# Whi3 function in G2/M transition

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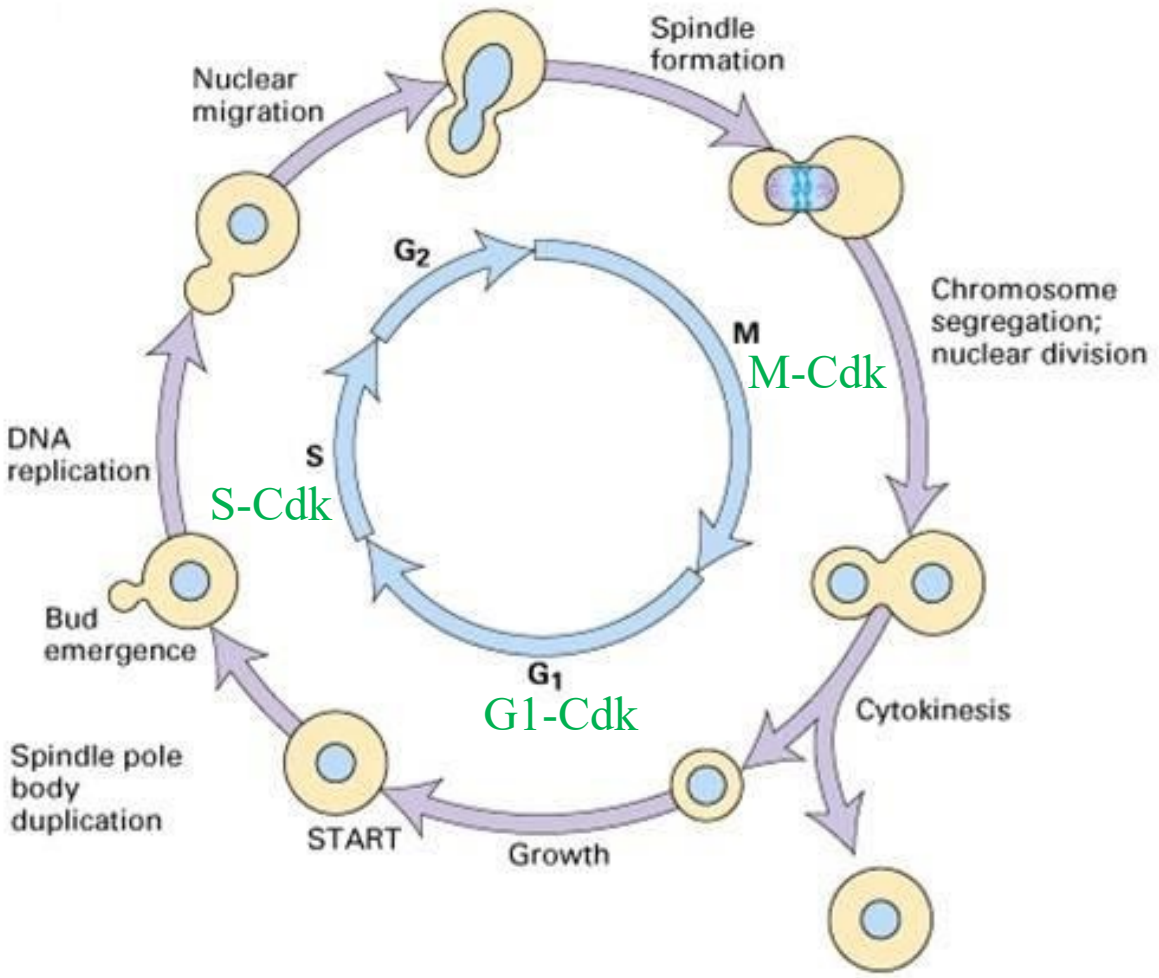
3/28/18

# START: Irreversible Cell Cycle Entry



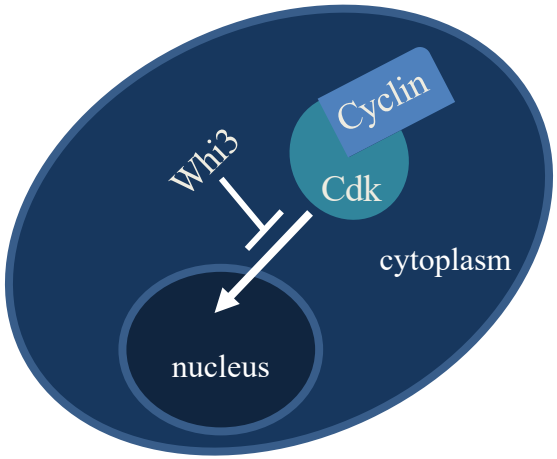

START is governed by nutrient availability, cell size, and Cdk activity.

https://voer.edu.vn/m/cell-cycle-regulation-in-the-budding-yeast/1ce212d4

# Swe1 Inhibits M-CDK to Ensure Timely Mitotic Entry

# Relevant Whi3 Mitotic Functions

- Cytoplasmic retention factor for Cdk1 and associated cyclins

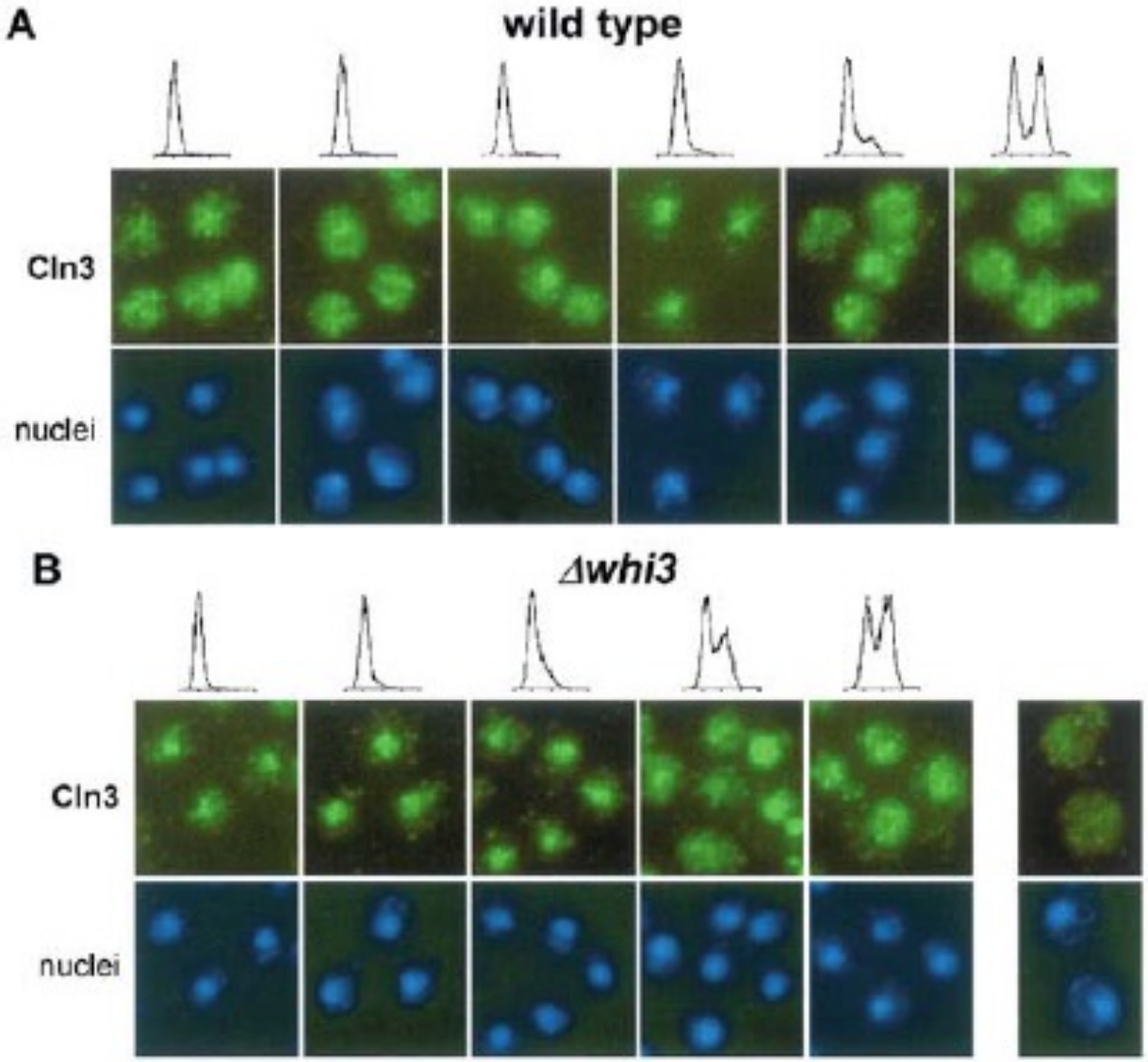



Both RNA and protein binding

Deletion forces the cells through START, which shortens the time of the cell cycle initiation

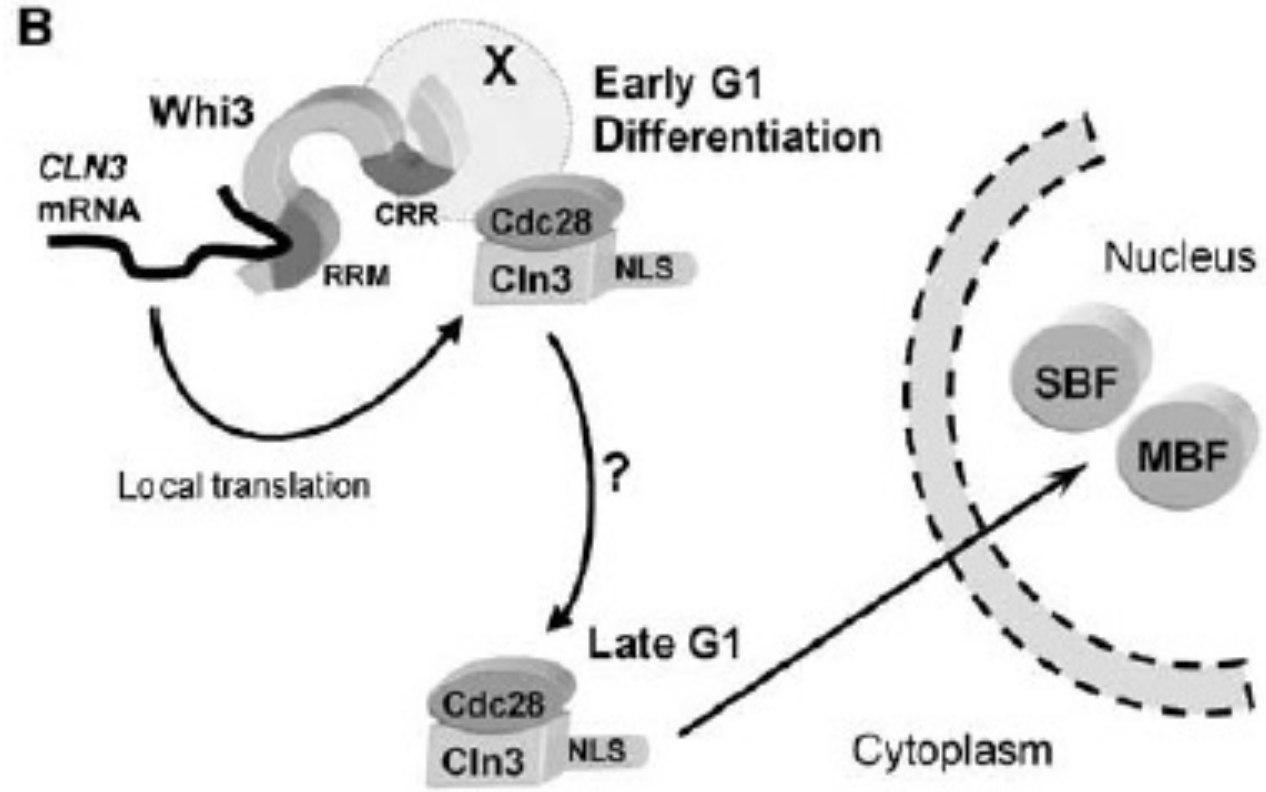
Wang et al., 2004; Columina et al., 2009

# Whi3 retains G1 Cyclin Cln3 in cytoplasm




Wang et al., 2004

# Proposed mechanism for Whi3 sequestration of Cln3




Wang et al., 2004

# Unanswered Questions

- Does Whi3 interact with B cyclins? Which ones? Where? When? Why? How?

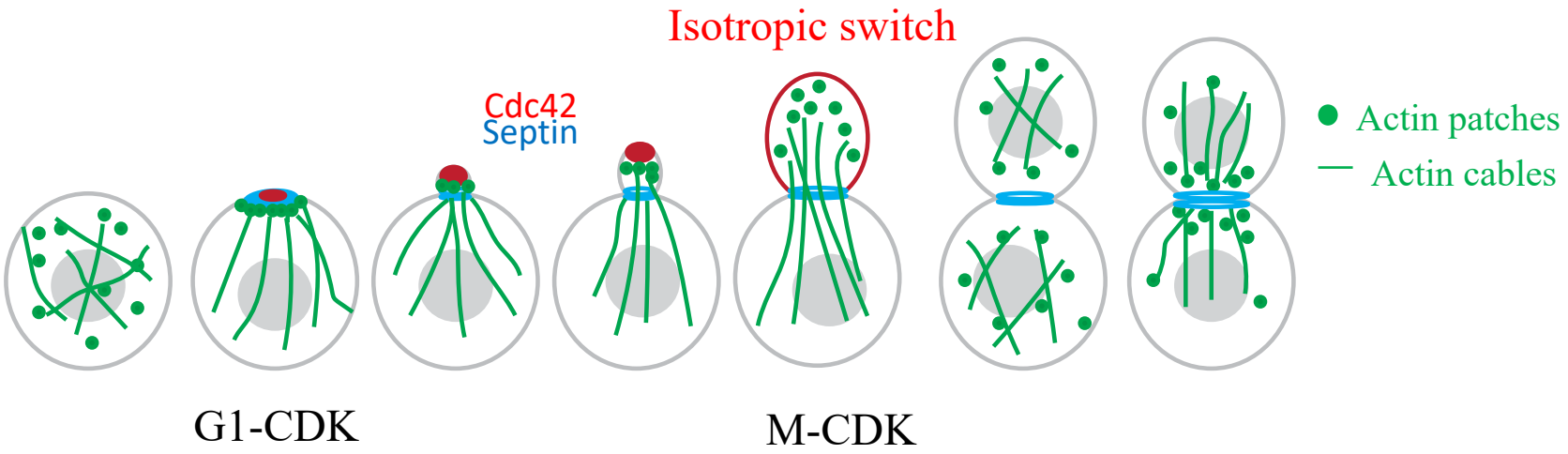
- **Does Whi3 affect cell cycle at the G2/M transition?**

# Initial Strategy

- Delete WHI3 in mutants that affect G2/M transition

- Assess cell cycle timing and M-Cdk activity in double mutants.

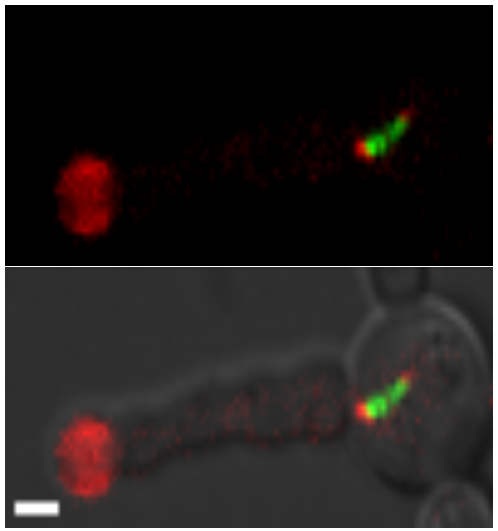
# Low M-Cdk leads to formation of elongated buds



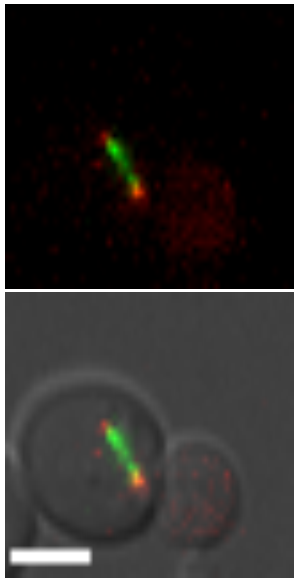

Lew and Reed, 1993

# Deletion of WHI3 Affects M-CDK Activity

*elm1Δ*



*elm1Δwhi3Δ*

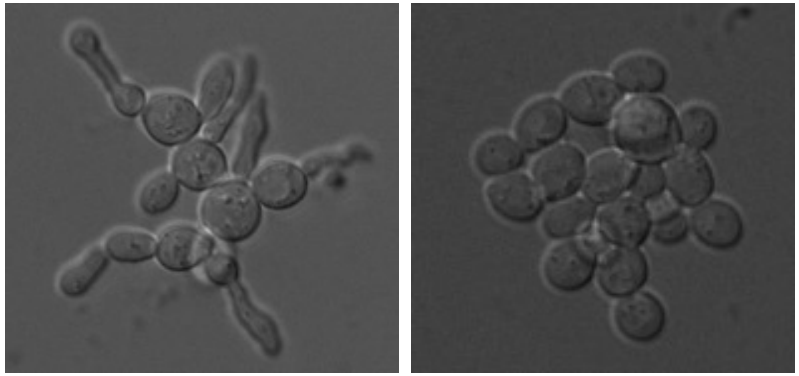


Cdc42
Spc42-mCherry
GFP-Tub1

Deletion of Whi3 promotes isotropic growth of the bud

# Deletion of WHI3 Affects M-CDK Activity

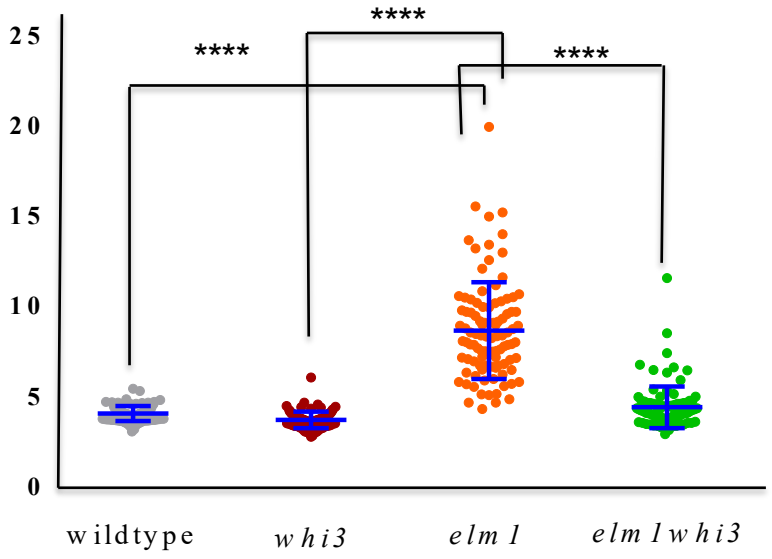
*elm1Δ* *elm1Δwhi3Δ*



Also rescues long buds in *gin4Δ*, *hsl1Δ*, *hsl7Δ*, and *cla4Δ* mutants

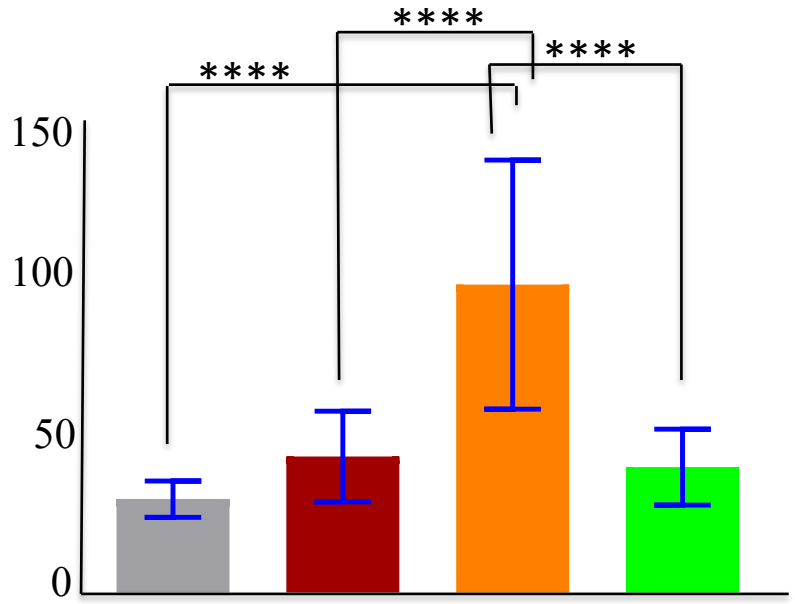
All the mutants are known as negative regulators of M-CDK

# Deletion of WHI3 Affects M-CDK Activity




Deletion of WHI3 rescues long buds in *elm1*

# Deletion of WHI3 Affects M-CDK Activity

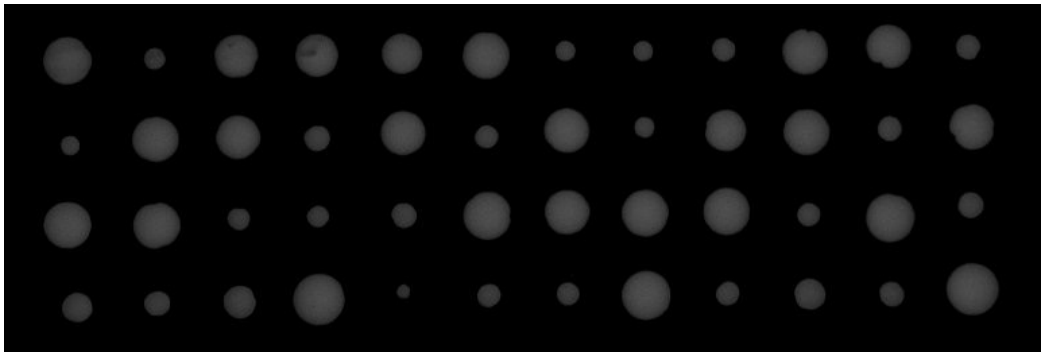



Deletion of WHI3 rescues anaphase delay in *elm1*

Deletion of WHI3 leads to increased M-CDK activity

How ?

# Deletion of WHI3 Affects M-CDK Activity



*elm1swe1whi3* X *swe1whi3*

Small spores are *elm1swe1whi3*

*elm1swe1whi3* cells are large and multinucleate

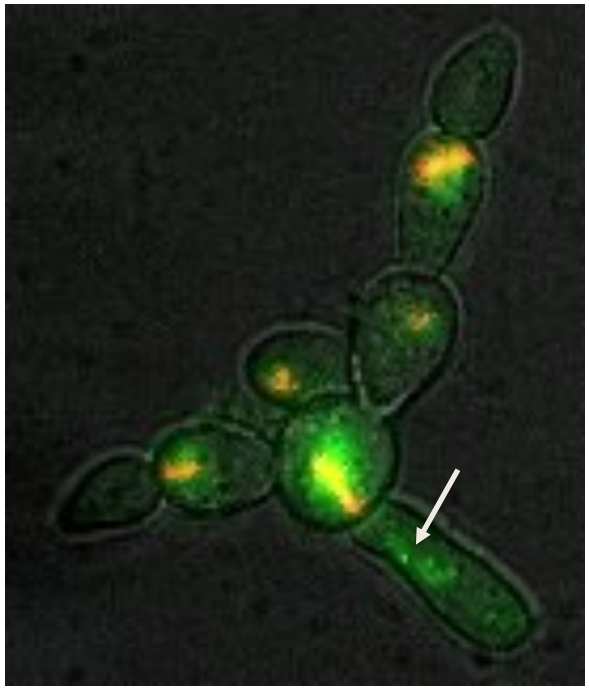
# Low M-CDK Activity affects B cyclin nuclear localization?

wildtype

*whi3*

Clb2-GFP
mCherry-Tub1

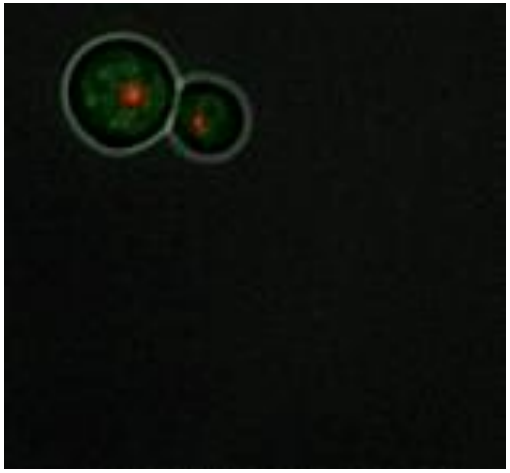
# Low M-CDK Activity affects B cyclin nuclear localization?



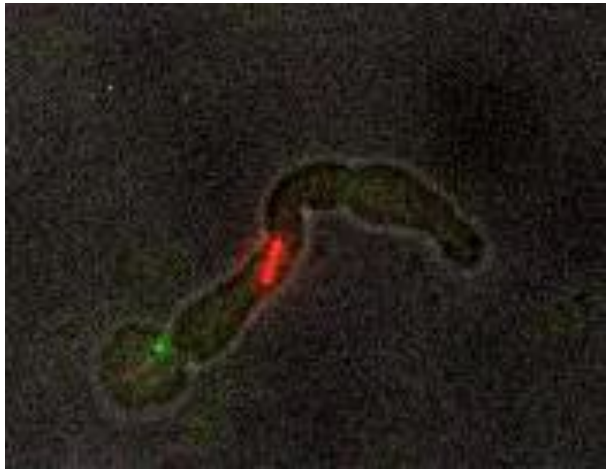
Clb2-GFP
mCherry-Tub1

# Low M-CDK Activity affects B cyclin nuclear localization?

GALpSWE1



GALpSWE1; glucose rescue



Clb2-GFP
mCherry-Tub1

# Future Directions

## Co-locolization of Whi3 and Clb2-GFP in GALpSWE1

Put SWE1 under a galactose inducible promotor

Tag Whi3 with mCherry

Tag Clb2 with GFP

Live Imaging

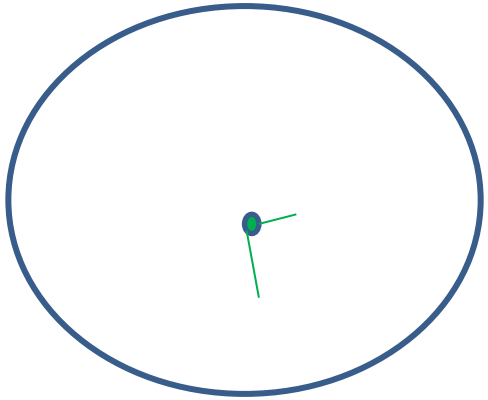
# Future Directions

## Overexpress Whi3 in exponentially growing cells

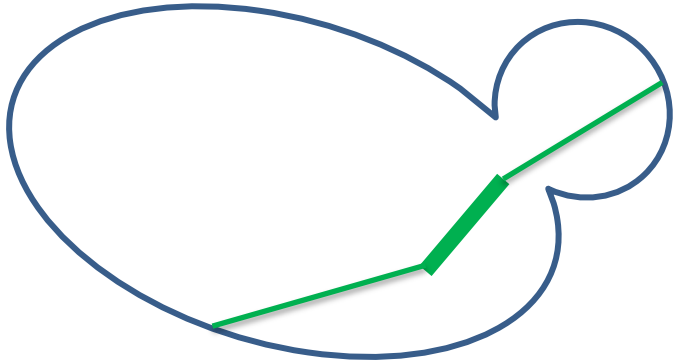
Put WHI3 under a galactose inducible promotor

Add galactose to exponentially growing cells

Monitor cellular outcome



Unbudded cells when galactose is added (Gari et al., 2001)

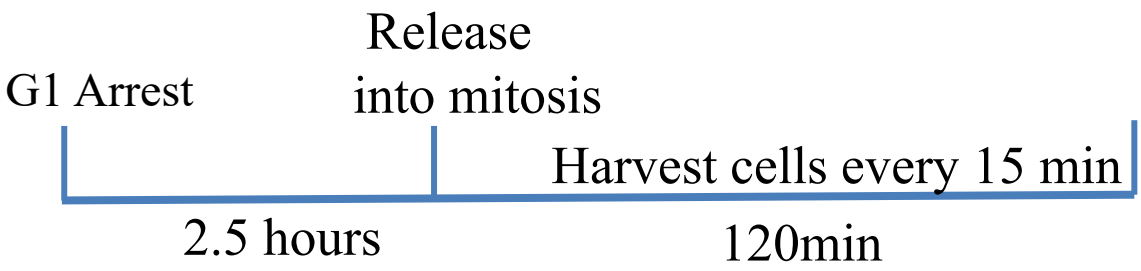


Budded cells when galactose is added
Hypothetical outcome: A metaphase arrest

# Future Directions

Assess the interaction of Whi3 with mitotic CDK complexes

Tag Whi3 with an HA tag and Cdc28 with a myc tag



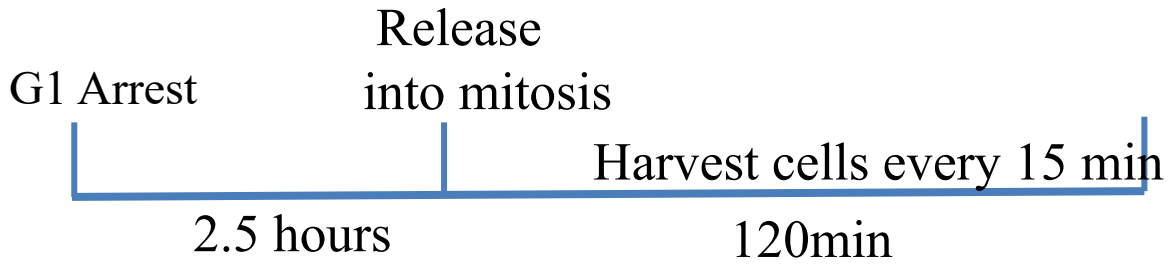

Pull down HA and blot for myc

Interaction with mitotic CDK would be indicated by a myc band at 34kDa between 60min and 120min

Repeat with Whi3-HA and B cyclin -myc

# Future Directions

Assess Swe1-dependent CDK phosphorylation in the absence of WHI3

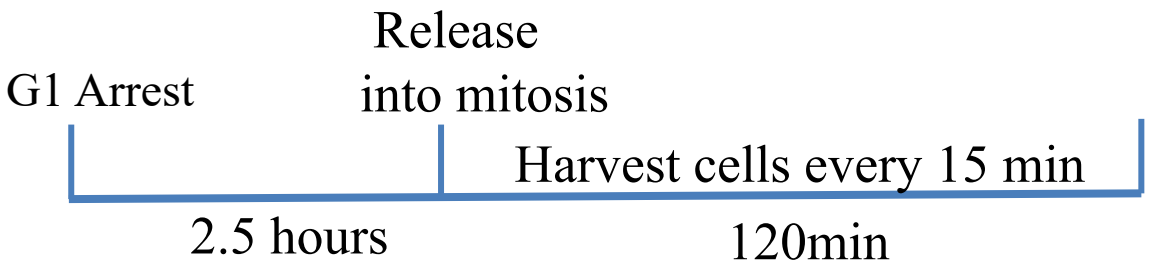



Western blots with anti Cdk Tyr19-P

Lower phosphorylation: increased CDK activity and decreased Swe1 activity

# Future Directions

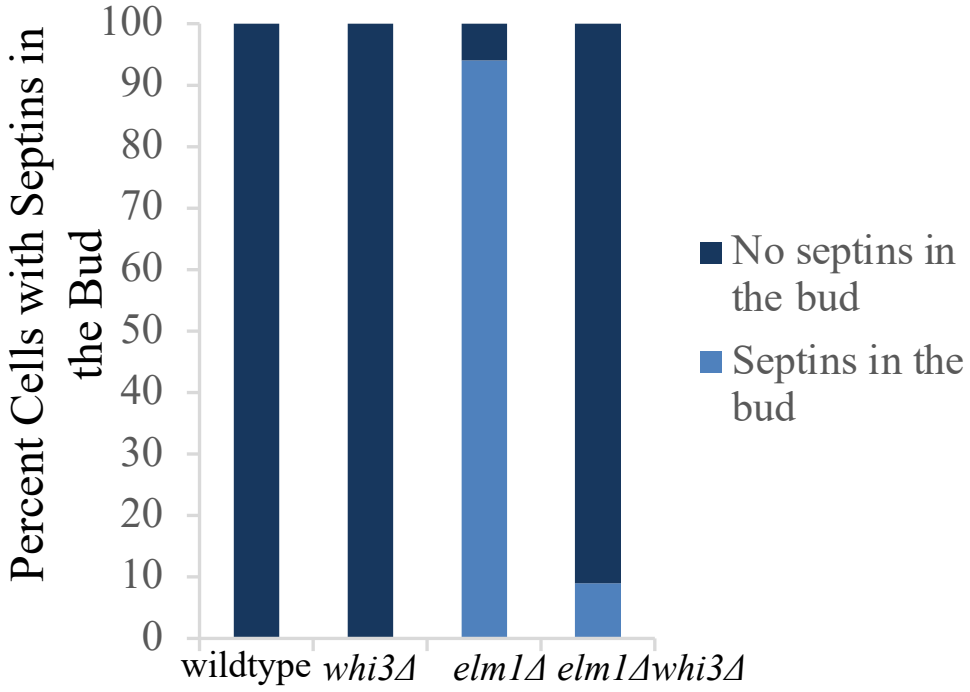
Assess Swe1 levels and phosphorylation in the absence of WHI3




Western blots with anti Swe1

Increased gel-mobility shift: increased phosphorylation
(Swe1 targeted for degradation)

# WHI3 Deletion Rescues Septin Localization Defects in elm1 Mutants




The G2/M delay is elm1 is linked to septin perturbation