WHI3 Regulation of Cyclin-Dependent Kinase Activity in *Saccharomyces cerevisiae*

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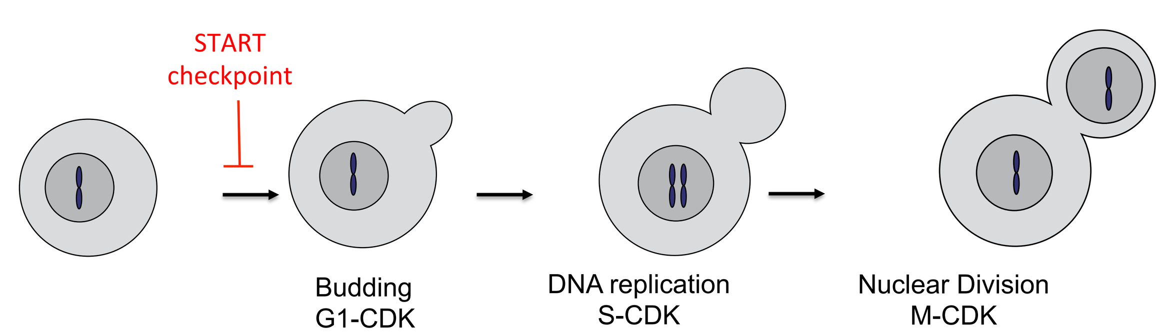
Background and Relevance

There are many diseases associated with malfunctions in the cell cycle. For instance, aneuploidy—when daughter cells have the abnormal number of chromosomes—results from improper cell division. Diseases that arise from chromosomal abnormalities range from Down Syndrome to Turner Syndrome to Patau Syndrome, all extremely debilitating afflictions. Another serious consequence of unregulated cell division is the development of cancers. One of the hallmarks of cancer cells is cell proliferation, which is a result of unregulated cell division. Studying cell cycle regulation in yeast, particularly budding yeast, Saccharomyces cerevisiae, allows for a better understanding of the human cell cycle. Many of the genes studied in my lab are conserved in humans, meaning that those yeast cell proteins also function in human cells.

Mitotic cell division—the type of division in which a mother cell produces two identical daughter cells—is regulated by a protein complex called cyclin-dependent kinase (CDK). This protein complex has been intensely studied by cell biologists, yet there is still much that is unknown about how it is controlled. CDK—which must be active in order for cell division to occur—is regulated by a protein in yeast called Swe1. Swe1 inhibits CDK when the cell is perturbed, thereby halting cell division. One way that the cell can be perturbed is by the deletion of the protein ELM1. When present, ELM1 regulates the cytoskeleton of the cell. However, when ELM1 is deleted, Swe1 is activated, which results in a delayed cell division and irregularly long buds. These long buds are also the result of septin perturbation. When functioning properly, septin proteins form a ring around the bud neck. We recently found that the deletion of another protein called WHI3 rescues the ELM1 deletion-induced cell division delay and long buds.

By utilizing fluorescent microscopy, we have been able to visualize cells lacking both WHI3 and ELM1. Our hypothesis is that WHI3 is somehow involved in regulation of CDK. In order to test this hypothesis, we are working on experiments to see what happens to CDK and other regulators of cell division when ELM1 is deleted, WHI3 is deleted, and when both WHI3 and ELM1 are deleted. I will be conducting other molecular biology experiments to measure the level of CDK activity in both the cytoplasm and the nucleus. My work will help to elucidate another mechanism by which CDK is regulated, which will contribute to our overall understanding of proper progression through the cell cycle.

START: Irreversible Cell Cycle Entry



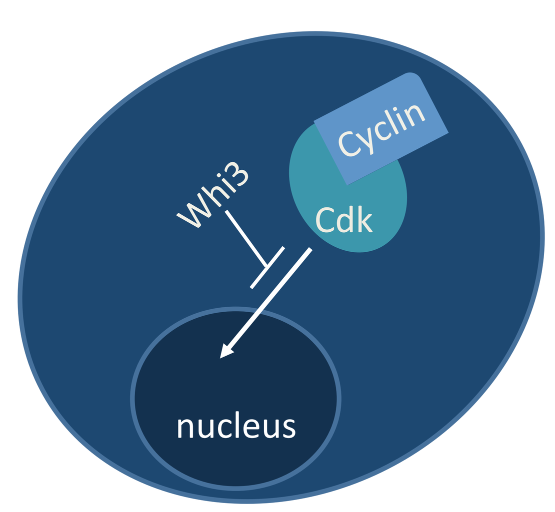
START is governed by nutrient availability, cell size, and Cdk activity.

Swe1 Inhibits M-CDK to Ensure Timely Mitotic Entry

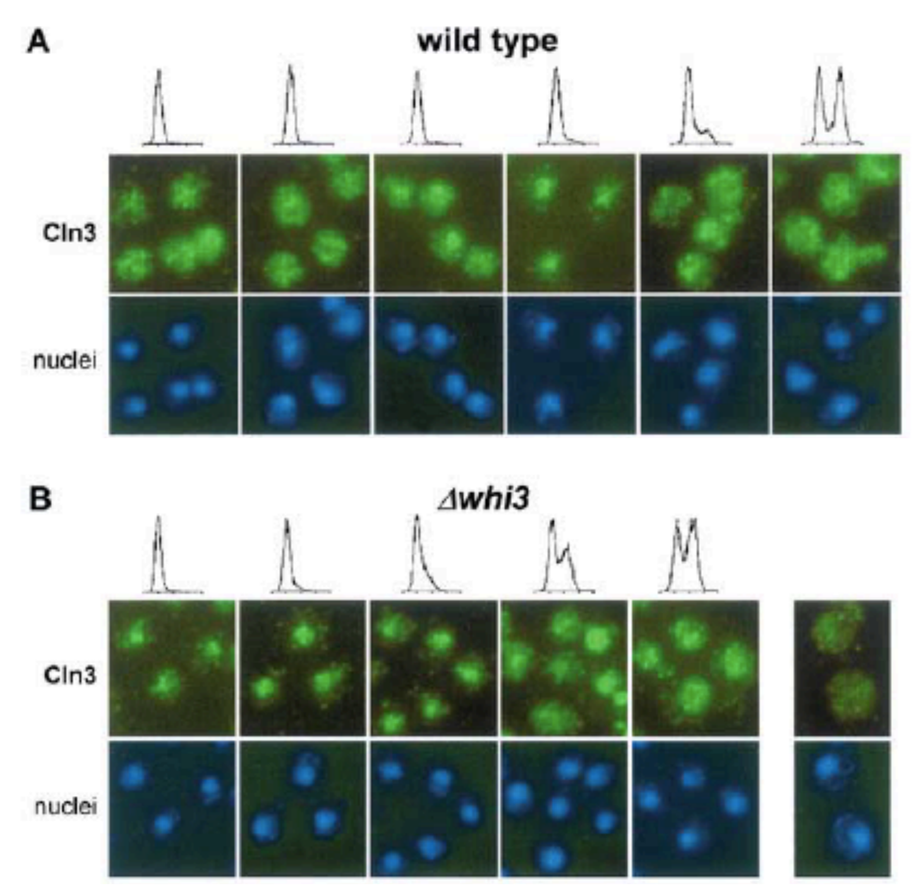
Whi3 is Involved in Cdk Regulation

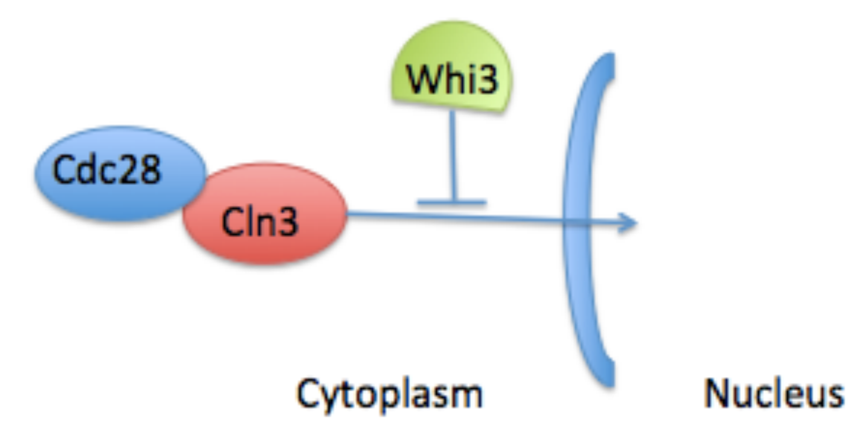
Relevant Whi3 functions:

- Cytoplasmic retention factor for Cdk1 and associated cyclins
- Both RNA and protein binding
- WHI3 deletion forces the cells through START, which shortens the time of the cell cycle initiation and yields small cells
- When Cdk is maintained in the cytoplasm, Whi3 is inactive



Whi3 Retains G1 Cyclin Cln3 in the Cytoplasm





Wang et al., 2004

Unanswered Question:

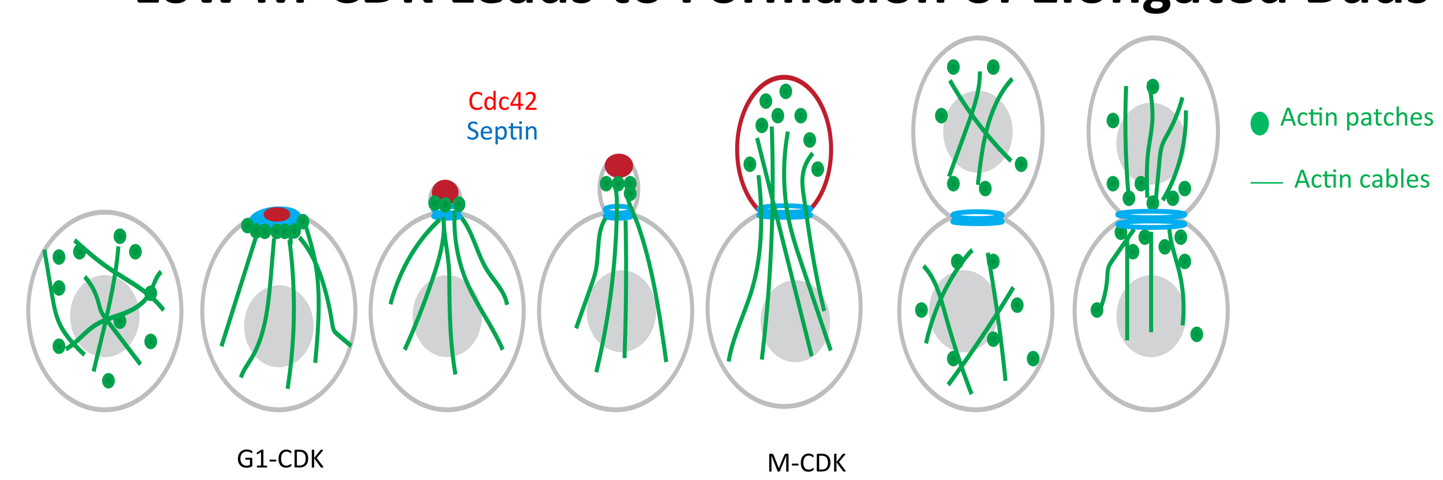
Does Whi3 affect the activity of M-CDK?

Strategy:

Delete WHI3 in mutants that affect M-CDK activity (i.e. *elm1Δ*).

Assess cell cycle timing and M-CDK activity in double mutants (i.e. *elm1Δwhi3Δ*).

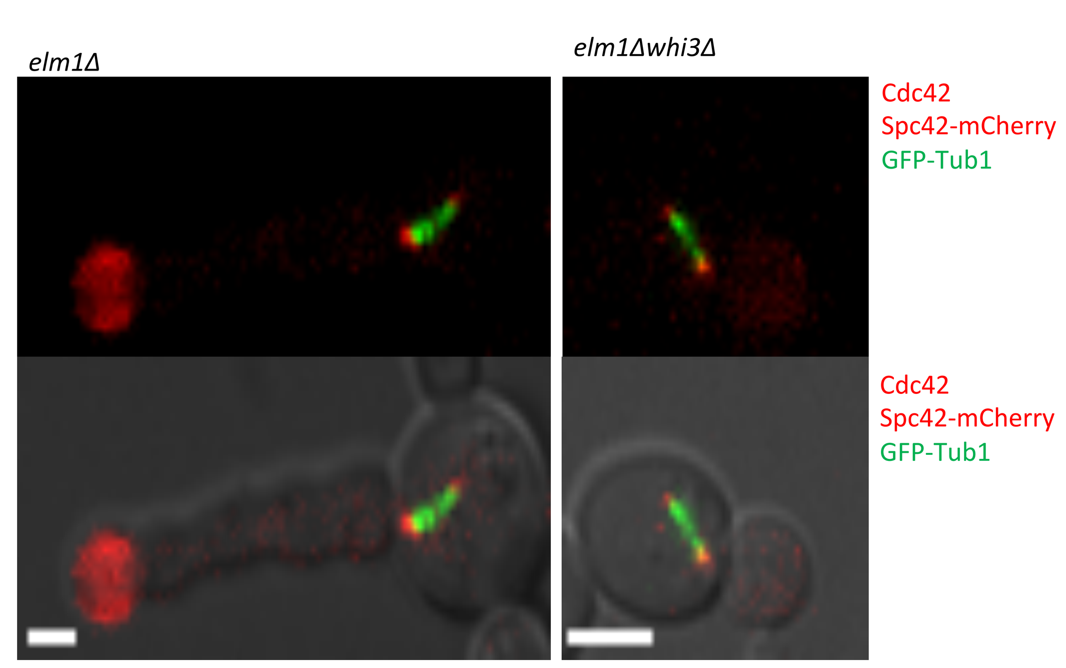
Low M-CDK Leads to Formation of Elongated Buds



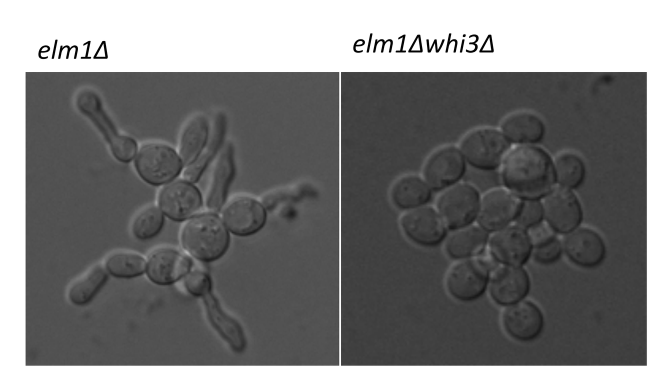
Lew and Reed, 1993

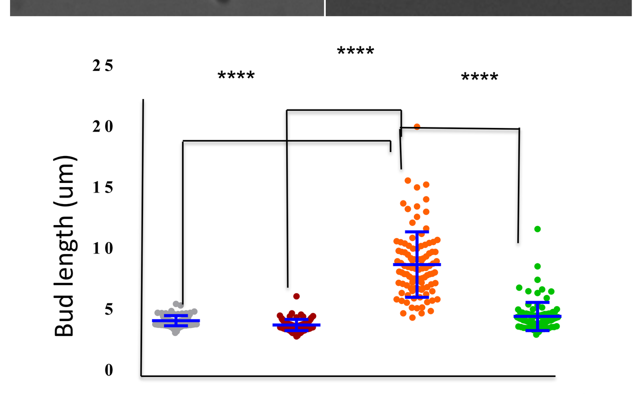
Deletion of WHI3 Affects M-CDK Activity

Deletion of WHI3 Promotes Isotropic Growth of the Bud



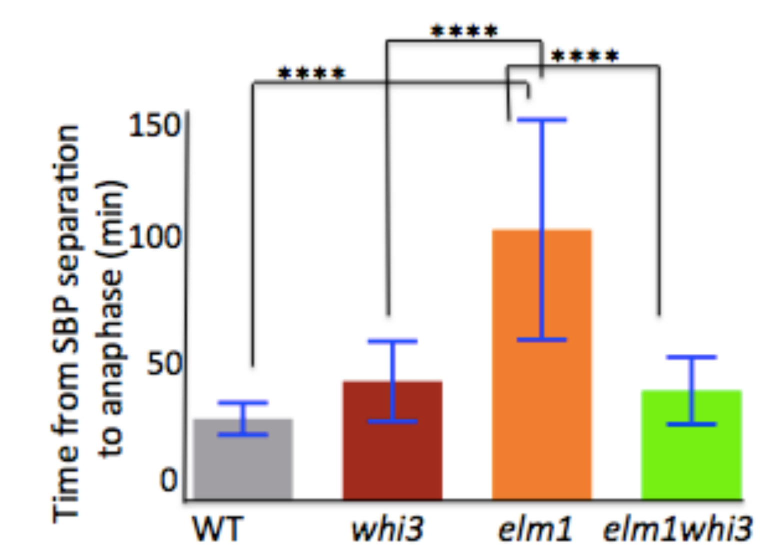
Deletion of Whi3 Rescues Long Buds in *elm1Δ*



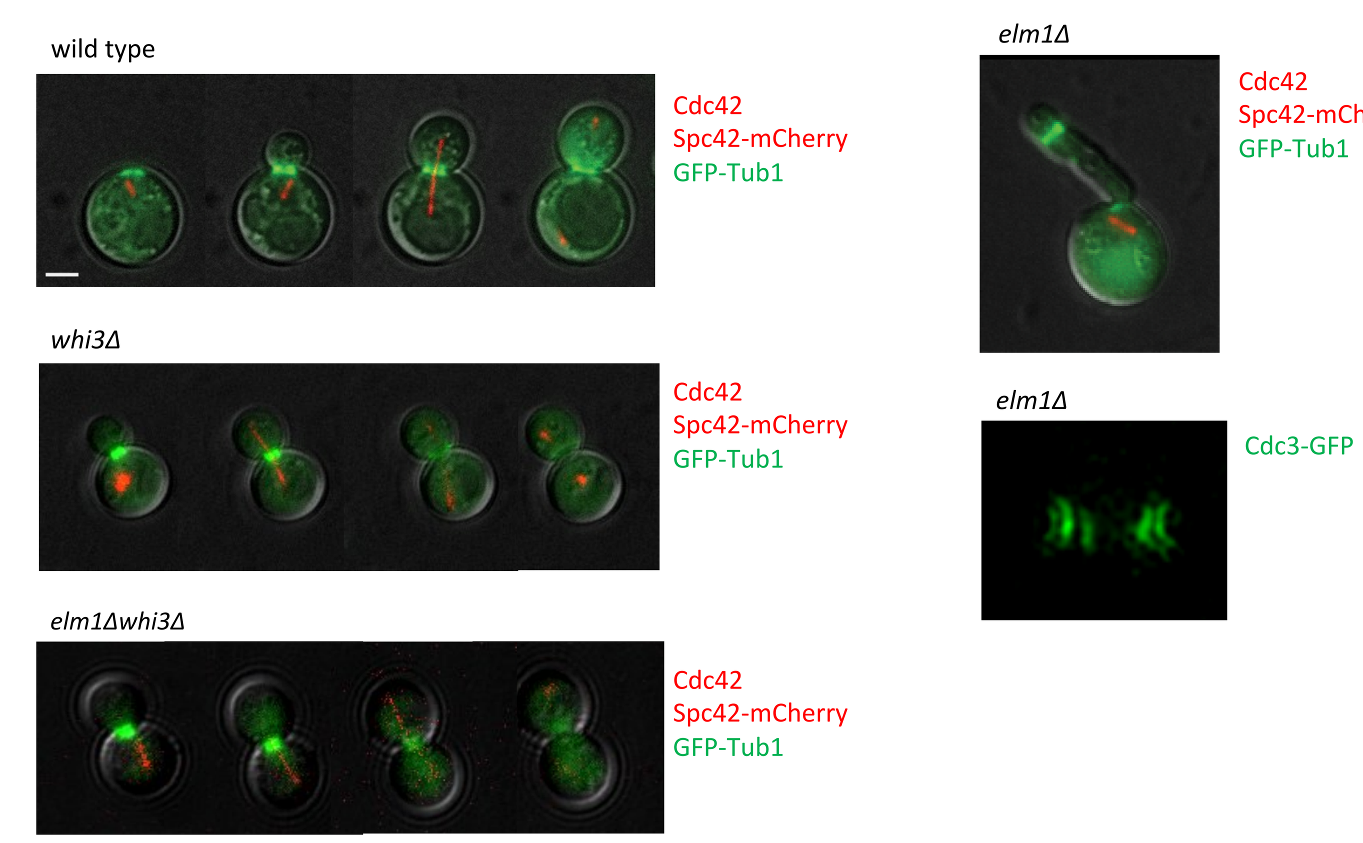


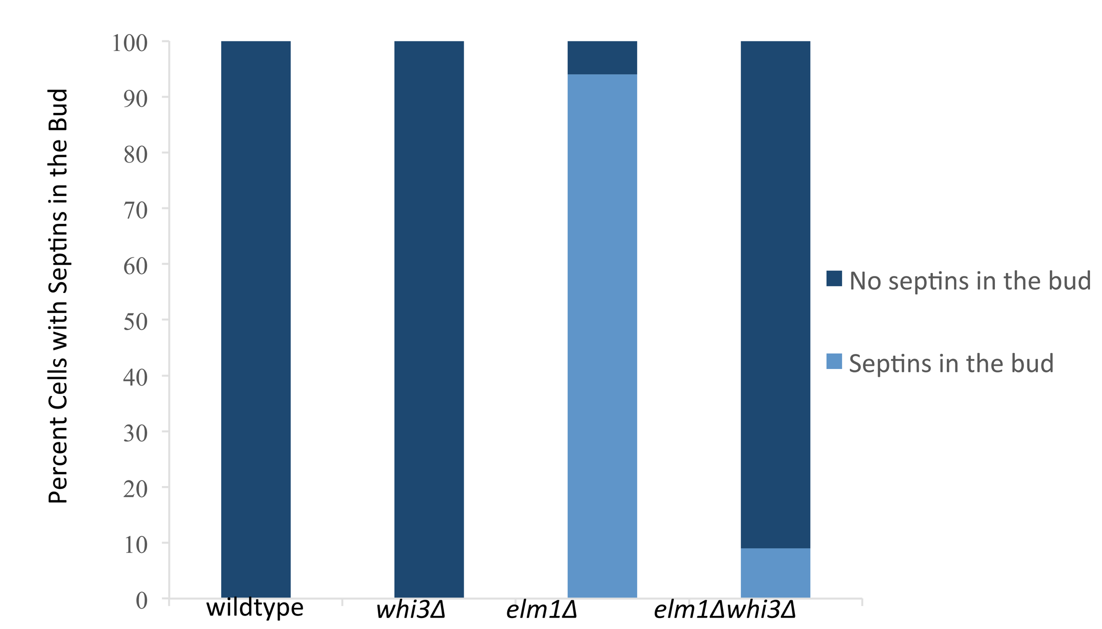
WHI3 deletion rescues long buds in *gin4Δ*, *hsl1Δ*, *hsl7Δ*, and *cla4Δ* mutants, negative regulators of M-CDK

Deletion of WHI3 rescues anaphase delay in *elm1*



Deletion of WHI3 Rescues Septin Perturbations





Future Directions

- Tag Clb2, a B cyclin involved in the mitotic pathway, with GFP and observe its activity.
- Measure fluorescence levels of Clb2 in the nucleus and the cytoplasm to determine where and how much it is present throughout cell division.
- Overexpress Swe1 while tagging Whi3 to determine its co-localization with Clb2.
- Place Whi3 under control of a galactose inducible promoter, thereby overexpressing it when placed in galactose media, and monitor the cellular outcome and Clb2 localization. We hypothesize that this will result in metaphase arrest because M-CDK will be inhibited by Whi3.

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