Cell Size Regulation for *S. pombe*

A Major Qualifying Project Report:
Submitted to the Faculty of
WORCESTER POLYTECHNIC INSTITUTE
In partial fulfillment of the requirements for the
Degree of Bachelor of Science
by

Lauren Guerrero
Biochemistry

Table of Contents

Abstract

Cell size control is required to maintain cells within a viable size range making it an essential cellular process. Cell-cycle regulatory proteins, such as Cdc13 and Cdc25, are expressed at cell size-dependent concentrations. This study extended observations of expressions of Cdc25 by developing a comparison model that demonstrates the accumulation of a control protein, GFP, which expresses time-dependency behavior. This will allow the exploration of the mechanisms of time-dependent expression and determine that Cdc25 is in fact a size-dependent protein.

Acknowledgments

I would like to thank Dr. Nick Rhind for providing me with this opportunity to perform my MQP at UMass Medical School. Thank you for being a supportive mentor and instilling trust in me to complete this project.

Thank you to Dr. Destin Heilman for his guidance and assistance throughout this project.

I thank Makoto Ohira, Scott Jiusto, Samirul Bashir, Wendy Kama, and Silvana Reid for their help and for creating a supportive and productive environment.

Introduction

The goal of this project is to obtain a further understanding of how cells regulate their size. Cell size control is required to maintain cells within a viable size range making it an essential cellular process. It has been shown that, in fission yeast, two cell-cycle regulatory proteins, Cdc13 and Cdc25, are expressed at cell size-dependent concentrations. Our working model is that these activators are expressed at size-dependent concentrations, such that small cells have low levels and thus cannot enter mitosis and larger cells have progressively higher levels, which allow for mitosis. A key test of this model is to show that the accumulation of Cdc13 and Cdc25 is in fact size-dependent and not just time-dependent. Size-dependent proteins have a directly proportional relationship between concentration and cell size. Time-dependent protein concentrations start at the same point in relation to time, making them size-independent. Our preliminary data from other students in the Rhind Lab suggest that Cdc13 and Cdc25 are size-dependent, but we do not have the proper controls to rigorously interpret our data. I will extend our observation of size-dependent expressions of Cdc13 and Cdc25, assayed as fluorescent protein fusions, to compare them to the expression of a control protein, GFP, which is expected to be expressed in a time-dependent, not size-dependent, manner. These experiments will allow me to explore the mechanisms of size-dependent expression and to determine if that expression has an important role in cell size regulation.

Background

Cell Size Importance for Unicellular Organisms

Unicellular organisms, such as budding and fission yeast, don't have the same survival rate as multicellular organisms due to their sensitivity to their surrounding environment. If placed in critical conditions, their cell cycle is compromised and must be changed in order to adapt to their new environment [11]. Protein concentration homeostasis is essential for cell function and survival because proteins are involved in all major cell processes, such as cell metabolism, cell separation, and changes in the cell's environment. The cell size for these organisms is crucial for the transport of particles and adapting to their environment. Moreover, these cells must grow at a certain size and rate depending on which environment they are under. However, when their cell cycle is compromised, cell size remains unchanged due to their ability to be aware of their cell size and how to regulate it [11]. This report will explore the cell size regulation process for these organisms.

Model Species

In this report, the fission yeast *Schizosaccharomyces pombe* was used. For this species, cell size regulation occurs at the G2 to Mitosis step. Also, there is an additional point of regulation which is at the G1-S step [9]. This report is focused on the G2 to Mitosis step during the cell cycle of *S. pombe*. *S. pombe* is a unicellular eukaryotic organism that has a rod shape that is typically 8 to 14 μm in length [11]. They reproduce by fission, which happens by cells getting longer and forming a division in the middle called the septum. At this septum, cells divide and create two new cells as you can see in Figure 1. *S. pombe* resembles the typical eukaryotic cell cycle and has a defined shape and features, making it an excellent model organism to study processes like the mitotic and meiotic cell cycle [11].

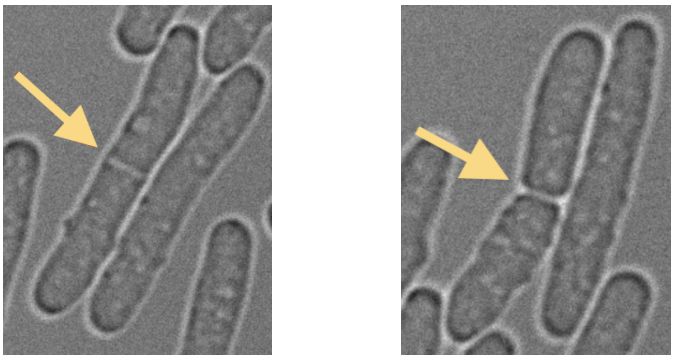

Figure 1. Septated cell dividing into two cells

This figure demonstrates an *S. pombe* cell undergoing septation. The septation line is depicted by the arrow in the left image. In the right image, you can observe the division caused by the septation process.

Canada albicans, a fungal pathogen, is also a great example of why understanding cell size regulation is so important [3]. It is also speculated that they exhibit the same role of Cdc25 in their cell regulation process. When there are low levels of Cdc25 in these cells, their shape transitions to an elongated filament, which causes them to go under cell cycle arrest, prohibiting cell growth development [3]. Furthermore, over-expression of Cdc25 also contributed to abnormal cell separation. These effects are similar to the effect S. pombe undergoes with changes in Cdc25 levels, playing an important role when it comes to a signal for the mitotic exit, cytokinesis, and cell separation [3].

Cell Size Regulation Process for *S. pombe*

The fission yeast cell cycle is seen in Figure 2. G1, S, and M phases are short while G2 is long, which is ~70% of the total time it takes to complete the cycle [10]. This is because size control operates in G2 in wild-type cells. Since septation requires about 20 minutes to appear, cells are not divided immediately [11]. During the septation process, the cells have two nuclei and are in a very short G1 phase, after which DNA replication occurs. The main cell size

regulation point in the *S. pombe* cell cycle is the G2 phase [10]. When cells reach a certain size at the G2 phase, the Cdc2 protein kinase is activated by Cdc13 cyclin binding and the Cdc25 phosphatase removal of the inhibitory phosphate from 15 tyrosine in Cdc2 [7]. This causes mitosis to occur. Once the cell with two nuclei divides, the cell will go into the S phase where DNA replication occurs. If the cell's environment is not well-suited for normal growth, the G1 phase will become longer and its growth rate will become slower [10].

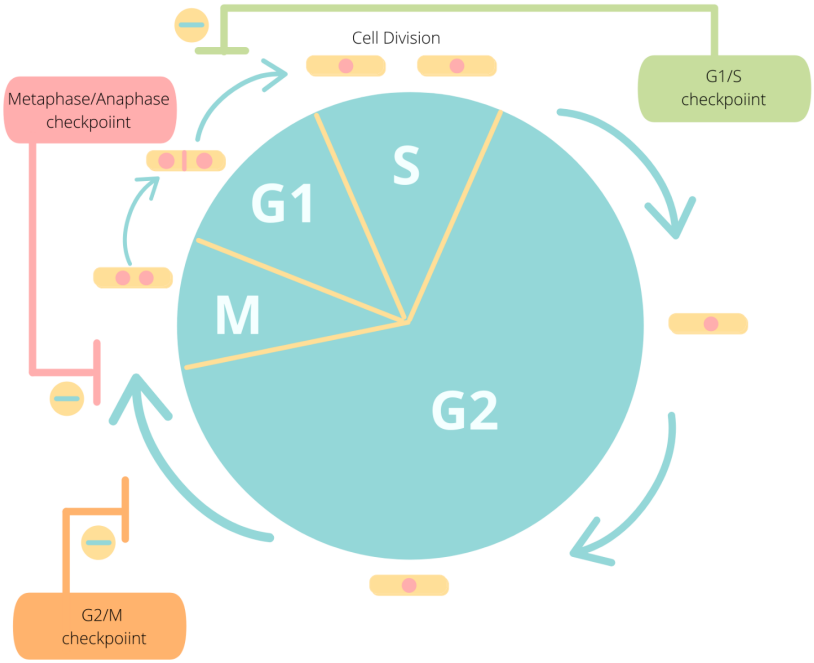

Figure 2. Cell Cycle for Fission Yeast Wild-type

Figure 2 illustrates the cell cycle for fission yeast. G2 phase is the longest and most essential process for size control. If cells reach a critical size, Cdc2 protein kinase is activated by cyclin binding, and the Cdc25 phosphatase removal of the inhibitory phosphate from Tyr15 of Cdc2, which allows the cell to enter mitosis. After nuclear division, the cell enters the S phase where it is completed by the time cell is completely divided. This causes G2 to be the longest step if cells have the appropriate conditions.

Shorter events are in M, G1, and S phases, with S occurring coincident with cytokinesis. If there are critical conditions, a size control step occurs at the G1 phase, causing the phase to be longer and the growth rate decreases.

Cdc2 is the main protein that regulates the G2 to Mitosis transition in *S. pombe*, which is the main cell size regulation point [4]. This protein is a cyclin-dependent kinase (CDK) and is inactivated by phosphorylation of its 15 tyrosine. This kinase makes a stable complex with the cyclin Cdc13 when it is active. The regulation of Cdc2 phosphorylation is facilitated by a pair of proteins; Cdc25 and Wee1. Cdc25 is a tyrosine phosphatase that dephosphorylates the tyrosine 15 from Cdc2. This dephosphorylation activates Cdc2 and Cdc13 [4]. Wee1, which remains constant during G2, does the opposite of Cdc25 and phosphorylates the tyrosine 15 from Cdc2, allowing the cell cycle to arrest at G2. Cdc13-Cdc2 phosphorylation activates Cdc25 and inhibits Wee1, which creates a feedback control. This design allows mitosis to occur when Cdc25 activity reaches a level that overcomes Wee1's activity [4]. This process is summarized in Figure 3, where we can observe that Wee1 levels are constant while Cdc25 and Cdc13 levels are increasing with concentrations.

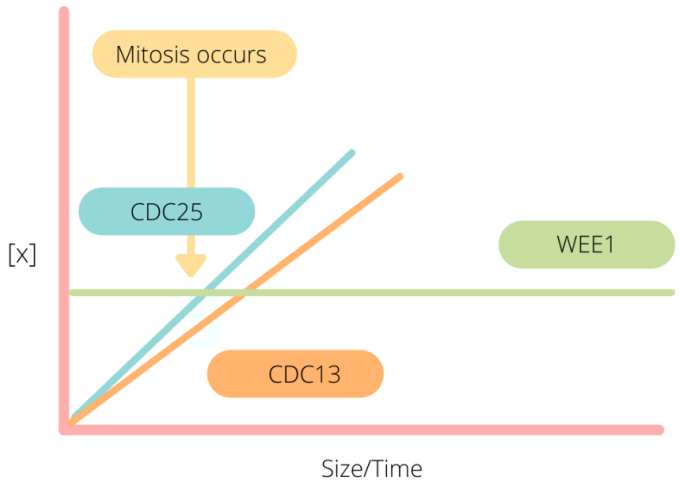

Figure 3. Cdc25 and Cdc13 as Size-dependent proteins

Figure 3 depicts the activity of Cdc25 and Cdc13 as size-dependent proteins. If Wee1 levels are doubled, cell size will increase because higher concentration of protein will inhibit mitosis from occurring.

Since *S. pombe* is rod-shaped it grows by tip extension, it divides through medial fission [8]. Through this process, microtubules appear at the cell tips in sites of polarized cell growth and deliver cell polarity factors. They also position themselves at the cell nucleus in the middle, which mark sites of cell division. Thus, the microtubule cytoskeleton is a main component for cell division for *S. pombe* by producing the pushing forces for nuclear positioning and division plane placement [8]. Moreover, it is also involved in initiating sites of polarized cell growth and thus determining cell shape. The actin cytoskeleton, however, directs cell growth independently of microtubules [8].

Size-dependent vs. Time-dependent

Cell size is regulated by a balance of Cdc25 and Wee1 levels. As mentioned before, Wee1 remains constant, while Cdc25's concentration increases until it reaches Wee1 levels, which activates mitosis. This raised the question of whether Cdc25's regulation depends on the size of the cell or the time it takes to complete the cell cycle. Based on previous studies from the Rhind Lab, Cdc25 was determined to be a sizer protein [4]. A sizer is a size-dependent protein, which has a concentration that is directly proportional to the size of the cell. For timer proteins, protein concentrations have a directly proportional relationship with time [2]. Therefore, timers' concentration goes up independently of size, making them size-independent [2]. As seen in Figure 4, the size-independent protein's initial concentration starts the same, while size-dependent proteins start at increasing concentrations as size increases.

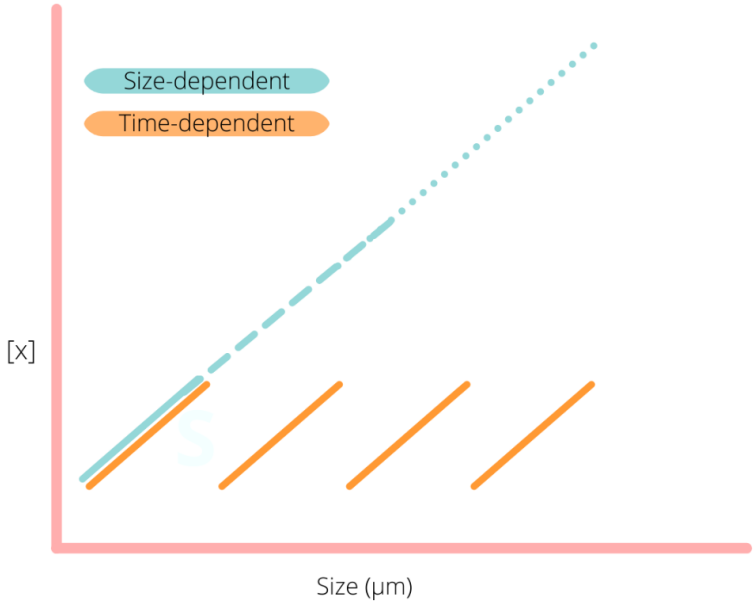

Figure 4. Size-dependent vs. Time dependent Protein Behavior

Figure 4 illustrates the behavior of size-dependent and time-dependent proteins (size-independent) proteins in relation to concentration and cell size. Four sets of cells are shown. The first set in blue exhibit size-dependent protein behavior where size increases throughout different populations cells, whereas in time-dependent protein cells shown in orange, these cells start at the same point and increases with time.

Question/Purpose

We previously asked the question of whether Cdc25 is a sizer or timer protein. Previous studies determined Cdc25 is a size-dependent protein, making it responsible for the cell size regulation of *S. pombe*. The experiments conducted in this report were analyzed to have control protein, GFP, demonstrate time-dependency.

Experimental Approach

A ZEV:GFP plasmid was created and integrated in the genome at his7 in the strain utilized, yFS1049. The ZEV transcriptional regulation system, which allows us to overexpress Wee1 to control cell length, was used to regulate cell size in these experiments. Cell length and concentration levels were measured by fluorescence microscopy using GFP.

To confirm GFP exhibits time-dependent behavior, a Wee1 temperature-sensitive system was used, which allows us to over-express Wee1 in a similar manner to the ZEV system. However, this strain contains the *Adh1:wee1-50* mutant, which is a temperature-dependent mutant that has a single mutation; its glycine at position 850 was substituted by glutamic acid [4]. This mutation allows for Wee1 to be regulated at temperatures of 25˚ C-35˚C. At 25˚C, the over-expressed Wee1 is active and inhibits mitosis. At 35˚C, Wee1 is inactive, enabling cells to divide at small sizes. By utilizing this system, Wee1-50 activity will be regulated with higher temperatures, making Wee1 less functional, allowing us to regulate cell size [4]. This system will allow the regulation of Wee1, allowing us to control cell size to confirm GFP expresses time-dependent behavior.

Materials and Methods

Strain Used

yFS1049: h- leu1-32::pFS461(leu1 adh1pr Z3EV) ura4-D18 his7-366::pFS462(his7 ZEV:GFP-NLS) wee1::pWAU-50 (ura3 adh1:wee1-50)

Preparation of Cultures

Cultures were created with a 10 mL tube filled with 5 mLs of YES media and a small collection of cells from the plate where the strain is growing. This was left overnight (8-9 hours) at either 32˚C, 30˚C, or 28˚C in a shaking incubator and was scaled up to a 50 mL culture with

an optical density of 0.5. Then, it got scaled up to a 500 mL culture to have an OD range of 0.4-1. Cells were then elutriated to collect 25 mLs of cells of approximately the same size.

Beta-estradiol

To turn on the strain's GFP protein, beta-estradiol was added to cultures after elutriation to create a 0.01% solution of beta-estradiol while the experiment's time course occurred. This system allows for a large dynamic range and linear dose response, based on the β-estradiol-regulated function of the human estrogen receptor, for use in *S. pombe* [6]. We demonstrate the utility of this system by regulating the mitotic inhibitor Wee1 to create a strain in which cell size is regulated by β-estradiol concentration. This promoter system will be of great utility for experimentally regulating gene expression in fission yeast [6].

Fixation

To arrest cells for analysis, a fixation method was utilized. Formaldehyde fixation was done by time and percentage titration. 3 mLs of the 25 mL solution after elutriation were extracted at each one-hour time point to make a 3.7% formaldehyde solution. They were left incubated at room temperature for ten minutes in a 3 mL culture tube. Then, cells were spun down and collected, and washed with water.

Temperature-dependent strain

The strain used to do a behavioral analysis regulated by the temperature, using the temperature-sensitive Wee1 protein. yFS1049 strain was grown in YES media overnight at 32˚C, 30˚C, and 28˚C, to mid-log phase (0.2-1 OD). Then, images of each temperature incubation associated with size were taken using the 60X immersion oil objective, at the Delta vision deconvolution fluorescence microscope. Images were taken from fixed cells with 3.7%

formaldehyde at room temperature with an incubation time of 10 minutes. Quantification was done from whole cells using PombeY program in MatLab.

MatLab PombeY

A program designed to measure the cell's size was formulated by Makoto Ohira. Images taken at the microscope were saved as .dv files which were then analyzed in the program, which would analyze different measurements according to the file's different channels that were seen in the microscope. The different measures include the length of the cell, the FITC (green channel) information, the shape of the cell was encircled taking the mean of fluorescence of this shape, secondly, the same shape is taken out of the cell to make the blank green channel (FITC) fluorescence. All this data is converted to generate graphs of relative fluorescence versus cell length to determine the relationship between GFP's concentration with cell size or time.

SCOPE (Sassetti DeltaVision WideField Deconvolution Fluorescence Microscope)

The Deltavision CoreDV Widefield Deconvolution System is a high-resolution widefield microscope for acquiring images of live or fixed samples [1]. It is constructed on on an Olympus IX71 inverted microscope with an LED transmitted light source for differential interference contrast and a 7-color solid-state illumination unit that allows for the excitation of fluorophores across the visible spectrum. In addition, it has polychronic beam splitters that are used to optimize imaging for Blue-Green-Red-FarRed fluorophore combinations in fixed cells or CFP/YFP and GFP/RFP combinations of expressed proteins in living cells. The system also has a Nikon CoolPix HQ cooled CCD camera to obtain images. For these experiments, the 60X immersion oil objective was utilized to view fixed samples [1].

Results

Beta Estradiol

A Beta-estradiol test was performed to observe whether GFP's fluorescent properties would turn on. As observed in Figure 5, the above images represent cells that were not treated with beta estradiol. Moreover, these cells experienced little to no fluorescent signal compared to the cells that had the addition beta estradiol (seen in the below images). This indicated that beta-estradiol indeed turned on the GFP protein, which allowed the continuation of the experiment for the size-dependency or time-dependency analysis of GFP.

Formaldehyde Fixation Test

A fixation titration was performed to analyze if cells would arrest their cell cycle in order to properly gather cells at their appropriate phase at different time points over a time course of four hours, where cells were obtained in each one hour increments. In Figure 6, this test is demonstrated. It demonstrates that after formaldehyde fixation, the fluorescent signal was not disturbed and cell growth ceased.

Time-Dependency Analysis

To examine the behavior of the control protein, GFP, an analysis at different temperatures was done to determine if it expresses in a size-dependent or time-dependent manner. By administering beta estradiol to turn on GFP and formaldehyde to arrest cells, cells were then able to be viewed under a high-resolution widefield microscope to collect images that were then turned into .dv files to obtain relative fluorescence and cell size measurements from each cell by utilizing a MatLab program, PombeY. With the collection of these measurements, graphs of relative fluorescence and cell length were constructed. Figures 8-10 represent these graphs. To

have a variety of cell size, cultures were grown overnight at different temperatures using a temperature-dependent strain developed by the ZEV system.

The largest size was obtained by growing the cultures at 28°C overnight. In figure 8, we can observe cells start out with a cell length of 10-15 micrometers, primarily around 15 micrometers, at the zero-hour timepoints and a relative fluorescence of 150-200. At the one-hour timepoint, cell length then increased to 15-20 micrometers at around the same relative fluorescence. For the second hour time point, cell length and relative fluorescence both increased to 20-25 micrometer and 200-250, respectively. For the three-hour timepoint, cell size increased up to 45 micrometers, but with its largest population being in the 25-30 micrometers. Its relative fluorescence stayed approximately the same as the two-hour time point. The four-hour timepoint had the largest values for relative fluorescence with a range of 250-350 and the majority of cells were in the 30-35 micrometer range for cell length.

Growing a culture at 30°C overnight allowed for a slightly smaller size for analysis. Compared to Figure 8, Figure 9 demonstrates cell size of the zero-hour time point tended to start more around the 11-13 micrometers cell size with a similar relative fluorescence to the 28°C's zero-hour timepoint of 150-200. The one-hour-time point had a larger cell size with a range of 14-20 with the same relative fluorescence as the zero-hour timepoint. At the second-hour timepoint, cells were usually at a cell length of 20-22 micrometers with a larger relative fluorescence of 200-300. The third hour timepoint experienced the same increase in both cell length and relative fluorescence. Its cells usually started at around 23 micrometers with the largest relative fluorescence range of 300-400. In the four-hour timepoint, the relative fluorescence range decreased to 350-200, but had an increase in cell length, with the majority of cells being at around 30 micrometers.

To obtain the smallest size for analysis, cultures were grown overnight at 32°C overnight seen in Figure 10. The zero-hour timepoint starts at 5 and ending at around 12 micrometers with cells usually having a length of approximately 8 or 9 micrometers. This timepoint also had similar relative fluorescence with a range of 150-200. At the one-hour timepoint, cell length then increased to 10-15, but cells tended to have a length of 11 or 12 micrometers with a slightly larger relative fluorescence of 180-220. Its cell length ranged from 13-16 micrometers and relative fluorescence ranged from 200-250. For the three-hour timepoint, cell length increased up to 20 micrometers with the relative fluorescence ranging from 250-300. Finally, the four-hour timepoint demonstrated the same increase of cell length and relative fluorescence. Cell length for this timepoint ranged from 17-25 micrometers with a relative fluorescence ranging from 250-320.

After collecting data points from each temperature, average intensities were calculated to assess whether GFP was exhibiting size-dependency or time-dependency behavior. Background fluorescence was removed from each temperature's relative fluorescence measurements. In Figure 11, all three temperatures' average intensities were plotted against cell length with all three demonstrating a linear relationship in their trendline. The 28°C trendline started at around 15 micrometers, having the largest cell length out of all three temperatures and having a relative fluorescence range of 0-70. 30 °C started at around 13 micrometers and its relative fluorescence ranged from 0-110 and 32°C had the smallest start with 9 micrometers with its relative fluorescence ranging from 0-100. Compared to Figure 4, this data is strongly suggesting GFP is exhibiting time-dependency behavior as it is demonstrating that regardless of cells having a larger cell length, GFP concentration starts at very similar concentrations, rejecting the size-dependency behavior tendency.

Discussion

Cdc25 and Cdc13 proteins are involved in the cell cycle, they are part of G2 to mitosis regulation [2]. Wee1, which inhibits the Cdc2-Cdc13 complex, maintains a stable concentration. Yeast's most important phase in its cell cycle is G2, where Cdc25 and Cdc13 need to surpass Wee1 levels in order for Cdc2-Cdc13 to be functional and start mitosis [2]. Cdc25 and Cdc13 are believed to be the proteins regulating the size of cells at this step. It is thought that the levels of Cdc25 and Cdc13 depend on the size, which means that the concentration of these proteins is directly proportional to the size of the cell [4]. Thus, when cells reach the appropriate size, Cdc25 and Cdc13 levels are higher than Wee1 levels allowing the activation of mitosis. Thus, it is believed that Cdc25 and Cdc13 levels depend on size. To determine if Cdc25 is indeed a size-dependent protein, a behavioral analysis of a control protein, GFP was performed.

A method was developed to check the dependency between a control protein's, GFP, concentration, and size. The strain utilized contained a ZEV:GFP plasmid that was integrated in the genome at his7. The method performed was primarily based on the control of the expression of a control protein, GFP. Firstly, beta estradiol was added to overnight grown cultures that were incubated and shook simultaneously at different temperatures to turn on the GFP protein. Then, a fixation titration was performed to arrest cells from completing the cell cycle. Moreover, cells were then washed with water and spun down to have a saturated sample in order to view under the high-resolution widefield microscope to collect images that were then turned into .dv files to obtain relative fluorescence and cell size measurements from each cell by utilizing a MatLab program, PombeY. These measurements were then plotted as relative fluorescence versus cell length graphs. In order to have cells start at different cell lengths, cultures were grown at three

different temperatures (28°C, 30°C, and 32°C) by utilizing a temperature-dependent strain that is controlled by the ZEV system.

Figures 8-10 illustrate the time course of over four hours of cultures grown overnight at 28°C, 30°C, and 32°C to demonstrate the relationship between GFP's relative fluorescence and cell length at five different timepoints. The 28°C (Figure 8) had the largest cell length for its zero-hour timepoint, which allowed for behavioral analysis since the ZEV-system was utilized to control cell size by the use of different temperatures. Moreover, 30°C (Figure 9) had the second largest cell length, while 32 °C (Figure 10) had the smallest cell length. Thus, the ZEV-system was successful in obtaining three different sizes in order to check the behavior of GFP. As for relative fluorescence, each temperature exhibited approximately the same intensities at the start and of the time course. The range that was usually observed was 150 for the zero-hour time point and 350 for the four-hour time point. Therefore, after all three temperature's relative fluorescence and cell length measurements were obtained and plotted, average intensities were then calculated to obtain trendlines and observe if GFP indeed behaves in a time-dependent manner. Figure 4 demonstrates both size-dependency and time-dependency behaviors to allow the comparison with the data collected. Size-dependent proteins have concentrations increase with size, while time-dependent proteins start at same concentrations and increase over time [2]. When comparing Figure 4 to Figure 11, the time-dependency behavior is observed because at different cell lengths, GFP concentration tends to have similar values, but then increase over time.

In conclusion, this strongly suggests that GFP expresses in a time-dependent manner because as time increased its concentration did as well. Thus, having confirmed GFP has time-dependent behavior, we can infer that GFP regulates the cell size by activation of mitosis based on the time it takes to complete the cell cycle. Moreover, it indicates Cdc25 does in fact behave as a size-dependent protein and regulates cell size in *S. pombe.*

Future Directions

Future directions of this project would be to analyze to find where in Cdc25 the size regulation domain is located. Moreover, this would allow further analysis of the mechanism of the Cdc25 protein's size-dependent regulation. It is known that Cdc25's size dependence is regulated transcriptionally, and its promoter is required and ample for its size-dependent expression, but it is unknown which parts of its promoter are essential for the cell size regulation process and what transcriptional regulators bind to it.

Appendix of Figures

Beta-Estradiol

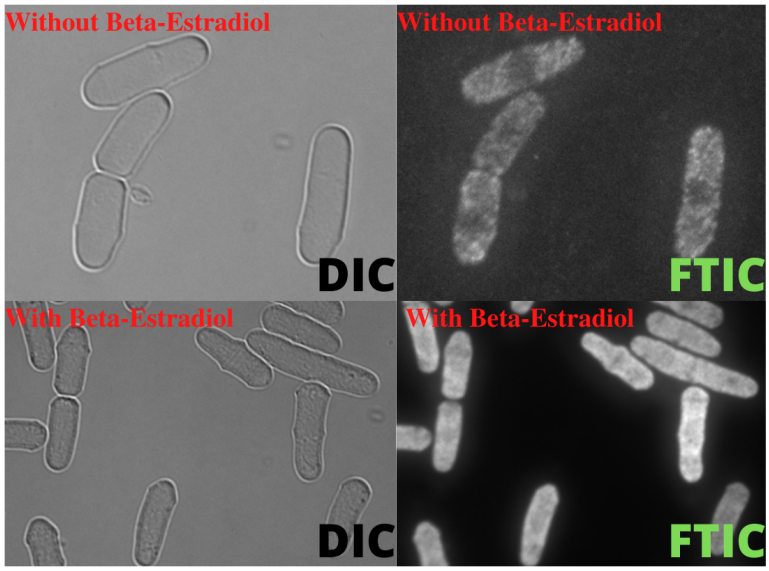

Figure 5 Beta-Estradiol Test

In this figure, images for yFS1049 strain using 10 nM of β-estradiol are shown. DIC show regular shape of live cells. FITC is from green channel which shows GFP protein tag. As observed, the bottom images demonstrate how beta-estradiol turns on GFP by making cells fluorescent. Compared to the FTIC figure for without beta-estradiol, the nuclear signal is nonexistent, and these cells are not as fluorescent as the ones with beta-estradiol.

Formaldehyde Fixation Test

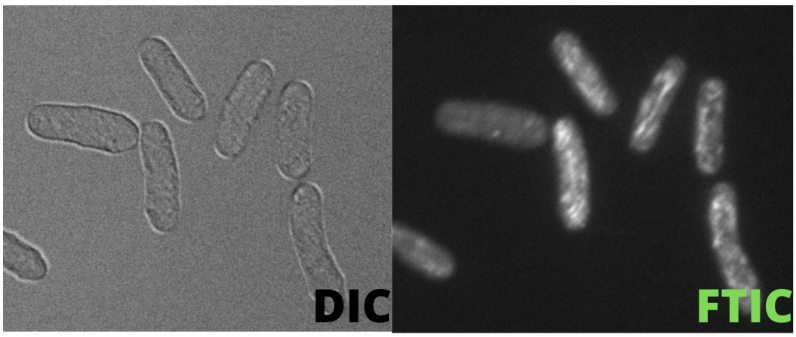

Figure 6 Formaldehyde Test

This figure demonstrates the fixation text utilized for yFS1049 strain. Strain was fixed with formaldehyde for ten minutes at room temperature. This allowed for cells to arrest at a certain phase in their cell cycle.

Time-dependency analysis of GFP

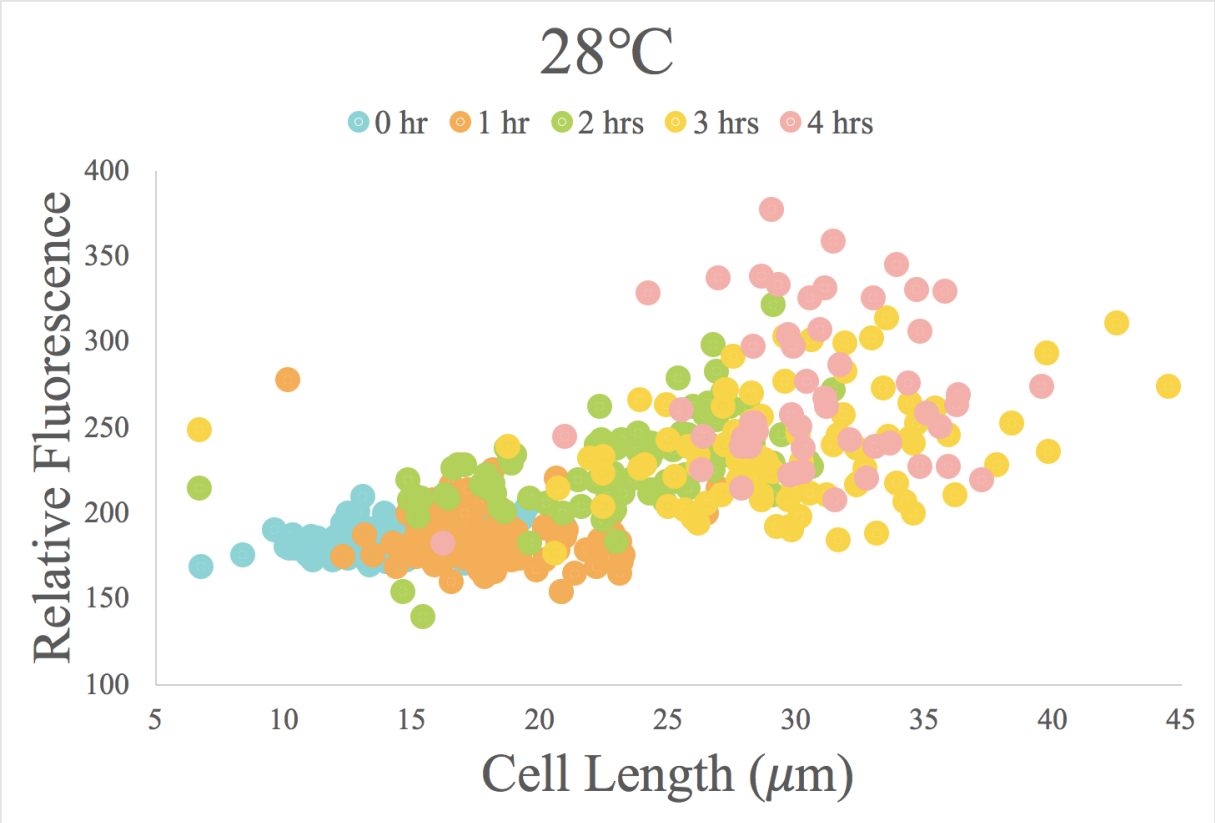

Figure 8 GFP protein concentration compared to cell length using Wee1 temperature sensitive at 28^{0}C over a time course of 4 Hours

Figure 8 demonstrates the relationship between relative fluorescence and cell length for the control protein, GFP using Wee1 stability. Every data point resembles an individual cell's GFP fluorescence measured from the images taken with the fluorescence microscope from formaldehyde-fixed cells and utilizing the MatLab program to analyze each cells' fluorescence. Each set of color resembles the set of cells collected at different timepoints staging at time zero to four hours.

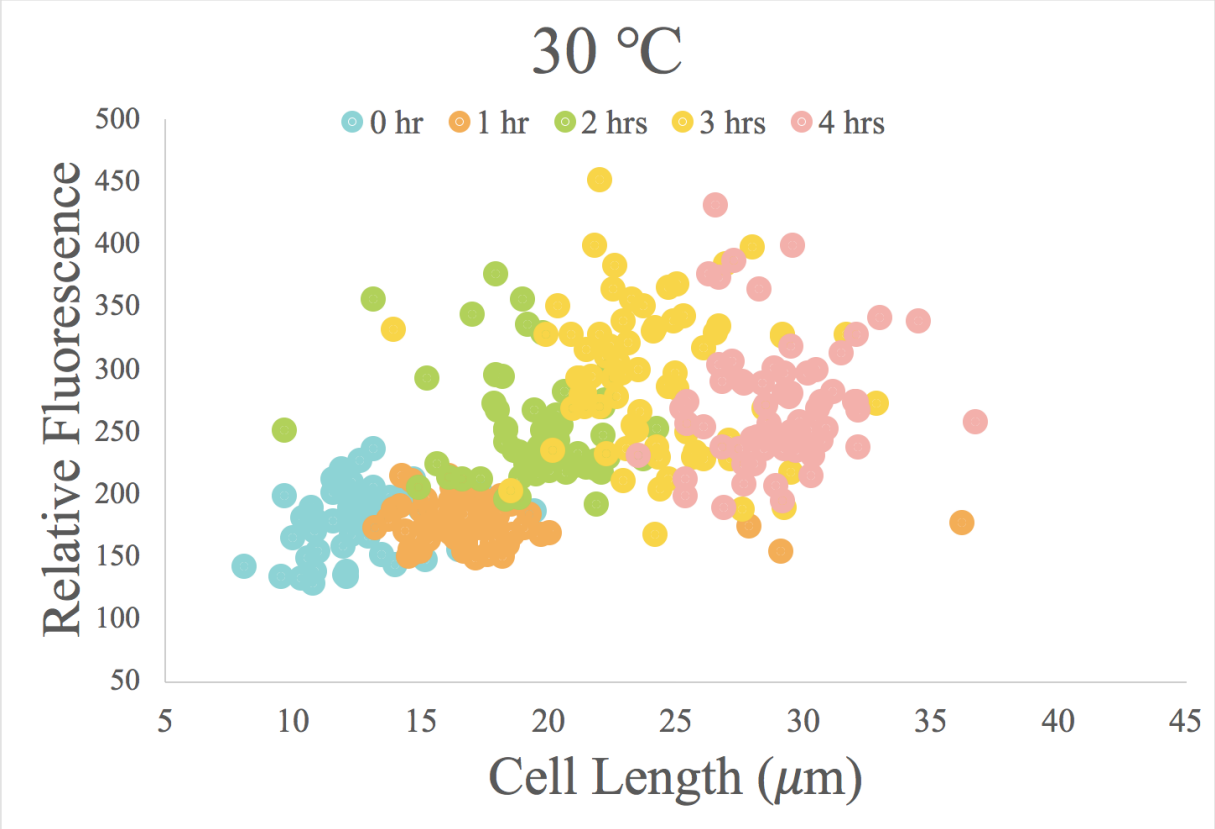

Figure 9 GFP protein concentration compared to cell length using Wee1 temperature-sensitive at 30^{0}C over a time course of 4 Hours

The figure above shows the directly proportional relationship between relative fluorescence and cell length for GFP using Wee1 stability. Every data point resembles an individual cell's GFP fluorescence measured from the images taken with the fluorescence microscope from formaldehyde fixed cells and utilizing MatLab program to analyze each cell's fluorescence. Each sets of color resembles the set of cells collected at different timepoints staging at time zero to four hours.

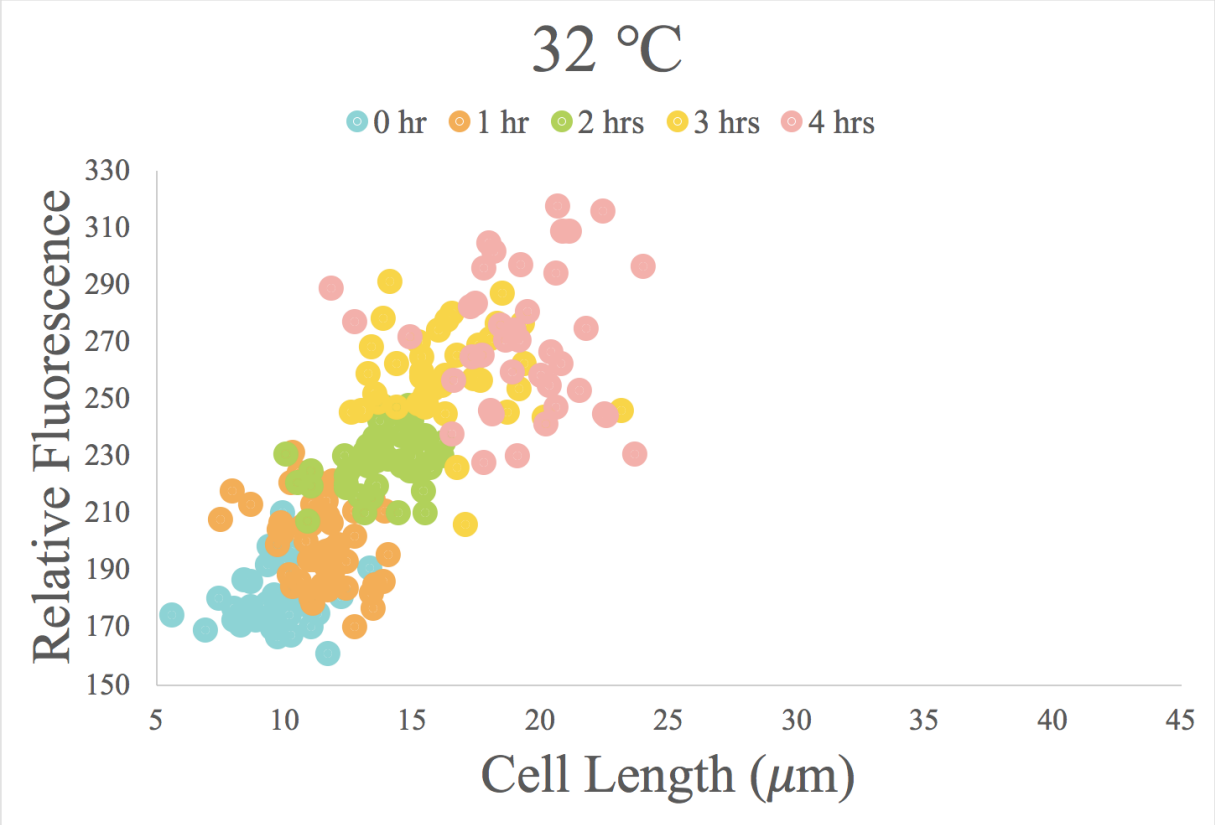

Figure 10 GFP protein concentration compared to cell length using Wee1 temperature-sensitive at 32^{0}C over a time course of 4 Hours

In Figure 10, relative fluorescence and cell length were compared using Wee1 stability to analyze the behavior of the GFP protein. Every data point resembles an individual cell's GFP fluorescence measured from the images taken with the fluorescence microscope from formaldehyde fixed cells and utilizing MatLab program to analyze each cells' fluorescence. Each sets of color resembles the set of cells collected at different timepoints staging at time zero to four hours.

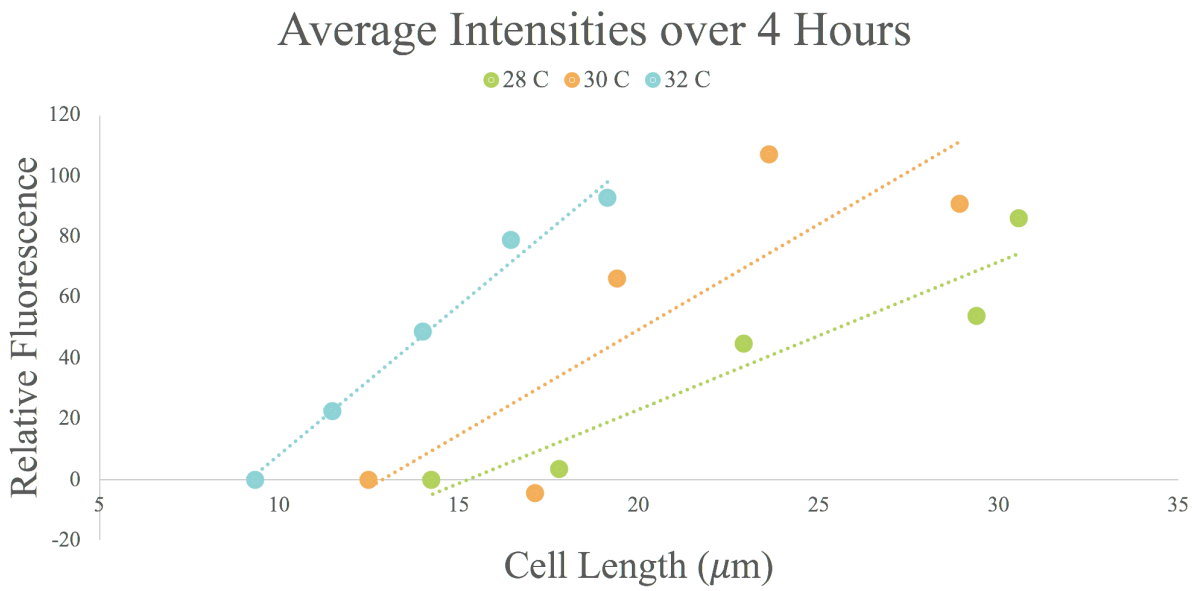

Figure 11 Average Relative Fluorescence values compared to cell length from each Temperature 28^{0}C, 30^{0}C, and 32^{0}C over a time course of 4 Hours

This graph demonstrates the time-dependency behavior of GFP protein. Each color represents the cultures that were grown overnight at three different temperatures (28^{0}C, 30^{0}C and 32^{0}C). Each data point resembles the average intensities of a certain timepoint, starting from the zzero hour timepoint to the four-hour timepoint. As observed, the 32^{0}C data set had the lowest relative fluorescence and shortest cell length. The highest relative fluorescence was the 28^{0}C data set with the longest cell length. Moreover, it illustrates that with varying cell size, GFP concentration starts at similar values and increases over time.

References

1. *Advanced light microscopy core*. OHSU. (n.d.). Retrieved April 14, 2022, from https://www.ohsu.edu/advanced-light-microscopy-core/delta-vision-widefield-scope-deconvolution
2. Amodeo, A. and Skotheim, J. (2015). Cell-Size Control. *Cold Spring Harbor Perspectives in Biology*, 8(4), p.a019083. Doi: 10.1101/cshperspect.a019083
3. Goldberg D, Marbach I, Gross E, Levitzki A, Simchen G. A Candida albicans homolog of CDC25 is functional in Saccharomyces cerevisiae. Eur J Biochem. 1993 Apr 1;213(1):195-204. doi: 10.1111/j.1432-1033.1993.tb17748.x. PMID: 8477693.
4. Keifenheim, D., Sun, X., D'Souza, E., Ohira, M., Magner, M., Mayhew, M., Marguerat, S. and Rhind, N. (2017). Size-Dependent Expression of the Mitotic Activator Cdc25 Suggests a Mechanism of Size Control in Fission Yeast. *Current Biology*, 27(10), pp.1491-1497.e4.Doi: 10.1016/j.cub.2017.04.016
5. Nurse, P. (1975). Genetic control of cell size at cell division in yeast. *Nature*, 256(5518), pp. 547-551.Doi: 10.1038/256547a0
6. Ohira, M., Hendrickson, D., Scott McIsaac, R., and Rhind, N. (2017). An estradiol-inducible promoter enables fast, graduated control of gene expression in fission yeast. *Yeast*, 34(8), pp. 323-334.Doi: 10.1002/yea.3235
7. Oliva, A., Rosebrock, A., Ferrezuelo, F., Pyne, S., Chen, H., Skiena, S., Futcher, B., & Leatherwood, J. (2005). The cell cycle-regulated genes of Schizosaccharomyces pombe. *PLoS biology*, *3*(7), e225. https://doi.org/10.1371/journal.pbio.0030225
8. Piel, M., & Tran, P. T. (2009). Cell shape and cell division in fission yeast. *Current biology: CB*, *19*(17), R823–R827. https://doi.org/10.1016/j.cub.2009.08.012

9. Turner, J., Ewald, J. and Skotheim, J. (2012). Cell Size Control in Yeast. *Current Biology*, 22(9), pp.R350-R359. Doi: 10.1016/j.cub.2012.02.041

10. Tyson J.J., Chen K.C., Novák B. (2013) Cell Cycle, Budding Yeast. In: Dubitzky W., Wolkenhauer O., Cho KH., Yokota H. (eds) Encyclopedia of Systems Biology. Springer, New York, NY. Doi:10.1007/978-1-4419-9863-7_16

11. Yanagida M. (2002). The model unicellular eukaryote, Schizosaccharomyces pombe. *Genome biology*, *3*(3), COMMENT2003. Doi: 10.1186/gb-2002-3-3-comment2003

https://www.nature.com/articles/s41598-018-27157-y