Connecting ultrastructure to molecules: Studying evolution of molecular components of cellular division in fungi from comparative genomics.

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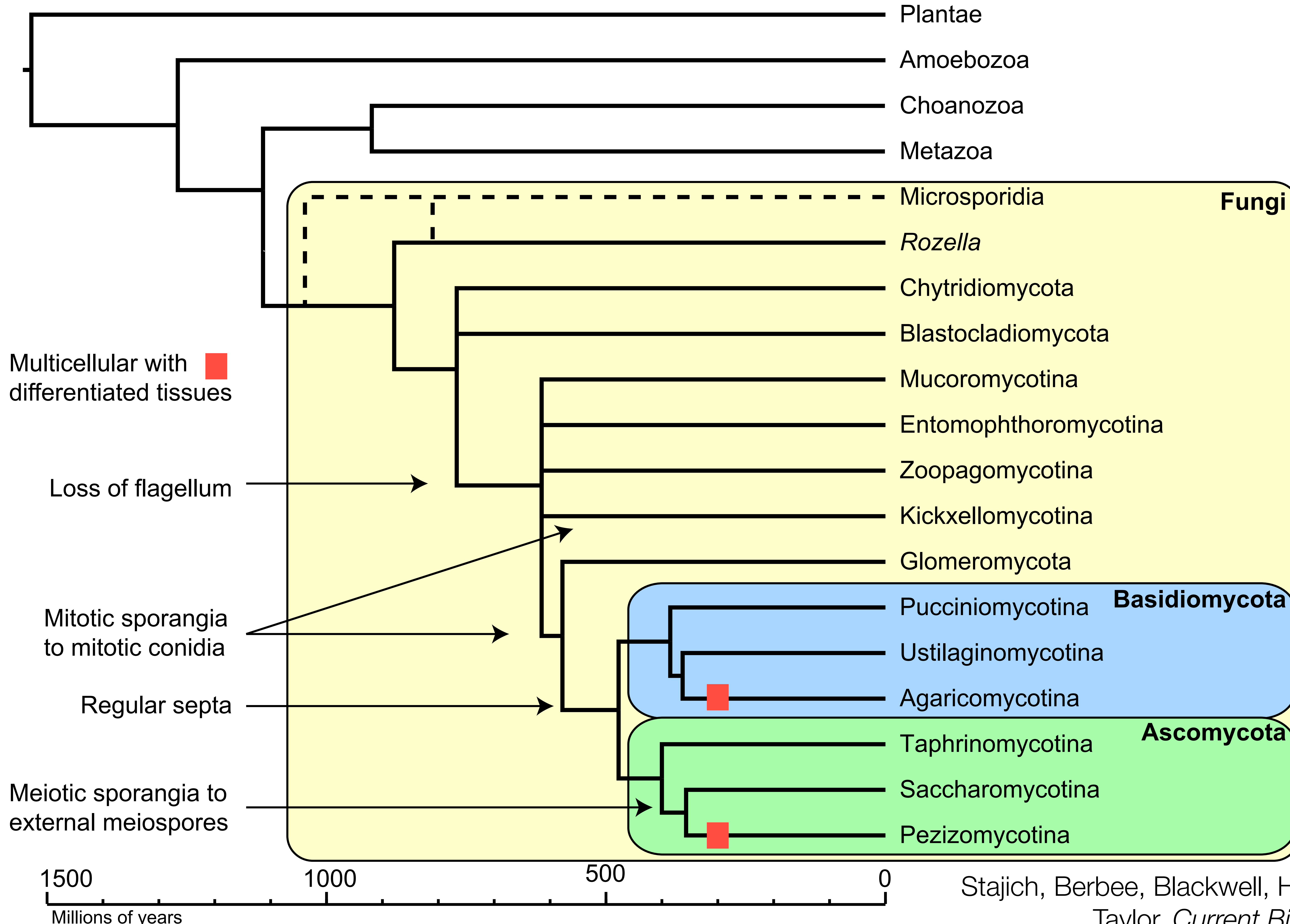
Evolutionary transitions in fungal cellular structure

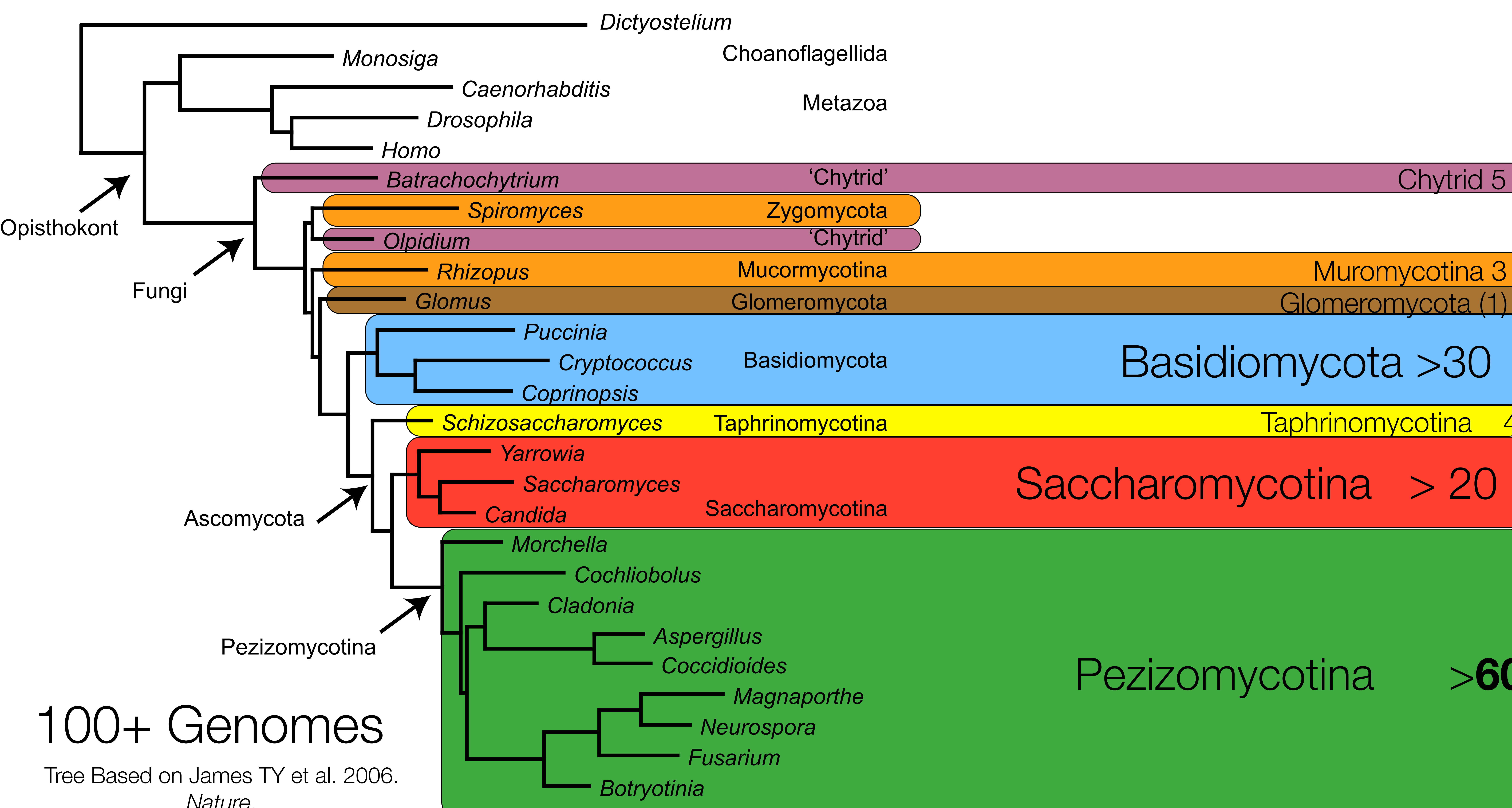


Stajich, Berbee, Blackwell, Hibbett, James, Spatafora, Taylor. *Current Biology*. *in press*

Connecting evolutionary transitions to molecules

- Comparative genomics can help inventory the genes shared or missing between species
- Tracing the evolution from a flagellated, aquatic ancestor to filamentous and
- Using genome sequences from Chytridiomycota, Mucormycotina, and Dikarya fungi can compare genes that are found only in Dikarya fungi and linked to the clade-specific phenotypes
- 3 transitions illuminated
  - Cellular division
  - Cell wall composition
  - Septa

Genome samples from fungi



100+ Genomes

Tree Based on James TY et al. 2006. *Nature.*

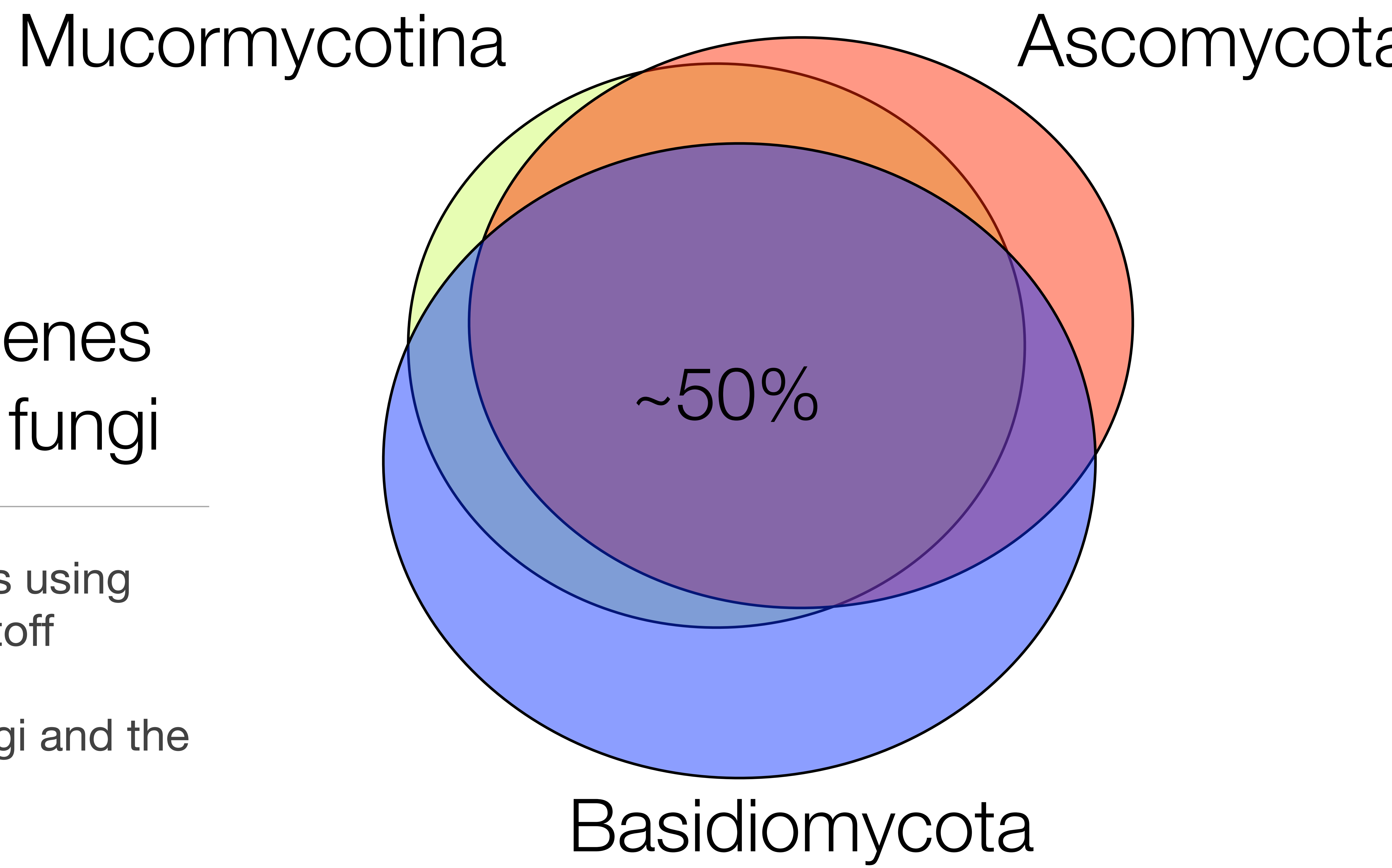
http://fungalgenomes.org/wiki/Fungal_Genome_Links

B.dendrobatidis genes shared with other fungi

Via all-vs-all similar searches using FASTP and 1e-5 E-value cutoff

Using genomes from 30 fungi and the *B. dendrobatidis* genome

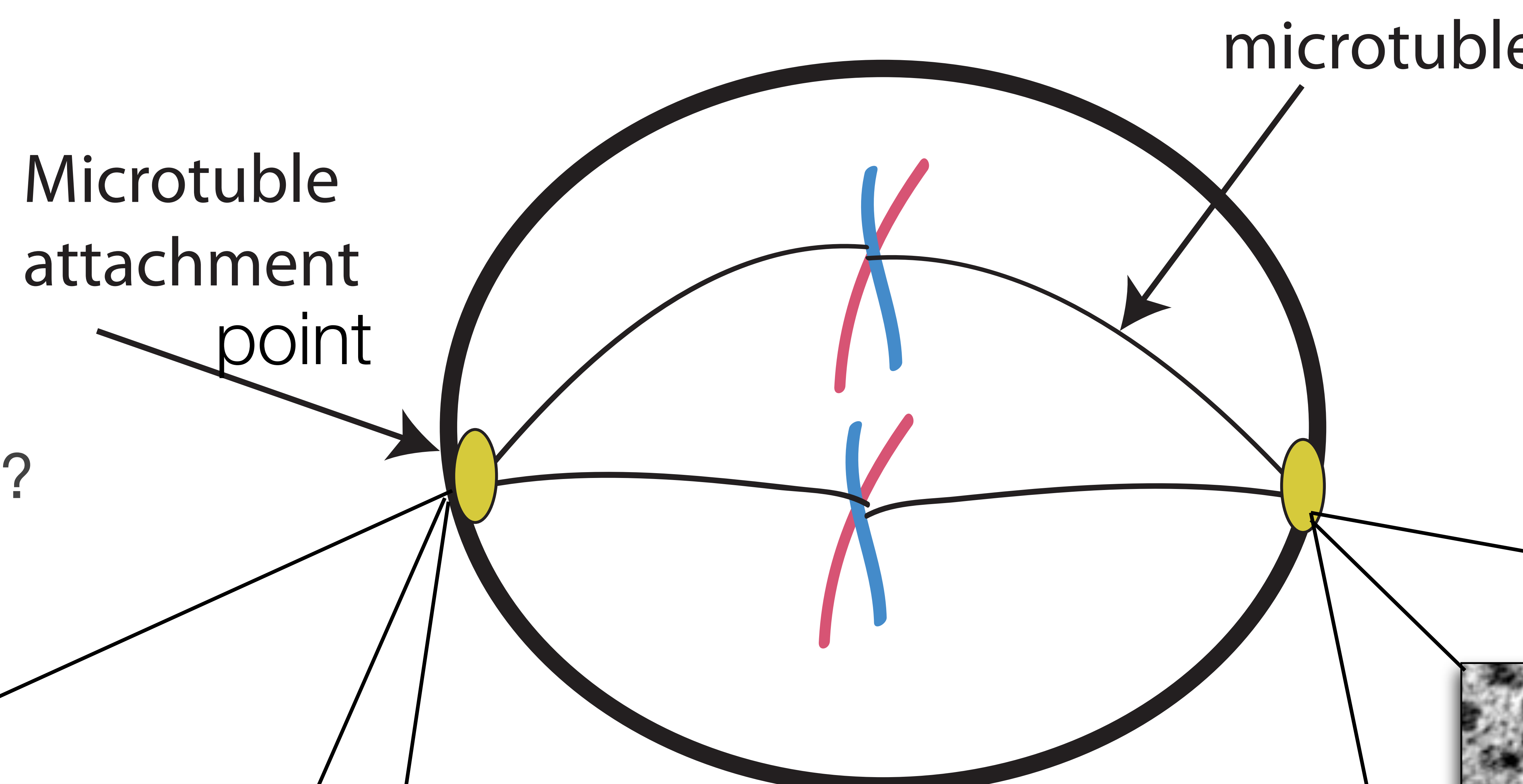
Identify the shared genes

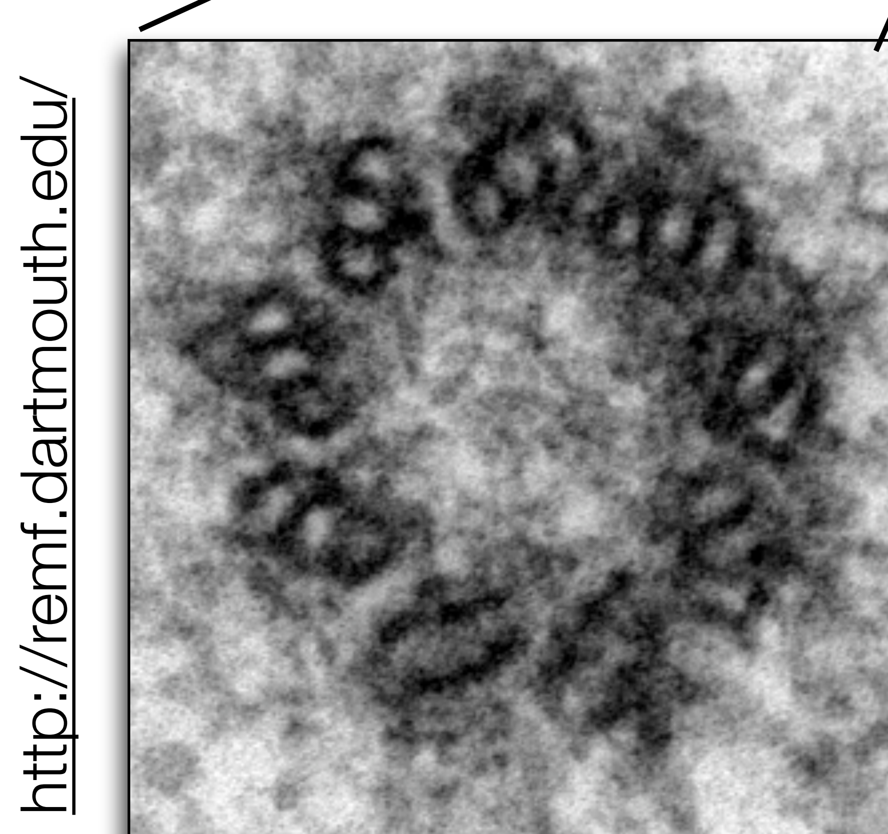


~8800 genes in *B. dendrobatidis*

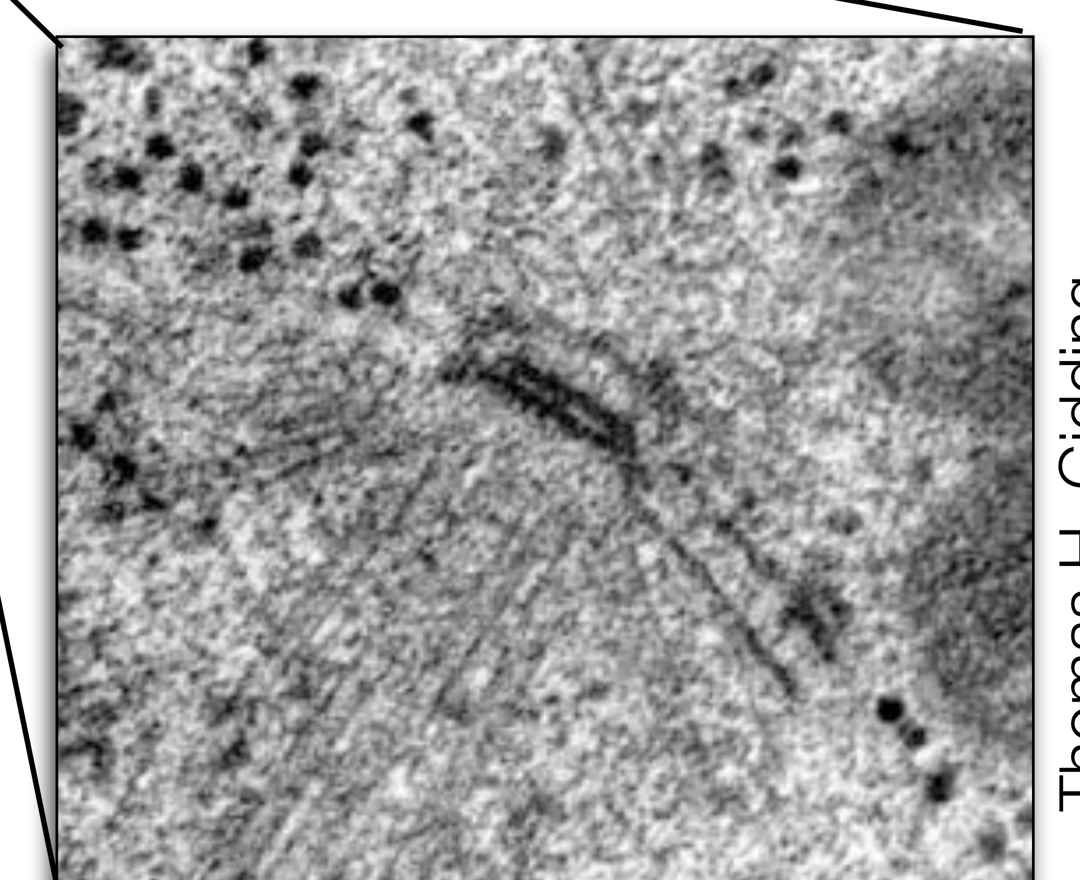
Differences between animal-like and fungal Microtubule attachment points

- Transition from flagellated cells with centrioles
- Spindle pole bodies in non-flagellated fungi
- How did this transition occur?
- Loss of flagellum coupled with transition in basal bodies?





Animal centriole



Yeast spindle-pole body

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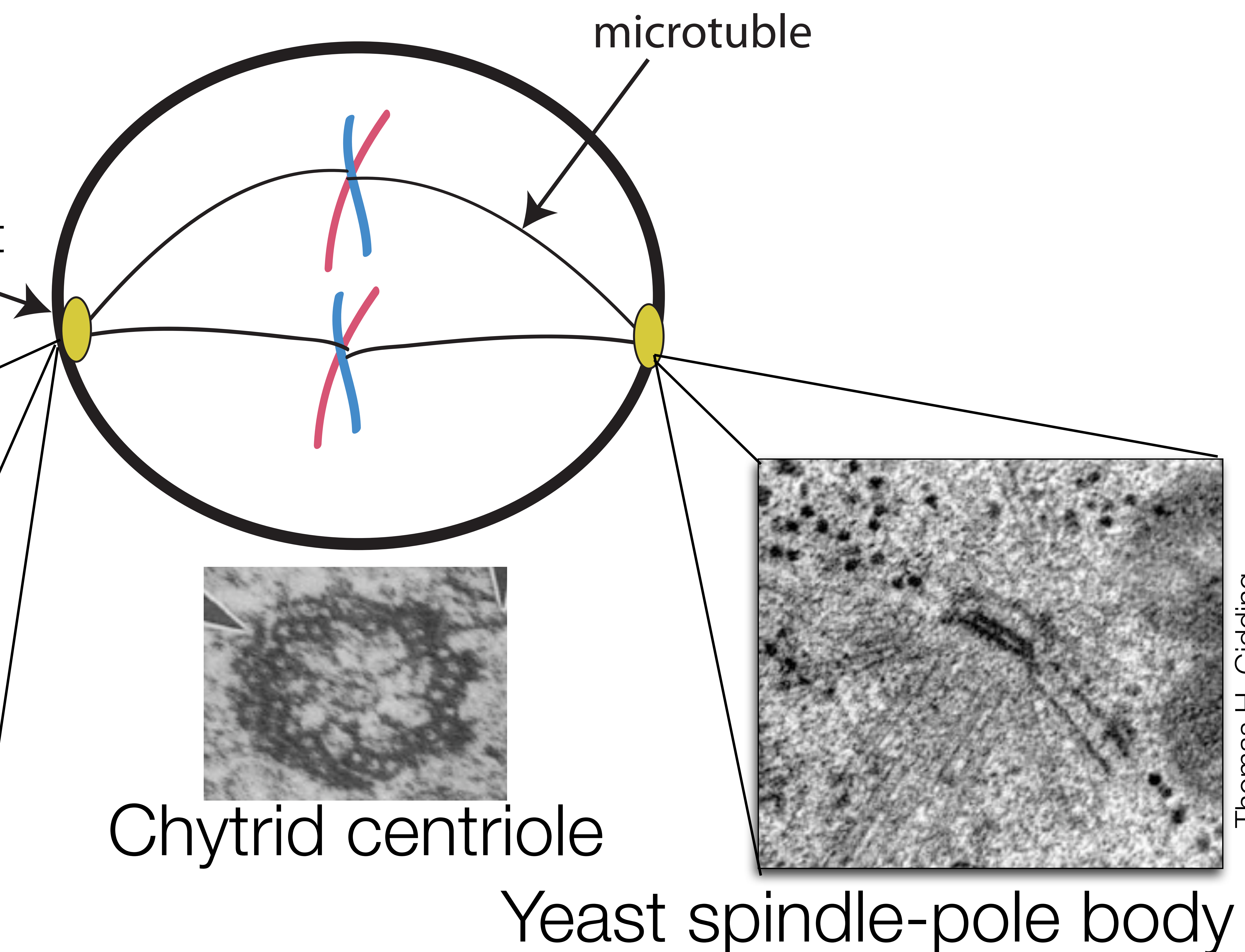
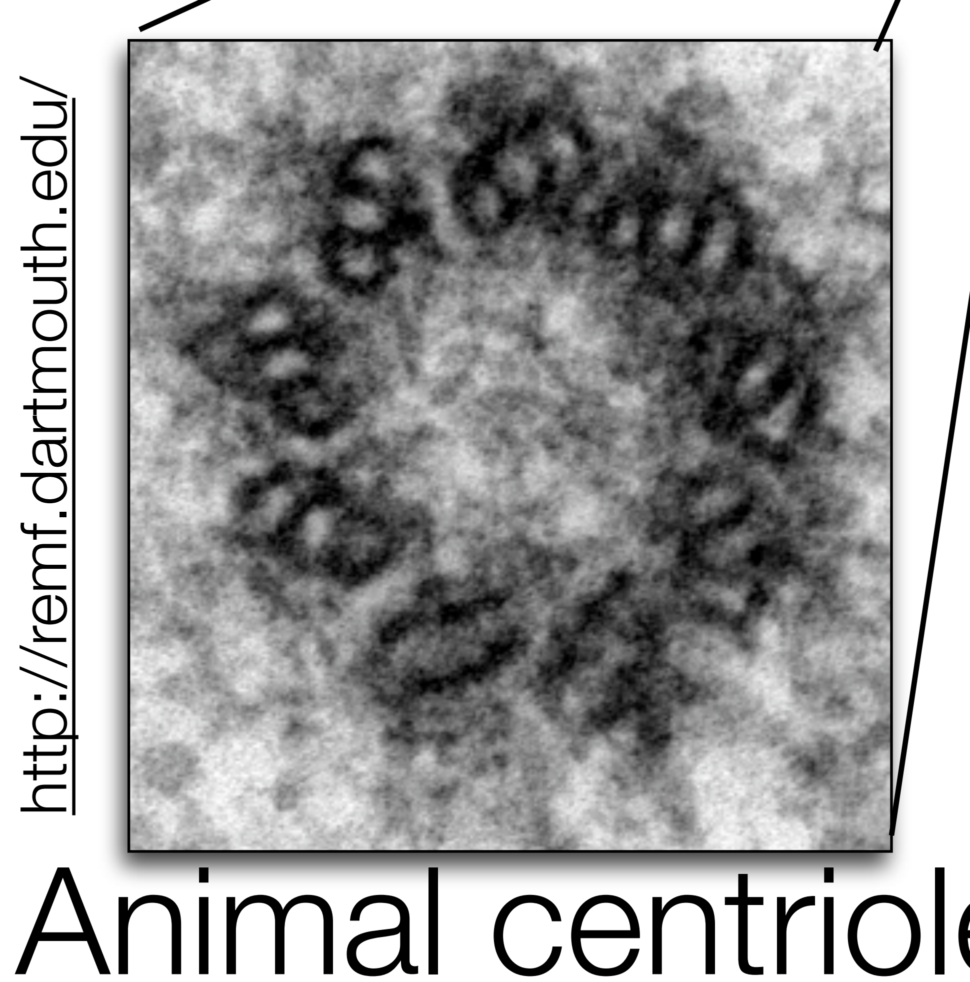
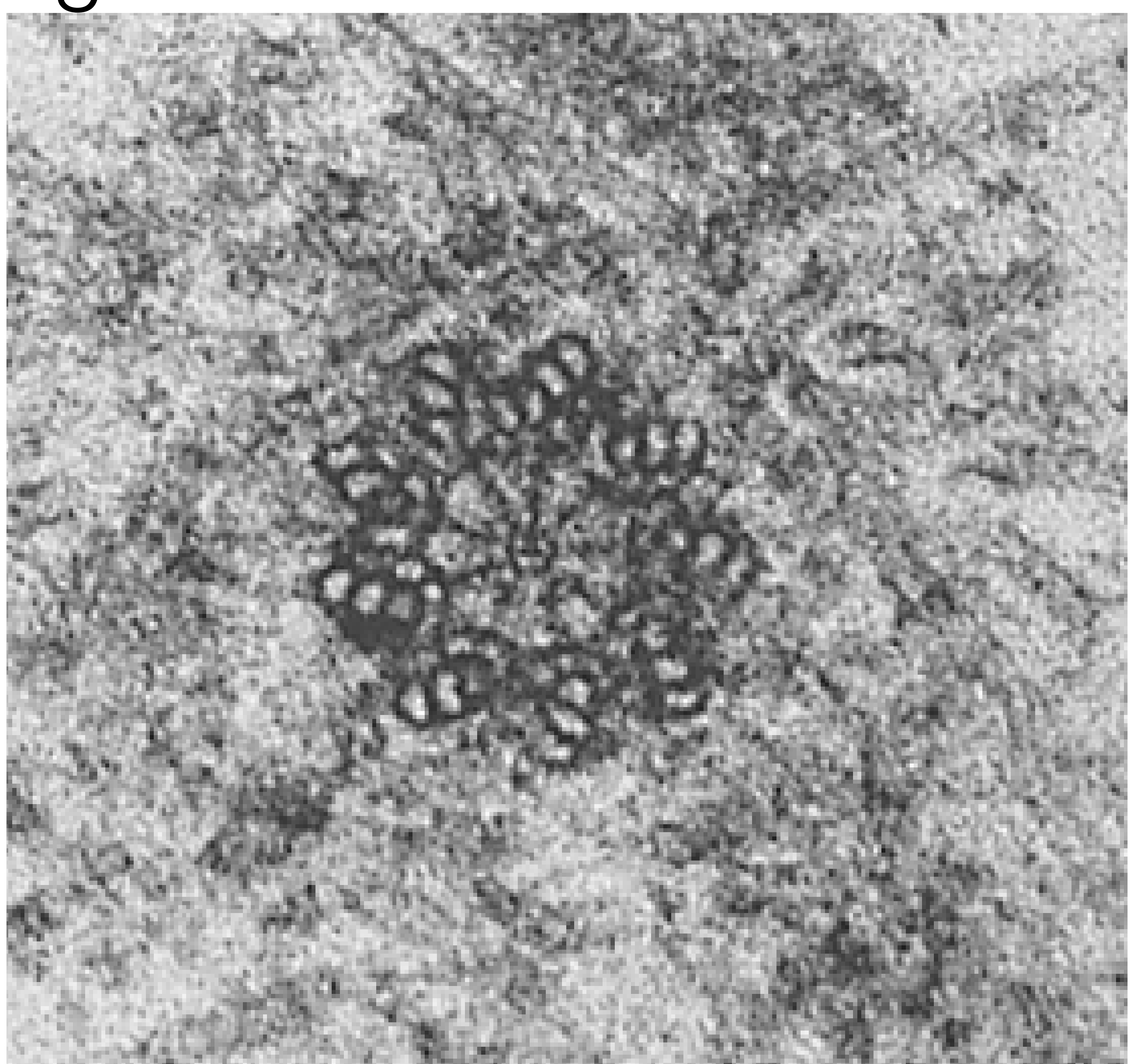




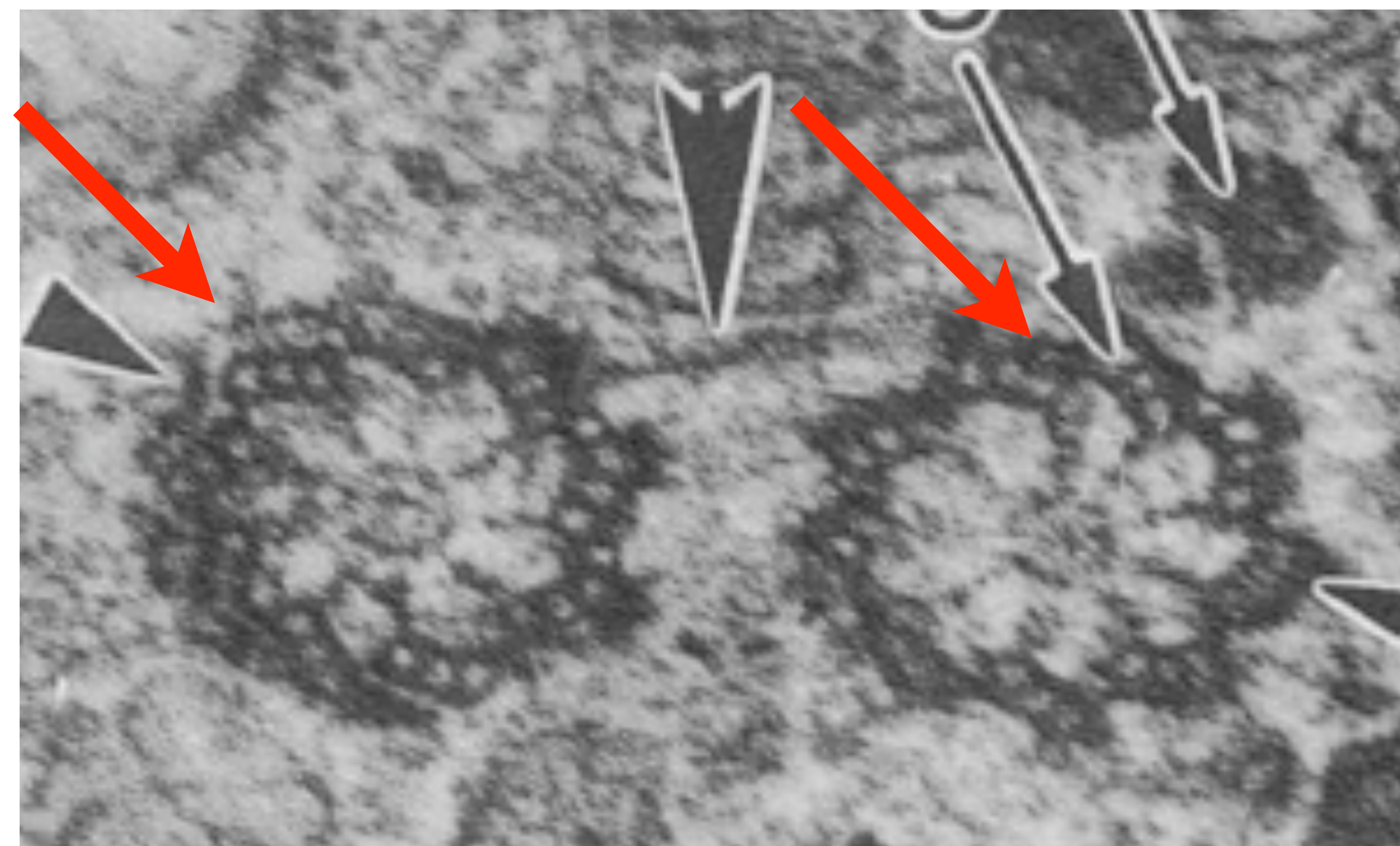
Centrioles in animals and chytrids are homologous structures

Cross-section of a rat kangaroo (Marsupial, Animalia) centriole region



Berns MW et al. *J Cell Biol* 1977

Chytrid zoospore cross section with 2 centrioles



Entophlyctis luteolus

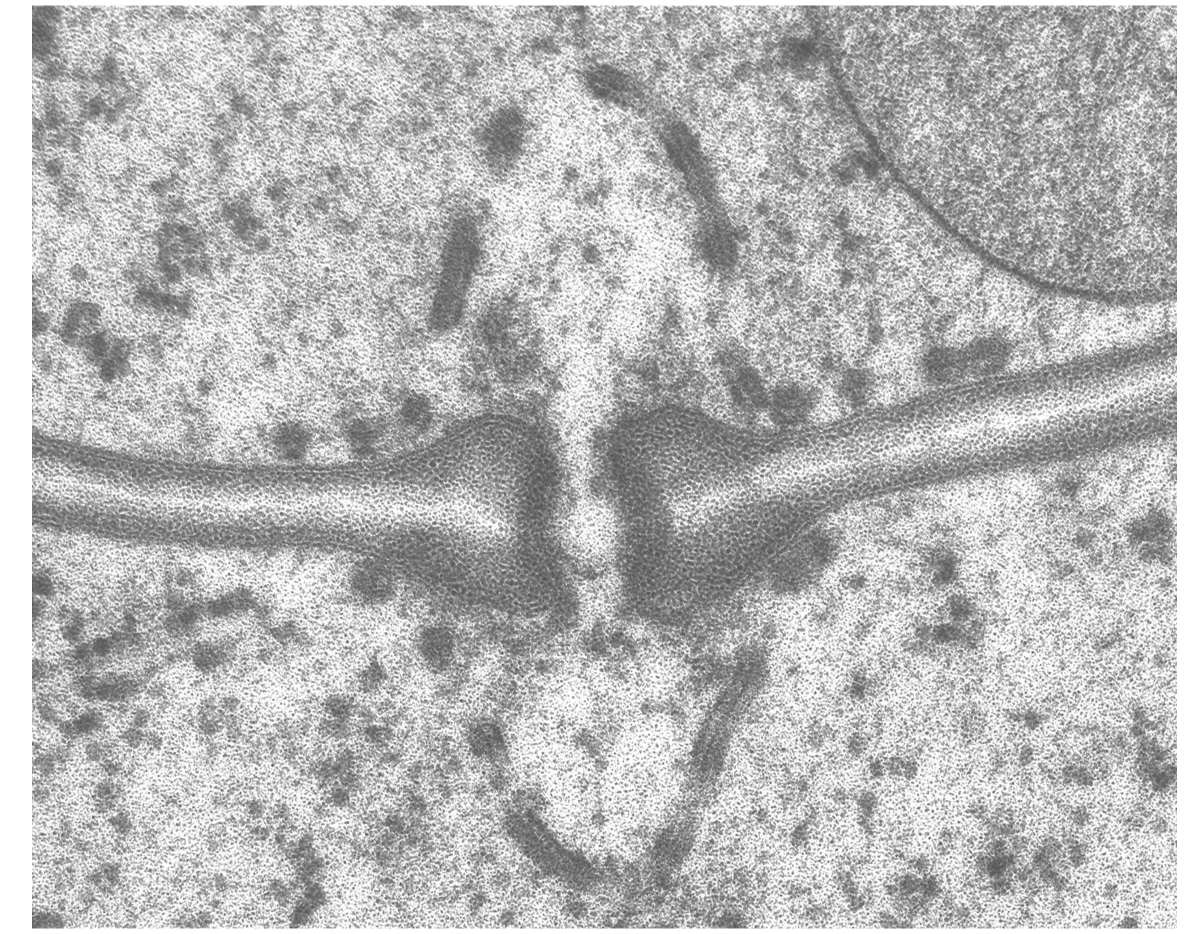
Longcore. *Mycologia* 1995

Chytrid genome is missing some chromosome segregation and mitosis related genes

- Missing genes in the *B. dendrobatidis* genome
- MSP3, KAR1, KAR2 for nuclear membrane fusion during karyogamy and Spindle-body duplication
- SPC42 - central plaque component of spindle pole bodies
- CEP3 - essential kinetochore protein
- CIN2 - Tubulin folding protein
- REC8 for sister chromatid cohesion
- DASH Complex for kinetochores coupling during mitosis (10 genes)

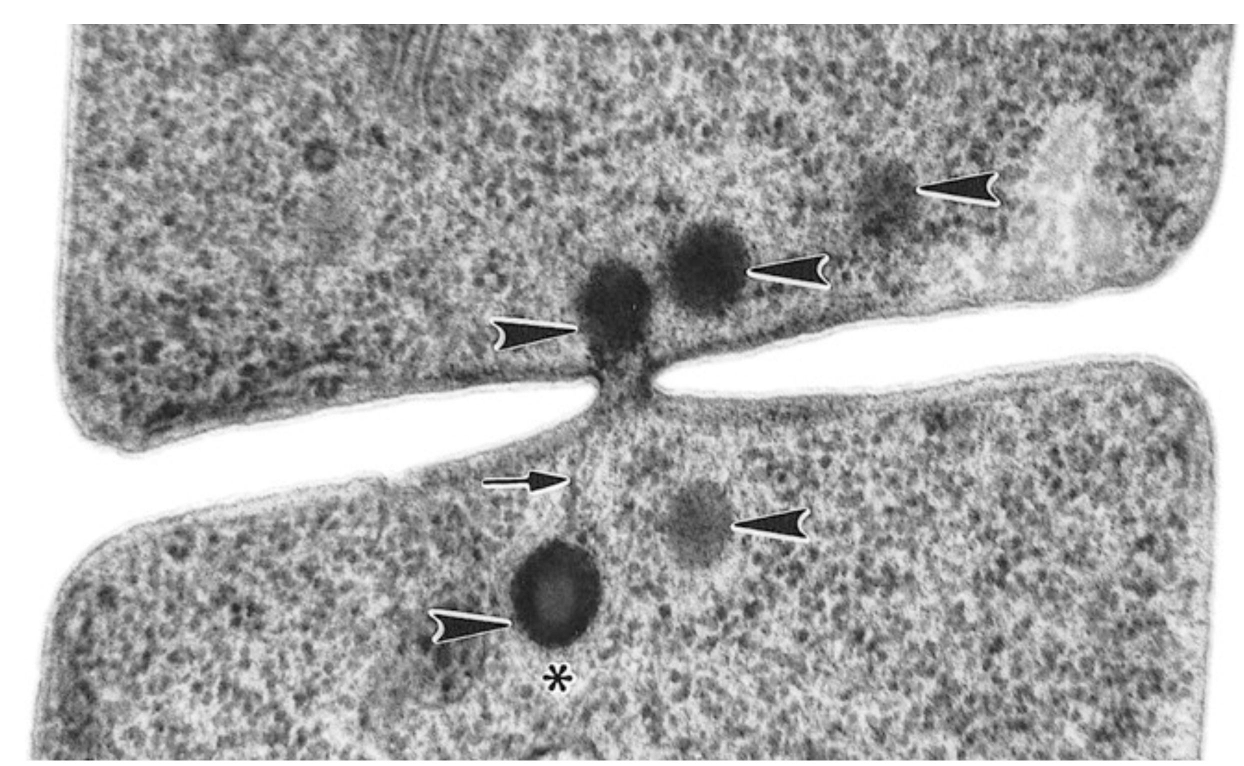
Septa

- Septa allow for separation of cells, isolate cytoplasm, allow for cellular differentiation.
- Regular septa in the Dikarya, but septa do exist in some pre-Dikarya
- Septa observed in the Kickxellomycotina (*Coemansia*)
- All 6 septin genes identified in Aspergillus are found in early fungi (*B. dendrobatidis*)



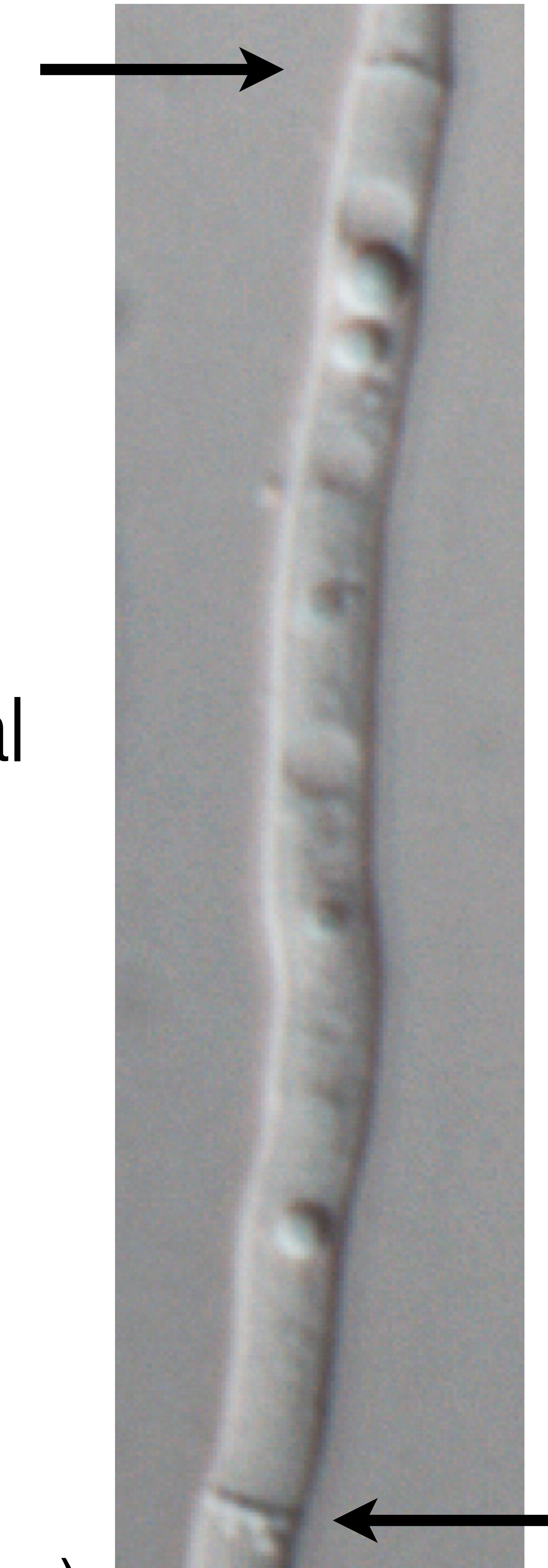
Auriscalpium vulgare (Agaricomycotina) hyphal septum.

Celio et al. Mycologia. 2007



A. nidulans (Pezizomycotina) septum and woronin body.

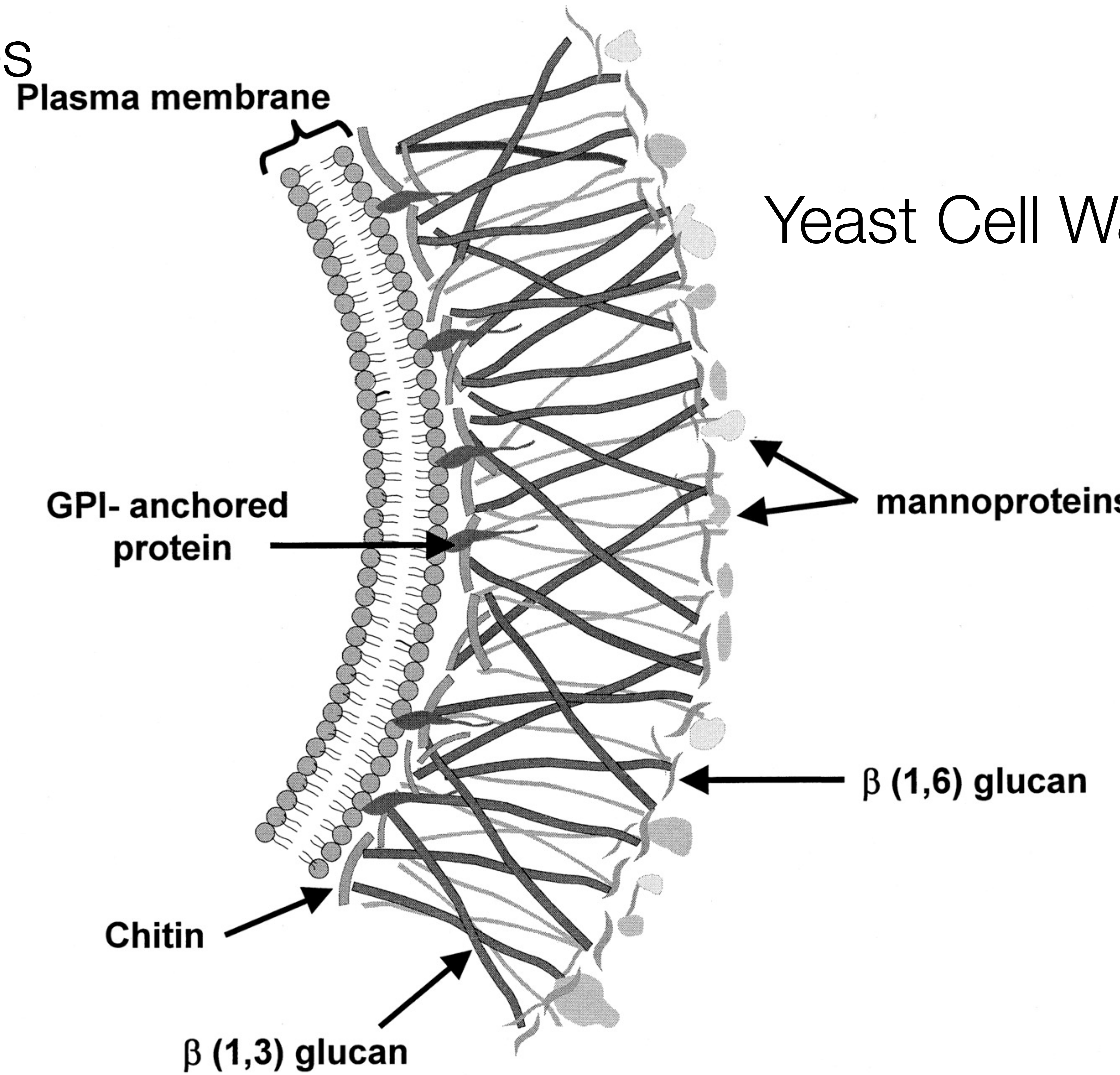
Momany et al. Mycologia. 2002



Coemansia

Stajich et al. *Current Biol*. in press

Fungal cell wall changes



Yeast Cell Wall

Selitrennikoff CP, AEM 2001

Cell wall changes from Early fungi

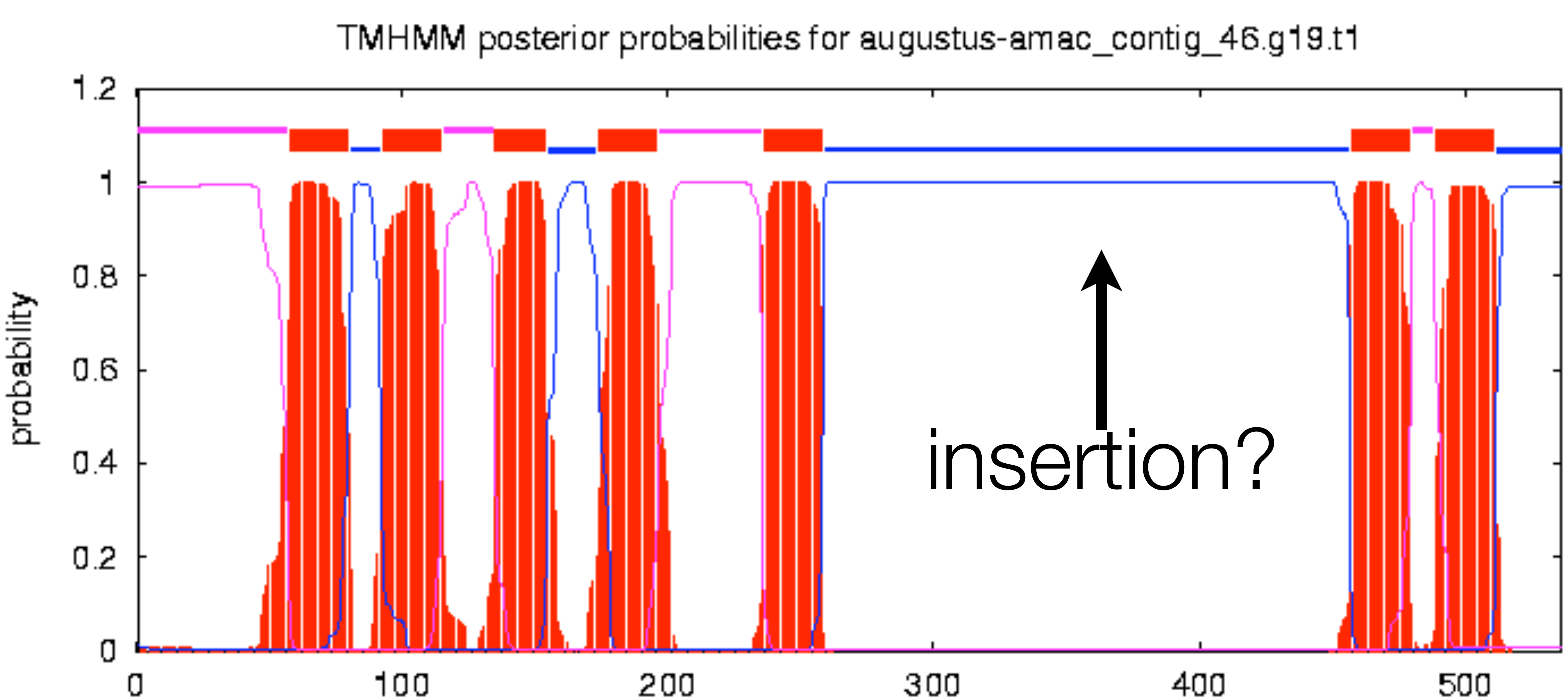
- Looking at the proteins known to make up cell wall in well studied yeast *Saccharomyces cerevisiae*
- Identified series of genes missing in Bd including major components of the 1,3-β glucan biosynthesis pathway (FKS1) and many of the KTH transporters
- With collaborators (JP Latge) we showed through a simple cell wall composition assay
    - Missing β(1,3)- & β(1,6)-glucans but contain β(1,4)- glucan: cellulose.
        - Few "higher" fungi have been documented to have cellulose in cell walls (Ascomycota)
- Bd is much less sensitive to Enchinocandin drugs which target FKS1
- Does gain of genes to make β(1,3)-glucan and/or loss of cellulose represent one of the major transitions from aquatic to terrestrial lifestyles for fungi?

Connecting molecules to evolutionary transitions

- Nuclear division changes
  - Basal body, centriole loss coupled with loss of flagellum
  - What happened first? Where did spindle-pole body genes evolve from - duplication or novel gene creation?
    Did some of flagellum & basal body genes evolve new functions?
- Multicellular development
  - Differentiation of multicellular tissue, septation, and signaling
- Cell wall changes
  - 1,3 Beta-glucan and 1,6 Beta-glucan synthesis evolved after Chytrid divergence
  - Was this a necessary transition with the aquatic to terresterial life changes?

Identifying an ancient photoreceptor

- Fungi have some light sensing molecules but no known rhodopsins have been cloned.
- Rhodopsin molecule implicated in zoospore phototaxis in *Allomyces* (by comparing wavelengths where cells responsed)
- Sequence similarity identifies a candidate 7-transmembrane protein, but has insertion found in both *Bd* and *Allomyces* (draft genome).



Rhodopsin guides fungal phototaxis

Rhodopsins act as the visual pigments of multicellular animals and also guide the swimming of green algae[1] towards or away from light (phototaxis). If these rhodopsin molecules have a common evolutionary origin, fungi, the sister group of animals[2], would also be expected to use a rhodopsin-based photoreceptor. Here we show that rhodopsin guides the zoospores of the fungus *Allomyces reticulatus*[3] towards the light, suggesting that the origin of vision might have been the phototaxis of their unicellular ancestors.

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NATURE | VOL 387 | 29 MAY 1997

Transitions inferred from genome comparisons

<u>AFTER CHYTRID SPLIT</u>

<u>Loss</u> of:

- Flagella, centrioles
- **<u>1,4 Beta-glucan synthesis</u>**

Gain of:

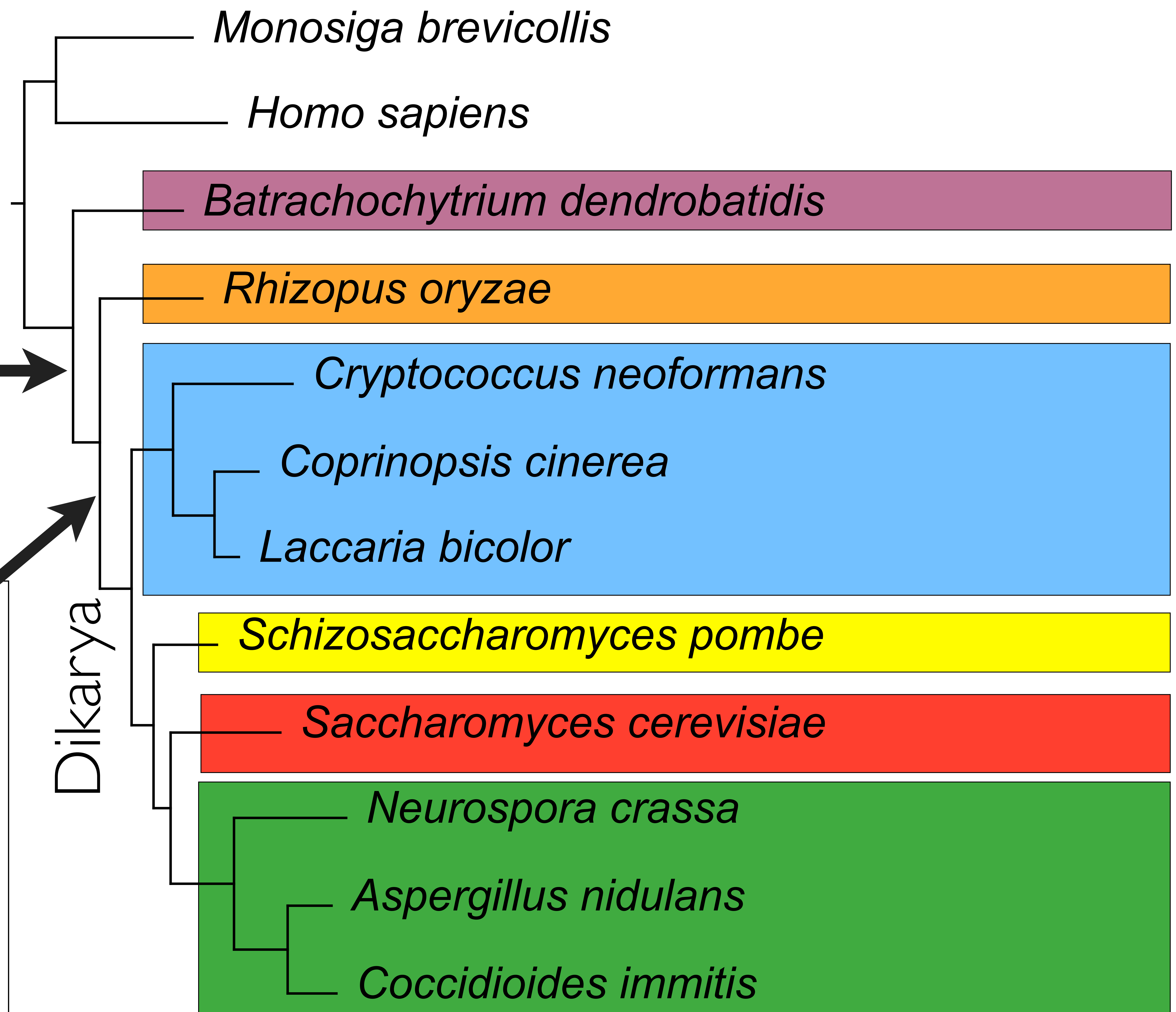
- STE50 adaptor
- Some glucan synthase and transferases
- **Rhodopsin-like 7TM**

<u>IN DIKARYA, Gain</u> of:

- Spindle-pole bodies
- <u>1,3 Beta glucan synthase</u>
- Thiamine biosynthesis
- STE3 pheremone receptor
- **DASH complex**
- **KTR mannosylphosphate family**

Loss of:

- Chitosanase



Thanks

John Taylor & Laboratory (Univ California, Berkeley)

Mary Berbee (Univ British Columbia)

UC Berkeley Imaging facility & Barbara Waaland

Miller Institute

B.dendrobatidis sequencing
Joint Genome Institute & Broad Institute

http://fungalgenomes.org/blog

New research starting at UC Riverside

- Interested in research in fungal genomics?
    - Evolution of fungal development
    - Post-transcriptional gene regulation and small RNAs
    - Fungal cell evolution in early diverging chytrid and 'zygomycete' fungi
- Bioinformatics and genome informatics of fungal comparative genomics

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