THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

THE EFFECT OF AGE ON *HO* EXPRESSION IN YEAST CELLS

BENJAMIN PASKOFF
SPRING 2017

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biochemistry and Molecular Biology
with honors in Biochemistry and Molecular Biology

Reviewed and approved* by the following:

Dr. Lu Bai
Professor of Biochemistry and Molecular Biology
Thesis Supervisor

Dr. Sarah Ades
Professor of Biochemistry and Molecular Biology
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Histones are an important component of chromatin structure that affects gene regulation. DNA in our cells is wound around an octamer of four core histone proteins that make up a nucleosome. Nucleosome occupancy plays a large role in regulating transcription by controlling the accessibility of DNA for transcription factors to bind and signal promoters downstream. Recent studies have linked lower histone concentration to aging and have even increased cell lifespan by overexpressing histones (1). Consequently, it was proposed that as cells lose histone concentration, nucleosome occupancy may be affected as well. This could create inappropriate cryptic transcription of the DNA when transcription factors are suddenly able to bind to the newly formed nucleosome depleted regions (2). Here we tested this hypothesis by analyzing *HO* expression in aged yeast cells. We chose the *HO* promoter because it is one of the best-characterized promoters in yeast and its activation is highly sensitive to nucleosome occupancy. If nucleosome occupancy is decreased in aged cells, we expect to see an increase in *HO* activation resulting from increased accessibility along the promoter for transcription factor binding. Our results showed that *HO* expression decreases as the cells age contradicting our original hypothesis. This result indicates that nucleosome loss does not appear to be occurring in the *HO* promoter. In addition, there appears to be a transcription activator or co-activator critical to driving *HO* expression that is losing efficacy in aged cells. Mating factors were tested for potential repression of *HO,* and SBF's binding capacity in older cells was evaluated in *CLN2* and *HO-Reb1**, however, neither appeared to be responsible for decreasing expression of *HO*.

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to thank everyone who has supported me throughout my undergraduate education and paved the way towards writing this thesis. First and foremost, I would like to thank my thesis advisor, principal investigator, and my incredible research mentor, Dr. Lu Bai. Her willingness to take a chance on me and accept me into her lab is the sole reason this thesis was possible. In addition, I would like to thank the awesome Ms. Manyu Du who helped me learn the ropes in lab and got me integrated into my project, and Mr. Chao Yan, who was always there with the answer if MATLab was acting up again. Also, I would like to thank all the other graduates and undergraduates I interacted with in the lab who created an amazing culture and learning environment. I could not have asked for a better group of young scientists to study alongside. Lastly, I would like to thank my honors advisor, Dr. Sarah Ades, without whom I would surely be missing several graduation requirements. Thank you everyone for all your support and guidance.

Chapter 1

Introduction

Background – Aging

Aging, like all biological processes, has both a molecular and genetic basis. In general, aging is thought to be caused by random damages and errors accumulated in the cell that lead to a gradual loss of fitness (3). The damages can happen in many places, including DNA, proteins and other macromolecules (3), and it is not clear which one is the dominant factor of aging.

Yeast has been a model for aging studies due to its simple genome and genetic capacity. In yeast, aging is usually defined as "replicative aging," or the number of times a cell replicates (4). Yeast cells undergo asymmetric cell division typically every 90 minutes where the mother retains a greater volume than the daughter (4). However, as the mother keeps dividing, the duration of cell cycles increases and mitotic division slows down. At approximately 25 cell cycles, mitotic division breaks down entirely and the mother cell stops dividing and dies (5). Occasionally, in old cells with a slow mitotic division time, symmetric division will occur (4). One proposed model for aging suggests that during the replication process there is a steady reduction of histones (1, 6). The relation between histones and the aging process was demonstrated by Feser et al. who overexpressed histones by 50% in yeast cells and recorded an increase in average cell life span (1).

Histones are protein complexes that are critical to organizing DNA. They are alkaline proteins that package DNA into a core unit known as nucleosome (7). Histones exist in five

major families: H1/H5, H2A, H2B, H3, and H4. H2A, H2B, H3, and H4 form the core of the nucleosome while H1/H5 link them together (7). Nucleosomes wrap approximately 147 bp of DNA for a total of 1.65 turns around the histone core (8). Because of their role in packaging DNA, nucleosomes play an important role in regulating transcription. For example, many components of cellular preinitiation complexes like SAGA and TFIIB have nucleosome binding subunits (8). Consequently, the positioning of nucleosomes might play a role in determining the transcription start site and the accessibility of DNA for binding by transcription factors (8).

Some studies have found that aging cells experience a decline in the concentration of core H3 and H4 histones (1, 9). This steady reduction could lead to a decrease in the nucleosome occupancy and therefore an increase in mis-regulated transcription (See figure 1-1). Since the histone loss is likely to be stochastic among different cells, we also expect to see an increase in cell-to-cell variability, or "noise," of transcription.

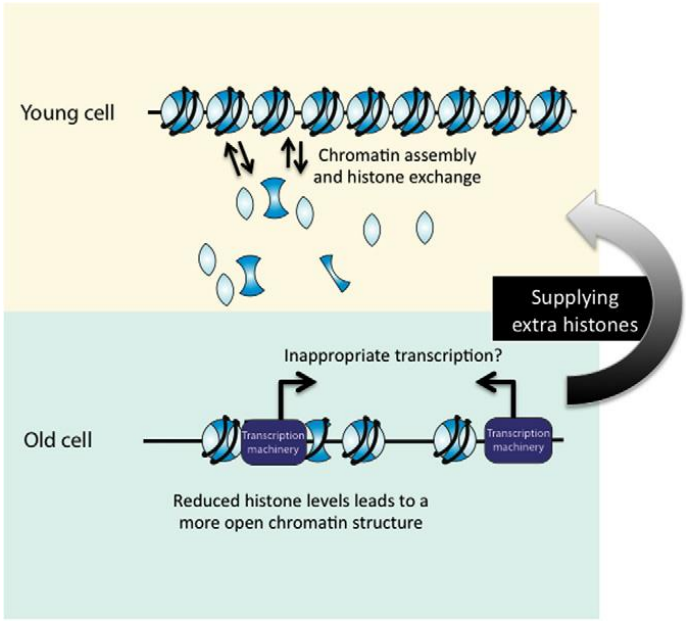

Figure 1-1: Model of histone loss associated with natural cell aging. Histone loss leads to a more open chromatin structure that creates additional opportunities for transcription factors to bind (Source, 1).

The *HO* Promoter

To test the idea that histone reduction increases transcription noise, we chose *HO* promoter in budding yeast as our model for the following reasons: 1) the *HO* promoter is well documented in literature and its regulatory path has largely been defined (10), 2) the transcriptional activity of the *HO* promoter is very sensitive to the nucleosome occupancy (10), and 3) *HO* expression tends to be highly stochastic among different yeast cells (11). Unlike most yeast genes that have small compact promoters, regulatory elements of the *HO* promoter extend to -1900 bp from the transcription start site (10). *HO* is divided into two regions: URS1 from -1900 to -1000, and URS2 from -900 to -200 (See figure 1-2) (10).

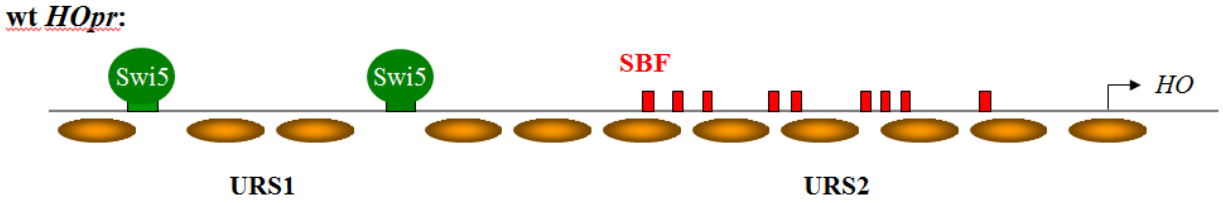

Figure 1-2: Diagram of the wildtype HO promoter. Swi5 is designated in green and its binding sites are located in nucleosome free regions at -1816 and -1305 in URS1 (10). Nine SBF binding sites are shown in red located in the URS2 region (10). Nucleosomes wrapping the DNA are depicted as orange ovals.

The process of activating *HO* is complex and involves sequential binding of transcription factors and coactivators (12). Swi5 and SBF are both cell cycle regulated transcription factors. SBF regulates gene expression during the G1/S transition of the cell cycle in yeast (13, 14) and Swi5 binds in URS1 as the cell transitions from anaphase in mitosis (11). Because SBF is only active during the G1/S transition of the cell, SBF only triggers HO activation over a short window of time every cell cycle (12). In addition, there is a daughter specific HO inhibitor,

Ash1, that prevents HO from being expressed in daughter cells, the first cell cycle after a cell is born (11, 15).

To activate *HO*, Swi5 binds to two nucleosome free sites located at -1816 and -1305 in URS1 (10). Once bound, Swi5 recruits three transcriptional coactivators: Swi/Snf, SAGA, and Mediator. Swi5 is then degraded while the SBF activator, composed of Swi4 and Swi6 subunits, binds to 9 different binding sites in the URS2 region and recruits the Swi/Snf, SAGA, and Mediator coactivators to this region of the promoter (10).

In order to create space for all these cofactors binding to the DNA, nucleosomes are evicted in waves proceeding in a left to right direction prior to transcription (10). If any wave fails to remodel the nucleosomes, transcription of *HO* fails. URS1 nucleosomes are depleted first by Swi5 and Swi/Snf (10). This is coupled with acetyltransferase activity of SAGA which facilitates binding of SBF (10). SBF can then recruit the FACT complex which evicts nucleosomes from the left end of URS2 (12). Lastly, the right end of the URS2 region undergoes nucleosome reduction by Asf1 H3/H4 histone chaperone. This finally culminates in nucleosomes being lost from the TATA region and RNA polymerase II binds and facilitates transcription. This process is condensed into four key steps shown in figure 1-3.

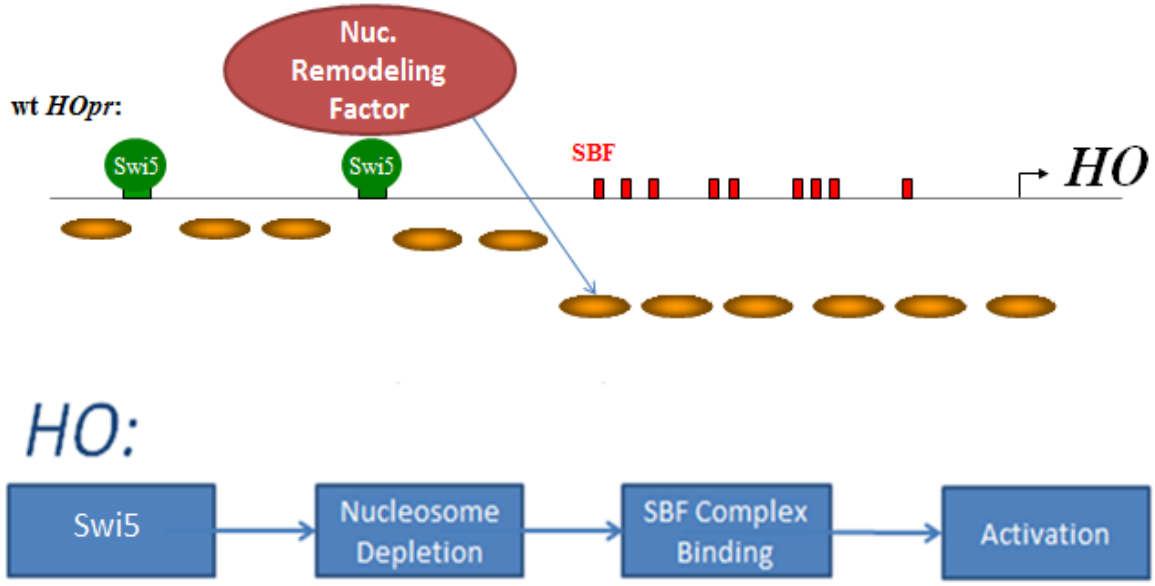

Figure 1-3: Process by which the HO promoter becomes activated. The HO gene presents layers of transcription factor binding regulation and high sensitivity to nucleosome occupancy.

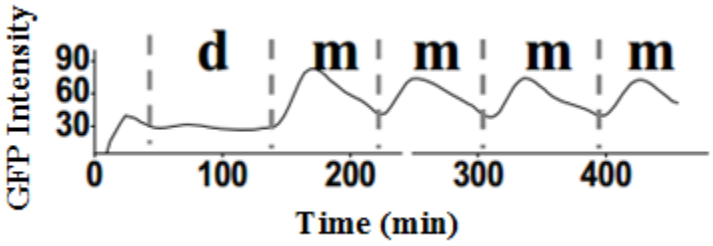

Figure 1-4: GFP intensity vs. time trace driven by the HO promoter in a single wildtype cell. The dashed vertical lines represent the cell division times. The low GFP intensity seen in the daughter cell cycle (d) is a result of HO expression being repressed by Ash1 (Source, 11).

The cell-cycle regulated and mother-specific activation of the *HO* promoter can be visualized in a strain containing *HO* promoter driving a destabilized green fluorescent protein

(GFP) using a time-lapse fluorescent microscope. Figure 1-4 shows a typical trace of GFP intensity as a function of time in a single cell. In the wild-type young cells, the *HO* promoter is activated in almost all the mother cycles, resulting in periodic changes in GFP intensity. For most daughter cycles (first cell cycle after the cell is born), *HO* promoter is not activated, and the GFP trace looks flat.

Another layer of *HO* regulation comes from the mating factors. All haploid yeast cells found in nature consist of two main mating types: a and α, which is determined by gene products from the mating locus (*MATa* or *MATα*) (16). A copy of both *MATa* and *MATα* also exist in the auxiliary mating loci, HMRa and HMLα (16), but expression from these sequences are inhibited by SIR2 (17). SIR2 is a histone deacetylase which condenses the nucleosomes making the two loci inaccessible to transcription (17). *MATa* or *MATα* alone do not affect *HO* expression; however, in diploid cells, both *MATa* and *MATα* are expressed, and the gene products a1 and α2 form dimers to repress *HO* expression (16, 17).

Research Hypothesis

Given previous studies that have shown histone concentrations decrease as they age, this experiment intended to observe the consequences of this depletion on a well-understood *HO* promoter. The hypothesis is that histone loss in aged yeast cells may create unplanned nucleosome depleted regions on the *HO* promoter over URS2, allowing SBF to bind and activate *HO* without help from Swi5. We also suspect that the expression level during the activated cycle would decrease due to the concentration of transcription machinery being distributed among increased genome-wide cryptic transcriptions.

Chapter 2

Materials and Methods

Growing Cells

Cells strains were obtained from Dr. David Stillman from the University of Utah. Samples were stored using a 15% glycerol solution and frozen in a -98^{o}C freezer. For use, strains were thawed in an ice bucket and plated on a YPD (Yeast Extract Peptone Dextrose) plate. The plate was then incubated for 24 hours in a 30^{o}C incubator. A small sample from the plate was extracted using a toothpick and placed in a liquid culture of YPD 2% glucose. The sample was then incubated again until cells reached OD around 0.1-0.2. If the cells had overgrown (OD 0.3-0.8), the sample would be diluted to 0.05 and grown back to 0.2. Anything beyond 0.8 was considered stationary phase and was discarded, and a new liquid culture was created from the previous plate.

Death Curves

The death curve data was generated by following the procedure outlined by Steffen et al., 2009 (18). Death curve data was obtained by observing cells divide to completion. This was done by plating liquid culture cells onto a YPD plate and monitoring growth on a dissection microscope. Once plated, daughter cells were selected from the plate and isolated. Care was taken during this step to ensure that the daughters selected were genuine daughter cells. The extracted cell was then carried via the dissection needle to a secluded portion of the plate.

Several more cells were also plated in this manner on the same plate and the entire plate was incubated in an incubator at 30^{o}C. Cells were checked every 90 minutes and new buds were removed and discarded using the needle. The number of times each cell produced a new daughter cell before dying was recorded. If the cells were still dividing at the end of the day, they were sealed with parafilm and placed in a refrigerator and the process resumed the following day. Each death curve was produced using 25-40 cells.

Making Movies

The movies were generated following the methodology outlined in Zhang et al., 2013 (11). Cells were grown until they divided 19 times using the methods outlined in the death curve procedure on YPD in a composite half-and-half YPD/SCD (SC-met + Glucose) plate. A composite plate was used so that the cells had the benefit of growing to an advanced age in a nutrient rich environment on the YPD half, but the SCD medium half of the plate was required to make the movie. This is due to the fact that SCD medium does not contain yeast extract like YPD and, consequently, has less background interference with the fluorescent microscope. Cells were then transferred to the SCD region and grown for one additional generation to make sure there were no issues during the transfer and that the cells were still growing on the new medium. The division time for this final generation usually took longer than 90 minutes because the cells had to acclimate to the synthetic medium that lacked the nutritional abundance found in the YPD plate. The cells were arranged in a 3x3 grid and then transferred to a microscope slide by cutting out the square of agar containing the cells (see figure 2-1).

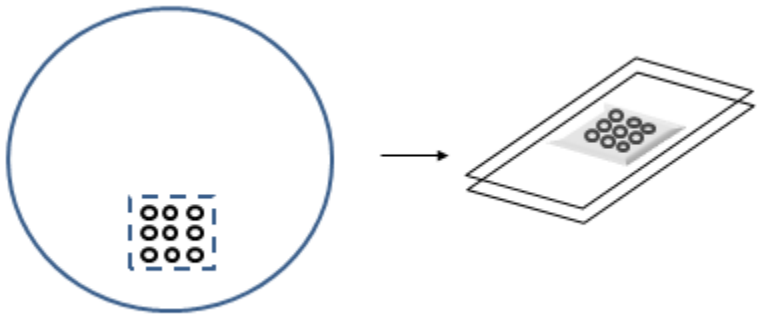

Figure 2-1: Procedure for extracting cells from plate to microscope slide for fluorescent analysis.

This slide was then taken to a Leica CTR 6500 fluorescent microscope, the positions recorded using a MATLab program, and fluorescent data was taken of each cell every five minutes for 8 hours. This data was analyzed using custom computer programs developed in lab to analyze fluorescent intensity within cell contours.

Chapter 3

Results

Death curve of the strain that contains the *HO* promoter driving GFP

In order to ascertain the effects of aging on cells, it was critical that we evaluate the lifespan of the strain we are using. Cells were grown continuously on a YPD plate in a 30^{o}C incubator during the day (at night they were stored in a 1.1^{o}C refrigerator), and the cell cycles were recorded by extracting daughter cells at the start of each new cell cycle using a dissection microscope (see Methods). This was done until all the cells stopped replicating. In this way, we were able to find the average lifespan of our strain and plot a death curve without losing the cell amidst thousands of its progeny (see figure 3-1).

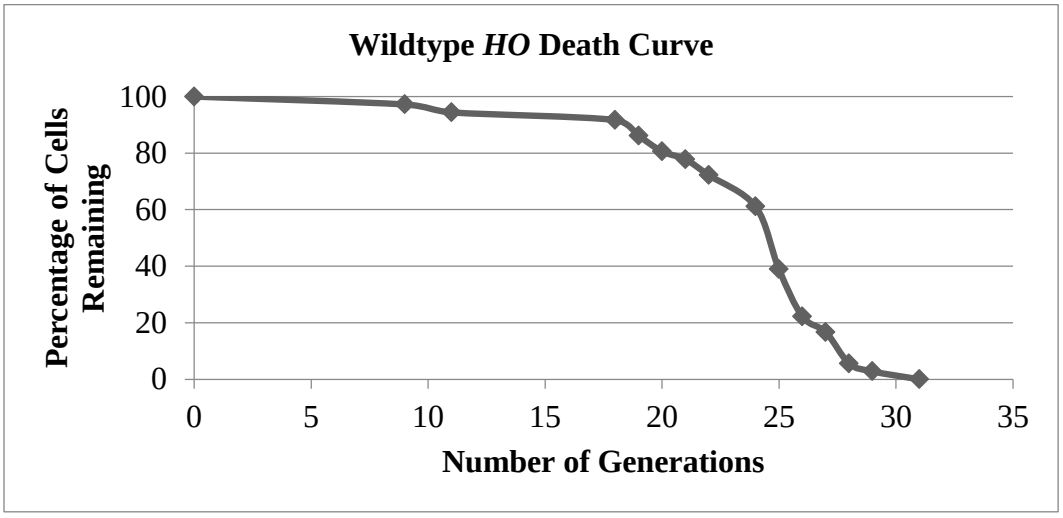

Figure 3-1: Percentage of cells still replicating plotted against the number of generations the cells underwent. Mean number of generations before death was found to be 23.86 ± 4.52.

The death curve shown in figure 3-1 was comprised of 34 individual cells which, when grown to completion, resulted in average lifespans of 23.86 cell cycles with a standard deviation of ±4.52. This number is comparable to 24.55 which was reported in other studies (5). As a result, we determined that when a cell reaches generation 20, it is likely old enough to experience some aging effects, but can still undergo a few additional cell cycles that can be observed under the microscope. Consequently, in all future experiments, this strain and similar strains were all grown to generation 20 on a plate using the dissection microscope to separate out daughter cells and ensure the exact age of the cell in order to effectively evaluate age-related expression changes.

_HO_ promoter with Swi5 binding site deletion does not show elevated expression in aged cells

As mentioned above, in order for *HO* promoter to be activated by SBF, Swi5 need to bind first and recruit co-activators to remove the nucleosomes covering the SBF binding sites. Without Swi5, *HO* can only be stochastically activated in a small fraction of cell cycles (REF). We hypothesized that, due to the decrease in histone concentration in aged cells, nucleosome coverage on the *HO* promoter will decrease, and SBF may be able to bind without Swi5. To test this hypothesis, we generated a mutant version of the *HO* promoter where both Swi5 binding sites are mutated (Swi5*) (figure 3-2). We used this promoter to drive destabilized GFP and integrate into a background strain. Given our hypothesis, we expected to see GFP activation in a larger fraction of cell cycles in aged cells compared with young cells.

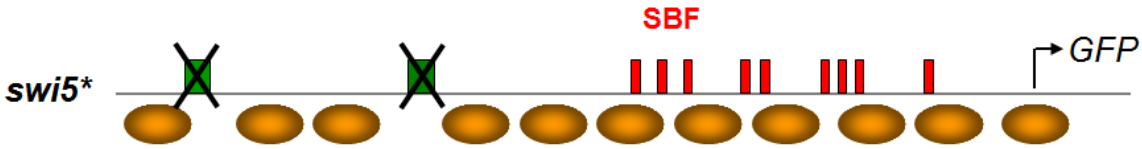

Figure 3-2: A diagram of Swi5 mutant. Swi5 binding sites are notated in green in the URS1 region (see figure 1-2 for reference to wildtype HO). Nucleosomes are denoted as orange ovals and nine SBF binding sites are designated as red rectangles in URS2.*

In figure 3-3, the fluorescent intensity from cells containing Swi5* promoter driving GFP. Young cells that have only replicated five times (G5) were compared to aged cells that have replicated twenty times (G20). The intensity of the light from GFP reflects the level of *HO* activation and the vertical dashed lines in figure 3-3A represent the beginning/end of a new cell cycle. The peak expression of GFP per cell cycle, or expression amplitude, was then plotted in a histogram in figure 3-3B for a direct comparison of the *HO* activity for each age bracket. Due to auto-fluorescence background, *HO* is not considered to express unless it surpasses an intensity level of 0.3 under the fluorescent microscope, i.e. any cell cycle that had *HO* expression over 0.3 was considered "on" and any cell cycle that had *HO* expression below 0.3 was considered "off." In contrast to our expectation, almost no GFP expression exceeded that threshold regardless of the age of the cell (see figure 3-3).

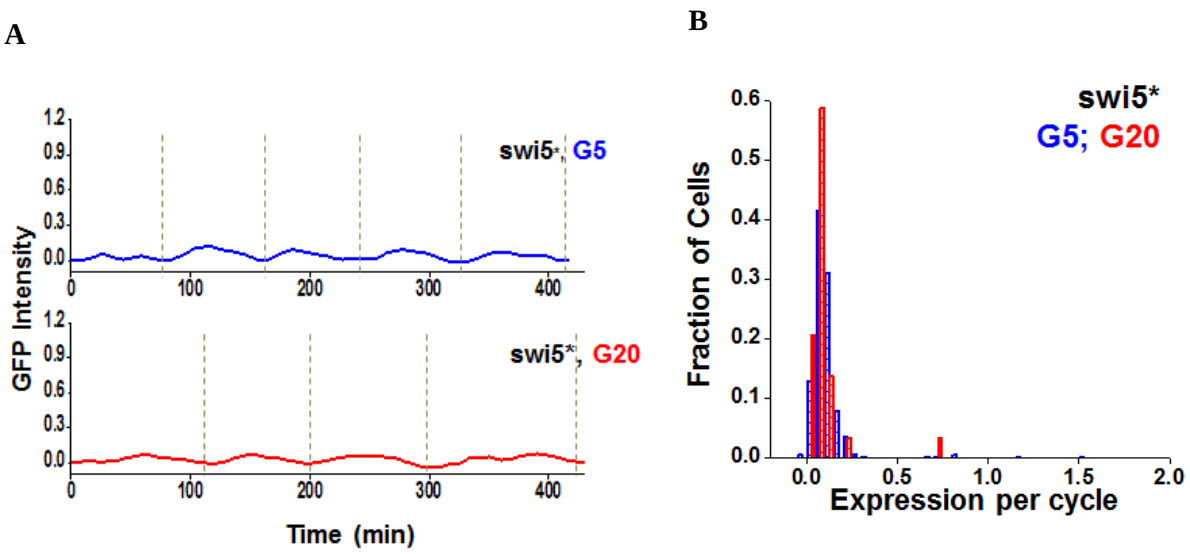

Figure 3-3: HO expression data for swi5 from young cells (Generation 5, G5) to old cells (Generation 20, G20). Panel A is representative cell traces recording GFP expression of one cell across multiple cell cycles. Panel B shows a chart of the frequency of GFP expression per cycle of approximately twenty cells ranging from G5-G20. The low amplitudes depicted indicate HO was not activated at all from cells at G5 – 20.*

The low activity of the Swi5* promoter, present in both young and old cells, indicated that histone loss was not occurring at a rate sufficient to create nucleosome depleted regions for SBF to bind.

Wildtype *HO* experiences sharp decrease in activation in aged cells

This discovery that the aged cells did not appear to increase *HO* activation conflicted with our original hypothesis and prompted us to run the same experiment for a strain containing the wild-type *HO* promoter driving GFP (see figure 3-4). Wildtype *HO* cells normally express *HO* at a near 100% frequency as opposed to the Swi5* strain that expresses <5% of the time.

Consequently, if key transcription factors were creating a decrease in expression as the cells aged, the trend would now be observable in wildtype *HO*.

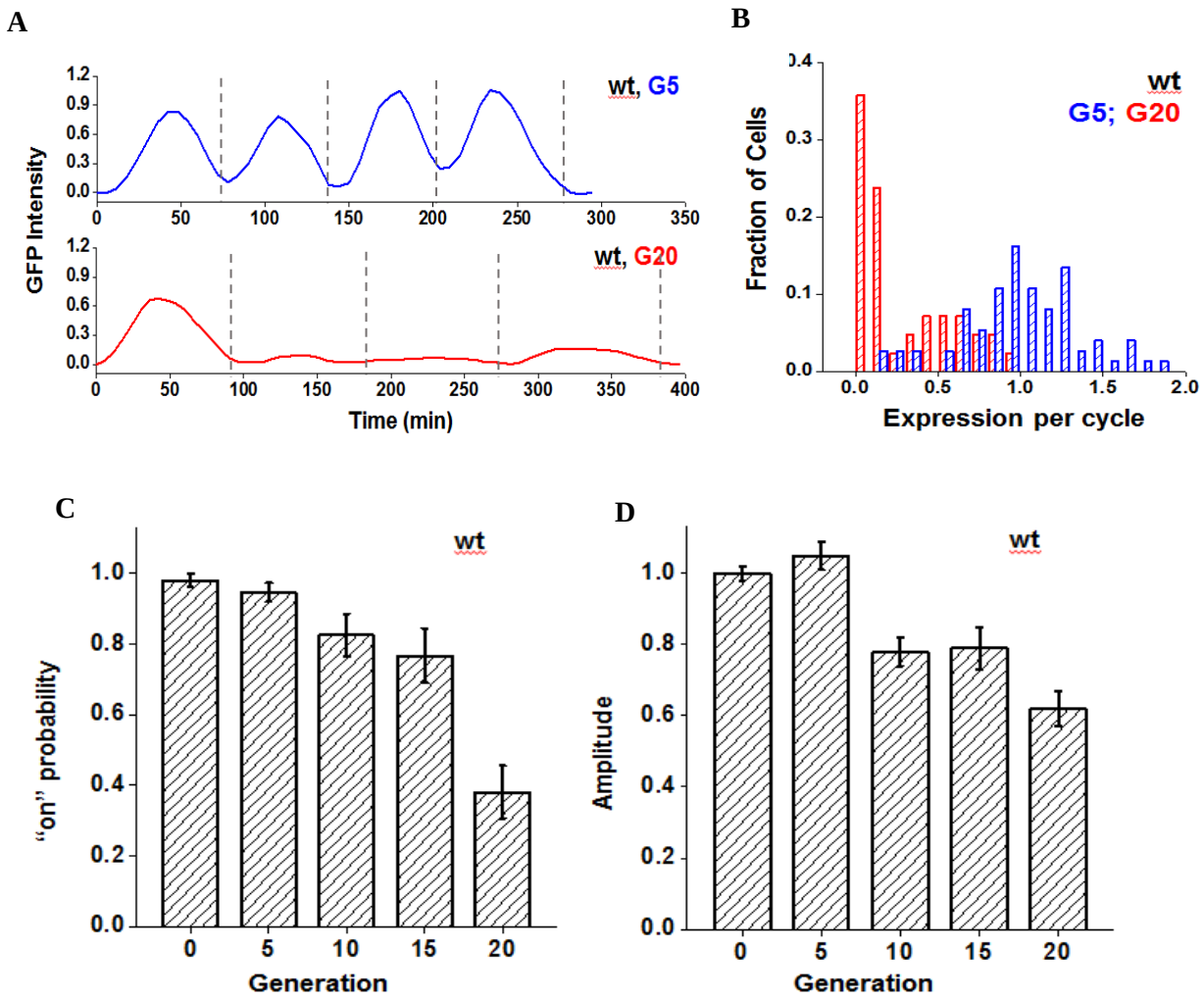

Figure 3-4: Panel A: Comparison of representative cell traces for wildtype HO for G5 and G20. Panel B: Expression per cycle of approximately twenty G5 and G20 cells. Panel C: "On probability" of HO Promoter per 5 generations. Panel D: Expression Amplitude of HO promoter per 5 generations.

As shown in figure 3-4, there was a clear decrease in the "on probability," or the probability that *HO* will be activated in a given cell cycle. In panel A, we see expression traces

typical of G5 and G20 cells. G5 cells were far more likely to have *HO* activation in each cell cycle than G20 cells which often formed a flat line indicating little or no *HO* expression. In panel B, we compared the histogram of *HO* expression from G5 to G20. Here we observed a clear downward shift in the average GFP light intensity of older cells. This indicates that *HO* is not being activated as often in G20 cells as it is in G5 cells. In panel C, we plotted the "on probability" of *HO* promoter across all generations in five generation increments. Again, this plot was generated by setting the threshold standard for *HO* activation at 0.3. Here we saw that the "on probability" drops steadily as cells age, from nearly 100% in new mother cells, to less than 40% in G20 cells. This is also represented by the decrease in average amplitude of GFP intensity plotted from the first generation to G20 in panel D.

The findings in Figures 3-3 and 3-4 show no evidence of nucleosome loss in aged yeast cells over the *HO* URS2 region. Rather, there are some other factors in aged cells that cause a decrease in *HO* expression. We next set out to determine the source of repression. By analyzing the transcription factors necessary to trigger *HO* activation, we tested two new hypotheses:

1) Yeast Mating factors HMRa and HMLα are driving *HO* repression
2) Reduced *HO* expression in aged cells is due to a decrease in SBF concentration

Yeast Mating Factors do not cause the decrease in *HO* expression

Previous studies have indicated that SIR2-mediated silencing is weakened in aged yeast cells (19). The de-silencing of HML and HMR would allow both mating factors a and α to be expressed in the same haploid cell. Since MATa/α inhibits *HO* expression, that would explain the decrease in *HO* activation.

To test for this, a strain of yeast was created so that both the HMRa and HMLα loci are removed. This allowed us to evaluate the *HO* expression in a condition that was unhampered by potential mating factor interference. Prior to running the experiment, a death curve was performed on this strain to validate that it underwent the same lifecycle as wildtype *HO* (see figure 3-5).

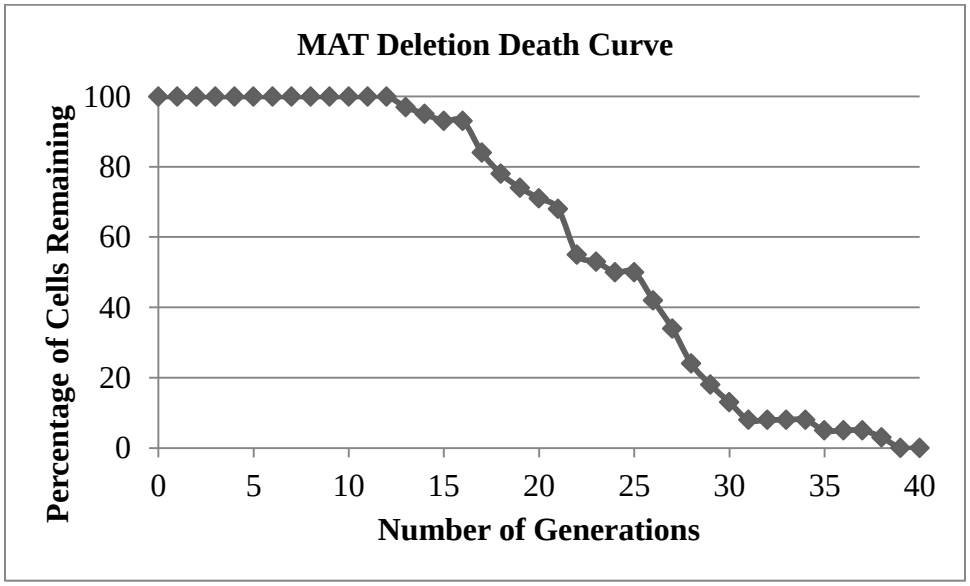

Figure 3-5: Death curve for MAT deletion HO cells. Curve was performed using 38 cells and produced an average lifespan of 24.55 ± 5.99 generations.

This death curve produced an average lifespan for each yeast cell of 24.55 with a standard deviation of 5.99. This is comparable to the wildtype *HO* average lifespan, so in all experiments with this strain, the cells were grown to the same generation 20 prior to placing them under a microscope.

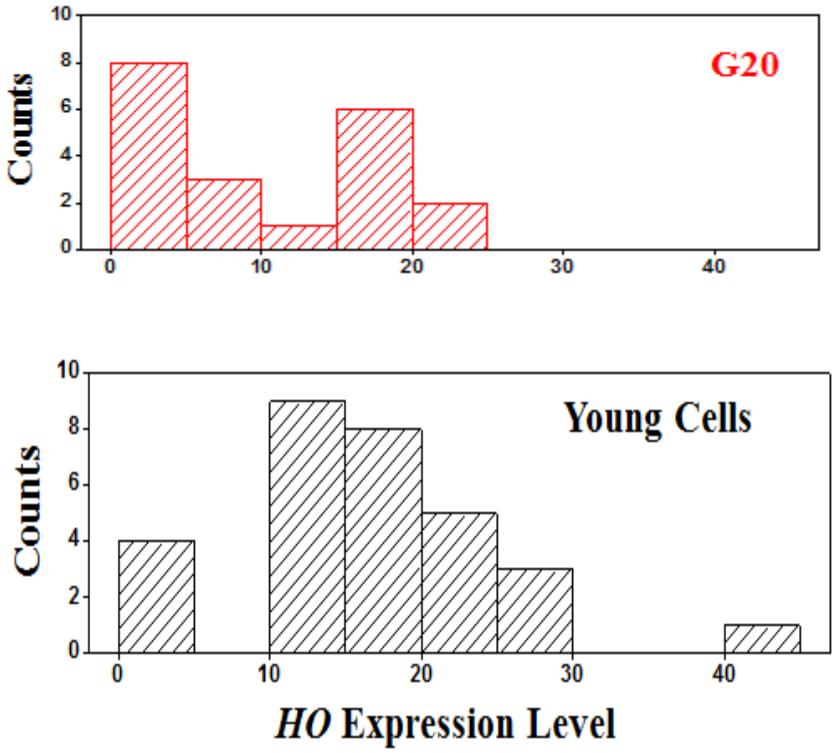

Figure 3-6: Histogram of light intensity from strain containing HO with mating type loci deletion driving GFP. G20 mother cells (red) and early generation young cells (black) were plotted. GFP light intensities were not normalized with young cells as was done for figures 3-3 and 3-4. Here, a GFP intensity value <10 was considered to have an inactive HO promoter.

As shown from figure 3-6, *HO* promoter in this strain is expressing approximately 90% of the time in the young cells, but this fraction drops to ~50% in G20 cells. Such decline in expression is comparable to what was seen in the strain containing the wildtype *HO* promoter driving GFP (see figure 3-4). Therefore, we concluded that HMRa and HMLα are not responsible for *HO* repression in aged cells.

SBF concentration reduction in aging yeast cells does not cause decrease in *HO* expression

We next investigated if the reduction of *HO* expression reflects a generic decrease of SBF activity in aged cells. To address this question, we compared the expression of the *HO* promoter to the *CLN2* promoter. *CLN2* is also activated by the same activator, SBF. However, unlike *HO*, the SBF binding site on the *CLN2* promoter is located in a 300bp nucleosome depleted region that is constitutive across the cell cycle (figure 3-7) (20). SBF can directly bind the *CLN2* promoter without the need for Swi5 to remodel nucleosomes (figure 3-7) (20). So if the change in expression occurs only in *HO*, but not to *CLN2*, this change is likely due to factors upstream of the SBF binding.

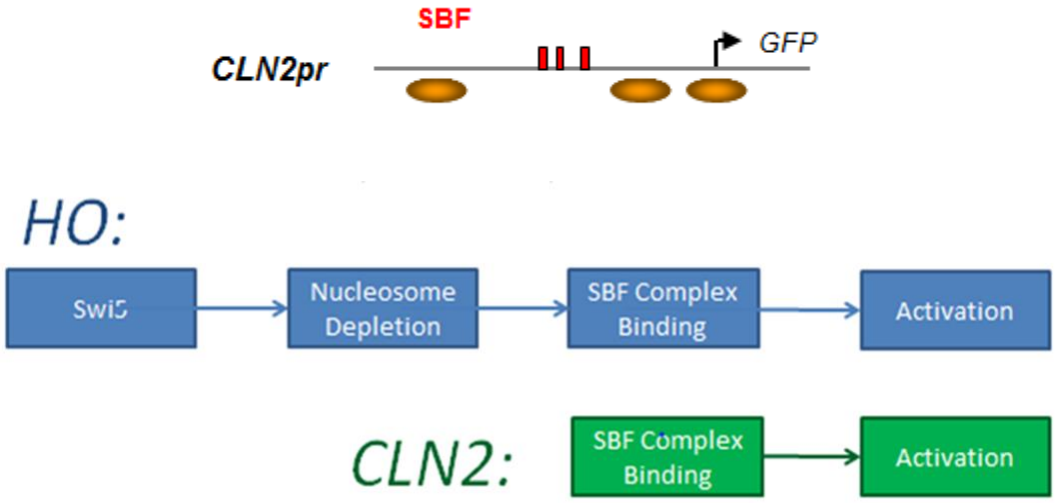

Figure 3-7: Diagram of the CLN2 promoter above and flowchart depicting the contrasting pathways to expressing HO vs. CLN2 below.

We then generated a strain containing *CLN2* driving GFP and measured the GFP expression in young (G5) cells and older (G20) cells (see figure 3-8).

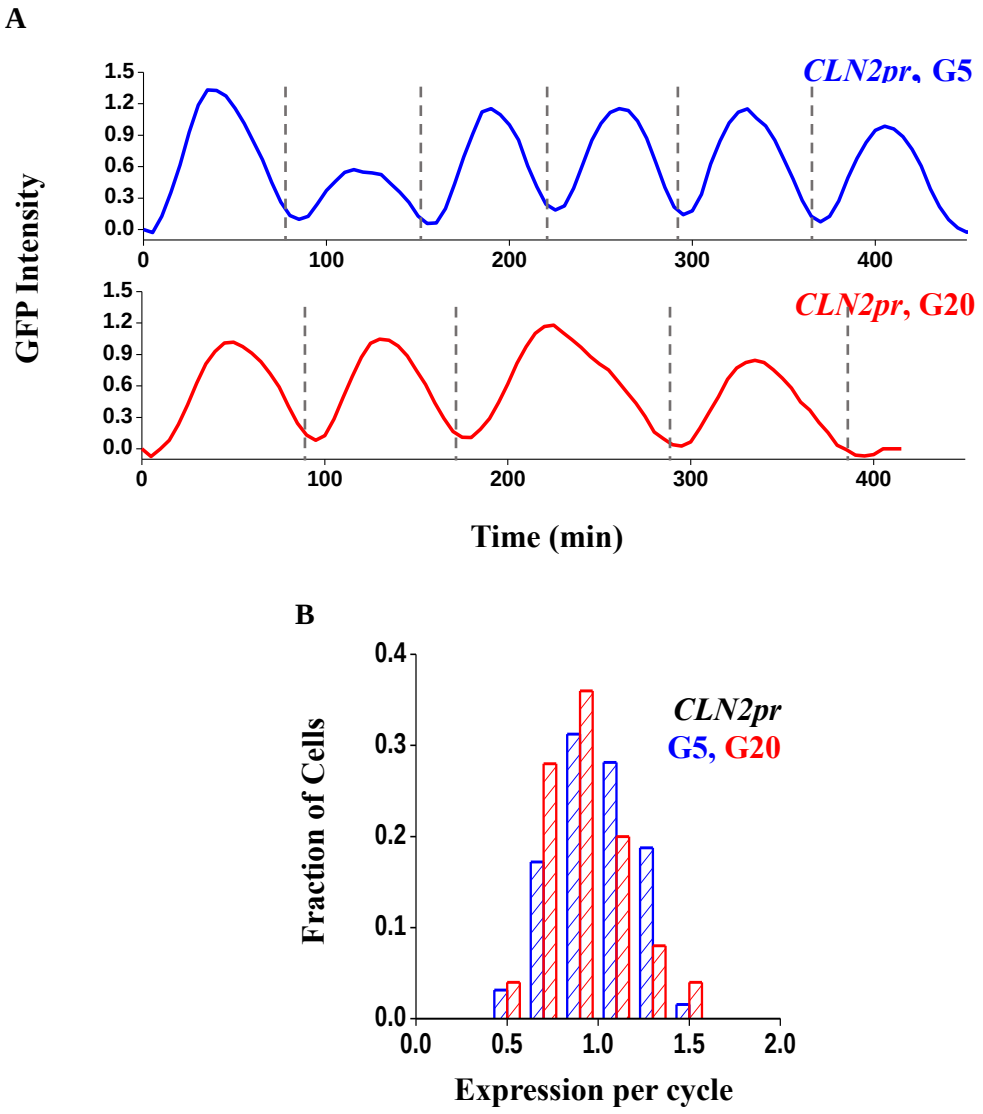

Figure 3-8: (A) GFP cell traces for CLN2 showing regular HO activation from G 5 cells and G20 cells. (B) Expression data for CLN2 cells. There was virtually no difference between the HO expression of CLN2 at G5 vs. G20. This is a stark contrast to the expression data found in wildtype HO (see figure 3-4B).

As shown in figure 3-8, *CLN2* showed 100% activation from G5 to G20 and GFP expression amplitude remained mostly the same despite the increase in age. This is different from what was observed in wildtype *HO* where we saw "on probability" drop under 40% in G20

cells and amplitude decrease to ~60%. This data suggests that SBF activity remains the same in aging cells and that it is not responsible for the decreased expression of aging *HO* yeast cells.

To further demonstrate the point above, we generated another version of the *HO* promoter with its Swi5 binding sites mutated and an artificial nucleosome depleted region inserted by designing a Reb1 binding site (consensus site 5'-TTACCCG-3' (21)) into the *HO* promoter (see figure 3-9). The Reb1 protein is a DNA binding protein whose main role is to antagonize nucleosome formation near its binding site (22). The theory behind this test is that Reb1 will deplete the nucleosomes adjacent to its binding site, creating a small area of accessible DNA for SBF to bind. This mutated *HO* promoter (*HO-Reb1**), similar to the *CLN2* promoter, will thus bind to SBF directly without help from Swi5. This additional test controls for factors that may be *CLN2* specific from interfering with the results.

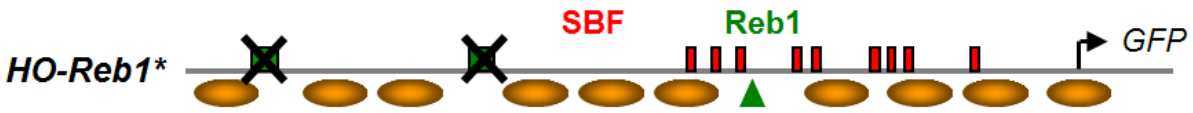

Figure 3-9: Diagram of the HO-Reb1 binding site. Swi5 binding sites are eliminated which stops nucleosome remodeling factors from being recruited. SBF is only able to bind after REB1 protein binds to the Reb1 binding site and repositions the nucleosomes to create space.*

The same procedure was carried out as before where G20 mother cells with *HO-Reb1** driving GFP were observed under a fluorescent microscope. The expression patterns of the aged G20 cells to younger G5 cells were compared (see figure 3-10).

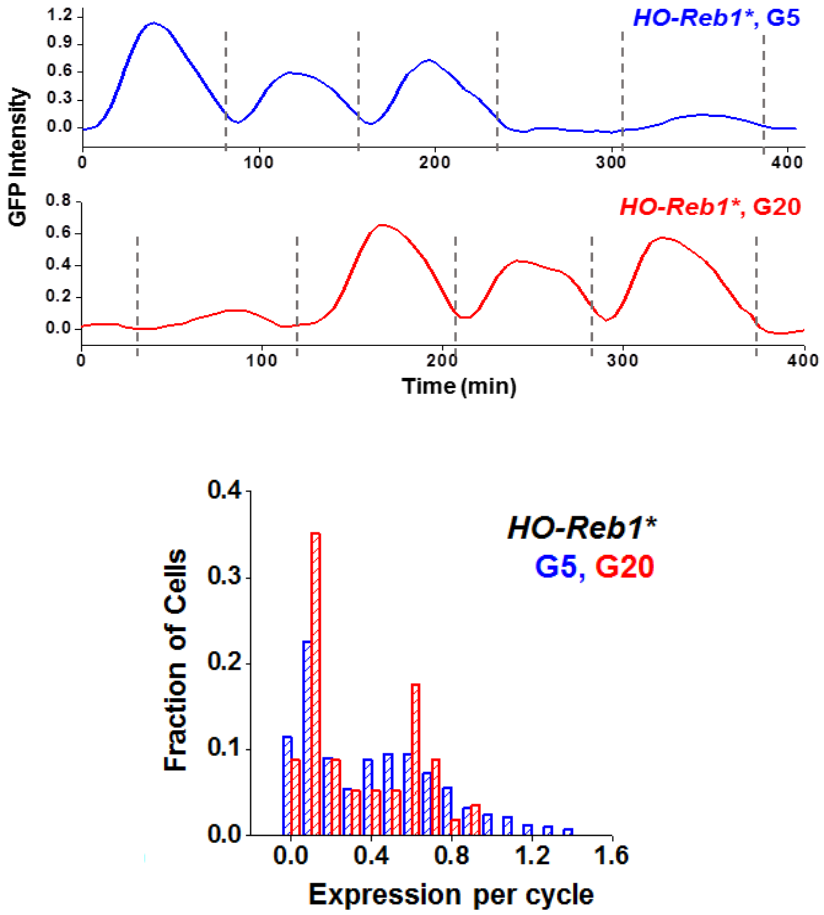

Figure 3-10: (A) HO-Reb1 cell traces for young cells (G5) and aged cells (G20). (B) Expression per cycle for G5 and G20 cells.*

From the data shown in figure 3-10, there is not much change in expression as the cell advances from G5 to G20. This result, combined with the data obtained from *CLN2*, indicates that once SBF binds to a promoter, it can activate equally well in old and young cells; *HO* repression in aged cells is likely to be caused by a step upstream from the SBF binding.

Chapter 4

Discussion of Results

Studies have shown that aging is linked to lower histone concentrations in yeast cells and that over-expression of histones can extend cell life (1). This is significant because it indicates that histone concentration is partially responsible for cell aging. The idea is that substantial histone loss in aged cells has the potential to expose sections of DNA that are intended to be inaccessible to transcription factors. This exposure could potentially undermine the cell's own epigenetic regulatory processes by creating unintended binding sites for transcription factors and machinery that ultimately leads to inappropriate transcriptions that limit cell life.

This study intended to address the question of whether or not this type of inappropriate transcription indeed happens in aged yeast cells using the *HO* promoter as a model. The hypothesis was that histone reduction would trigger a loss of nucleosomes over the URS2 of the *HO* promoter that would allow SBF binding and promoter firing without Swi5. However, in the absence of Swi5 binding sites, *HO* showed no signs of increased activation. In fact, figures 3-3 and 3-4 illustrate a sharp downward trend in *HO* expression as the cells advanced in age. Based on these results, we tested two new hypotheses: 1) Yeast mating factors were undergoing simultaneous a and α activation creating an *HO* repressor, or 2) SBF concentration was decreasing in the aging cells and was making SBF binding to activate *HO* infrequent in aged cells.

Hypothesis 1 was tested by deleting yeast mating loci HMRa and HMLα. This prevented both a and α factors from becoming activated in the first place, completely eliminating the potential for a a/α repressor to be formed. *HO* expression in this strain also decreased as cells

aged, similar to the wild-type strain. Therefore, we reasoned that mating factors were not the primary contributor to decreased *HO* expression.

We tested hypothesis 2 by comparing wildtype *HO* data to data obtained by running the same experiment on a *CLN*2 promoter or *HO-Reb1** promoter driving GFP. These two promoters contain constitutive nucleosome free sites, allowing SBF to activate transcription without requiring Swi5. This design allowed us to determine if reduced SBF concentration was responsible for the downward trend of *HO* expression in wildtype *HO.* However, neither the *CLN2* expression nor the *HO-Reb1** expression appeared to change when older mother cells were compared to young cells. This indicates that, despite increasing age, SBF is still just as functional in old cells as it is in younger cells.

Since none of these hypotheses appeared to identify the responsible factor behind the decrease of *HO* expression in wildtype cells, more experimentation needs to be done to control for the other transcription factors involved in the *HO* activation pathway. One idea is that repression of a global transcription factor like Swi5 might be causing the decline in wildtype *HO* expression. If Swi5 concentration is decreasing or is no longer capable of reaching the Swi5 binding sites efficiently or is failing to recruit nucleosome remodeling factors, SBF does not bind and the promoter remains inactive. To test to see if this transcription factor was responsible for the decrease in expression of aging *HO* cells, we could engineer a diploid yeast strain with a GFP reporting *HO* promoter and a YFP reporting *HO* promoter simultaneously (see figure 4-1). The rationale here is that Swi5 concentrations should be largely consistent across the cell. If Swi5 concentrations are decreasing and causing miscues in the expression frequency, there should be a high correlation between the two promoters within the cell. If this is not the case, Swi5 cannot be responsible for decreasing *HO* expression.

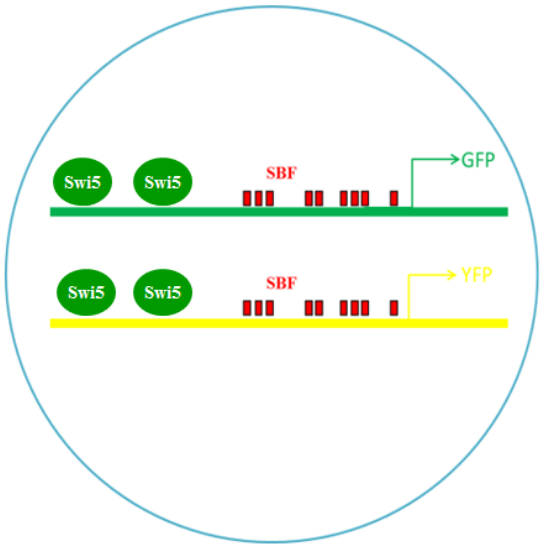

Figure 4-1: A diploid HO cell where one strand would be engineered to fluoresce GFP and the other strand would fluoresce YFP allowing us to distinguish between the two under a fluorescent microscope.

Lastly, it is also possible that nucleosome remodeling factors lose their ability to effectively remodel the nucleosomes and fail to create sufficient space for SBF to bind. We could test for this possibility by using an *HO* strain with the nucleosome remodeling factors greatly over-expressed to ensure that there is always a nucleosome remodeling factor for Swi5 to recruit. If this corrects the low expression frequency of *HO* in later generations, then we can conclude that nucleosome remodeling factors are the main factors behind older cells being unable to activate *HO*.

The results of this study, and continued exploration of *HO* expression in older cells, have several implications in our understanding of aging mechanisms. While histones may play a role in regulating DNA accessibility and initiating certain epigenetic changes that can lead to age-related symptoms (1), histone loss did not appear to have any impact on *HO* expression. Rather, instead of seeing an increase in *HO* expression due to increased accessibility to the promoter, *HO*

expression decreased, likely as a result of one of the remaining untested transcription factors like Swi5 or nucleosome remodeling factors. The findings of this study suggest that changes to the efficacy of key transcription factors may be responsible for decreasing expression of *HO* with age, but, as of now, it is unclear exactly which factor is accountable.

BIBLIOGRAPHY

1. Feser, Jason et al. "Elevated Histone Expression Promotes Life Span Extension." *Molecular Cell* 39.5 (2010): 724-735.
2. Berretta, Julia, and Antonin Morillon. "Pervasive Transcription Constitutes A New Level Of *Eukaryotic Genome Regulation*." EMBO reports 10.9 (2009): 973-982.
3. Gladyshev, Vadim N. "Aging: Progressive Decline In Fitness Due to the Rising Deleteriome Adjusted by Genetic, Environmental, and Stochastic Processes." *Aging Cell* 15.4 (2016): 594-602.
4. Steinkraus, K.A., M. Kaeberlein, and B.K. Kennedy. "Replicative Aging In Yeast: The Means To The End." *Annual Review of Cell and Developmental Biology* 24.1 (2008): 29-54.
5. Jo, Myeong Chan et al. "High-Throughput Analysis of Yeast Replicative Aging Using a Microfluidic System." *Proceedings of the National Academy of Sciences* 112.30 (2015): 9364-9369.
6. Rando OJ, Winston F. "Chromatin and Transcription in Yeast." *Genetics* 190.2 (2012): 351-387.
7. Nelson, David et al. Lehninger Principles of Biochemistry. 1st ed. New York, NY: W.H. Freeman, 2005. Print.
8. Jiang, Cizhong, and B. Franklin Pugh. "Nucleosome Positioning And Gene Regulation: Advances Through Genomics." Nature Reviews Genetics 10.3 (2009): 161-172.
9. Dang, Weiwei et al. "Histone H4 Lysine 16 Acetylation Regulates Cellular Lifespan." *Nature* 459.7248 (2009): 802-807.
10. Stillman, David J. "Dancing the Cell Cycle Two-Step: Regulation of Yeast G1-Cell-Cycle Genes By Chromatin Structure." Trends in Biochemical Sciences 38.9 (2013): 467-475.
11. Zhang, Qian et al. "Stochastic Expression And Epigenetic Memory At The Yeast *HO* Promoter." *Proceedings of the National Academy of Sciences* 110.34 (2013): 14012-14017.
12. Cosma MP, Tanaka T, Nasmyth K (1999) Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell* 97: 299–311.

13. Koch, C., Moll, T., Neuberg, M., Ahorn, H. & Nasmyth, K. “A role for the transcription factors Mbp1 and Swi4 in progression from G1 to S phase.” Science 261, 1551-1557 (1993)
14. Koch, C. & Nasmyth, K. “Cell cycle regulated transcription in yeast.” Cell Biol. 6, 451-459 (1994)
15. Sil, Anita, and Ira Herskowitz. "Identification Of An Asymmetrically Localized Determinant, Ash1p, Required For Lineage-Specific Transcription Of The Yeast HO Gene". Cell 84.5 (1996): 711-722.
16. Madhani, Hiten D. From a to α 1st ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2007. Print.
17. Smeal, Tod, et al. "Loss of Transcriptional Silencing Causes Sterility in Old Mother Cells of S. Cerevisiae." *Cell* 84.4 (1996): 633-642.
18. Steffen, Kristan K., Brian K. Kennedy, and Matt Kaeberlein. "Measuring Replicative Life Span in the Budding Yeast." *Journal of Visualized Experiments*, 28 (2009):
19. Wierman, Margaret B., and Jeffrey S. Smith. "Yeast Sirtuins And The Regulation Of Aging". *FEMS Yeast Research* 14.1 (2013): 73-88.
20. Bai, Lu, Andrej Ondracka, and Frederick R. Cross. "Multiple Sequence-Specific Factors Generate the Nucleosome-Depleted Region on CLN2 Promoter." Molecular Cell 42.4 (2011): 465-476.
21. Afek, Ariel, and David B. Lukatsky. "Positive And Negative Design For Nonconsensus Protein-DNA Binding Affinity In The Vicinity Of Functional Binding Sites". *Biophysical Journal* 105.7 (2013): 1653-1660.
22. Radman-Livaja, Marta, and Oliver J. Rando. "Nucleosome Positioning: How Is It Established, And Why Does It Matter?" *Developmental Biology* 339.2 (2010): 258-266.

Academic Vita

Benjamin L. Paskoff
0154 Simmons Hall University Park, PA 16802 blp177@psu.edu

EDUCATION
Bachelor of Science in Biochemistry and Molecular Biology – Cell Biology Option
Piano Performance Minor
Pennsylvania State University - Schreyer Honors College – University Park, PA (2014 – 2017)

College of Southern Maryland – La Plata, MD
Dual-enrollment during high school (2012 – 2014)

WORK HISTORY

Undergraduate Researcher at Bai Lab, Penn State University (2015 – Current)
- Developed research project to observe aging effects on yeast gene expression
- Experienced in operating fluorescent microscope & dissection microscope
- Proficient in data analysis using Matlab
- Maintained and grew cell cultures

Teaching Assistant at Penn State University for BMB 428 Physical Chemistry for Biologists (Fall 2016)
- Held weekly hour-long office hour sessions
- Scheduled individual appointments with students on an as-needed basis
- Graded homework for all 80 students in the class
- Proctored exams

AWARDS AND AFFILIATIONS

Dean's List 2014, 2015, 2016 Fall Semester at Penn State University
Dean's List all semesters (2012 – 2014) at College of Southern Maryland
National Society of Leadership and Success Member
Phi Eta Sigma Honor Society Member