The Role of H2A.Z in Chromosome Segregation in

*Saccharomyces cerevisiae*

by

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Introduction

The nucleosome is the basic unit of chromatin.

DNA in any eukaryotic nucleus must be packaged into chromosomes in order to keep genetic data organized. Chromosomes are composed of DNA and protein, which is known as chromatin. There are two types of chromatin: euchromatin and heterochromatin. Active regions in the genome where chromatin is less condensed is referred to as euchromatin, while inactive regions where chromatin is more condensed are known as heterochromatin or silent chromatin (Rusche, Kirchmaier, & Rine, 2003).

The basic component of chromatin is the nucleosome. The nucleosome is composed of 147 base pairs of DNA that is wrapped 1.67 times around a cluster of eight proteins, known as the histone octamer. The core of this octamer is made up of the histones H2A, H2B, H3 and H4. Histones H2A and H2B form two heterodimers and histones H3 and H4 form a heterotetramer. Each nucleosome is separated by linker DNA containing a linker histone, H1, which does not resemble the four core histones. Linker DNA can range from an average of 18 base pairs in *Saccharomyces cerevisiae* to about 38 base pairs in humans. The whole unit is thought to resemble “beads on a string”, which is shown in Figure 1. To condense chromatin, the nucleosome folds into a 30 nm fiber, which in turn folds into undefined higher order packaging units that organize into a chromosome (Jansen & Verstrepen, 2011; Peterson & Laniel, 2004; Zlatanova & Thakar, 2008).
The packaging of DNA into chromatin not only restricts access to genes, but also affects many processes such as transcription, replication, repair, and homologous recombination. Thus, chromatin structure must be altered in efficient and reversible processes to allow access to certain genes. One way to achieve this is through post-translational modifications of the amino-terminal residues of the core histones. Examples of these modifications are methylation and acetylation of lysine and arginine residues, phosphorylation, poly(ADP)-ribosylation, and ubiquitylation (Krogan et al., 2004; Peterson & Laniel, 2004). Acetylation of the amino-terminal tail of core histones, for example lysine 16 of histone H4, is generally associated with open and actively transcribed genomic regions, whereas deacetylation of these tails is linked to transcriptional silencing (Rusche et al., 2003).

The chromatin fiber is highly heterogeneous (Peterson & Laniel, 2004). Hence, in addition to modifications of the histones, altering nucleosome composition through

![Figure 1: The beads on a string model.](image-url)

the use of histone variants achieves this purpose. The histone H2A, for example, has five different forms: canonical H2A, H2A.X, MacroH2A, H2ABbd, and H2A.Z (Raisner & Madhani, 2006). H2A.Z is conserved from yeast to humans more than its counterpart H2A (Eirin-Lopez & Ausio, 2007; Guillemette & Gaudreau, 2006). Its function is essential in organisms, such as *Drosophila, Tetrahymena*, and mice (Dryhurst, Thambirajah, & Ausio, 2004). However, in *Saccharomyces cerevisiae*, the deletion of the gene for H2A.Z, *HTZ1*, is nonlethal providing a model system to study the variant’s function.

**H2A.Z distribution genome wide**

Studies have shown that H2A.Z is dispersed throughout all yeast chromosomes, including the centromeres and telomeres (Eirin-Lopez & Ausio, 2007; Guillemette & Gaudreau, 2006; Zlatanova & Thakar, 2008). H2A.Z nucleosomes have been found at both euchromatin and heterochromatin regions (Dryhurst et al., 2004) and at genes that are transcribed by RNA polymerase II (Eirin-Lopez & Ausio, 2007).

Furthermore, H2A.Z-containing nucleosomes have been mostly identified at the promoter regions of the genes it occupies. All yeast genes contain nucleosome free regions (NFR) that span about 150 base pairs and are about 200 base pairs upstream from the ATG start codon. In about two thirds of all genes, these NFR regions are flanked by two nucleosomes containing H2A.Z, indicating a link between the histone variant and the transcription initiation sites. This suggests that H2A.Z
could help prepare genes for transcription (Eirin-Lopez & Ausio, 2007; Guillemette & Gaudreau, 2006; Jansen & Verstrepen, 2011; Zlatanova & Thakar, 2008).

**H2A.Z deposition onto the chromosome: The SWR1 and NuA4 Complexes**

In *S. cerevisiae*, H2A.Z is deposited onto the chromatin by the SWR1 remodeling complex, which is named after its catalytic subunit Swr1. The complex recruits H2A.Z to various regions of the genome (Krogan et al., 2004). As depicted in Figure 2A, an H2A/H2B dimer is then exchanged for an H2A.Z/H2B dimer (Guillemette & Gaudreau, 2006). Furthermore, after incorporation of H2A.Z into the genome, the variant and the SWR1 complex remain co-localized (Krogan et al., 2004).

The Swr1 complex shares four of its thirteen subunits (Yaf9, Arp4, Act1 and Swc4) with the NuA4 complex (Krogan et al., 2004; Raisner & Madhani, 2006). The NuA4 complex is a histone acetyltransferase (HAT) made of ten subunits, and is found to acetylate the lysine residues on the amino terminal tail of histones H4 and H2A.Z. The catalytic subunit of NuA4 is Esa1, which is essential for cell viability in yeast and acetylates H2A.Z at lysine 14 (Eirin-Lopez & Ausio, 2007). In more complex eukaryotes, the SWR1 and NuA4 complexes seem to be fused into a single entity (Guillemette & Gaudreau, 2006; Krogan et al., 2004; Raisner & Madhani, 2006). Specifically, the Tip60 and SrCAP complexes in humans seem to be a fusion of the Swr1 and NuA4 (Raisner & Madhani, 2006).
**Figure 2: Model for H2A.Z at the promoter regions.** A shows the SWR1 complex (yellow) depositing the H2A.Z/H2A.B dimer (red/green) onto the chromosome. H2A.Z nucleosomes are depicted in red. B depicts upon gene activation, H2A.Z nucleosomes are evicted, which in turn recruits RNA polymerase II (blue) to the site. H2A.Z is thought to be acetylated prior to its eviction by the NuA4 complex. *Source:* Guillemette, B., & Gaudreau, L. (2006). Reuniting the contrasting functions of H2A.Z. Biochem Cell Biol, 84(4), 528-535.

**Relationship between H2A.Z Distribution and Transcriptional Activity**

Since H2A.Z is found at the promoter regions in a highly organized manner, it may possess a relationship with gene transcription (Eirin-Lopez & Ausio, 2007). This hypothesis was supported by an association between the carboxy-terminal domain of H2A.Z and RNA Polymerase II (Raisner & Madhani, 2006). However, a study quantifying H2A.Z at the promoters of transcriptionally active genes showed virtually no amounts of this variant. This raised the possibility that H2A.Z may be ejected from the chromatin during gene activation, facilitating the binding of RNA Polymerase II (shown in Figure 2B)(Eirin-Lopez & Ausio, 2007; Guillemette & Gaudreau, 2006; Zlatanova & Thakar, 2008). This suggests an inverse correlation between transcriptional activity and H2A.Z enrichment.

Additionally, acetylated H2A.Z at lysine-14 has been found near active promoters, while the unacetylated histone is associated with inactive genes. This
implies that acetylation of H2A.Z may play a role in preparing genes for transcription (Guillemette & Gaudreau, 2006; Zlatanova & Thakar, 2008). Another study has shown that H2A.Z is localized at a recently repressed promoter of a gene, INO1. In contrast, at either activated or long-term repressed promoters, H2A.Z was not found in significant amounts. Hence, H2A.Z may be recruited to recently repressed regions in order to rapidly reactivate them (Zlatanova & Thakar, 2008).

The variant appears to poise the promoter, which may then facilitate initiation (Eirin-Lopez & Ausio, 2007). However, a deletion of HTZ1 has shown that cells have only a slight defect in transcriptional initiation. Therefore, H2A.Z may play a role in induction, but it is not necessary for survival and may be redundant with other factors in the cell (Raisner & Madhani, 2006). For example, in the absence of H2A.Z, there is a significant increase in the requirement of the SWI/SNF nucleosome-remodeling complex, which is another transcriptional activator (Santisteban, 2000 #608). This finding is consistent with the partial redundancy of H2A.Z in gene activation.

**H2A.Z is found near centromeric regions**

As previously mentioned, the SWR1 complex in yeast recruits H2A.Z to various regions of the chromosome, including the centromere. The centromeric DNA is linked by a kinetochore complex, which serves to connect chromosomes to the spindle microtubules (Krogan et al., 2004). It has been found that H2A.Z is located adjacent to the inner kinetochore at the pericentric heterochromatin regions (Rangasamy, Berven, Ridgway, & Tremethick, 2003). Furthermore, studies in mice have shown H2A.Z interacting with HP1α and INCENP (inner centromere protein), both of which have important functions in chromosome segregation (Guillemette &
H2A.Z and the subunits of the SWR1 and NuA4 complexes have been independently linked to kinetochore components and are important for chromosome stability. It has also been found that they all have synthetic interactions with both kinetochore and mitotic spindle mutants (Krogan et al., 2004). Additionally, there is an increase in chromosome loss rates in *htz1Δ* mutants as compared to wild type cells. These findings suggest that H2A.Z may have a direct role in proper centromere function, which then affects chromosome segregation (Krogan et al., 2004; Raisner & Madhani, 2006).

**Silencing in Yeast**

Budding yeast control the expression of certain genes through silencing. This mechanism forms a condensed chromatin structure, known as heterochromatin, that inhibits expression of most genes within this domain. Silencing in *S. cerevisiae* is mediated by silent information regulator (Sir) proteins. There are three distinct silenced loci that are identified in yeast: the two mating type loci, telomeres, and ribosomal DNA repeats (Rusche et al., 2003; Sun, Hatanaka, & Oki, 2011).

In order to spread heterochromatin, the Sir proteins first interact with silencers, which serve as nucleation sites that determine which regions are silenced, and then with the histone tails of nucleosomes (Rusche et al., 2003; Sun et al., 2011). Silencing at loci in yeast correlates with decrease in histone H3 and H4 acetylation. For example, deacetylation of lysine 16 of H4 is important for the spreading of the Sir-protein complex (Shahbazian & Grunstein, 2007). Therefore, hypoacetylated
nucleosomes and continuous distributions of Sir proteins at silenced regions form the condensed and transcriptionally restrictive structure of heterochromatin. It is important to note that various studies have proved that in order to establish silencing in yeast cells, progression through the cell cycle is required (Rusche et al., 2003; Sun et al., 2011).

In *S. cerevisiae*, H2A.Z acts as a barrier to heterochromatin.

The spreading of silent chromatin is limited by the presence of barrier factors. H2A.Z has been found to act as a barrier to heterochromatin at the telomeres and *HMR* locus in yeast cells (Krogan et al., 2004). Structurally, biochemical studies have suggested that nucleosomes containing H2A.Z only partially compact into higher order structures, therefore, enhancing chromosome dynamics (Peterson & Laniel, 2004). Additionally, in the absence of H2A.Z, cells exhibit silencing beyond normal boundaries (Raisner & Madhani, 2006).

Figure 3A shows that in *htz1Δ* mutants, heterochromatin spread has been found beyond normal boundaries. Further evidence is found in studies that have shown that genes within 35 kilobases from the telomeres required H2A.Z for normal expression (Zlatanova & Thakar, 2008). Therefore, the variant is required to maintain an anti-silenced expression state and prevent the spread of heterochromatin. To observe if the repression was Sir-dependent, one of these proteins, Sir2, was deleted in an *htz1Δ* mutant. The result showed that this double deletion rescued the defective repression of the telomere-proximal genes (Dryhurst et al., 2004; Eirin-Lopez & Ausio, 2007).
Figure 3: H2A.Z acts as a barrier to heterochromatin spreading. A depicts the spreading of the Sir protein complex (orange) to adjacent nucleosomes (blue) in the absence of H2A.Z. This induces silencing of genes and formation of heterochromatin. B shows in the presence of acetylated H2A.Z (gray), there is an antagonizing effect that prohibits the spread of Sir-dependent silencing and heterochromatin. Adapted from: (Zhou et al., 2010)

It was determined that histone H4 that was acetylated at lysine 16 was important for the boundary of heterochromatin. Cells lacking H2A.Z showed a decrease in acetylated histone H4. Furthermore, mutation of the usually acetylated lysine residues on H2A.Z’s amino-terminal tail showed defective boundary formation (Eirin-Lopez & Ausio, 2007). Therefore, it can be concluded that acetylated H2A.Z prevents the spreading of Sir complexes into euchromatin (shown in Figure 3B).
Cell-cycle dependent establishment of transcriptional silencing at the telomeres is controlled by H2A.Z.

In *S. cerevisiae*, it has been found that progression through metaphase is necessary for the establishment of silencing. Cells arrested at either G1 phase or beginning of metaphase do not indicate Sir-dependent silencing at the telomeres and prevent histone modifications required for silencing. However, cells that were arrested in telophase showed silencing (Martins-Taylor et al., 2011).

In wild type cells, there are factors that act as barriers to heterochromatin spreading. H2A.Z, Scc1, and Sas2 are such factors. After their removal by either gene deletion or inactivation, yeast cells bypassed cell cycle dependent silencing at the telomeres and silencing was established during all phases. Furthermore, H2A.Z was shown to come off the genome during telophase (Figure 4) supporting that cell-cycle dependent establishment of silencing does not occur until progression though metaphase (Martins-Taylor et al., 2011).
Sister Chromatid Cohesion

All organisms need a mechanism to hold their replicated DNA together after S-phase until chromosome segregation in anaphase. The replicated chromatin copy and the original are linked together and known as sister chromatids (seen in Figure 5). During S phase, sister chromatids are identified and paired, a process called cohesion. This pairing is maintained from the establishment of cohesion to chromosome segregation by the cohesin complex (Skibbens, 2009).

The model for sister chromatid pairing is not well defined, but the cohesin complex is thought to form rings around the sister chromatids. Components of the cohesin complex include Smc1, Smc3, Scc3, and Med1/Scc1. The Scc2-Scc4 complex loads cohesin onto the chromatin during S phase (Skibbens, 2009). During

Figure 4: H2A.Z comes off the chromosome genome-wide. This figure shows a ChIP assay that indicates H2A.Z comes off the chromosome during telophase at non-telomeric loci. The genes tested are known to be associated with H2A.Z. Source: (Martins-Taylor, Sharma, Rozario, & Holmes, 2011)
anaphase, the separase enzyme is required to cleave the cohesin complex that is holding sister chromatids together. Throughout other points of the cell cycle, separase is inhibited by securin (Musacchio & Salmon, 2007).

Scc1 is one essential subunit of the cohesin complex. Both H2A.Z and Scc1 are proteins that are widely dispersed throughout the chromatin and affect DNA transcription, repair, and chromosome segregation (Krogan et al., 2004; Zlatanova & Thakar, 2008). They both also act in a similar manner to regulate cell cycle dependent establishment of silencing suggesting suggest a possible link between the two (Martins-Taylor et al., 2011).

Figure 5: Sister chromatid cohesion by cohesin. The above figure shows a single chromosome (orange) during G1 phase. After replication during S phase, there are two copies of the chromosome, called sister chromatids. They are linked by cohesin rings (green), however, the mechanism of this link is not well known. Scc1 is part of the cohesin complex. Source: Alberts, B., et. al. (2010). Essential cell biology, third edition. New York: Garland Science. Figure 18-15.
Questions Addressed in This Study

In more complex eukaryotes, the absence of H2A.Z is thought to cause genomic instability resulting in lethality. However, the deletion of the variant in *Saccharomyces cerevisiae* does not result in a lethal phenotype, allowing the study of its function. H2A.Z has been linked with kinetochore components, is important in chromosome segregation, and dissociates off the chromosome during telophase. Hence, it appears that the variant possesses a critical role during M-phase. This study aims to respond to the question: What is H2A.Z’s role in chromosome segregation?

*Do major subunits of the Swr1 complex or NuA4 complex play a role in removing H2A.Z from the chromatin?*

Our lab has discovered that H2A.Z broadly dissociates from the genome during telophase, but the removal mechanism of H2A.Z is unknown. H2A.Z is put onto the chromosome by the Swr1 complex and acetylated by the NuA4 complex. As mentioned previously, studies show an interaction between these three factors and kinetochore components, which may contribute to chromosome instability observed in cells that have mutated H2A.Z or NuA4/ SWR1 subunits. We investigated the possibility that the essential function of components in the Swr1 or NuA4 complex is the removal of H2A.Z from the chromosome.

*What are the consequences of H2A.Z inactivity in the absence of checkpoint proteins?*
In yeast, *htz1Δ* mutants are viable but have an increased rate of chromosome loss, however, the exact contribution of H2A.Z is unclear. The removal of the mitotic checkpoint proteins Mad2, Bub1, and Mcm21 were all found to have a lethal phenotype in strains with *htz1Δ* (Bandyopadhyay et al., 2010; Measday et al., 2005). This suggests that these proteins may interact in a parallel pathway. We hypothesized that loss of H2A.Z causes a defect in the cell, which is recognized and repaired by the checkpoint proteins. In the absence of these proteins, the damage is not fixed resulting in inviability. Therefore, we attempted to observe the specific consequences of H2A.Z inactivation in cells lacking the proteins Bub1, Mad2, and Mcm21.

*Does H2A.Z play a role in sister chromatid cohesion?*

H2A.Z affects chromosome segregation in an unknown way. In addition to the prior experiments in which we attempt to determine the specific defect caused by the absence of H2A.Z we also tested the specific hypothesis that H2A.Z influences sister chromatid cohesion. As previously stated, H2A.Z and the Scc1 cohesin subunit have been shown to regulate the cell cycle dependent establishment of silencing at the telomeres in a similar manner. This suggests the proteins may have related functions. The main role of Scc1 in the cell is to maintain sister chromatid cohesion. Since H2A.Z is also determined to affect chromosome segregation, this study tests the hypothesis that the variant may interact with Scc1 in sister chromatid cohesion.
Materials and Methods

Media / Plates Used

YPD media (1% Bacto yeast extract, 2% Bacto peptone extract, and 2% dextrose, solid) was used to grow most of the strains. For solid media, 2% bacto agar was added. Drugs, such as geneticin (100 µg/mL) and hygromycin (300 µg/mL), were added to the media to select for yeast colonies that contained the drug resistant genes. CuSO₄ (100 µg/mL) was added to the media of strains containing the temperature sensitive degron allele in order to ensure that HTZ1 was transcribed.

Sporulation plates (1% Potassium Acetate, 0.1% Bacto yeast extract, 0.05% Dextrose, 2% Bacto Agar) were used to sporulate cells.

SDC-HIS (0.8 % SDC-HIS Yeast Drop-Out Mix, 2 % Dextrose, 2 % Bacto agar) plates were used to select for Haploids.

Strains

A list of yeast strains that are used in this study are shown in Table 1. All strains were grown at 30 °C with the exception of YSH991, YSH1063, YSH1064, and YSH1022, which were grown at 23 °C. YSH991 and YSH1012 were gifts from Dr. Mitchell Smith and Dr. Robert Skibbens respectively.
Table 1: List of Strains Used in this Study

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>YSH474</td>
<td>his3Δ, leu2Δ, met15Δ, ura3Δ</td>
</tr>
<tr>
<td>YSH991</td>
<td>his3Δ, leu2Δ, met15Δ, ura3Δ, UBR1::HIS3, bar1Δ::NAT, htz1-ts::URA3</td>
</tr>
<tr>
<td>YSH1022</td>
<td>his3Δ, leu2Δ, met15Δ, ura3Δ, UBR1::HIS3, bar1Δ::NAT, htz1-ts::URA3, mad2Δ::HYG</td>
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<tr>
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<tr>
<td>YSH657, YSH950</td>
<td>his3Δ/his3Δ, leu2Δ/leu2Δ, met15Δ/met15Δ, ura3Δ/ura3Δ, +/Can1Δ::STE2pr, +/MFA1pr-HIS5, +/lyp1Δ, Δhtz1::kanMX/+</td>
</tr>
<tr>
<td>YSH1034</td>
<td>his3Δ/his3Δ, leu2Δ/leu2Δ, met15Δ/met15Δ, ura3Δ/ura3Δ, +/Can1Δ::STE2pr, +/MFA1pr-HIS5, +/lyp1Δ, Δhtz1::kanMX/+, Δesa1::hygMX/+</td>
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<tr>
<td>YSH1048</td>
<td>his3Δ/his3Δ, leu2Δ/leu2Δ, met15Δ/met15Δ, ura3Δ/ura3Δ, +/Can1Δ::STE2pr, +/MFA1pr-HIS5, +/lyp1Δ, Δhtz1::kanMX/+, Δmad2::hygMX/+</td>
</tr>
<tr>
<td>YSH1012</td>
<td>HIS3:LacI GFP, CLONAT:KAN:LacO:Tel IV, PDS1-12myc:TRP1</td>
</tr>
<tr>
<td>YSH1068</td>
<td>HIS3:LacI GFP, CLONAT:KAN:LacO:Tel IV, PDS1-12myc:TRP1, htz1Δ::HYG</td>
</tr>
</tbody>
</table>
Primers/ Plasmids

Table 2 shows a list of primers that were used and their function. The plasmid, pAG32 (V160), contained the \textit{HygMX} gene.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP470</td>
<td>Forward primer that binds to \textit{HTZ1} and \textit{MX}, used with SP471 to knockout \textit{HTZ1}</td>
</tr>
<tr>
<td>SP471</td>
<td>Reverse primer that binds to \textit{HTZ1} and \textit{MX}, used with SP470 to knockout \textit{HTZ1}</td>
</tr>
<tr>
<td>SP1220</td>
<td>Forward primer that binds to \textit{ESA1} and \textit{MX}, used with SP1221 to knockout \textit{ESA1}</td>
</tr>
<tr>
<td>SP1221</td>
<td>Reverse primer that binds to \textit{ESA1} and \textit{MX}, used with SP1220 to knockout \textit{ESA1}</td>
</tr>
<tr>
<td>SP1224</td>
<td>Forward primer that binds to \textit{SWC4} and \textit{MX}, used with SP1225 to knockout \textit{SWC4}</td>
</tr>
<tr>
<td>SP1225</td>
<td>Reverse primer that binds to \textit{SWC4} and \textit{MX}, used with SP1224 to knockout \textit{SWC4}</td>
</tr>
<tr>
<td>SP1228</td>
<td>Forward primer that binds to \textit{MAD2} and \textit{MX}, used with SP1229 to knockout \textit{MAD2}</td>
</tr>
<tr>
<td>SP1229</td>
<td>Reverse primer that binds to \textit{MAD2} and \textit{MX}, used with SP1228 to knockout \textit{MAD2}</td>
</tr>
<tr>
<td>SP1243</td>
<td>Forward primer that binds to \textit{BUB1} and \textit{MX}, used with SP1244 to knockout \textit{BUB1}</td>
</tr>
<tr>
<td>SP1244</td>
<td>Reverse primer that binds to \textit{BUB1} and \textit{MX}, used with SP1243 to knockout \textit{BUB1}</td>
</tr>
<tr>
<td>SP1255</td>
<td>Forward primer that binds to \textit{MCM21} and \textit{MX}, used with SP1256 to knockout \textit{MCM21}</td>
</tr>
<tr>
<td>SP1256</td>
<td>Reverse primer that binds to \textit{MCM21} and \textit{MX}, used with SP1255 to knockout \textit{MCM21}</td>
</tr>
<tr>
<td>SP1251</td>
<td>Forward primer that binds to \textit{RAD9} and \textit{MX}, used with SP1252 to knockout \textit{RAD9}</td>
</tr>
<tr>
<td>SP1252</td>
<td>Reverse primer that binds to \textit{RAD9} and \textit{MX}, used with SP1251 to knockout \textit{RAD9}</td>
</tr>
<tr>
<td>SP101</td>
<td>Reverse primer that binds to the \textit{hygMX} marker and was used to check for the knockout for any HYG-replaced gene</td>
</tr>
<tr>
<td>SP501</td>
<td>Forward primer used with SP101 or SP307 to check for \textit{HTZ1} deletion</td>
</tr>
</tbody>
</table>
Gene deletion/ knockout

To study H2A.Z the yeast genome needed to be manipulated. Using PCR-based gene disruption, strains lacking the gene HTZ1 was created.

The plasmid pAG32 contained the HygMX gene. Using primers that had homology to the 5’ and 3’ regions outside the target gene’s open reading frame, the HygMX gene was amplified. The PCR was done using one of two methods. The first utilized the standard Taq polymerase protocol. The annealing temperature was 50 ºC and the PCR was run for thirty cycles. The second method used the standard BD
protocol, which used an annealing temperature of 50 °C and was run for thirty-five cycles. Some of the fragment was run on a 1% agarose gel to make sure it was the correct size.

Using the standard yeast transformation protocol, homologous recombination allowed the wild-type cells to incorporate the PCR fragment with the HygMX gene into their genome in place of HTZ1. The cells were spread onto YPD plates containing hygromycin in order to select for the wild-type colonies that integrated the HygMX gene. After incubating at 30 °C for three to four days, the plates were removed and colonies were selected as potential candidates.

From these colonies, the genomic DNA was extracted and a Taq-polymerase PCR with the above-mentioned conditions was performed to check for the replacement of HTZ1 with the HygMX gene. A 1% agarose gel confirmed the presence or absence of the gene.

The genes ESA1, SWC4, BUB1, MAD2, MCM21 and RAD9 were also deleted in this manner using the HYG marker. In these cases, the wild-type strain contained at deletion of HTZ1 with the KanMX gene. Thus, to avoid the HYG marker replacing the KanMX gene, the cells were grown on YPD containing both hygromycin and geneticin to ensure both genes were replaced.

**Viability Assays**

To observe the possible connection of H2A.Z with other proteins, viability assays were done. A diploid strain, containing a deletion of HTZ1 with the KanMX gene was used. The gene SWC4 was deleted in the strain with the hygromycin drug
resistance marker. The strain was grown on YPD plates for 36-48 hours at 30 °C and then single colonies were streaked out on sporulation plates, which were grown at 23 °C for seven to ten days. After being sporulated for over a week, the cells were checked for tetrads. Under a light microscope, tetrads appear in a tetrahedral shape and small spores could be observed. Each set of sporulated strains contained made of about 15 to 20% spores.

Random spore analysis was done by suspending sporulated cells in 25 µL Zymolase-100T (1 mg/mL in 1M sorbitol). The cells were then incubated for thirty minutes at 37 °C to ensure sufficient digestion. 500 µL of sterile water was added to the zymolase-cell suspension and vortexed vigorously to break up the tetrads. They were then plated onto SDC – HIS plates to select for haploids. The diploid strain contained a HIS3 gene under the regulation of an MFA1 promoter. The MFA1 gene codes for a protein by the same name, which is only activated in haploid a mating type cells. The promoter activates the HIS3 gene only when the cell is of a type. In cases where the mating type is α or α/a, the promoter is not activated and the HIS3 gene will not be transcribed. Yeast require histidine to grow. Therefore, SDC- HIS plates select for haploid a type cells that would have this gene transcribed.

Forty colonies were then chosen from the plates and diluted in 100-µL of sterile distilled water. Using a multichannel pipette, they were grown onto YPD, YPD with hygromycin, YPD with geneticin, and YPD with hygromycin and geneticin. The cells were grown for two to three days at 30 °C and then analyzed. Similar to SWC4, a viability assay was done for the genes ESA1, BUB1, MAD2, MCM21 and RAD9.
Serial Dilution Assay

To test the proper functionality of the H2A.Z temperature sensitive allele, a serial dilution was done. The \textit{htz1-ts} allele was part of the strain YSH991. The strains YSH1022, YSH1063, and YSH1064 were tested in this assay and YSH474 was used as a control. Cells were grown in an overnight 2 mL culture at 23ºC in YPD media containing 0.1 M copper sulfate. One-milliliter samples of each strain were then washed from the media and re-suspended in 1 mL sterile water. The cells were then approximately counted under a light microscope and diluted according to the strain with the least amount of cells. Thus, each group had the same number of cells.

100 µL of each strain was transferred into a 96-well plate. The adjacent four columns were filled with 90 µL of sterile water and ten-fold serial dilutions were made by adding 10 µL of culture from the previous well. The cells were then spotted onto both control and selective plates using a multichannel pipette. The plates were incubated at either 23 ºC or 37 ºC and monitored over three to five days. They were then taken out and analyzed.

Cell Counting: Bud Morphology

Cell cultures in 20 mL YPD containing 0.1 M CuSO\textsubscript{4} were grown overnight at permissive temperature, 23 ºC. At least fifteen hours of growth was necessary for YSH1064 (YSH991 \textit{bub1Δ}). The optical density \((\text{OD}_{600})\) was checked using a spectrophotometer and the cells were diluted to 0.10. None of the cells had an \text{OD}_{600} greater than 1.00. Next, the cells were divided into two different flasks with 10 mL of freshly diluted cells in each. One half of the cells were washed from the YPD + 0.1M
CuSO₄ media and 10 mL of YPD was added. The cells containing the YPD media were transferred to non-permissive temperature, 37 °C, while the others remained at 23 °C as a control.

After waiting two hours to ensure complete H2A.Z degradation, 50 µL samples were collected every twenty minutes for eighty minutes, and fixed with 50 µL of cell fixation solution (3.7% Formaldehyde, 10 mM Tris-HCl pH 8, 100 mM EDTA pH 8). Before viewing, the cells were sonicated at setting 2 for ten seconds. Five microliters of the cells were then put on a slide and observed under a light microscope. The bud morphologies of the yeast cells indicated whether they were in G₁ (no bud), S (small bud), or G₂/M (large bud) phase. Cells that had a bud larger than half of its body were considered to be in G₂/M phase. A total of 200 cells were counted for this experiment for the strains YSH474, YSH991, YSH1064, YSH1063, and YSH1022.

**FACS Analysis**

Yeast cells were prepared for flow cytometry using a protocol adapted from (Hutter & Eipel, 1978). FACS samples were harvested similarly to the bud morphology experiment and the same strains were used. Samples were grown to log phase at 23 °C in YPD + CuSO₄ media. The cells were then separated into two groups, one that remained at 23 °C in YPD + CuSO₄ media and one that was washed of YPD +CuSO₄, re-suspended in YPD, and incubated at 37 °C. After waiting two hours, 1 mL samples were harvested every thirty minutes. They were washed in 0.2 M TRIS-HCL buffer, pH 7.5, fixed in 70 % ethanol, and directly stored at 4 °C.
On the day of FACS, fixed cells were collected by microfuging at 7000 rpm for 2 minutes. The samples were washed with TRIS Buffer and re-suspended in TRIS Buffer containing 1 mg/mL RNAse. After incubating for one hour at 37 °C, the cells were washed and re-suspended in pepsin solution (0.05% pepsin in 0.55N HCl). They were then incubated for five minutes at room temperature. After washing with TRIS Buffer, 1 mL of staining buffer (0.18 M TRIS-HCl pH 7.5, 0.19 M NaCl, 70 mM MgCl₂) containing 50 µg/mL propidium iodide was added to the cells and cells were kept on ice until analyzed.

FACS analysis was performed using a FACS Caliber instrument at the Yale Cell Sorter Facility. Data was analyzed using CellQuest and FlowJo software and data presented was created by the Dean/Jet/Fox model.

**Sister Chromatid Cohesion: Slide making and Microscope Use**

To observe sister chromatid cohesion, the strain YSH1012 was used, which contained a fusion protein GFP-Lac repressor and Lac operator arrays located at telomere IV (source: Skibbens’ Lab Group). The GFP-Lac repressor binds to the lac operator arrays, allowing visualization of sister chromatids at this site. YSH1068 had an identical genotype to YSH1012, except it had a deletion of HTZ1 with *HygMX*. Creation of the slides and immunostaining were done following a protocol by Upasna Sharma adapted from Dr. Amy MacQueen (Rockmill, 2009).

A 10 mL culture was grown at 30°C to log phase (OD₆₀₀ 0.2-0.4). The culture was then split into two 5 mL cultures and the OD₆₀₀ of each one was equalized to 0.2. One culture was blocked in G₁ by using α-factor (10 µg/mL) and the other in G₂/M
by using nocodazole (15 µg/mL). After waiting between two and three hours for α-factor and around six hours for nocodazole, the cells were checked for a minimum of 85% cell cycle arrest in G₁ and G₂/M respectively.

To fix the cells in their current state, freshly prepared 37% paraformaldehyde was added to a concentration of 4%. This was carried out at room temperature for 1 hour to 1.5 hours on a rotor drum. The cells were then washed with 1 mL 1% KAc /1M sorbitol solution (3000 rpm for 4-5 minutes). The pellet was re-suspended in 500 µL 1% KAc/ 1M sorbitol solution, 10 µL 1M DDT and 20 µL of 10 mg/mL Zymolyase. The suspension was then incubated at 37 ºC with constant agitation for 30 minutes. To stop digestion, 100 µL of cold 1X MES/ 1M sorbitol solution was added to the cells. After spinning, the cells were washed in 1 mL of 1X MES/ 1M sorbitol. Finally, the pelleted cells were re-suspended in 80 µL of cold 1X MES solution and 200 µL of 1% paraformaldehyde. The cells were immediately spread over a glass slide and stored at -20ºC.

The slides then underwent immunostaining using the mouse c-myc antibody to bind to Pds1-13myc and Alexa Fluor® 568 anti-mouse as a secondary antibody. Pds1 is known as the protein securin, which arises in the cell during S-phase and degraded after metaphase. The staining of Pds1 ensured that the chromosomes had not segregated. The slides were then stained with Dapi mount and were looked at under a microscope. Softworx software, in conjunction with the Deltavision RT imaging system (Applied Precision) and adapted to the Olympus (IX70) microscope was used to obtain Z-stacked images. Independent experiments were performed for each cell in order to collect data. Cells were counted based on the number of GFP
spots seen, most commonly one spot (establishment of sister chromatid cohesion) or two spots (loss of sister chromatid cohesion). To eliminate experimenter’s bias, data was collected without the experimenter’s knowledge of the strain being analyzed. At least 78 cells for each strain were analyzed and those that showed two nuclei or no securin co-localization were excluded from the data.
Results

H2A.Z is found to have an important role during mitosis. In this study, we observe the consequences of \textit{HTZ1} deletion to investigate what removes H2A.Z off chromatin, to observe its interactions between mitotic proteins, and to determine whether it plays a role in sister chromatid cohesion.

\textbf{H2A.Z removal is not an essential role of the SWR1 or NuA4 complexes.}

Our lab has shown that H2A.Z dissociation from chromatin occurs during anaphase. However, the removal mechanism of the variant is unknown. We investigate whether the SWR1 or NuA4 complexes are involved in H2A.Z removal from chromatin. One of the SWR1 complex’s functions is to exchange the H2A/H2B dimer for a H2A.Z/H2B dimer, hence adding H2A.Z into the nucleosome. The SWR1 complex has many components, one being the protein Swc4. The deletion of the gene encoding the protein, \textit{SWC4}, results in a lethal phenotype. Since the SWR1 complex loads H2A.Z onto the chromatin, another function could be to unload it. Perhaps the lethality of cells lacking Swc4 is related to the inability to exercise its function of removing H2A.Z. Thus, a double deletion of both \textit{HTZ1} and \textit{SWC4} could result in a rescue of the lethality.

To test this, a diploid strain was used so that deletion of an essential gene could be possible. The diploid strain contained a deletion of \textit{HTZ1} with the \textit{KanMX} gene. \textit{SWC4} was deleted with the \textit{HygMX} gene using PCR-based gene disruption. The
A diploid strain was then sporulated. The strain had a haploid specific promoter in front of a HIS3 gene, which selects for cells of a mating type on SDC-HIS plates.

Forty haploids that grew on the SDC-HIS plates were chosen to be tested. Each haploid colony that was chosen was diluted in 100 µL of sterile distilled water. Using a multichannel pipette, the cells were spotted on four different plates: YPD, YPD containing hygromycin, YPD containing geneticin, and YPD with both hygromycin and geneticin. Theoretically, all haploid cells should grow on YPD. YPD with geneticin selected for colonies that had a successful deletion of HTZ1 and double deletion of HTZ1 and SWC4. Thus, 50% growth should be seen if the double deletion is viable and only 25% growth should be seen if the double deletion is inviable. YPD with hygromycin selected for colonies that had a successful deletion of solely SWC4 and the double deletion of the gene with HTZ1. It was expected to get 25% growth of colonies if the double deletion was nonlethal and 0% growth if the double deletion was lethal. Finally, YPD containing hygromycin and geneticin selected for cells that had the double deletion of HTZ1 and SWC4. If the double deletion rescued the lethal phenotype, 25% growth should be seen while no growth should be seen if the deletion was lethal. The result of this assay is portrayed by Figure 6.B.

Figure 6.B.i shows that all forty colonies grew successfully on YPD. A mating assay showed that all the colonies mated, indicating the original cells were all haploids (results not shown). Figure 6.B.ii shows that 35% of the yeast colonies grew on YPD containing the drug geneticin. Both YPD containing hygromycin and YPD containing hygromycin and geneticin had 0% growth as shown in Figure 6.B.iii and 6.B.iv. These results suggest that the essential function of Swc4 and the SWR1
complex in general is not limited to removing the H2A.Z/H2B dimer off the chromosome, but rather has another important role in the cell.

Figure 6: Results of the viability assay for $htz1\Delta$ $swc4\Delta$ double mutant haploids. YSH1037 was sporulated to produce four genetically distinct haploids [A]. The haploids were then grown on YPD [B.i], YPD + geneticin [B.ii], YPD + hygromycin [B.iii], and YPD + geneticin + hygromycin [B.iv] plates. The percentages were calculated for each plate and areas where only a single colony grew were not considered as growth.

The NuA4 complex has similar subunits to the SWR1 complex in yeast, including Swc4. These complexes are fused in more complex eukaryotes. The main function of this complex in the cell is to acetylate H2A.Z. However, it may also be a candidate for removal of H2A.Z off the chromosome because of the similar subunits to the SWR1 complex. Furthermore, one model depicts that H2A.Z eviction from the nucleosome is a result of acetylation by NuA4 (Guillemette & Gaudreau, 2006). One essential component of the NuA4 complex is the protein Esa1, coded by the gene $ESA1$. A deletion of the gene was inserted into the starting diploid strain mentioned.
previously and created the strain YSH1034. The same method was used in the viability assay as described with the Swc4, and results are portrayed in Figure 7.B.

![Diagram of the viability assay](image)

Figure 7: Results of the viability assay for \(htz1\Delta esa1\Delta\) double mutant haploids. YSH1034 was sporulated to produce four genetically distinct haploids [A]. The haploids were then grown on YPD [B.i], YPD + geneticin [B.ii], YPD + hygromycin [B.iii], and YPD + geneticin + hygromycin [B.iv] plates. The percentages were calculated for each plate and areas where only a single colony grew were not considered as growth.

Figure 7.B.i again shows that all forty colonies grew on YPD. The mating assay proved that the colonies were haploids since all the colonies mated (results not shown). Figure 7.B.ii shows that 12.5% of the yeast colonies grew on YPD with geneticin. Areas that showed single colony growth were not included in the percentage. Both YPD with hygromycin and YPD with both the hygromycin and geneticin had 0% growth as shown in Figure 7.B.iii and 7.B.iv respectively. These
results also suggest that the essential function of Esa1 and the NuA4 complex is not to remove H2A.Z off the chromosome.

**Linking H2A.Z to Mitotic Checkpoint Proteins**

Previous studies have shown that H2A.Z comes off chromatin by telophase, implying that there may be an important role for the variant during anaphase. Additionally, since $htz1\Delta$ mutants show both chromosome loss and instability, chromosome segregation during anaphase specifically may be affected. Prior reports suggested that double deletion of $HTZ1$ and certain genes encoding mitotic proteins, such as $BUB1$ (Bandyopadhyay et al., 2010), $MAD2$, and $MCM21$ (Measday et al., 2005), result in lethality of the cell. Bub1 and Mad2 are both components of the mitotic spindle assemble checkpoint, and Mcm21 is a component of the kinetochore involved in chromosome maintenance.

**In the Holmes’ Lab strain background, $\Delta bub1 \Delta htz1, \Delta mad2 \Delta htz1$, and $\Delta mcm21$ $\Delta htz1$ mutants are inviable.**

Bub1 and Mad2 are both part of the spindle assembly checkpoint and Mcm21 is a component of the kinetochore. Since H2A.Z is important in chromosome segregation, the absence of both the histone variant and mitotic proteins may be lethal in the cell. To examine whether previous results that found double deletions with $HTZ1$ and $MAD2$, $BUB1$, or $MCM21$ were true, tests for viability were done. $RAD9$, which codes for a protein that is involved in recognizing DNA damage at all checkpoints (Longhese, Foiani, Muzi-Falconi, Lucchini, & Plevani, 1998), was
included in addition to \textit{BUB1}, \textit{MAD2} and \textit{MCM21} to observe if the Rad9 protein also followed a parallel pathway with H2A.Z.

To determine this, viability assays were done. The diploid strain strategy from the \textit{Esa1/Htz1} and \textit{Swc4/Htz1} viability assays was used in these experiments as well. All of the genes tested were deleted with the \textit{HygMX} gene in diploids heterozygous for the \textit{HTZ1} gene using PCR-based gene disruption. Yeast colonies that incorporated the desired knockouts into their genome were sporulated for seven to ten days. All the cells were comprised of at least 15% of spores before undergoing zymolase treatment. Haploids of mating type \textit{a} were selected on SDC-His plates. Forty haploids that grew on these plates were selected for the viability assay. The cells were diluted and spotted on YPD and selective plates using a multichannel pipette.

The strain \text{YSH1035} had the gene \textit{BUB1} deleted. Theoretically, there should be 100% growth on the YPD plates. YPD plates with the geneticin drug selected for \textit{Δhtz1} mutants and \textit{Δhtz1 Δbub1} mutants. If the double deletion were inviable, there would be an expected 25% growth on the plate. On the contrary, if the double deletion was viable there should be 50% growth on the plate. The same growth percentage is expected for YPD plates with the hygromycin drug, which selected for \textit{Δbub1} mutants and \textit{Δbub1 Δhtz1} mutants. To select solely for \textit{Δbub1 Δhtz1} mutants, cells were grown on YPD plates containing the drugs, geneticin and hygromycin. The expected result if the double deletion was lethal was no growth on these plates and 25% growth if the deletion was nonlethal. These results are shown in Figure 8.B.
The strains YSH1048, YSH1040, and YSH1036 corresponded to \( \Delta \text{mad2} \), \( \Delta \text{mcm21} \), and \( \Delta \text{rad9} \) respectively. The same theoretical outcome was expected for all three strains and results are shown in Figures 9.B, 10.B and 11.B.

**Figure 8: Results of the viability assay for htz1\( \Delta \) bub1\( \Delta \) double mutant haploids.** YSH1035 was sporulated to produce four genetically distinct haploids [A]. The haploids were then grown on YPD [B.i], YPD + geneticin [B.ii], YPD + hygromycin [B.iii], and YPD + geneticin + hygromycin [B.iv] plates. The percentages were calculated for each plate and areas where only a single colony grew were not considered as growth.

All strains had complete growth on YPD plates as shown in Figure 8.B.i, 9.B.iE, 10.B.i, and 11.B.i. YSH1035 (\( \Delta \text{bub1}/\text{BUB1} \ \Delta \text{htz1}/\text{HTZ1} \)) haploids had 32.5% growth and 20% growth on YPD with geneticin and YPD with hygromycin respectively (Figure 8.B.ii and 8.B.iii). The plate selecting for solely \( \Delta \text{htz1}\Delta \text{bub1} \) mutants showed 2.5% growth (Figure 8.B.iv). A mating assay done showed that all the haploids grew on YPD. It was thought that the one colony that grew on YPD with
hygromycin and geneticin may have had a mutation somewhere else in the genome that rescued the lethal phenotype. Another error that may have occurred is that the HygMX gene incorporated into another area of the chromosome. Therefore, although there was one colony that grew on the Δbub1 Δhtz1 selective plate, it was most likely not the genotype expected. It was concluded that the double deletion was lethal in the cell.

Figure 9: Results of the viability assay for Δhtz1Δ mad2Δ double mutant haploids. YSH1048 was sporulated to produce four genetically distinct haploids [A]. The haploids were then grown on YPD [B.i], YPD + geneticin [B.ii], YPD + hygromycin [B.iii], and YPD + geneticin + hygromycin [B.iv] plates. The percentages were calculated for each plate and areas where only a single colony grew were not considered as growth.

The haploids of YSH1048 (Δmad2/MAD2 Δhtz1/HTZ1) had 20% growth and 50% growth on YPD with geneticin and YPD with hygromycin plates respectively (Figure 9.B.ii and 9.B.iii). Figure 9.B.iv shows that only one colony completely grew on this plate. These haploids were grown for five days instead of two to three days like the rest of the strains. Thus, more growth was seen on YPD with hygromycin and
geneticin. Furthermore, thirteen of the colonies that had minimal growth on YPD with hygromycin and geneticin showed no growth on YPD with geneticin, and were thus excluded as being counted as viable double deletions. Three slow growing colonies on the selective plate also grew on both YPD with solely hygromycin and YPD with solely geneticin. Since a mating assay was not done for this strain, these three colonies may have been diploids. Additionally, they may have had another mutation that suppressed the lethal phenotype or the HygMX gene incorporated at another location in the genome. Therefore, these cells may not be the exact genotype they are thought to be. Despite these three colonies, in general a double deletion of MAD2 and HTZ1 was also lethal in the cell.

**Figure 10: Results of the viability assay for \( \text{htz1}\Delta \text{mcm21}\Delta \text{double mutant haploids.} \)** YSH1040 was sporulated to produce four genetically distinct haploids [A]. The haploids were then grown on YPD [B.i], YPD + geneticin [B.ii], YPD + hygromycin [B.iii], and YPD + geneticin + hygromycin [B.iv] plates. The percentages were calculated for each plate and areas where only a single colony grew were not considered as growth.
YSH1040 (Δmcm21/MCM21 Δhtz1/HTZ1) haploids showed a similar phenotype to both YSH1035 and YSH1048 haploids. They had 25% growth on YPD containing geneticin, about 50% growth on YPD containing hygromycin, and 2.5% growth on YPD containing both drugs (Figure 10.B.ii, 10B.iii, and 10.B.iv respectively). Therefore, this double deletion was also lethal in yeast.

YSH1036 (Δrad9/RAD9 Δhtz1/HTZ1) haploids showed 35% growth on YPD with geneticin, 58% growth on YPD with hygromycin, and 25% growth on YPD with both drugs (Figure 11.B.ii, 11.B.iii, and 11.B.iv respectively). These values corresponded to the theoretical percentages when the double deletion did not result in lethality. A mating assay ensured that all of the colonies were haploids. Therefore, it was concluded that the double deletion of RAD9 and HTZ1 was nonlethal.

**Figure 11: Results of the viability assay for htz1 Δ rad9 Δ double mutant haploids.** YSH1036 was sporulated to produce four genetically distinct haploids [A]. The haploids were then grown on YPD [B.i], YPD + geneticin [B.ii], YPD + hygromycin [B.iii], and YPD + geneticin + hygromycin [B.iv] plates. The percentages were calculated for each plate and areas where only a single colony grew were not considered as growth.
The viability assay demonstrated that the absence of all the proteins except Rad9 were lethal when combined with a deletion of \textit{HTZ1} in the Holmes’ Lab strain background. Rad9 plays a major role in the DNA damage checkpoint (Longhese et al., 1998). Since the double deletion of \textit{RAD9} and \textit{HTZ1} is not synthetically lethal, perhaps H2A.Z does not play a role in DNA damage. Instead, there may be some parallel pathway specifically during chromosome segregation between checkpoint proteins and H2A.Z.

**Immediate effects of on cell upon inactivation of H2A.Z in checkpoint deficient strains**

Perhaps a loss of H2A.Z causes a defect in chromosome segregation that mitotic checkpoint proteins are required to repair. When the cells lose these proteins, the cell may not be able to correct the problem resulting in lethality. We wished to determine the specific defect caused by loss of H2A.Z. To achieve this a strain containing a temperature sensitive allele of H2A.Z, YSH991, was used. The gene \textit{HTZ1} is under a copper promoter, which activates the gene only in the addition of copper sulfate. Furthermore, in front of \textit{HTZ1} there is a heat inducible degron fused to the gene, which will cause H2A.Z to undergo degradation at non-permissive temperature (37 °C). Thus, cells growing on plain YPD media incubated at 37°C will be depleted of H2A.Z. A deletion of \textit{BUB1}, \textit{MAD2}, or \textit{MCM21} was incorporated into this strain creating YSH1064, YSH1022, and YSH1063 respectively. Unlike the strains in the viability assay, in this case we are able to control the degradation of H2A.Z in the cell and study the effects of its absence by using this temperature
dependent allele. By examining the cells soon after H2A.Z loss we hoped to
determine the specific defect in chromosome segregation caused by H2A.Z’s absence.

In order to test for the effectiveness of the temperature sensitive strain, a 10X
serial dilution assay was done to attempt to re-create the lethal phenotype observed in
the viability assays. To do this, cells were grown overnight at 23 °C in YPD-CuSO₄
media. During this time, it was observed that YSH1064 (Δbub1) was slow growing
and needed to grow for at least 15 hours to get an OD₆₀₀ of about 0.2. The cultures
were then adjusted so that each strain started with the same amount of cells. Five 10X
serial dilutions were done and using a multichannel pipette, spotted onto two sets of
YPD-CuSO₄ and YPD plates. One set of each plate was incubated at 23 °C
(permissive temperature) and the other set at 37 °C (non-permissive temperature).
YSH474 was used as a control. It was expected that at non-permissive temperature,
the strains lacking the mitotic proteins would not grow, since H2A.Z would be absent.
This would confirm that the degron was functioning correctly. The result is shown in
Figure 12.
YPD+CuSO$_4$ at 23ºC

Wild type

*Htz1-ts*

*Htz1-ts Δbub1*

*Htz1-ts Δmad2*

*Htz1-ts Δmcm21*

YPD at 37ºC

Wild type

*Htz1-ts*

*Htz1-ts Δbub1*

*Htz1-ts Δmad2*

*Htz1-ts Δmcm21*

YPD at 23ºC

Wild type

*Htz1-ts*

*Htz1-ts Δbub1*

*Htz1-ts Δmad2*

*Htz1-ts Δmcm21*

YPD +CuSO$_4$ at 37ºC

Wild type

*Htz1-ts*

*Htz1-ts Δbub1*

*Htz1-ts Δmad2*

*Htz1-ts Δmcm21*
Figure 12: 10 X Serial dilution assay shows some function of the degron allele. The first and second columns represent two separate trials of this assay. The first row represents the control conditions of the degron strains, the second row represents the degron strains at non-permissive temperature, and the third and fourth rows represent other controls. The wild type was the strain YSH474. The htz1-ts is YSH991. The genes encoding for mitotic proteins are shown as the bottom three dilutions.

The results of this assay were not as expected. The H2A.Z degron strain grew the same amount as the wild type at 23 °C and grew less at 37 °C. This is consistent with the observation that cells that exhibit a deletion of HTZ1 grow slower than normal. YSH1064 (htz1-ts ∆bub1) shows significantly less growth on all plates. It has been seen that ∆bub1 mutants are extremely slow growing but perhaps, they were also sensitive to any change in function caused by the degron attached to H2A.Z. Both YSH1022 (htz1-ts ∆mad2) and YSH1063 (htz1-ts ∆mcm21) show slightly less growth as compared to the wild type at 23 °C. At 37 °C, both strains have even less growth. Both trial one and two show that there is less growth when compared to the wild type and htz1-ts strain. However, the phenotype is not as concrete as the viability assays suggest, since there is still growth. A reason for this may be the time it takes for the H2A.Z-degron to be depleted and fully nonfunctional allows for some cells to continue growing. This is not completely consistent with a Western done showing that it takes about two hours for H2A.Z to be degraded in the degron strain (Figure 13). Therefore, perhaps the copper promoter is still allowing some H2A.Z to be transcribed despite the absence of copper sulfate.
Figure 13: Western blot analysis of YSH991 (htz1–ts) grown at permissive temperature and non-permissive temperature. Copper sulfate was removed from half of the culture that was being shifted to non-permissive temperature. Samples were taken at 80 minutes and every 40 minutes after that. Tubulin served as a control. The amount of H2A.Z at 37 °C is very minimal. Source: Upasna Sharma, 2010, Unpublished Data.

Exploring the consequence of H2A.Z inactivation and checkpoint protein deletions.

Although the serial dilution assay did not give ideal results, it still showed a stunted growth defect in the htz1-ts strains. Since Mcm21, Mad2, Bub1, and H2A.Z all affect the cell during metaphase, maybe upon inactivation of H2A.Z, defects in the cell cycle will be observed. For example, the absences of both proteins may immediately cause an accumulation of cells in one part of the cell cycle. To observe this, a bud morphology experiment that quantified cells at G1, S, and G2/M stages in the cell cycle was done. This would allow us to define if there is a certain time after H2A.Z removal where cells start to exhibit slower growth at one stage of the cell cycle.

The first step was to grow cells to log phase in YPD-CuSO4 media at 23 °C. The cells were then diluted to an OD600 of 0.1. Half of each culture was removed and washed of the YPD-CuSO4 media and re-suspended in YPD media. This culture was
put at 37 °C. After allowing two hours for H2A.Z to be degraded, 50 µL samples were taken and fixed every twenty minutes. The cells were then sonicated at a low frequency to remove any adhesion. Under a light microscope, two hundred cells were counted as G1, S, or G2/M. The G1 cells had no buds; the S cells had small buds; the G2/M cells had large buds (Figure 14). Buds that were larger than half of the body were considered G2/M. Results of each mutant strain at G2/M phase are shown in Figures 15, 16, and 17.

![Cell Cycle Diagram](image)

**Figure 14: Bud morphologies of S. cerevisiae at different stages of the cell cycle.** Depicted above is a map of the cell cycle. During G1 (blue) phase, the yeast cell appears round and no replication of DNA has occurred. S-phase (red) is where a small bud starts to appear, and DNA becomes replicated. After complete replication, the cell enters G2/M (purple/green) phase. The cells appear as largely budded. Source: Alberts, B., et. al. (2002). Molecular biology of the cell, fourth edition. New York: Garland Science. Figure 17-3.
Figure 15: Graphs depicting the percentage of YSH1064 (Δbub1 htz1-ts) and controls during G2/M phase from the bud morphology assay. The percentage of cycling cells at G2/M at both permissive temperature (left) and non-permissive temperature (right) is shown. The x-axis represents the time the sample was collected after two hours of growing at non-permissive temperature. Samples were taken every twenty minutes. The y-axis shows the percent of cells that were large budded and counted as G2/M phase. The green line is the experimental strain, YSH1064, the light blue line is YSH991, and the dark blue line is YSH474.

Figure 16: Graphs depicting the percentage of YSH1022 (Δmad2 htz1-ts) and controls during G2/M phase from the bud morphology assay. The percentage of cycling cells at G2/M at both permissive temperature (left) and non-permissive temperature (right) is shown. The x-axis represents the time the sample was collected after two hours of growing at non-permissive temperature. Samples were taken every twenty minutes. The y-axis shows the percent of cells that were large budded and counted as G2/M phase. The green line is the experimental strain, YSH1022, the light blue line is YSH991, and the dark blue line is YSH474.
Figure 17: Graphs depicting the percentage of YSH1063 (Δmcm21 htz1-ts) and controls during G$_2$/M phase from the bud morphology assay. The percentage of cycling cells at G$_2$/M at both permissive temperature (left) and non-permissive temperature (right) is shown. The x-axis represents the time the sample was collected after two hours of growing at non-permissive temperature. Samples were taken every twenty minutes. The y-axis shows the percent of cells that were large budded and counted as G$_2$/M phase. The green line is the experimental strain, YSH1063, the light blue line is YSH991, and the dark blue line is YSH474.

We expected to see a significant change in cell cycle progression, such as a sudden buildup of cells in one phase and a drastic decrease in another phase. However, this was not observed in any of the three strains mutant strains as compared to wild type and the original htz1-ts strain. Furthermore, there was no change in the cell cycle between permissive and non-permissive temperatures. These inconclusive results suggest there may have been a problem with the sensitivity of the bud morphology assay. Thus, FACS analysis was done as a more sensitive assay in determining cells at different stages of the cell cycle.

FACS samples were collected in a similar manner as the bud morphology assay. Samples were grown at 23 °C to log phase (OD$_{600}$ 0.2-0.4) in YPD-CuSO$_4$ media and diluted to an OD$_{600}$ of 0.1. Half the cells were washed, re-suspended in YPD, and shifted to 37 °C. FACS samples were then taken every thirty minutes after
waiting two hours for H2A.Z to be completely degraded in the cell. The collected cells were washed in Tris-HCl buffer and fixed in 70% ethanol. The samples were prepared and stained for FACS and then taken to be analyzed. A representative result of YSH1022 is shown in Figure 18.

**FACS Analysis for YSH1022 (htz1-ts, Δmad2)**

![FACS Analysis](image)

![FACS Analysis](image)

**Figure 18: Representative FACS analysis for YSH1022.** FACS analysis was carried out as a more sensitive assay to see if there is a change in the cell cycle. The samples were taken four hours after the cells were shifted to 37 °C and DNA content was measured by propidium iodide staining. The y-axis shows the cell count and the x-axis is the DNA content. The Dean/Jett/Fox mathematical model is shown as the green line. The above picture shows FACS analysis at permissive temperature and the below picture shows analysis at non-permissive temperature.
FACS analysis of YSH1022 showed an unexpected phenotype. At 23 ºC the control cells should have shown about 70% cells in G\textsubscript{1} phase and the rest in S phase and G\textsubscript{2}/M phase. A study that showed FACS in Δmad2 cycling cells showed this type of phenotype (Lai, Seki, Ui, & Enomoto, 2007). However, the cycling cells show a high percentage of G\textsubscript{2}/M phase, which is not consistent with findings where cells lacking Mad2 are mitotic arrest defective (Straight, Belmont, Robinett, & Murray, 1996). The \textit{htz1-ts Δbub1} and \textit{htz1-ts Δmcm21} mutants also had similar phenotypes, while wild type and \textit{htz1-ts} showed a small percentage increase in G\textsubscript{1} (results not shown). Even in the controls, the cycling cells always had less than fifty percent of cells in G\textsubscript{1}.

As compared to permissive temperature cells where H2A.Z was present, H2A.Z degraded cells did not show an accumulation at another part of the cell cycle. This analysis was more sensitive than the bud morphology observation. However, other factors could have influenced this result. The degron-ts allele attached to H2A.Z may have caused improper function at permissive temperature resulting in similar results as the non-permissive temperature. Another possibility is that at non-permissive temperature, H2A.Z may have not been entirely degraded resulting in a similar phenotype as the permissive temperature. H2A.Z may continue to be transcribed at non-permissive temperatures in the absence of CuSO\textsubscript{4} as a result of a “leaky” copper promoter. These factors could be resolved by using a different temperature sensitive allele of H2A.Z.

The H2A.Z degron may not be the only problem in this assay. The experimental setup may have affected the results. Perhaps there was not a long
enough time period to see any dramatic changes in the cell. Therefore, taking samples at a later time may show effects on the cell. The inability of reproducing the original synthetic lethal phenotype observed using the degron allele both on plates and in cultures caused the data of this experiment to be inconclusive.

**H2A.Z’s influence on sister chromatid cohesion**

In *Saccharomyces cerevisiae*, the cohesin complex is bound to chromosomes in metaphase but lost from chromosomes during anaphase (Guacci, 2007). Separse is an enzyme that cleaves the complex at the Scc1 subunit. Recent data shows that H2A.Z and an essential cohesion subunit Scc1 play a similar role in cell cycle dependent silencing at the telomeres. Therefore, since H2A.Z has been shown to play a role in chromosome segregation, one role may be in sister chromatid cohesion. A sister chromatid cohesion assay was done in the absence of H2A.Z at the telomeres.

To test the possibility of cohesion loss, cohesion was monitored in the absence of H2A.Z. A strain that had a GFP-Lac repressor fusion protein bound to Lac operator arrays located around 9.7 kilo-bases from the telomere of Chromosome IV was used to visualize sister chromatid cohesion (YSH1012). A diagram of this is shown in Figure 19A. Cells were observed not only in metaphase, but also in G₁ in order to check for aneuploidy. Nocodazole was used to arrest cells in metaphase, while α-factor was used to block cells in G₁. The slides were looked at without the experimenter’s knowledge of the strain being observed. The number of GFP spots in the cell was then determined. To ensure the metaphase-arrested cells had not entered anaphase, Pds1 staining was used. Pds1 codes for the protein, securin, which is
produced during S-phase and rapidly degraded at the onset of anaphase.

Representative cells are shown in Figures 20 and 21.

**Figure 19: Experimental Design of Sister Chromatid Cohesion Assay.** [A] shows the Lac operator array new telomere IV. A Lac repressor fused to a GFP marker binds to the array illuminating the area. [B] shows the difference of one spot and two spots. When the sister chromatids are attached by cohesin rings, only one spot and two spots. When there is cohesion failure, two GFP spots will be seen, since they’ll be far away from each other.
Figure 20: Pds1 staining shows cells are pre-anaphase but have their DNA replicated. The efficiency of Pds1 is shown above. Cells arrested in G\textsubscript{1} phase and had Pds1 staining [A] show no presence securin. Cells arrested in M-phase [B] show securin co-localization to the DNA in the nucleus. Most cells showed this kind of staining. Both cells were taken from the wild type strain. Cells that were in G\textsubscript{2}/M that showed no co-localization of Pds1-staining and DAPI were not included in the data collection since they were post-metaphase cells. Additionally, only large budded cells were counted in metaphase-arrested slides.
Figure 21: GFP spots in metaphase cells. The difference between one spot and two spots was observable by GFP. [A] shows a representative cell showing one GFP spot and DNA-Securin co-localization and indicates proper sister chromatid cohesion. A representative cell with two GFP spots and DNA-Securin co-localization indicates that the sister chromatids are separated [B]. Both of these cells were taken from the \( \Delta htz1 \) mutant strain. Only large budded cells were counted in metaphase-arrested slides. Occasionally, there would be a cell that had two spots touching on the flattened image. These were be counted as separated sister chromatids.

Figure 20A shows the Pds1 staining in a G\(_1\) arrested cell. As expected, there is no co-localization with the nucleus because securin should appear during S-phase. Figure 20B shows a large budded cell arrested in G\(_2\)/M that has Pds1 co-localization with the DNA mass, which is indicative of pre-anaphase cells. Cells arrested in G\(_2\)/M that did not show Pds1-DNA co-localization were not included as well as cells that had more than one DNA mass. For G\(_2\)/M, a total of 81 cells were counted in the \( \Delta htz1 \) mutant (YSH1068) and 85 cells were counted in wild type (YSH1012). Very few cells exhibited more than two spots and were included in the overall cell count. For
G₁, a total of 97 cells were counted in YSH1068 and 78 cells were counted in YSH1012. The majority of G₁ arrested cells exhibited a single GFP spot showing minimal aneuploidy in the early-cell cycle. Additionally, most cells exhibited a co-localization of securin and DNA, confirming that G₂/M arrested cells had not entered anaphase. Figure 21 depicts the difference between one spot (A) and two spots (B) in G₂/M arrested cells. The percentage of two GFP spots is shown in Figure 22.

![Sister Chromatid Cohesion at the Telomere](image)

**Figure 22:** Sister chromatid cohesion is lost at the telomeres in cells lacking H2A.Z. This figure shows an increase in the percentage of separated sister chromatids at telomere IV when HTZ1 is deleted as compared to wild type cells (YSH1012). The x-axis shows what strains were used. The y-axis shows the percent of cells with two GFP spots, which represents the percent of separated sister chromatids. Light blue bars correspond to G₁ arrested cells and royal blue bars correspond to G₂/M arrested cells. YSH1068 shows a significant increase of sister chromatid cohesion loss at the telomere.

Figure 22 shows that about 2.6% of wild type cells and 5.2% of Δhtz1 mutants had two spots at G₁. At G₂/M, 5.8% of wild type cells exhibited loss of sister
chromatid cohesion, which was not a significant difference compared to the G1 cells. In contrast, 38.3% of cells lacking H2A.Z exhibited separated sister chromatids. Fisher’s exact test showed a p-value less than 0.0001. Thus, at metaphase arrest, Δhtz1 mutant cells show a significant increase of the percentage of two GFP spots as compared to the wild type cells.

Another sister chromatid cohesion assay had been done to monitor cohesion at the centromere in the absence of H2A.Z. The result shows that at the centromere there is a significant loss of sister chromatid cohesion in cells lacking H2A.Z as compared to wild type (Figure 23).

\[\text{Sister Chromatid Cohesion at the Centromere}\]

\[\text{Figure 23: Sister chromatid cohesion is lost at the centromere in the absence of H2A.Z. This figure shows that there is an increase in the percentage of separated sister chromatids at Centromere V in the absence of H2A.Z as compared to the wild type cells. The x-axis is the type of strain used. The wild-type strain was YSH1015. The y-axis is the percent of separated sister chromatids, which were cells that exhibited two spots instead of one spot. G1 shows a small percentage of two spots in the cell. Both Δhtz1 deletion strains show significant increase of sister chromatid cohesion loss at the centromere. Source: Upasna Sharma, 2012, Unpublished Data.}\]
It was concluded that sister chromatid cohesion is affected by H2A.Z at the centromere. This result is consistent with the telomere cohesion assay and it may be suggested that premature loss of cohesion occurs throughout the length of the chromosome and at different chromosomes.
**Discussion**

H2A.Z is an important histone variant in all organisms; general roles of the variant include maintaining genomic integrity, acting as a barrier to silencing, and activating genes. However, despite being widely studied, the specific functions and mechanisms of H2A.Z have remained unclear. This study investigated what the consequences in the cell were upon deletion of HTZ1. Studies have shown that the deletion of HTZ1 results in chromosome loss as compared to wild type cells. This role is conserved across many species. For example, cells lacking H2A.Z in fission yeast have observed increased chromosome instability (Guacci, 2007; Krogan et al., 2004) and mammalian H2A.Z plays a role in chromosome segregation (Krogan et al., 2004). These functions of H2A.Z inspired our lab to study the role of H2A.Z in mitosis.

**The sole essential function SWR1 or NuA4 complex is not to remove H2A.Z.**

Mutations in H2A.Z, the SWR1 complex, and the NuA4 complex result in defects in chromosome segregation. This is consistent with interactions observed between kinetochore proteins and these three macromolecules (Krogan et al., 2004). H2A.Z has been found to dissociate off the chromosome during telophase (Martins-Taylor et al., 2011). However, what complex does this and the mechanism by how this occurs remains unclear. Since H2A.Z and the NuA4 and SWR1 complexes may have a common pathway that regulates centromere function (Krogan et al., 2004), the dissociation of H2A.Z off the chromatin may be related to one of these two complexes.
Through viability assays, we showed that the essential function of the NuA4 and SWR1 complexes is not to take H2A.Z off the chromatin. Despite this, these complexes may still play a role in removing H2A.Z, however, they have additional essential functions that lead to lethality.

**INO80 is structurally similar to the SWR1 complex and plays a role in removal of H2A.Z.**

Another nucleosome remodeling complex that is similar to the SWR1 complex is the INO80 complex. It was originally found to be involved in H2A.Z eviction at double strand breaks (van Attikum, Fritsch, & Gasser, 2007). Furthermore, a deletion of the gene encoding the catalytic subunit, *INO80*, resulted in H2A.Z being mis-localized throughout the genome, causing genomic instability. A recent study showed that it is involved in H2A.Z eviction. In fact, *ino80Δ htz1Δ* mutants show synthetic lethality. The mechanism for eviction in not known, but the authors of this study suggest two possible untested models. The first one suggests that the SWR1 complex incorporates an excess amount of H2A.Z near a target promoter. INO80 may remove excess H2A.Z and reinforce the deposition at the promoter region (Papamichos-Chronakis, Watanabe, Rando, & Peterson, 2011). The second model stems from the finding of INO80 association with replication forks, contributing to stability and elongation. At this location, INO80 may remove H2A.Z that was mis-localized during chromatin assembly. This case also suggests that INO80 promotes the SWR1 complex to deposit H2A.Z in the proper location (Papamichos-Chronakis et al., 2011). Overall, although it was found that the NuA4 and SWR1 complex were
not solely responsible for removal of H2A.Z, the similar INO80 complex may do this function.

**Cells lacking H2A.Z are sensitive to the spindle assembly checkpoint.**

This study found that Δhtz1 Δbub1, Δhtz1 Δmad2, and Δhtz1 Δmcm21 mutants all resulted in synthetic lethality of the cell. The viability assay allowed us to observe this in a more rigorous way as compared to large-scale studies. On the other hand, Δhtz1 Δrad9 mutants did not exhibit this phenotype. Rad9 is an important protein in the DNA damage checkpoint. This is consistent with a result where mutations in genes encoding DNA damage checkpoint proteins have not seen any synthetic growth defects in the absence of H2A.Z (Dhillon, Oki, Szyjka, Aparicio, & Kamakaka, 2006). This provides evidence that H2A.Z does not play a role in DNA damage, but rather chromosome segregation. In the absence of H2A.Z, cells exhibit defects in chromosome segregation, which may be repaired partially by the mitotic checkpoint proteins.

Using a temperature dependent allele of H2A.Z, we attempted to define the specific defect caused by the absence of H2A.Z. However, through serial dilution assays, bud morphology and FACS analysis, we were unable to define these pathways. Since the serial dilution assays did not show the expected synthetic lethality as observed in the viability assays, the temperature sensitive HTZ1 allele might have been insufficiently unstable at non-permissive temperature.

To test the functionality of the htz-ts strain, deletions of just the mitotic proteins should be done in the wild-type background. A serial dilution assay of these
strains compared to the temperature dependent allele may show whether solely the deletions of the mitotic proteins were causing a growth defect or if H2A.Z inactivation had some role. Additionally, another strain containing an experimentally proven functioning *HTZ1* temperature dependent allele may be used to observe if similar results are obtained.

If the synthetic lethality in the cell is reproduced using a temperature sensitive allele, the FACS analysis and bud morphology experiments may be replicated. If the samples are taken for a longer time period, perhaps the cells will show a defect in cell cycle position or aneuploidy. Cells lacking Bub1 exhibited very slow growth as compared with wild type, cells lacking Mad2, and cells lacking Mcm21, making it hard to study. Therefore, it may be excluded from this analysis, since cells may already be sick.

**H2A.Z is important to maintain sister chromatid cohesion.**

Genomic integrity is reliant on flawless chromosome segregation. Sister chromatid cohesion is one way to ensure that chromosomes separate efficiently and correctly. Sister chromatids prematurely separating could lead to aneuploidy or chromosome loss (Diaz-Martinez, Gimenez-Abian, & Clarke, 2008).

We have found that in the absence of H2A.Z there is a loss of sister chromatid cohesion at the telomere and centromere. Hence, H2A.Z affects sister chromatid cohesion throughout the whole length of the chromosomes. Therefore, it can be concluded that at least one of the roles H2A.Z plays in chromosome segregation is in sister chromatid cohesion. Our lab also has observed that an unacetylable mutant of
H2A.Z results in a loss of sister chromatid cohesion, which indicates that sister chromatid cohesion is dependent of H2A.Z acetylation (Sharma, 2011, unpublished data).

Although both Scc1 and H2A.Z have been shown to come off the chromosome during telophase, a ChIP assay on metaphase cells has shown that cells lacking H2A.Z do not show less Scc1 association with chromatin at the centromere (Sharma, 2011, unpublished data). Therefore is not clear H2A.Z whether loss of sister chromatid cohesion is an indirect or direct effect of the histone variant. Another study in our lab using an H2A.Z degron allele attributed a direct role of H2A.Z in sister chromatid cohesion. Thus, H2A.Z is thought to maintain cohesion during metaphase (Sharma, 2011, unpublished data).

**How may H2A.Z affect cohesin?**

H2A.Z is found to maintain sister chromatid cohesion but upon deletion of *HTZ1*, Scc1 binding to chromatin is not affected. Both electron microscopy and biochemical studies have shown that cohesion forms a ring-like structure and Scc1 controls the rings opening and closing (Guacci, 2007). One way H2A.Z may affect sister chromatid cohesion is by actively interacting with and stabilizing the ring. Alternatively, H2A.Z may inhibit factors that destabilize sister chromatid cohesion.

Recent studies have shown that condensin has a role in the removal of sister chromatin cohesion (Cuylen & Haering, 2011; Vas, Andrews, Kirkland Matesky, & Clarke, 2007). Condensin is enriched at the chromosome arms and the kinetochore. It is required for the assembly of compacted chromatin and plays a role in chromosome segregation (Cuylen & Haering, 2011; Tada, Susumu, Sakuno, & Watanabe, 2011). A
study in fission yeast has shown that condensin association with the chromatin depends on an interaction with H2A.Z (Tada et al., 2011). Therefore, perhaps H2A.Z is indirectly affecting cohesin through its interaction with condensin. In general, our lab has found that H2A.Z is important in maintaining sister chromatid cohesion. However, the specific process of how this occurs is unknown.

**Future Directions**

To further investigate the function of H2A.Z in chromosome segregation, we would like to conduct experiments based on the data we collected. We would like to first determine what takes H2A.Z off the chromosome and refine the checkpoint protein analysis. We then hope to extend our study on how H2A.Z affects sister chromatid cohesion.

The INO80 complex is thought to remove H2A.Z off the chromosome (Papamichos-Chronakis et al., 2011). It would be beneficial to determine if this occurs during telophase and what subunits of the INO80 complex are involved. Studies suggest that H2A.Z is acetylated and then evicted from the nucleosome during gene activation (Guillemette & Gaudreau, 2006). Studies have noted that the INO80 complex takes off unacetylated H2A.Z, however, it is unclear whether it takes off the acetylated version of the variant (Papamichos-Chronakis et al., 2011). Therefore, it would be interesting to test if the INO80 complex is involved in the removal of acetylated H2A.Z.

In this study we found synthetic lethality between deletions of genes encoding checkpoint proteins and H2A.Z. To investigate the role of H2A.Z in
chromosome segregation, the experiments testing specific defects of the cell as it
starts to lose H2A.Z should be repeated. Because we did not see conclusive data on
the serial dilution assay, bud morphology analysis, and FACS analysis, a different
and functional htz1-temperature sensitive allele should be used during repeats for our
experiments. These experiments may then show a shift in cell cycle position or
aneuploidy in the strains lacking the mitotic checkpoints proteins, indicating what
type of defect occurs as H2A.Z is lost.

We want to determine the influence of H2A.Z on sister chromatid cohesion.
Since Scc1 is found in abundance at the centromere, an association with chromatin
even after H2A.Z removal may not be surprising. However, Scc1 is not found overly
abundant at telomeres (Martins-Taylor et al., 2011). Thus, it is important to observe
whether Scc1 is present at the telomeres in the absence of H2A.Z through the use of a
chromatin immunoprecipitation (ChIP) assay. If we see a decrease in the enrichment
of Scc1 at the telomeres, the cohesin ring may be prematurely removed in the absence
of H2A.Z resulting in cohesion failure. On the contrary, no change in the enrichment
of Scc1 at the telomeres may provide evidence against the current static ring model,
where the cohesin complex does not dissociate from the sister chromatids until it is
cleaved during anaphase.

It has been shown that in *S. pombe*, H2A.Z interacts with condensin, which is
required for normal sister chromatid cohesion. Therefore, an assay to determine
whether the deletion of *HTZ1* results in the loss of condensation of the chromosome
should be done. If we see a loss in chromosome condensation, H2A.Z may affect
sister chromatid cohesion through an interaction with condensin proteins.
Furthermore, studies have shown that in *S. cerevisiae* chromosome decondensation may be dependent on Mad2 (Vas et al., 2007). This suggests that both H2A.Z and Mad2 have crucial roles in chromosome condensation, which may define their specific parallel pathway.

In this study we observed the role of H2A.Z in chromosome segregation in *S. cerevisiae*. Although the specifics are unclear, H2A.Z and mitotic checkpoint proteins have a parallel function, which is essential in cell viability. We have also demonstrated that H2A.Z is important in sister chromatid cohesion throughout the length of the chromosome. This study has provided a foundation for further exploration of this histone variant’s function in chromosome segregation.
References


