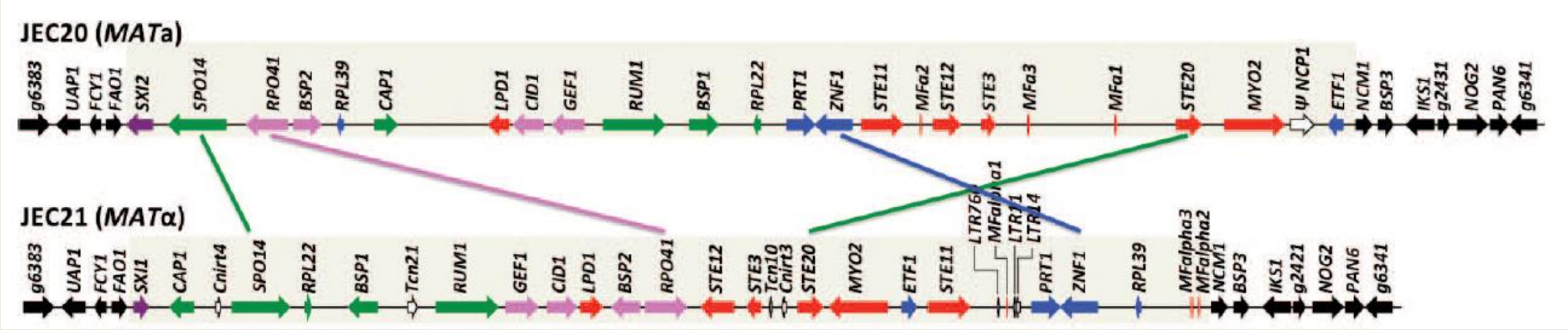


FIGURE 4 | Sample bipolar mating systems in basidiomycete fungi. While the two mating type loci of these organisms have become physically linked by chromosomal arrangement, the composition of the mating type loci remains similar to that found in tetrapolar systems. Boxes of the same color represent orthologous components in the different mating types and across species.

- Basidiomycete
  - Basidiospores
- Meningitis - Inflammation of the meninges
  - Protective layer that covers the spinal cord and brain

Mating Type Loci

- *Cryptococcus neoformans*
  - *MATa and MATα*

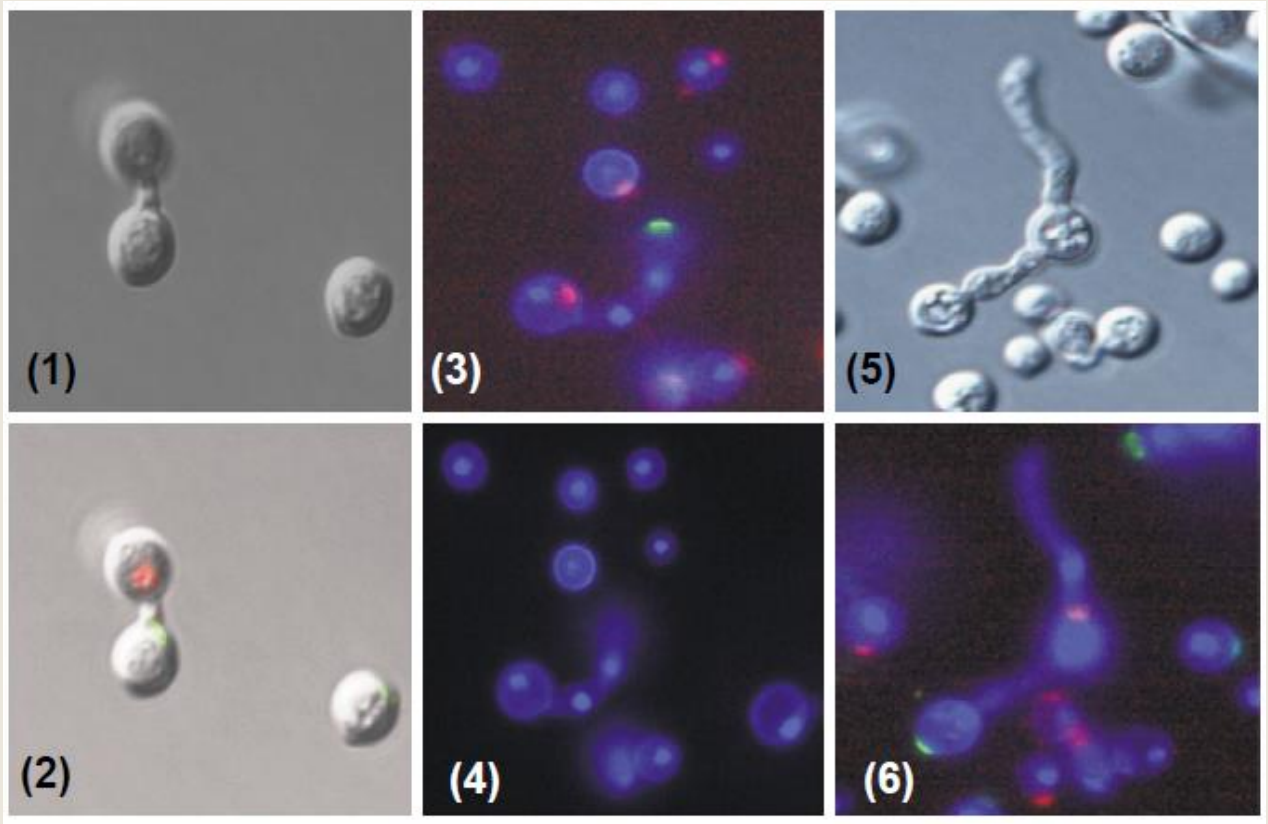


Black - flanking genes
Red - genes within the ancient stratum are in red
Green/Blue - genes within the intermediate strata
Pink - genes within the recent stratum

Chang et al., 2000

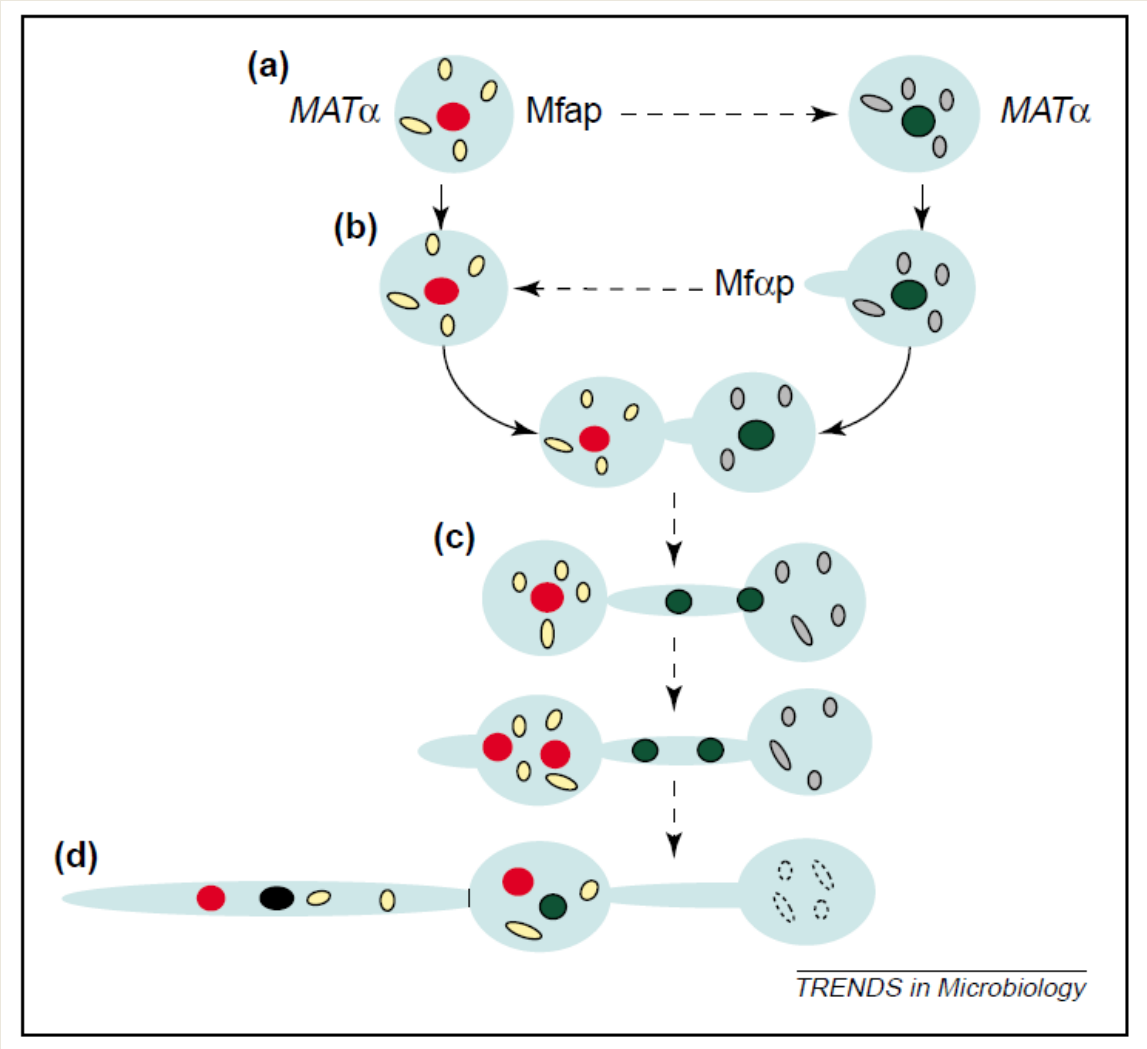
Sexual Reproduction - Pheromones

- MATa produces a pheromone (*MFa*) in response to environmental stimuli
  - *Nitrogen starvation*
  - *Desiccation*
- *MFa* binds to its cognate receptors on MATα
  - *Activates a heterotrimeric G protein ⇨ MAPK cascade ⇨ activates STE12α*
- MATα produces *MF*α and a conjugation tube that
- Fuses with MATa
  - *Plasmogamy*



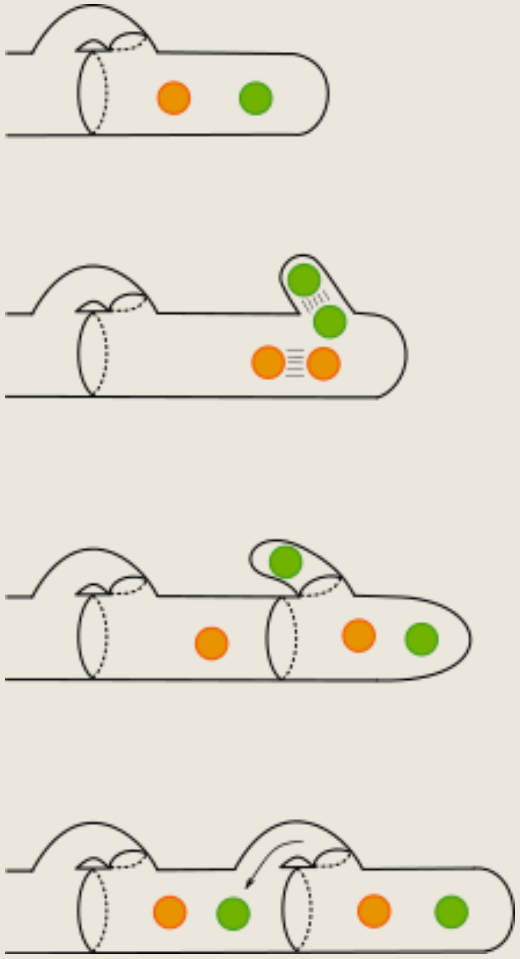
Sexual Reproduction - Hyphae

- MATα nucleus divides and move into the conjugation tube
  - *Unidirectional DNA flow*
- MATa nucleus divides and the cell initiates hyphal growth
  - *Ccn1, Cdc42, Chs3p, VSC, tugor, Golgi*
- One of each nuclei migrate into the hypha
  - *Only MATa mitochondria are transferred*
- Septum is formed between the yeast cell and the hypha
  - *Chs1p and Chs2p*



https://en.wikipedia.org/wiki/Clamp_connection

Sexual Reproduction – Clamp Connections



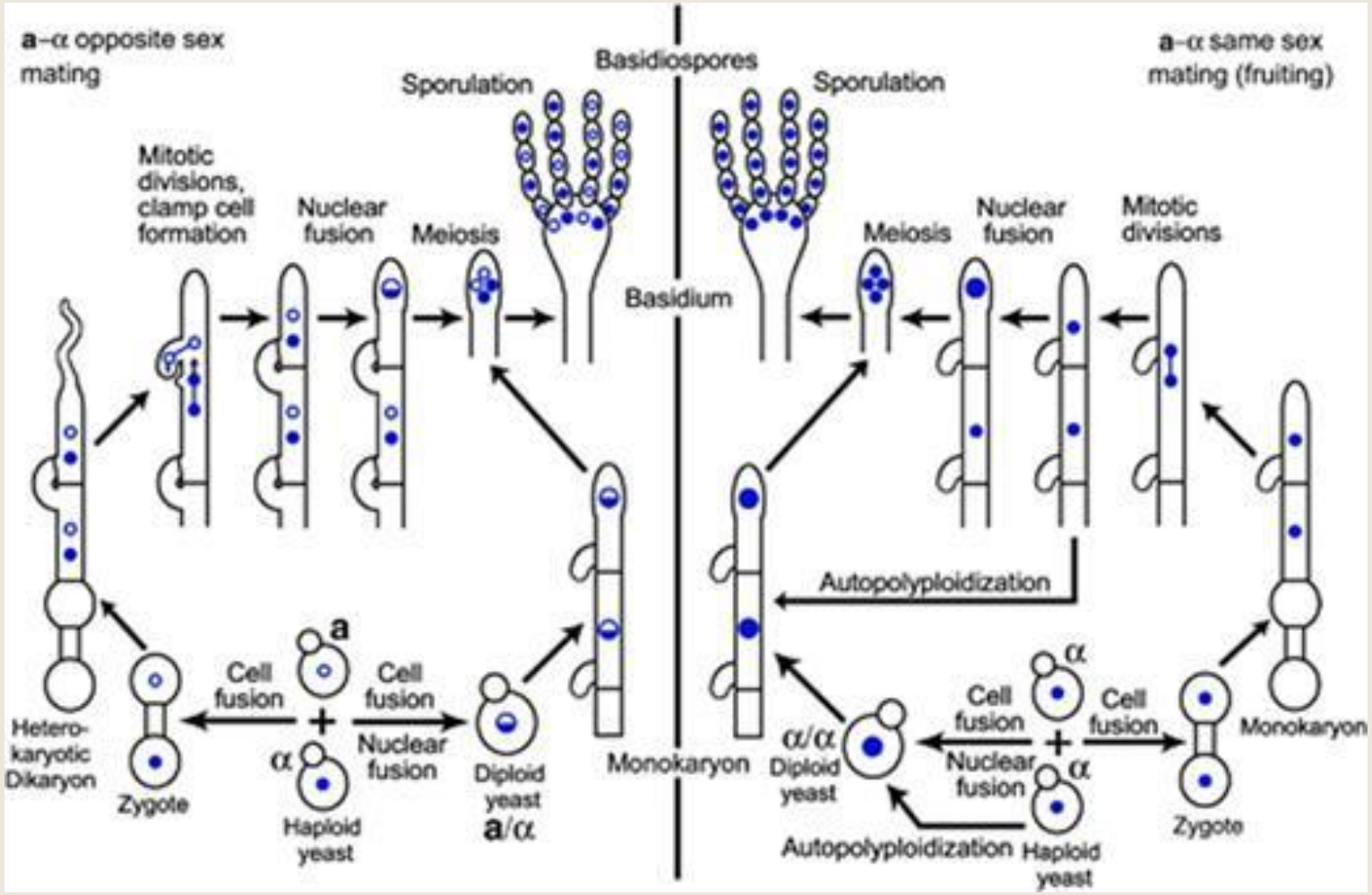
- Tip growth not linked to nuclear division
- Septa form along the hyphae
  - *Risk losing the nuclei*

- Nuclei localize to the apex and replicate
- Backwards clamp connection is formed
  - *Nuclear migration bridge*
- Nuclei migrate into the clamp connection and relocate the rear compartment

- Crucial in maintaining a dikaryotic mycelium

Kozubowski et al., 2011

Sexual Reproduction – Clamp Connections



Spore Dispersal

- Animals, insects, wind and water
- Attracting animals and insects
  - *Smell*
  - *Taste*
  - *Colour*
  - *Bioluminescent*

NEXT TIME…

…TUT – How to read a research article