Regulation of cellular morphogenesis during development in *Aspergillus nidulans*

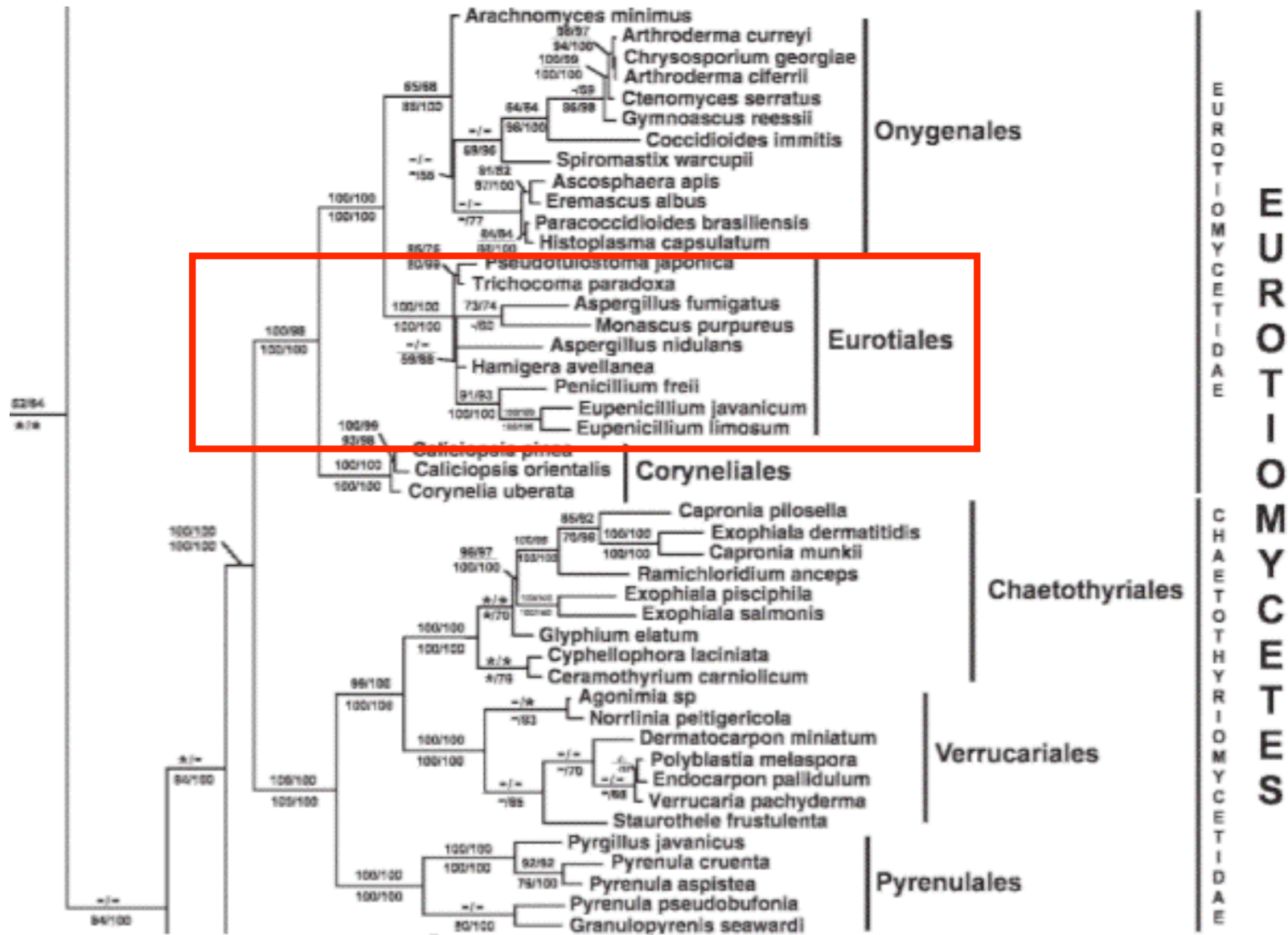## Steven D. Harris

Center for Plant Science Innovation/Dept. Plant Pathology

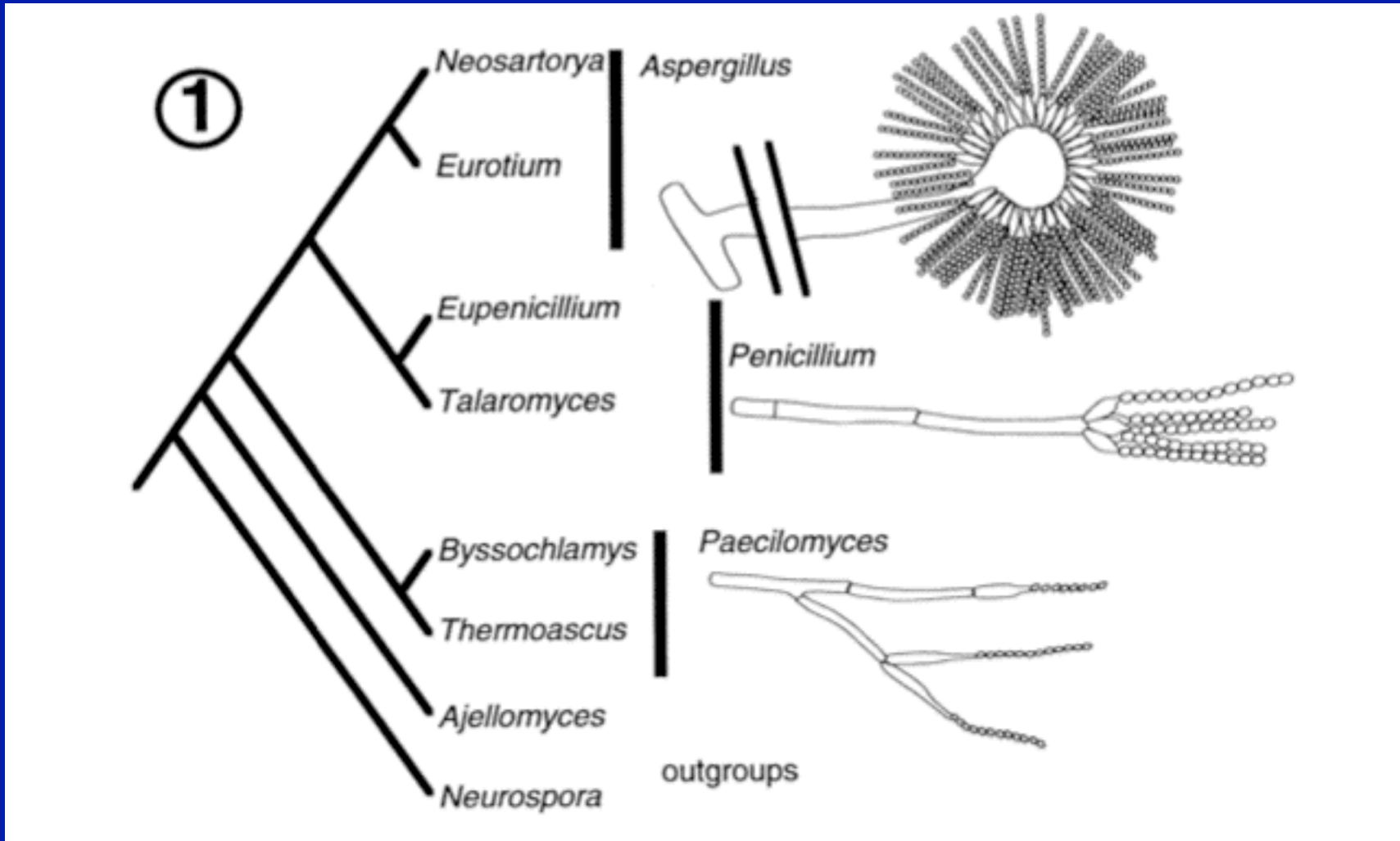## University of Nebraska

Lincoln, NE

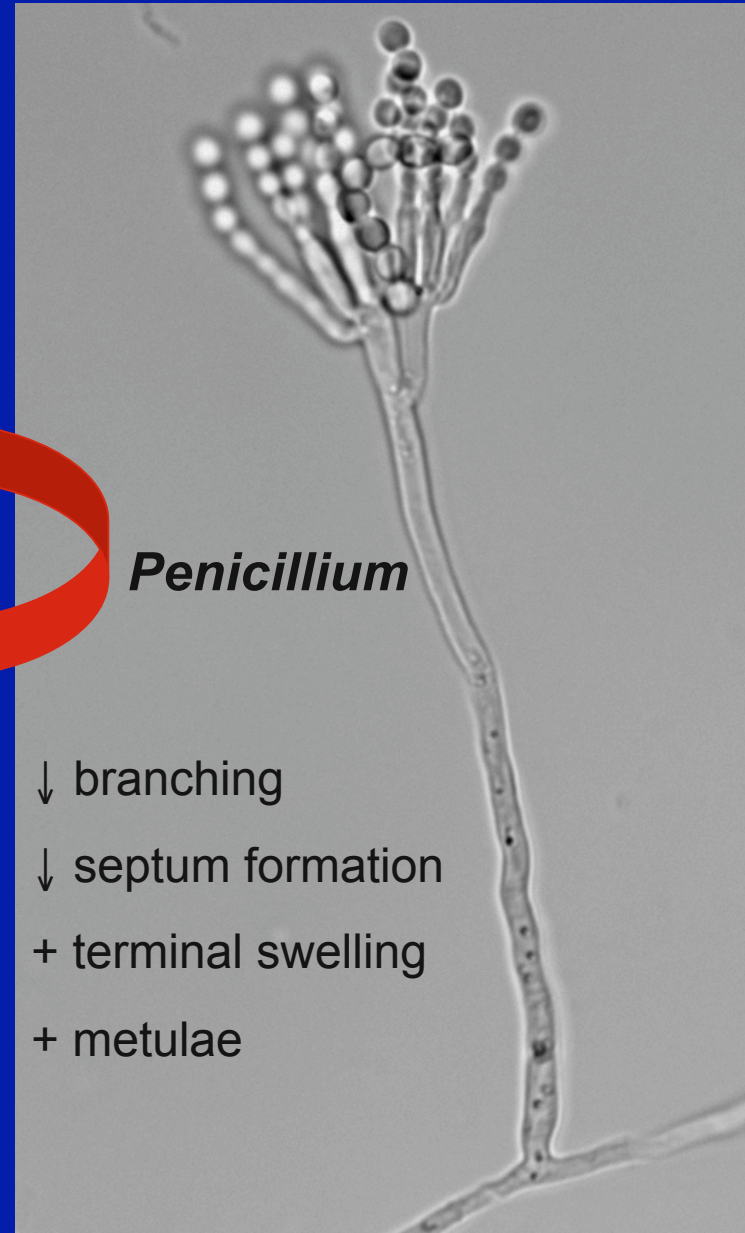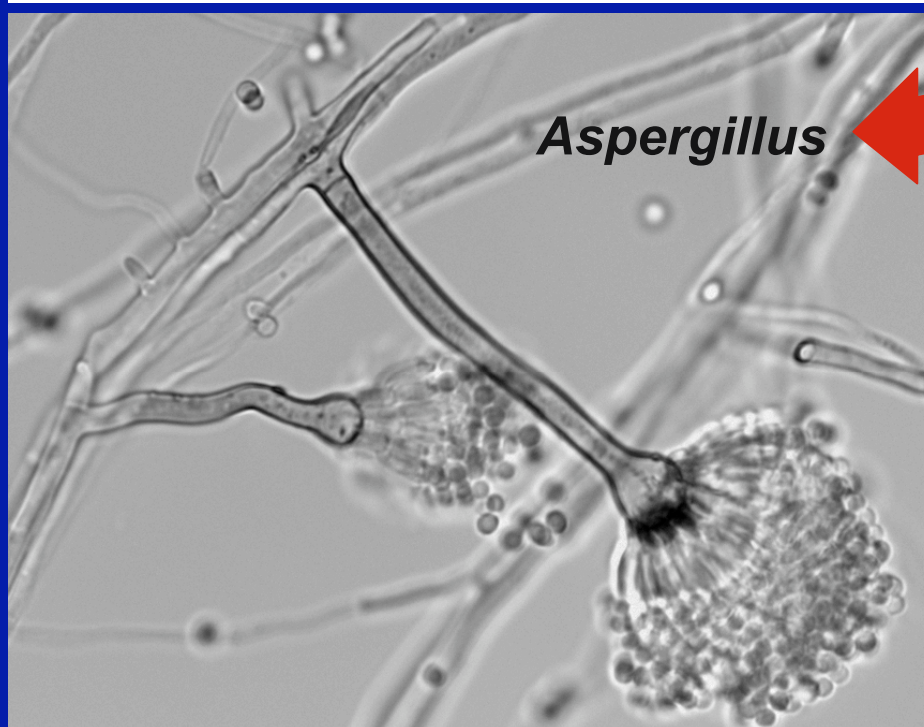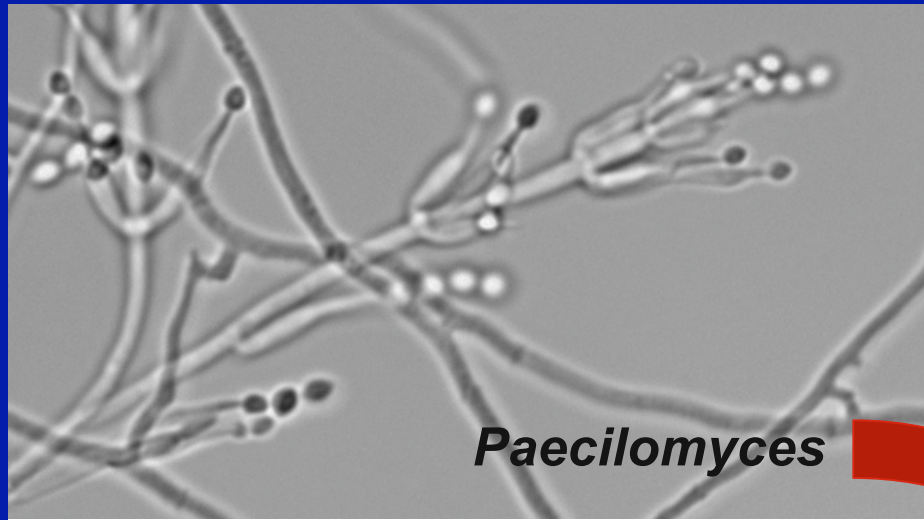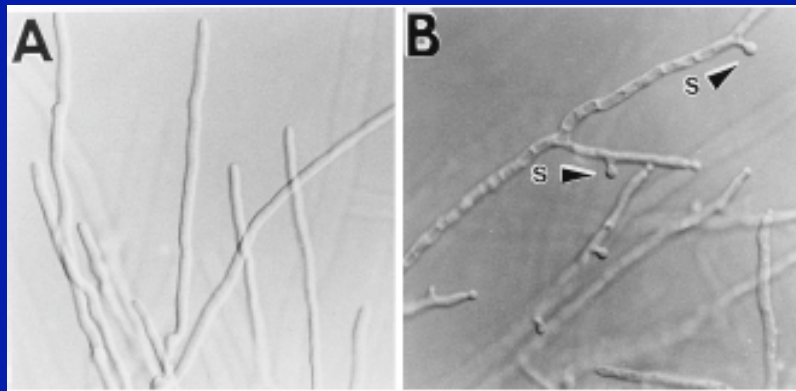# Evolution of conidiophore morphology in the Eurotiales
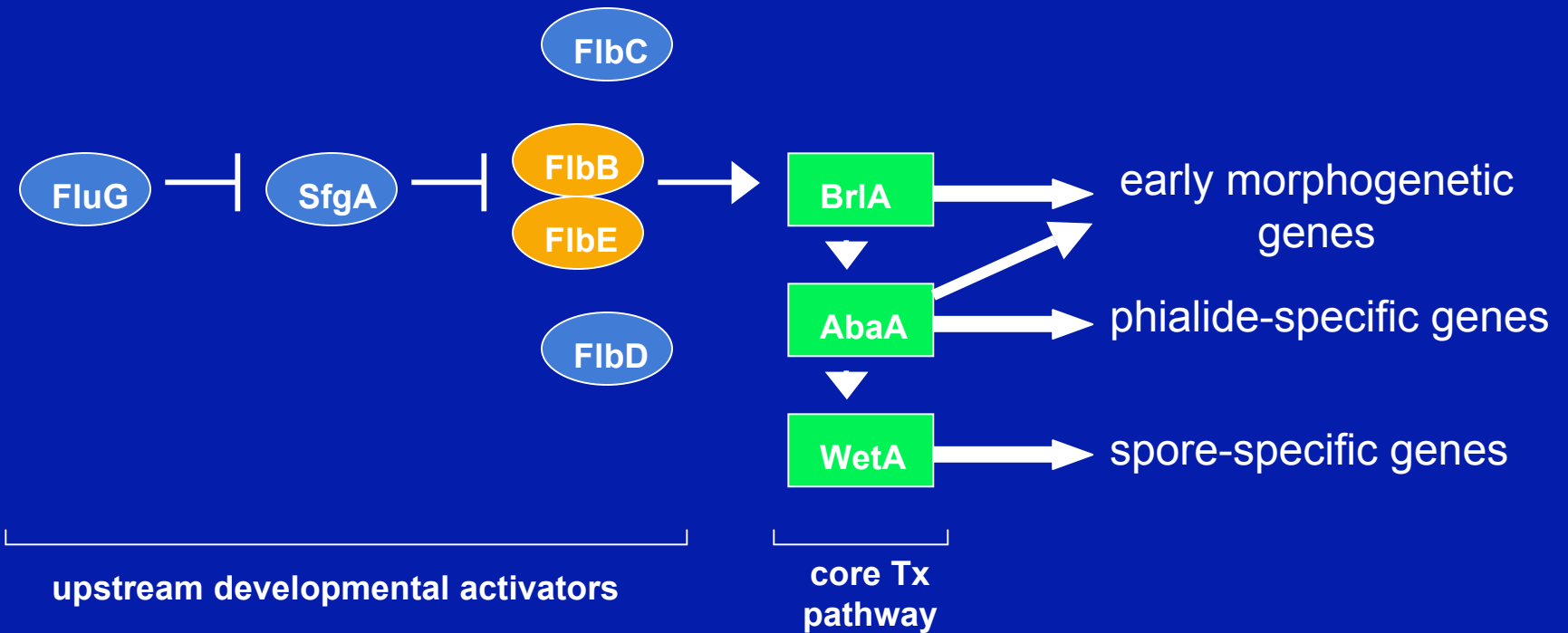
Evolution of conidiophore morphology in the Eurotiales

Increasing complexity of conidiophore architecture

Paecilomyces

Penicillium

Aspergillus

↓ branching

↓ septum formation

+ terminal swelling

+ metulae

Regulation of conidiophore development in *A. nidulans*

FlbC

FluG ⊣ **SfgA** ⊣ **FlbB** / **FlbE** → **BrlA** → early morphogenetic genes

FlbD

BrlA → **AbaA** → phialide-specific genes

AbaA → **WetA** → spore-specific genes

upstream developmental activators

core Tx pathway

expression of *alcA*(p)::*brlA* drives formation of spores in submerged hyphae

How are cell cycle and morphogenetic functions integrated within this regulatory network?

# Potential factors underlying the evolution of conidiophore morphology

1. Cell cycle regulation
   Wee1 tyrosine kinase; Ace2/Swi5 C2H2 transcription factor

2. Spatial regulation of cellular morphogenesis
   Cdc42 GTPase module

3. Integration with UDAs and core transcriptional regulatory network
   BrlA; AbaA

Why?
-- improved understanding of conidiophore morphogenesis
-- understand the molecular events involved in the evolution of conidiophore morphology
-- identification of potential strategies for the control of sporulation

Progress ➜

Cell cycle regulation: Wee1

AnkA^{Wee1}

Y15P

NimX^{CDK1}

G2 ⟶ M

⟹

NimT^{Cdc25}

Y15

NimX^{CDK1}

G2 ⟶ M
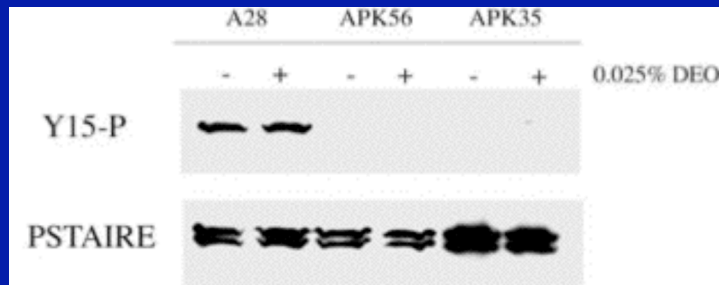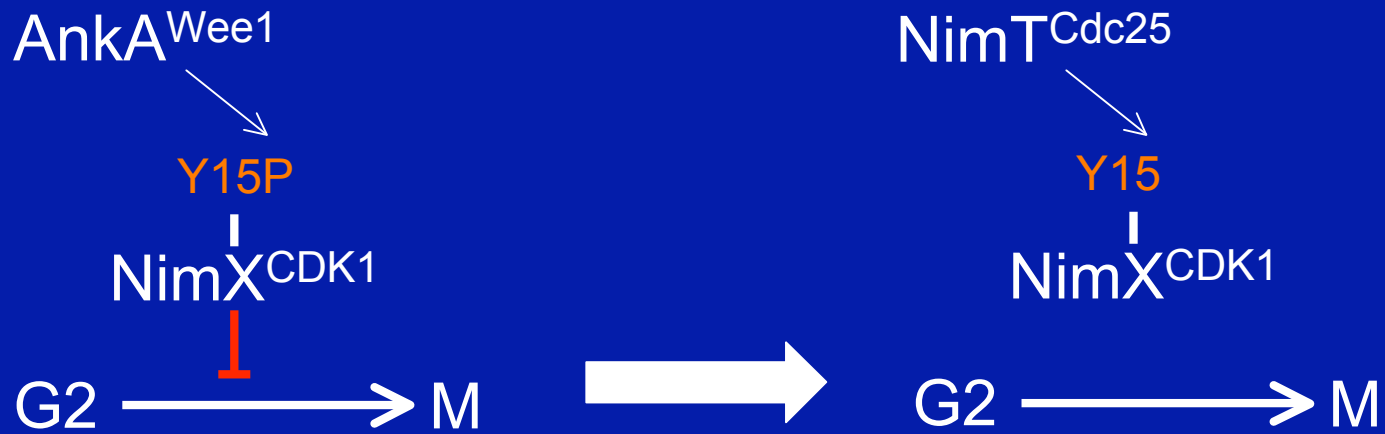
Kraus and Harris, 2001
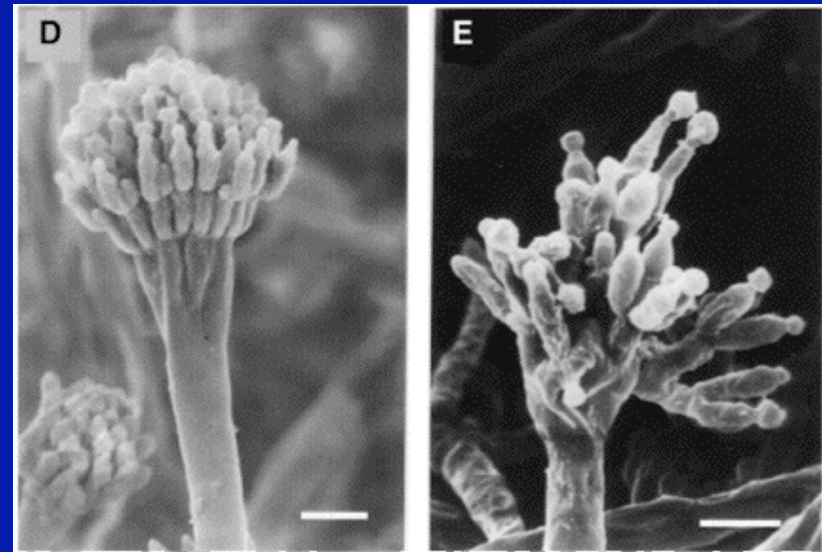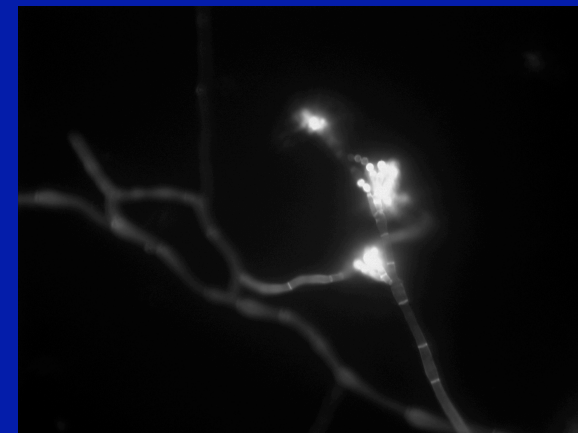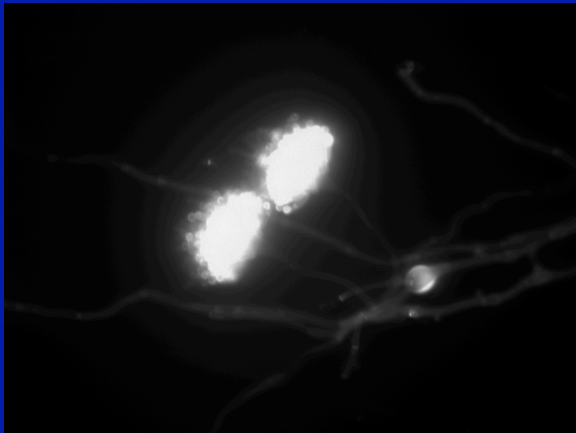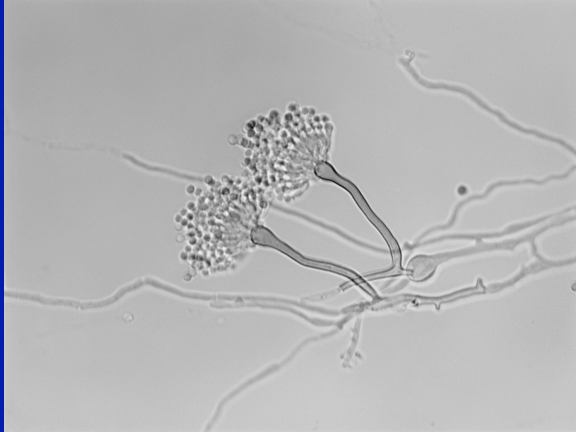

Ye et al., 1999

Cell cycle regulation: Wee1

wildtype   *ankA2* (formerly *sntA1*)

Loss of Wee1 function (i.e., NimX^{CDK1}Y15 Pn) causes;

septum formation in stalk, loss of vesicle, spurious branch formation, defective hyphal➔budding transition

Cell cycle regulation: Ace2/Swi5

Ace2 and Swi5 = paralogous C2H2 transcription factors in yeast

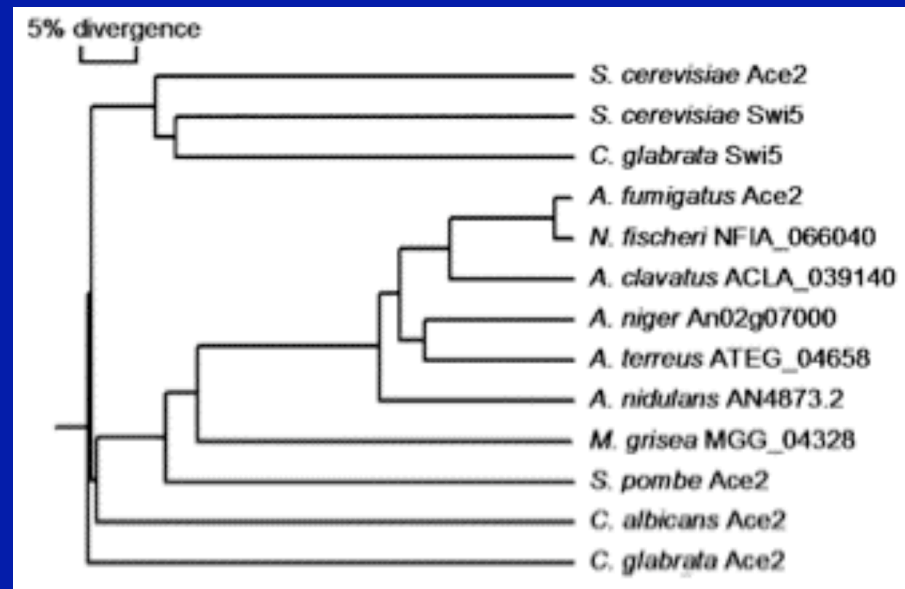Ace2 ➔ septum degradation following cytokinesis
Swi5 ➔ transcription of genes expressed at M/G1 transition and in G1

Afum Ace2 (Afu3g11250) required for phialide formation, cell wall architecture, and virulence (Ejzykowicz et al., 2009)
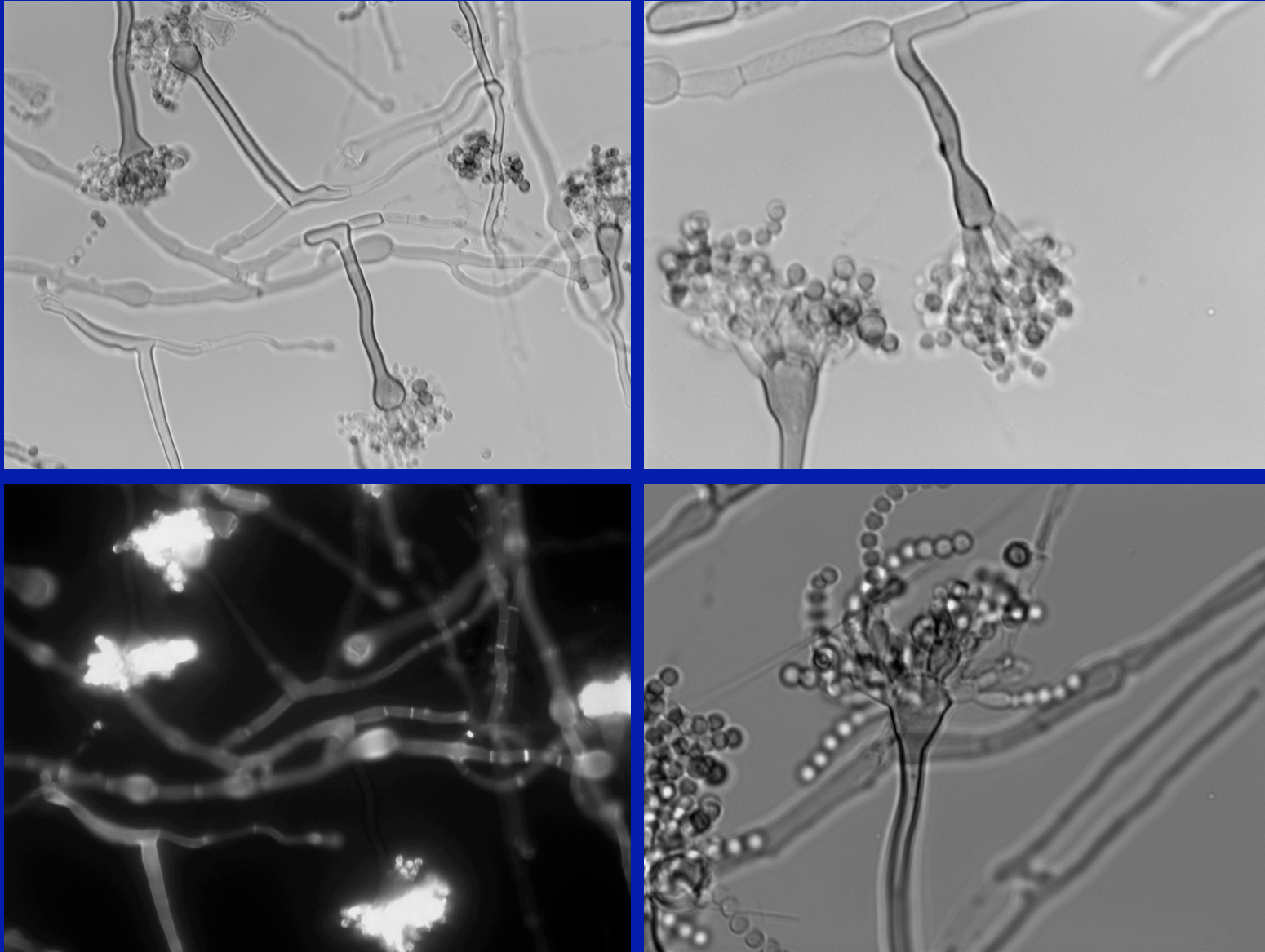
ANID_04873.1

51%ID/71%S over 781 AA – Afum
55%ID/65%S over 110 AA – Swi5

generated Δace2::pyroA mutants and screened for conidiation defects

5% divergence

- S. cerevisiae Ace2
- S. cerevisiae Swi5
- C. glabrata Swi5
- A. fumigatus Ace2
- N. fischeri NFIA_066040
- A. clavatus ACLA_039140
- A. niger An02g07000
- A. terreus ATEG_04658
- A. nidulans AN4873.2
- M. grisea MGG_04328
- S. pombe Ace2
- C. albicans Ace2
- C. glabrata Ace2

Cell cycle regulation: Ace2/Swi5
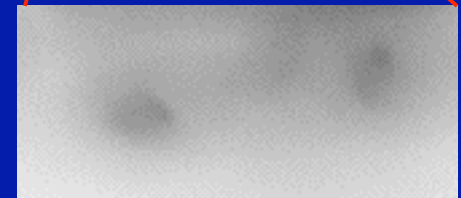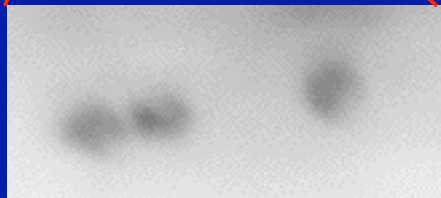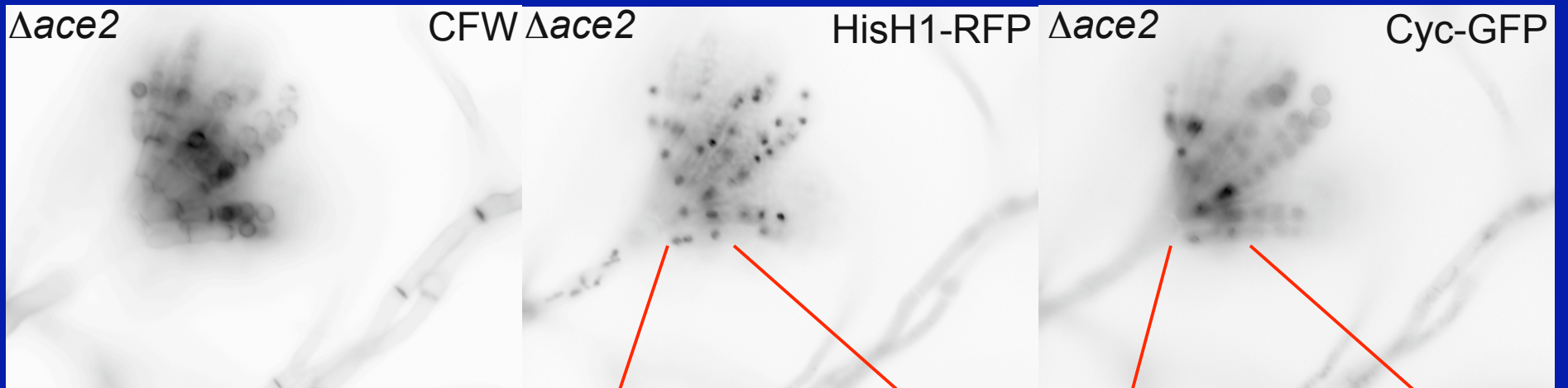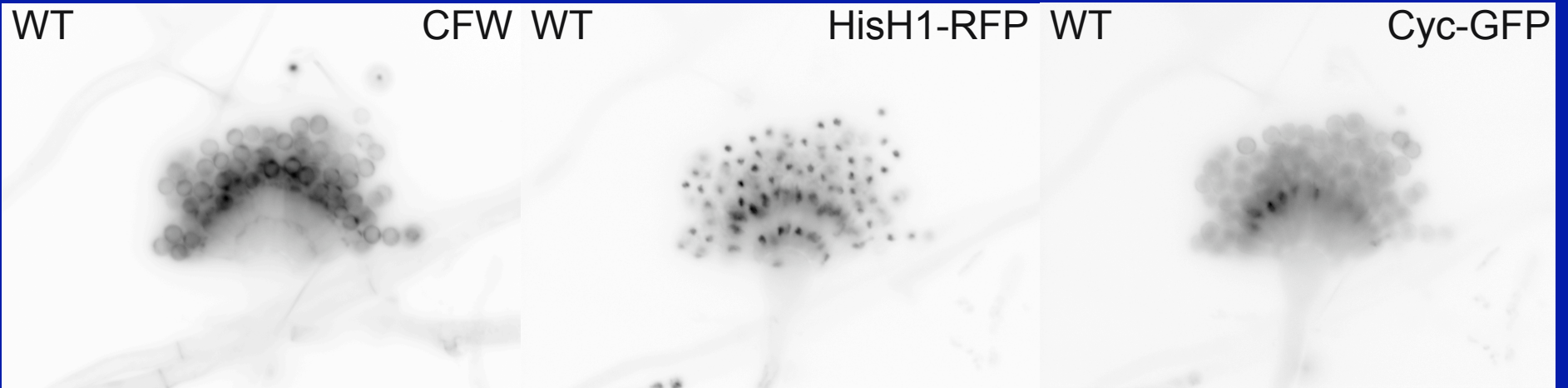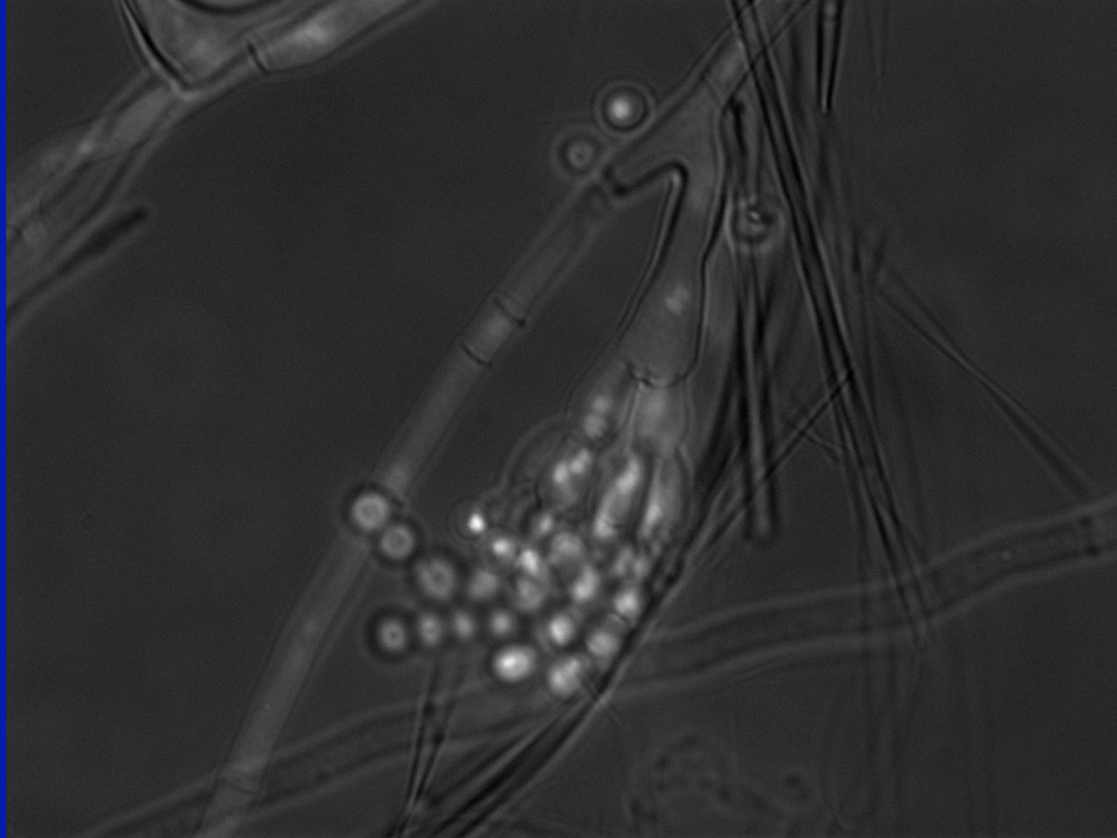

normal stalk + vesicle; defective transition from vesicle to metulae (hyphal➔budding)

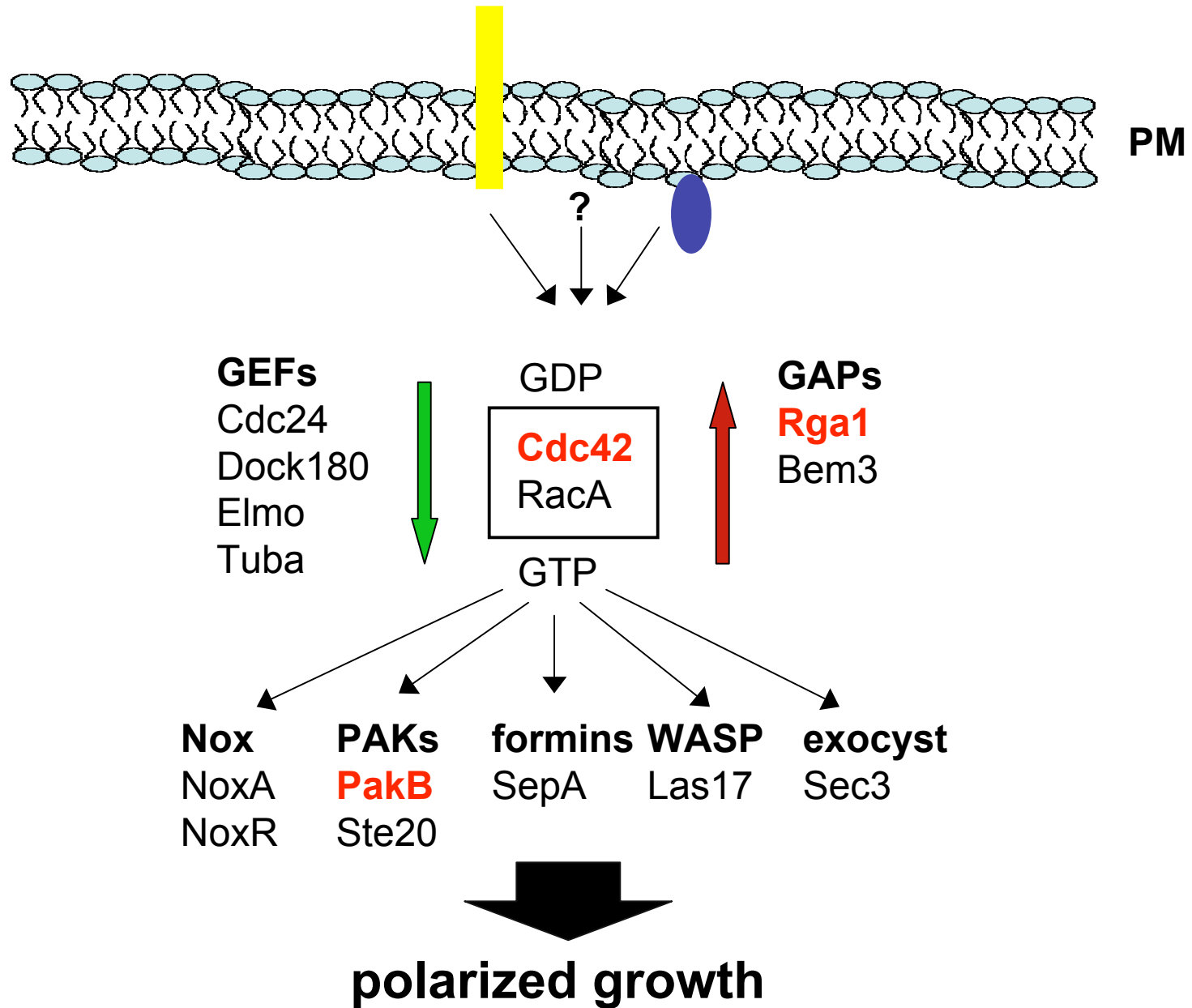# Cell cycle regulation: Ace2/Swi5

Cell cycle regulation: Ace2/Swi5

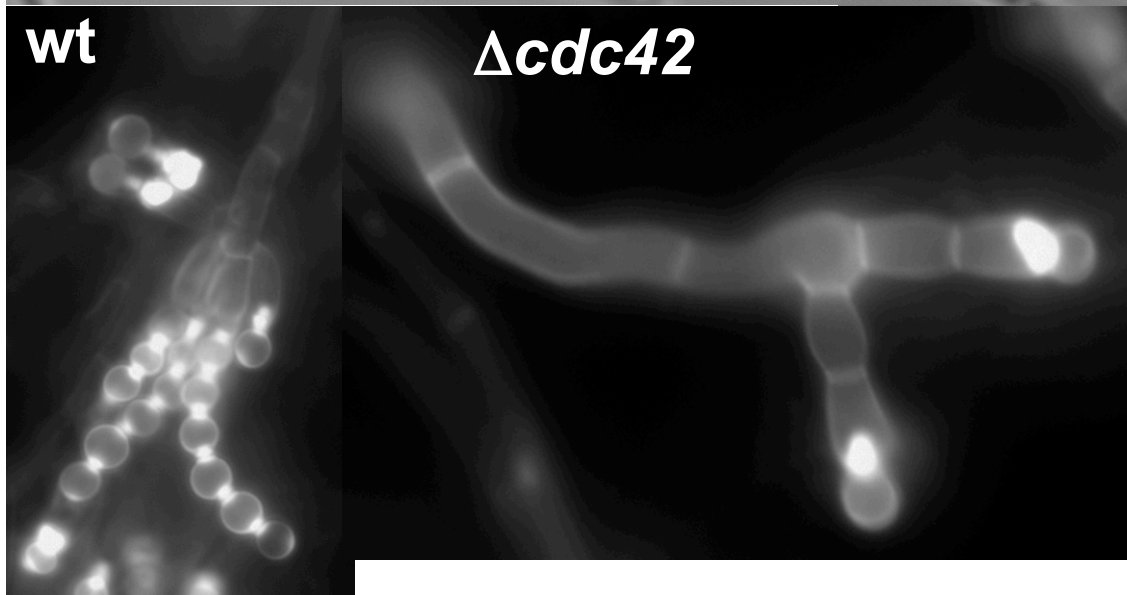Ace2/Swi5 is required for orderly transition from vesicle➔metulae

absence results in multinucleate hybrid cells that appear to undergo asynchronous mitosis

coordinated cell cycle regulation underlies morphological transitions

Cdc42/Rac GTPase module

PM

GEFs
Cdc24
Dock180
Elmo
Tuba

GDP

Cdc42
RacA

GTP

GAPs
Rga1
Bem3

Nox
NoxA
NoxR

PAKs
PakB
Ste20

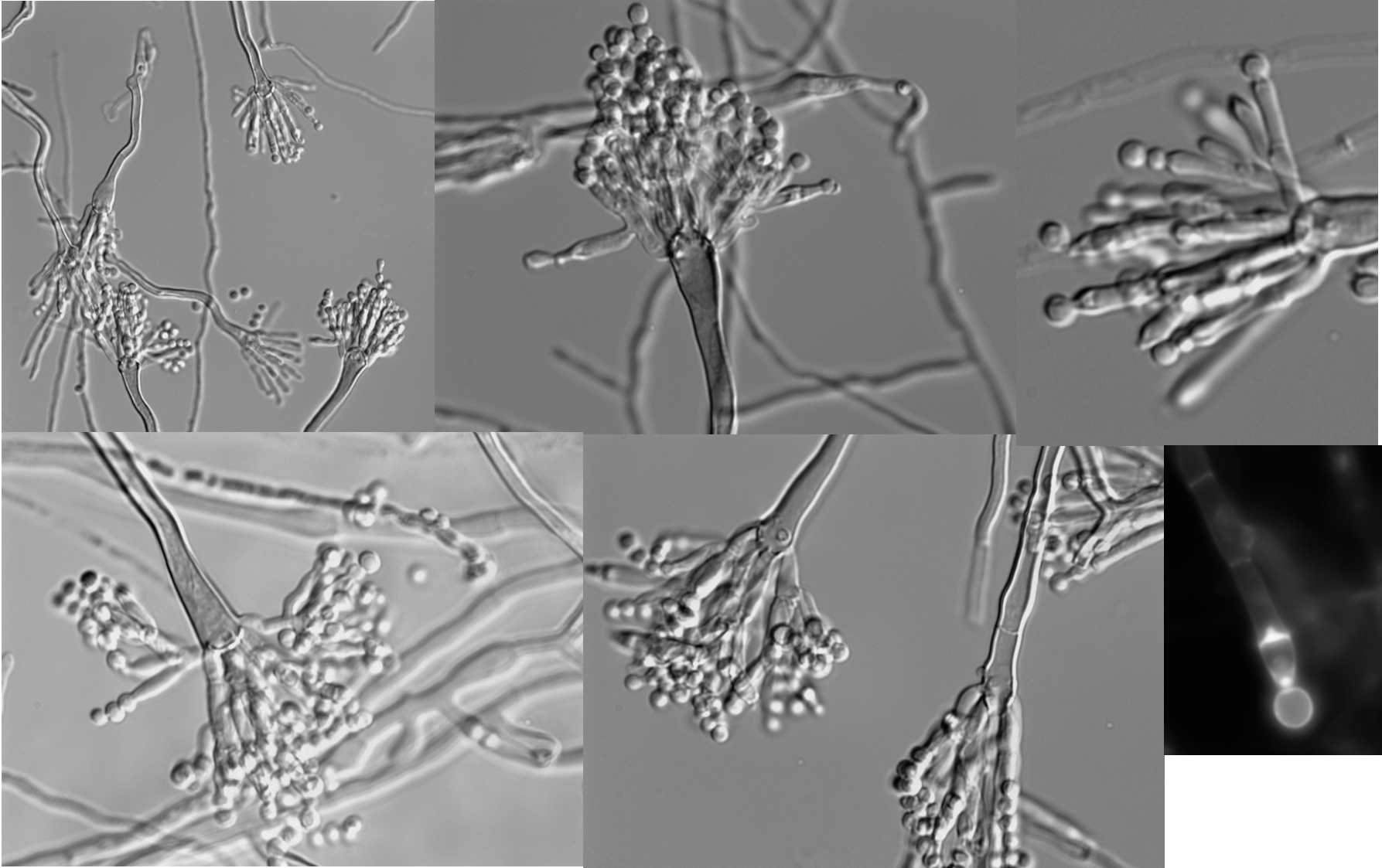formins
SepA

WASP
Las17

exocyst
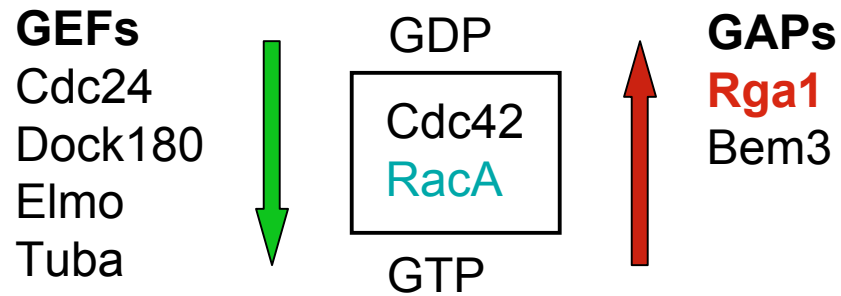Sec3

polarized growth

Conidiophore development: Cdc42
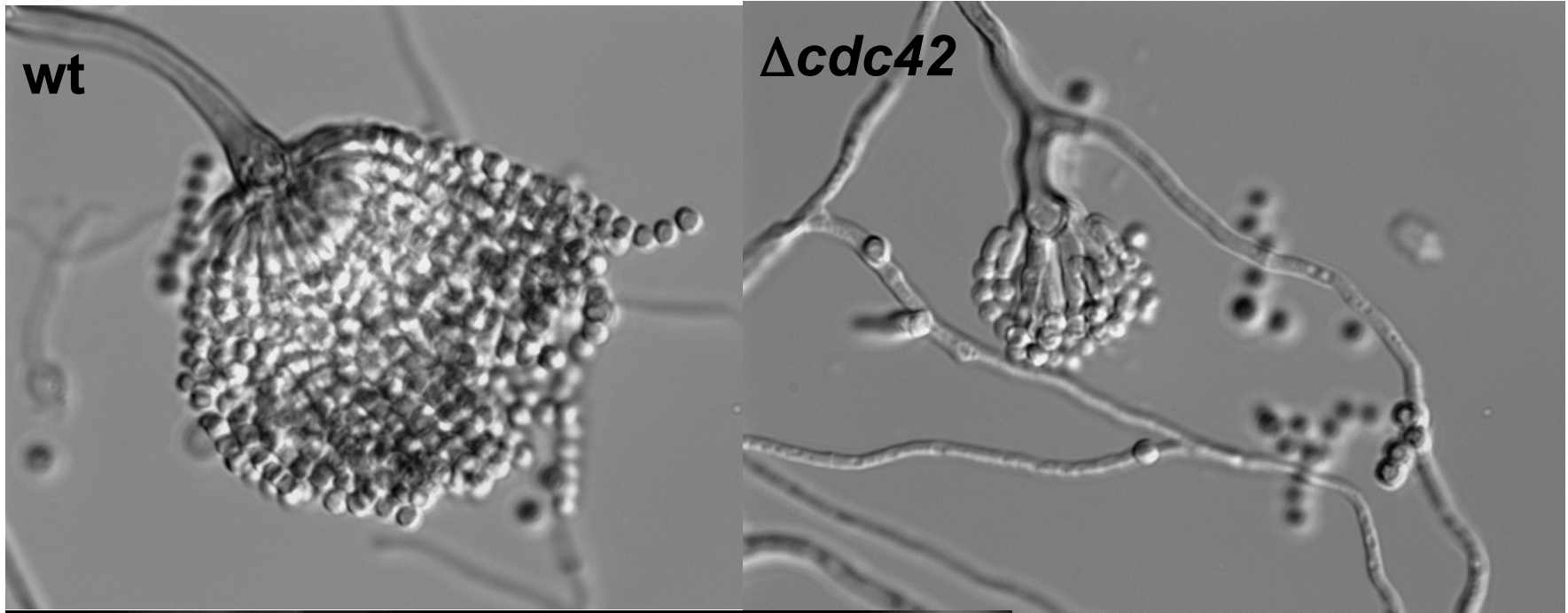
shorter conidiophores

wider cells

abnormal phialide-
spore junctions

Conidiophore development: Rga1

Δ*rga1* -- no obvious vesicles; elongated metulae and phialides; random branching of metulae

Conidiophore development: Rga1

GEFs		GDP		GAPs
Cdc24				**Rga1**
Dock180		Cdc42		Bem3
Elmo		RacA		
Tuba		GTP		

Does the Δ*rga1* phenotypes reflect activation of Cdc42 or Rac1?

Δ*rga1*   Δ*rga1*Δ*rac1*   Δ*rga1*Δ*cdc42*

Deletion of *cdc42* suppresses Δ*rga1* conidiophore defects.
Thus, at least during conidiation, the primary target of Rga1
is Cdc42 (and Δ*rga1* defects are due to abnormally active Cdc42)

Conidiophore development: PakB
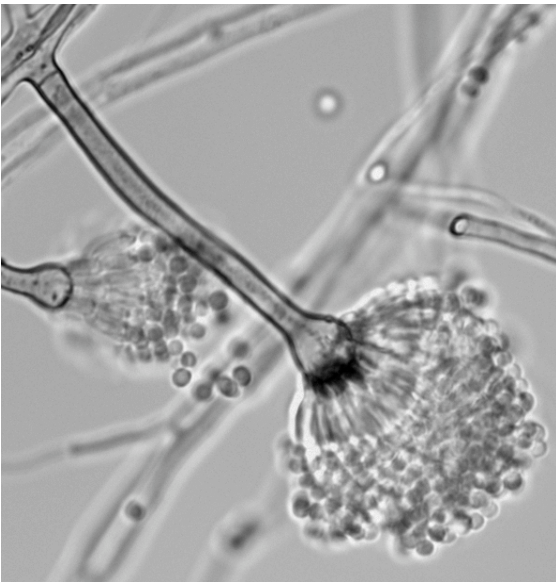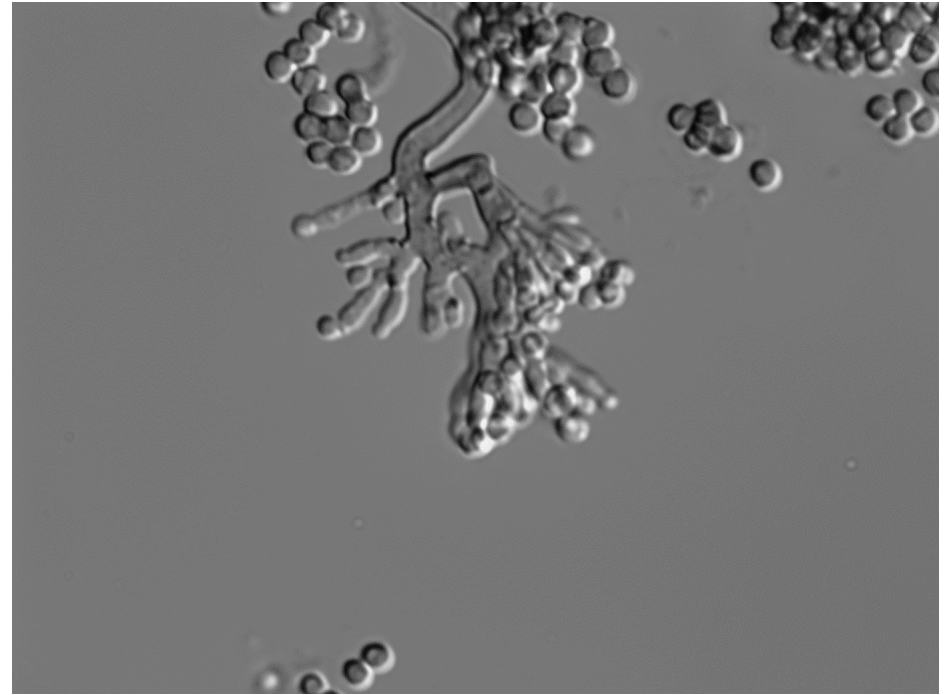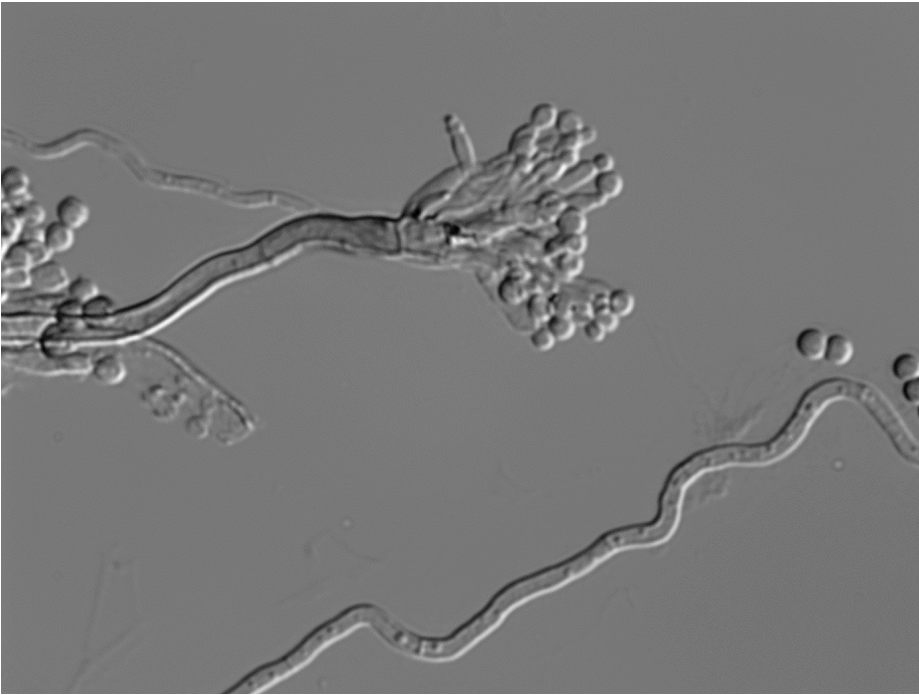
PakB;

suppresses conidiophore branching, formation of vesicle
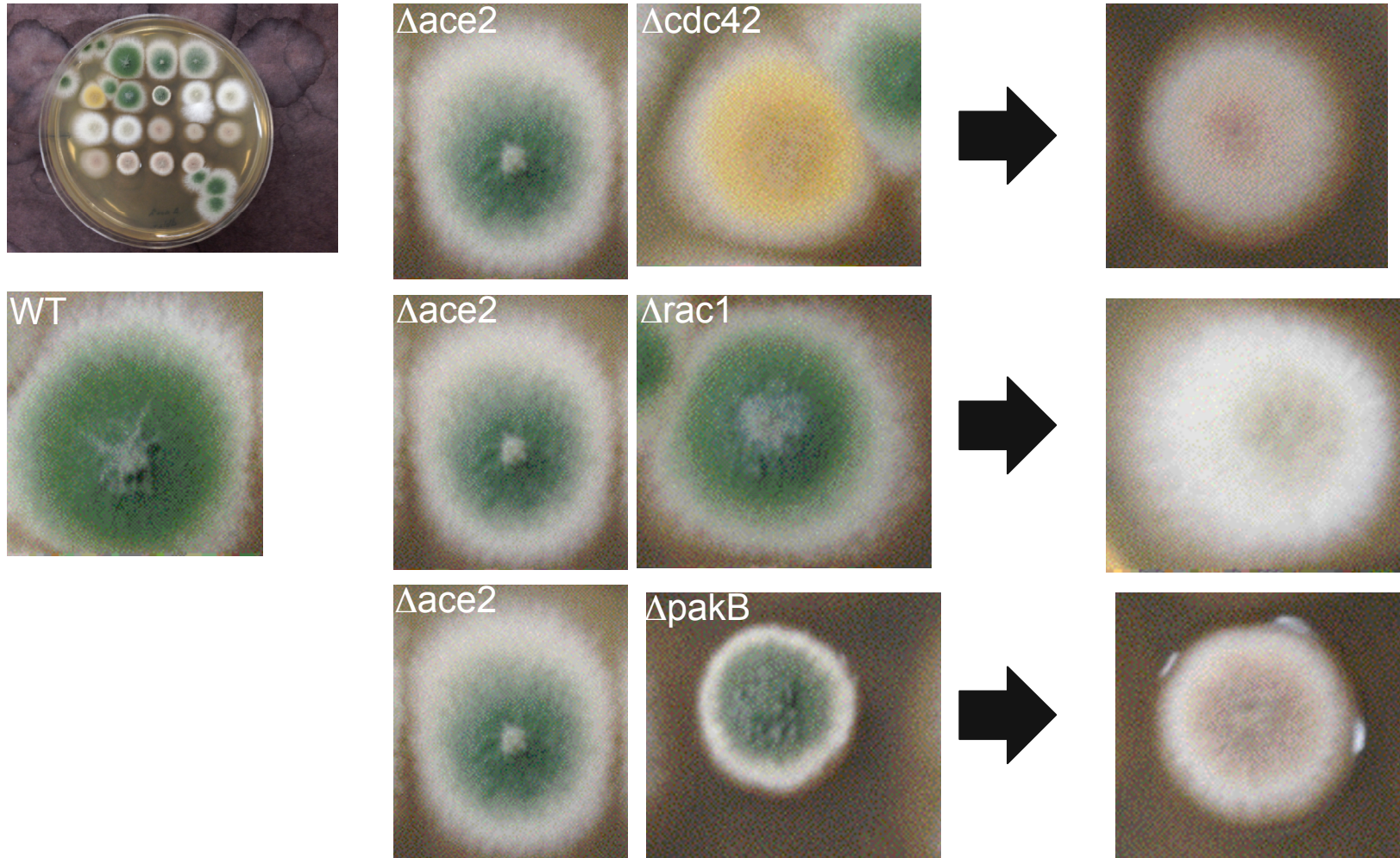
Cdc42/Rac GTPase module

Rga1 and PakB suppress lateral branching during stalk formation --- proper spatial control of Cdc42 activation confines growth to tip and enables vesicle formation?

Rga1 is required for normal transition from hyphal to budding growth --- Cdc42 activity must be down-regulated to enable budding of metulae from vesicle?

Do cell cycle regulation and the Cdc42/Rac GTPase modules function in the same pathway?

Test double mutants for epistasis

Genetic interaction analysis

Enhanced conidiation defects imply that Ace2/Swi5 and the Cdc42/Rac1 GTPase module act via different pathways to regulate development

Integration of cell cycle functions and Cdc42/Rac GTPase module with the core transcription pathway (BrlA)

Forced expression of *brlA* triggers formation of conidia (Adams and Timberlake, 1988)

glucose   ethanol
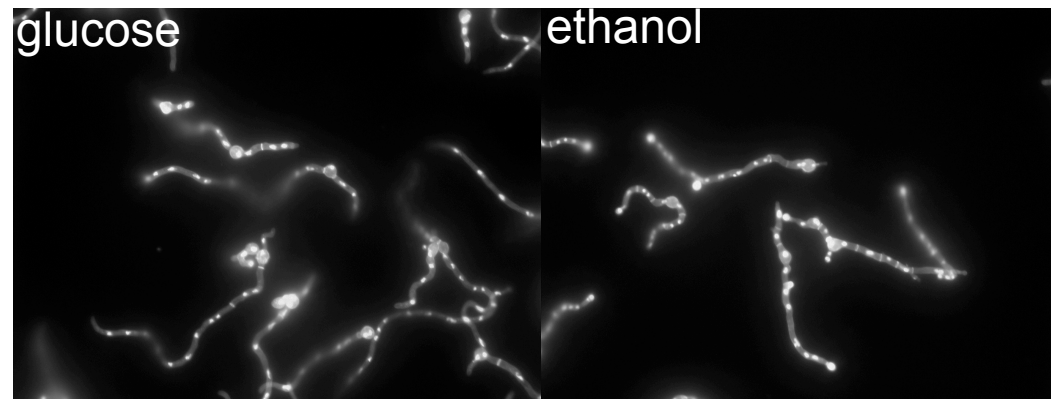
alcA(p)::*brlA*

RNA-Seq was used to examine transcript profiles following *brlA* induction;

cell cycle   nimX (CDK1)   ↑2.5x
                   ankA (Wee1)   ↑2.2x
                   nimE (G2 cyclin) ↑5.4x
                   cln1 (G1 cyclin) ↑2.2x
                   pclA (G1 cyclin) ↑4.9x
                   ace2/swi5   ↑3.8x

Suggests that expression of cell cycle functions is regulated by BrlA➡AbaA

stronger induction in response to *abaA* induction

Cdc42/Rac1 GTPase module – no response; post-transcriptional?

# Model for conidiophore morphogenesis in *A. nidulans*

AnkA
PakB

AnkA
Rga1

AnkA
Ace2
Rga1

Bud4

Bud4
Axl2

septins
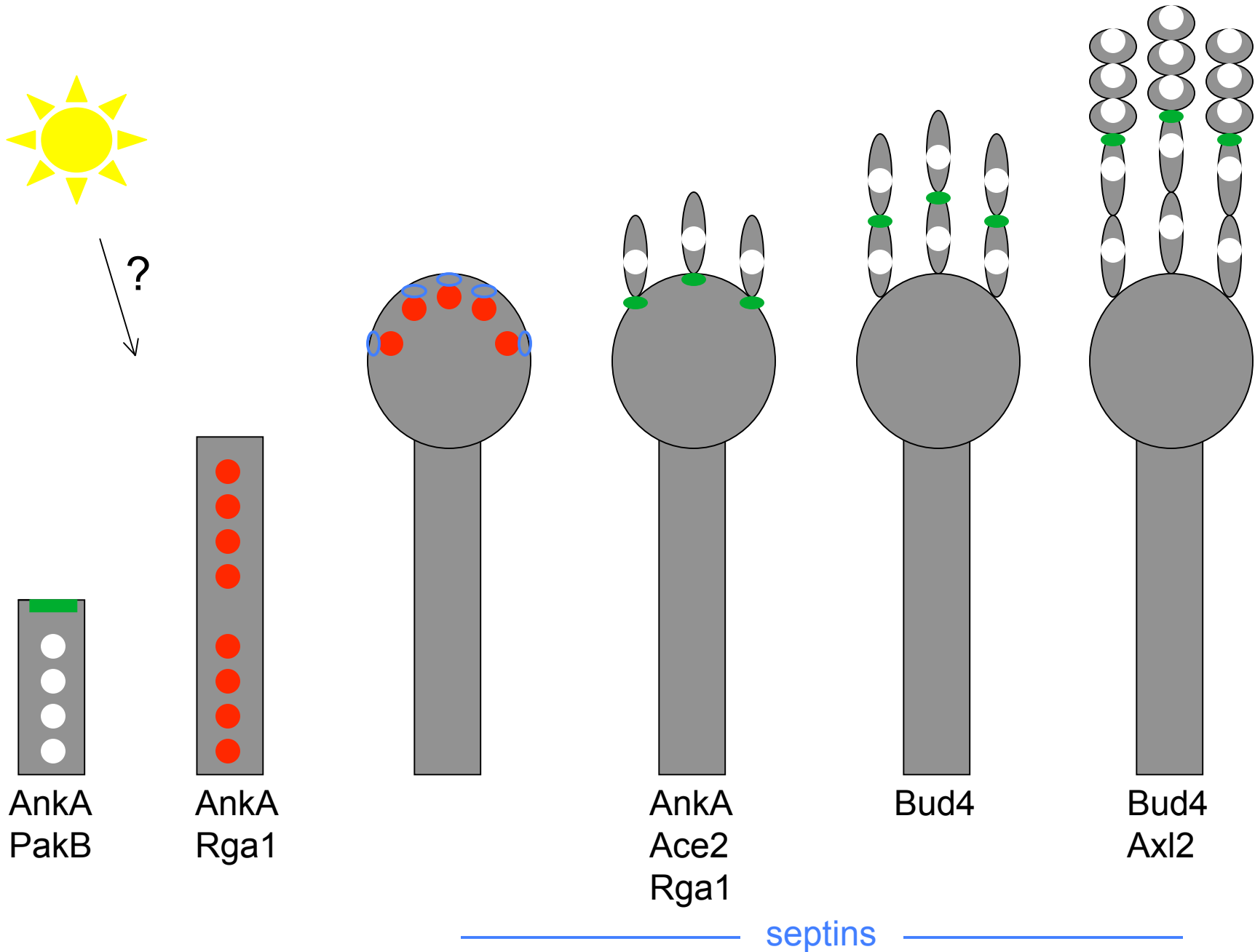
Evolution of conidiophore morphology

-- suppression of branching and septum formation in stalk
-- termination of polarized growth to form vesicle
-- switch to budding growth

Targets of selection?
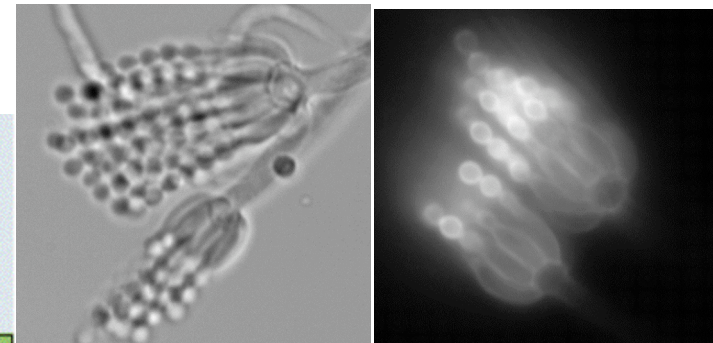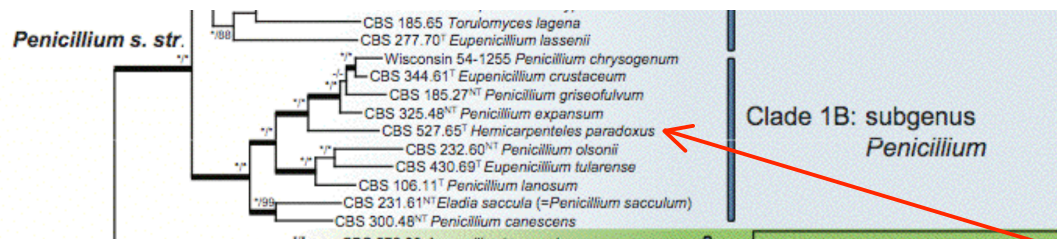expression of *ankA*, *ace2/swi5*, cyclins, or other cell cycle regulators?
post-transcriptional control of Cdc42 GTPase activity (phosphorylation of GEFs, GAPs)?
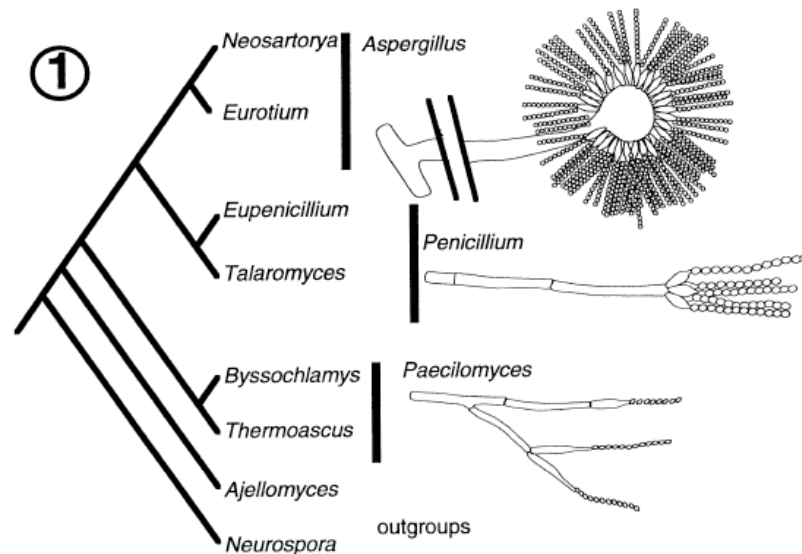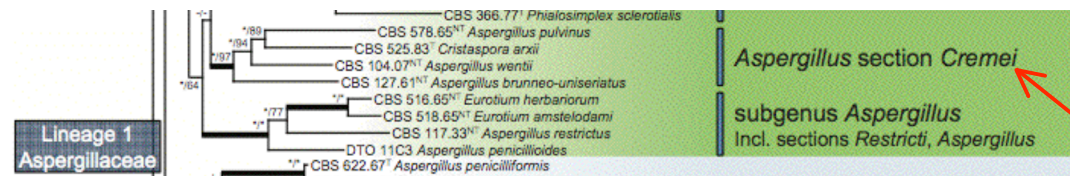
Evolution of conidiophore morphology

Test by;
1. Comparative gene expression analysis (e.g., *A. nidulans* vs. *P. chrysogenum*) during staged conidiation
2. Examine morphologically aberrant/transitional species

an "aspergilliod" *Penicillium*?

Penicillium paradoxus

a "penicilliod" *Aspergillus*?

Penicillium inflatum

Aleks Virag

Haoyu Si

Bill Rittenour

Brad Downs

Rennes Nassrat

Lakshmi Yerra