University of Arkansas, Fayetteville
ScholarWorks@UARK

Theses and Dissertations

5-2009

Understanding the Role of Histones during Chromosome Segregation: A Study of the effects of Histone Gene Dosage in Saccharomyces cerevisiae

Rebecca Leigh Danforth
University of Arkansas, Fayetteville

Follow this and additional works at: http://scholarworks.uark.edu/etd

Part of the Molecular Biology Commons

Recommended Citation
Danforth, Rebecca Leigh, "Understanding the Role of Histones during Chromosome Segregation: A Study of the effects of Histone Gene Dosage in Saccharomyces cerevisiae" (2009). *Theses and Dissertations*. 42.
http://scholarworks.uark.edu/etd/42

This Dissertation is brought to you for free and open access by ScholarWorks@UARK. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of ScholarWorks@UARK. For more information, please contact scholar@uark.edu, ccmiddle@uark.edu.

UNDERSTANDING THE ROLE OF HISTONES DURING CHROMOSOME SEGREGATION: A STUDY OF THE EFFECTS OF HISTONE GENE DOSAGE IN *SACCHAROMYCES CEREVISIAE*

UNDERSTANDING THE ROLE OF HISTONES DURING CHROMOSOME SEGREGATION: A STUDY OF THE EFFECTS OF HISTONE GENE DOSAGE IN *SACCHAROMYCES CEREVISIAE*

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Cell and Molecular Biology

By

Rebecca Leigh Danforth
University of Arkansas
Bachelor of Science in Chemistry, 2004

May 2009
University of Arkansas

ABSTRACT

Chromatin plays a role in all cellular functions that involve DNA. These include, but are not limited to replication, recombination, transcription, and chromosome segregation. Chromosome segregation is an extremely well conserved cellular process and is essential for maintaining the genetic integrity of a cell. There is very strong evidence indicating that chromatin structure is critical for maintaining the fidelity of chromosome transmission, but its specific role(s) in this process remains unclear. Chromatin is comprised of arrays of nucleosomes that serve to compact DNA. These nucleosomes consist of 146 bp of DNA that is wrapped around a histone octamer; two each of histones H2A, H2B, H3, and H4.

The overall goal of this project has been to elucidate and understand the function of histones during chromosome segregation. Previous work has shown that a mutation in histone H2A, *hta1-300* can cause both increase in ploidy and increase in chromosome loss, and that these defects correlate with an altered chromatin structure at the centromere.[1] Suppressor analysis of this allele has identified a mutation in one of the two genes that encode histone H3 (*hht1*) is able to suppress the increase in ploidy phenotype.[2] This suppression has been confirmed by deletion of the *hht1* allele, and it has also been found that deleting the accompanying histone H4 allele (*hhf1*) suppresses the increase in ploidy caused by *hta1-300*. A new phenotype for the *hta1-300* allele has been identified through mass spectrometry and western blotting; there is a marked increase in acetylation of lysine 12 of histone H4 (H4K12) in strains carrying the *hta1-300* allele. Interestingly, the *hht1Δ* allele has a decrease in acetylation on H4K12. To further characterize these mutations at the centromere in order to understand their function in chromosome

segregation, chromatin immunoprecipitation was done using an antibody against H4 acetylated at lysine 12. The increase in acetylation caused by *hta1-300* was observed around the centromere, but not the decrease in acetylation caused by the *hht1Δ* allele.

In contrast to these data, increasing the expression of *HHT1, HHF1*, or the gene pair results in severe growth phenotypes. Overexpression of the single genes in the presence of *hta1-300* leads to a synthetic sickness, whereas overexpression of both leads to cell death. Previous work described an increased rate of chromosome loss as a result of high copy H3-H4 in a WT background,[3] suggesting an additive effect of chromosome instability as a cause for the inviability of the H2A mutant strain.

Taken together, these results stress the sensitivity of the *Saccharomyces cerevisiae* cell to histone gene dosage and histone pair stoichiometry. The data presented here suggest that histone modifications are altered in the H2A mutant and deletion of either H3 or H4 genes suppresses by restoring a balance in histone modifications. Also, these data support hypotheses that for proper cell function, histone genes must be stoichiometrically balanced as well as stoichiometrically balanced in their modifications across chromatin and that histone gene ratio has a function in the maintenance of histone post-translational modifications.

This dissertation is approved for
Recommendation to the
Graduate Council

Dissertation Director:

____
Dr. Inés Pinto

Dissertation Committee:

____
Dr. Jeannine M. Durdik

____
Dr. Kenneth L. Korth

____
Dr. Wesley E. Stites

DISSERTATION DUPLICATION RELEASE

I hereby authorize the University of Arkansas Libraries to duplicate this dissertation when needed for research and/or scholarship.

Agreed ________________________________
Rebecca L. Danforth

Refused ________________________________
Rebecca L. Danforth

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. Inés Pinto for her outstanding guidance. She was immensely helpful and patient in my learning process. I cannot imagine having a better advisor for my graduate education. Thank you.

I would also like to thank the other members of our research group both past and present, including Dr. David McNabb for all of their helpful criticism. I could not have gotten this far without someone to listen to me through my frustrations and successes. I especially want to acknowledge Marsha Rhoads for her help in completing the ChIP experiments as well as for keeping the lab running and all of the infinite wisdom she has provided.

I would like to thank the members of my advisory committee for keeping me on course.

Credit also needs to go to the University of Arkansas and to the Graduate School for providing me with the resources to get where I am today and for being the place I will always think of as home.

Lastly, I would like to thank parents. They have always been supportive of my education and I am eternally grateful. Their support, both emotional and financial, has made this process much easier. I also want to thank my dad for leading me to science and never leading me to believe that girls aren't smart enough.

This dissertation has been brought to you by the letter "H."

TABLE OF CONTENTS

I. LITERATURE REVIEW 1
- A. Overview of chromosome segregation 2
- B. Chromatin 6
- C. Histones 8
- D. Histone modifications 11
- E. *ARP4* and the NuA4 Complex 18
- F. Centromere 20
- G. Kinetochore 25
- H. Histone genetics 31

II. INTRODUCTION 34

III. METHODS 40
- A. Yeast strains, genetic methods, and media 40
- B. Bacterial strains 40
- C. Construction of overexpression plasmids 44
- D. Construction of histone gene deletions 49
- E. Construction of *ARP4* temperature sensitive mutants 49
- F. Canavanine assay for ploidy 50
- G. Flow Cytometry 51
- H. Growth curves 51
- I. Chromosome loss and recombination assay 52
- J. Mass spectrometry 53
- K. Histone purification 53
- L. Whole cell extracts and western blotting 54
- M. Preparation of nuclei and Indirect-end labeling analysis 56
- N. Chromatin immunoprecipitation 57

IV. RESULTS 59
- A. Overexpression of H3/H4 genes in *hta1* mutant strains causes synthetic dosage sickness and synthetic dosage lethal phenotypes 59
- B. Development of H3 and H4 gene deletions 64
- C. H3 and H4 gene deletions do not suppress the increased-in-ploidy phenotype 67
- D. H3 and H4 gene deletions do not suppress the increased rate of chromosome loss 69
- E. Chromatin structure remains altered at the centromere 70
- F. Whole histone protein amounts are not altered 72
- G. Histone H4 has altered amounts of acetylation on lysines 5 and 12 73
- H. Chromatin immunoprecipitation shows an overall increase in H4 acetylation at lysine 12 in *hta1-300* mutants 77
- I. Construction of *ARP4* temperature sensitive mutant 80
- J. An *ARP4* mutant suppresses the cold sensitive and increase-in-ploidy phenotypes of *hta1-300* 81

V. DISCUSSION 84

VI. REFFERENCES 91

I. LITERATURE REVIEW

During mitosis, the sister chromatids of a cell must separate and move to opposite ends of the cell to allow for cell division, thus maintaining the genetic integrity of both mother and daughter cells. This process has been termed chromosome segregation. The chromatids are pulled apart by microtubules, which attach to large proteinacious structures called kinetochores. Kinetochores are located at the centromeric regions of each chromatid and facilitate the attachment of the microtubule and the separation of the chromatid pair. This is a very dynamic process that is highly conserved evolutionarily across all eukaryotes and is essential to cell division as well as gene conservation. Improper chromosome segregation can lead to loss of genetic material or to excess chromosomes in a given cell (increase in ploidy). The precise molecular details of this process remain a challenging mystery within the fields of genetics and cell biology. This dissertation will outline the process of chromosome segregation as it is currently understood. Specifically, it will outline what is currently known of the role(s) of histones, a class of proteins responsible for DNA compaction, in that process using the model organism *Saccharomyces cerevisiae*, or, commonly, budding yeast.

In all eukaryotes, proper chromosome segregation is necessary for propagation of genetic information. Chromosome missegregation events can lead to increase in ploidy and chromosome loss, resulting in polyploidy or aneuploidy, respectively. The continuance of life, whether simple or complex, absolutely requires proper chromosome replication and segregation.[4] Aneuploidy, a hallmark of tumor cells,[5] can be deleterious to cells not only due to the potential loss of genetic information, but also because alterations in chromosome number can disrupt gene dosage, and thus cellular functions.[6]

In mammalian cells, polyploidy later in life most often results in tumor development,[7] but polyploidy events early in development most frequently are inviable, though occasionally result in severe birth defects,[8] including trisomy 21 (Down's syndrome).

Yeast are ideally suited for studies of chromosome segregation because aneuploidy and polyploidy are not always lethal events, though frequently they are accompanied by a delay in the cell cycle. *Saccharomyces cerevisiae* can exist in either a haploid (one copy of each chromosome) or a diploid (two copies of each chromosome) and can switch between the two through the mating and meiotic processes.[9] Yeast has a fully sequenced genome,[10] grows easily and rapidly in culture, and has fully developed methods for genetic manipulation.[9] For chromosome segregation studies, *S. cerevisiae* has a biological system that is much simpler than its metazoan counterparts, providing an ideal model for the study and understanding of the basic molecular machinery required for chromosome segregation and cell division.[11, 12]

A. Overview of chromosome segregation

During cell division, cells first must replicate their entire genome; this is called the S phase of the cell cycle. The cell then proceeds into the G2 phase in which materials required for spindle formation and chromosome segregation are assembled. Sister chromatids are also 'glued' together so that they remain properly paired. This is followed by mitosis: the first step is prophase in which chromosomes condense and microtubules begin to form. The nuclear envelope then dissolves (prometaphase) and microtubules begin a "search and capture"[13] process in which they oscillate back and forth from the spindle to the region where chromosomes are gathered until a chromosome is 'captured' through the binding of the microtubule to the kinetochore.[14] A kinetochore is a large

proteinacious structure that builds on the centromere of each chromosome and serves as a link between the chromosome and the microtubules. Once all of the chromosome pairs are bound to a microtubule at each centromeric region, they are aligned such that the sister chromatids face opposite spindle poles (metaphase). During anaphase, the centromeres lose adhesion and the sister chromatids are pulled apart. Finally, the nuclear membrane reforms, the spindles are dissolved (telophase), and the mother and daughter cells can completely separate from one another and proceed into the G1 phase in which both cells actively grow. These events must happen in that sequence to maintain a full copy of the genetic information in both mother and daughter cell. It is important to note here that, in budding yeast, the nuclear envelope never dissolves, but instead the nucleus itself divides by fission, a process called endomitosis.

Understanding this process in full molecular detail has been one of the key problems in biology for decades, but has also been one of the slowest progressing ones due to the complexity of the process as well as the lack of molecular and microscopic tools, which have only been developed relatively recently.[14] A full understanding of the mechanisms of chromosome segregation will require the identification of all of the protein and DNA components of both centromeres and kinetochores and each of their specific functions in the process.[12] This has been a daunting task since as many as 75 different protein subunits of the kinetochore can be identified in even the simplest of organisms.[14]

The *cis*-acting centromere,[15] in conjunction with the *trans*-acting inner kinetochore (which binds to the centromere), outer kinetochore (which binds to the microtubule), and central kinetochore (which serves as a linker between the inner and

outer domains) are components known to be required for accurate chromosome segregation. The centromere of budding yeast can be defined as the DNA sequence necessary and sufficient for accurate chromosome segregation.[12] However, in most organisms, the centromere is a defined region of the chromosome with no specific sequence that is either necessary or sufficient. Regardless of centromere complexity, a single kinetochore must assemble on each centromere; missing kinetochores or multiple kinetochores lead to defective chromosome segregation.[16] The kinetochores must be oriented in a bipolar fashion such that the kinetochores of a sister chromatid pair are facing opposite poles. This is aided by the fact that sister chromatids are bound together by a protein called cohesin immediately following S phase.[17] Once proper orientation and kinetochore assembly have been achieved, the kinetochore can then capture an oscillating microtubule and if the mitotic checkpoint senses no damage, chromatid cohesion is lost, and mitosis may proceed.[12] It is well known that the fidelity of chromosome segregation depends on both the interactions between centromeres and kinetochores and the interactions between kinetochores and microtubules, but the mediation and regulation of these interactions are still unclear.[18]

As long as there are unattached kinetochores, the mitotic checkpoint holds the cell in mitotic arrest until proper attachment may be achieved.[19] It is known that the mitotic checkpoint is comprised of the yeast *MAD* and *BUB* genes which are silenced only when all chromatid pairs have achieved bipolar attachment.[20] This process is amazingly efficient and it is unclear how chromosome missegregation events occur in cells in which the mitotic checkpoint genes have not been disrupted. It is possible that the cell reaches a point during mitotic arrest at which it releases the arrest in a survival attempt. It may also

be possible that there are some mutations that create too many errors for the checkpoint to correct, thus causing a phenotypic loss of the checkpoint, resulting in chromosomal instability.

Since it is still unclear exactly what the checkpoint is sensing, the mitotic checkpoint has also been referred to as the spindle assembly checkpoint, the metaphase checkpoint, and the kinetochore attachment checkpoint.[4] The study of this checkpoint has been a key focal point of many cancer researchers since chromosomal instability and aneuploidy can be found in nearly all tumor types, resulting in the loss or gain of whole chromosomes as well as whole regions of chromosomes.[21] It has been suggested that there is a fundamental link between chromosome segregation and tumorigenesis, though it remains unclear if aneuploidy arises early, marking a starting point for tumorigenesis, or if it arises late as a result of a general breakdown of cell cycle control.[22] A few recent studies have found that chromosome instability events occur very early in human breast, bladder, and aggressive prostate cancers.[23-25] In addition to aneuploidy in cancer, there is growing evidence that chromosome missegregation during development can lead to birth defects, and many aneuploidy events are known to be embryonic lethal when they occur in early development.[23]

A recent review by Y. Wang[23] has outlined how chromosome segregation studies in yeast have implications to the study of human cancer. Wang hypothesizes that loss or gain of chromosomes can lead to either down-regulation of tumor-suppressor genes or up-regulation of oncogenes. Since yeast is a genetically tractable model organism, and chromosome segregation is a conserved cellular process, studies in yeast are able to lay a foundation for the understanding of human mitosis.[23] Since *S. cerevisiae* has one of the

simplest versions of a very complex cellular machine, it is sensible to work toward a full understanding of its kinetochore and apply that knowledge to the kinetochores of more complex organisms.[26] Yeast currently has the most well characterized kinetochore[27] and is an ideal model for understanding the kinetochore-microtubule binding mechanism because it attaches only a single microtubule.[28] Also, the yeast centromere is very small and lacks the long heterochromatic regions and inverted repeats that accompany the centromeres of more complex organisms, making it more suited to genetic manipulations and the study of the basic units of chromosome segregation.[2]

B. Chromatin

Before discussing further the process of chromosome segregation, it is necessary to first discuss the nature and arrangement of the chromosomes themselves. All eukaryotic chromosomes contain immense amounts of genetic material that must be packaged into a comparably small nucleus. This packaging problem was described rather distinctly by Peterson and Laniel[29] when they wrote: "Imagine trying to stuff about 10,000 miles of spaghetti inside a basketball. Then if that was not difficult enough, attempt to find a unique one inch segment of pasta from the middle of this mess, or try to duplicate, untangle and separate individual strings to opposite ends. This simple analogy illustrates some of the daunting tasks associated with transcription, repair and replication of the nearly two meters of DNA that is packaged into the confines of a tiny eukaryotic nucleus." Not only must the DNA be packaged, but all cellular processes that involve DNA must have access to chromatin. Usually structural modifications must be made such that the DNA is accessible to the cellular machinery required for DNA replication, recombination, transcription, and repair.

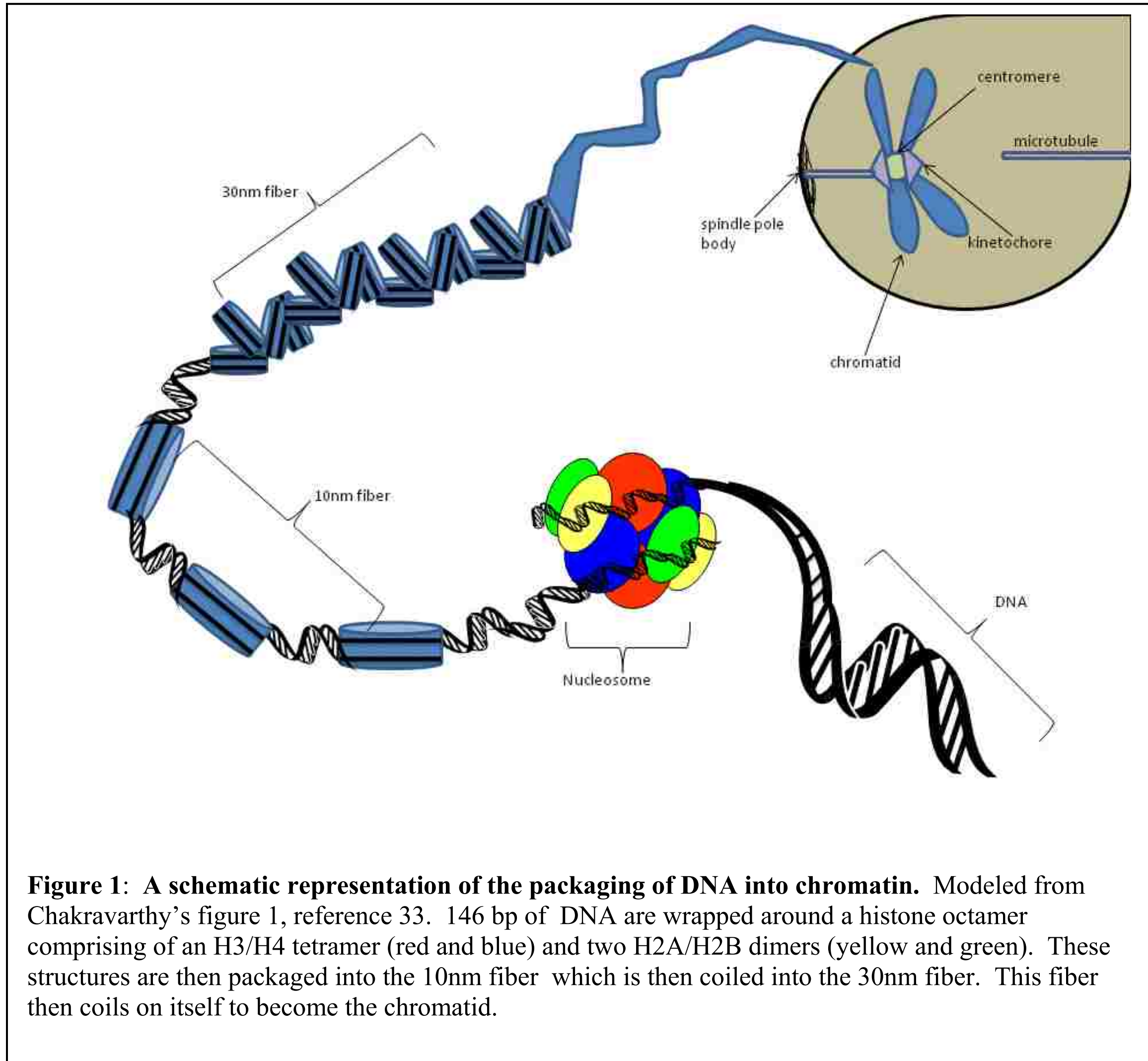


Figure 1: **A schematic representation of the packaging of DNA into chromatin.** Modeled from Chakravarthy's figure 1, reference 33. 146 bp of DNA are wrapped around a histone octamer comprising of an H3/H4 tetramer (red and blue) and two H2A/H2B dimers (yellow and green). These structures are then packaged into the 10nm fiber which is then coiled into the 30nm fiber. This fiber then coils on itself to become the chromatid.

In 1975 Oudet *et al.* presented evidence that chromatin structure is comprised of a repeating unit that they described as "beads on a string".[30] Those 'beads' are now known as nucleosomes, which are small protein discs that DNA (the 'string') wraps around. These nucleosomes are separated by 10-60 bp of linker DNA[29] and coil to form an array along the DNA that is about 11nm in diameter.[31] This array is then supercoiled into a 30nm fiber,[32] which winds upon itself into the microscopic unit known as a chromosome (see **Figure 1**[33]). The nucleosome is comprised of 146 bp of DNA wrapped 1.7 times around a histone octamer, comprised of a histone H3/H4 tetramer flanked by two

H2A/H2B dimers. Nucleosomes serve to package the DNA while still allowing access for gene transcription and DNA replication and repair.[31] In addition to the electromicrographs showing beads on a string, nucleosomes were also identified by digestion of chromatin with nucleases resulting in approximately 200-bp ladders[34] as well as by centrifugal isolation of 11.5S nucleoprotein complexes.[35] It has long been thought that the behavior of chromatin is a direct result of the properties of these nucleosomes.[32] Chromatin is now known to consist of DNA and histones, as well as a plethora of other protein complexes that assist in the DNA-related cellular functions. Histones play both structural and functional roles in these processes, which include replication, repair, recombination, transcription, and segregation.[36]

C. Histones

Histones consist of a globular carboxy-terminal domain and an unstructured amino-terminal tail. The central core of each of the proteins consists of a histone fold domain (a long α-helix known to mediate protein-protein interactions), flanked by shorter helices and loops that electrostatically interact with DNA.[37] The N-terminal tails range from 15 to 35 amino acids that extend outward from the nucleosome particle.[38] Histone tails are subject to almost every known protein modification, and these modifications are thought to alter histone-DNA, histone-histone, and inter-nucleosome interactions such that the structure of chromatin is altered, allowing access of cellular machinery, as well as serving as markers or flags that signal for the recruitment of other cellular machinery. Though originally thought to be a static building block of chromatin, it is now known that nucleosomes and, as such, histones are highly dynamic structures that can be regulated by posttranslational modifications and enzymatic functions.[35] Interestingly, even with their

highly dynamic nature, histones are among the most invariant proteins known.[37] Histones from yeast have long been known to be nearly identical to those of chicken and bovine calf,[39] and that similarity is now known to extend through all eukaryotes.

Though histones were first characterized over a century ago,[40] their abnormal biochemical behavior has greatly hampered their study. Each histone is small (11-16 kDa) and positively charged, and purified histones can be found in a wide variety of aggregates: individually as well as in various complexes with one another and with DNA.[37] The view of the nucleosomal unit comprised of a histone octamer wrapped twice by 146 bp of DNA was not proposed until 1974,[41] and not confirmed until the nucleosome crystal structure was published in 1997,[42] although immense amounts of data in support of this hypothesis were published in the intervening years (reviewed by Kornberg[37]).

The dynamic nature of histones is surprising given the fact that there are more than 120 direct atomic interactions between histones and DNA; in fact, histones are able to 'slide' along the DNA over rather large distances.[43] The interactions are primarily tight hydrogen bonds between the amide of the protein and the phosphate oxygen of the DNA.[44] The structure of histones directly regulates their function, as even the slightest alteration to the core can abolish proper function. It is now clear that the core of histone structure is primarily responsible for the compaction of DNA into chromatin and the amino-terminal tails are responsible for interactions with non-chromatin proteins, dictating the recruitment of and interactions with DNA replication and transcription machinery. The histone tails do not contribute significantly to the structure or stability of nucleosomes, but they do function to control the folding of individual nucleosomes into

the higher-order chromatin structures.[29] Most current research on chromatin structure and histones is focused on post-translational modifications to the N-terminal tails of histones, mostly in their relationship to transcription.

Histone proteins are known to be essential to cellular function and each of the histones are transcribed from two separate genes in yeast, more in higher eukaryotes, making genetic analysis difficult. However, it is possible to disrupt one of the two genes without causing excessive damage to the cell, and the genes may be put under the control of conditional promoters, allowing depletion or overexpression of the proteins.[15, 45-47]

The requirement for histones in DNA replication and segregation has been shown by experiments in which cells were depleted for histone protein, resulting in arrest at the G2-M transition point.[45, 46] It is important for cells to have an equal ratio of H3:H4 and H2A:H2B. Histone stoichiometry has long been shown to be important in mitosis, transcription, cell cycle, stress response, and gene inactivation.[47] It has been shown that overexpressing the genes encoding either H2A and H2B or H3 and H4 causes an increase in chromosome loss and an altered chromatin structure.[3] Deletion of genes encoding H2A and H2B can affect mitosis and cellular stress response.[48] Also, it has been shown that kinetochore integrity is dependent on maintaining a normal ratio of H3:H4.[49]

Though most histone depletion events result in cellular arrest at the G2/M boundary, experiments using synchronously growing cells show that the first lethal events caused by histone depletion occur in S phase, indicating a defect in DNA replication.[50] During DNA replication, histones already on chromatin are randomly separated onto the lagging and leading strands at the replication fork, and new histones are deposited almost immediately behind the replication fork.[51] Histone genes are

transcribed in late G1 and early S phases such that an abundant supply of histones are available for deposition as the DNA is synthesized.[51] However, even a slight excess of histones at this step has been shown to disrupt synthesis since histones have such a high affinity for DNA.

An imbalance in histone stoichiometry has also been shown to alter the sensitivity of chromatin to digestion by nucleases, specifically micrococcal nuclease (MNase).[52] Yeast centromeres were originally defined by a 150-160 bp region of DNA protected from digestion by these nucleases.[34] Depletion of either H2B or H4, as well as a mutation in H2A, have been shown to disrupt the nuclease resistant centromeric region.[1, 11, 15] Though it is not surprising that altering the histone balance has effects on the chromatin structure as a whole, the implication made by altering centromeric chromatin structure is that this plays an important role in chromosome segregation.[15] This phenotype is unique to histone mutants, as a variety of cell cycle mutants, nuclear division mutants, and stationary phase mutants were found to cause no change in chromatin structure at the centromere even though they share other phenotypes with histone mutants.[53]

D. Histone modifications

As discussed above, histone N-terminal tails are subject to a variety of post-translational modifications that include acetylation, phosphorylation, methylation, ribosylation, ubiquitylation, and sumoylation. These modifications have been shown to have functions in a variety of cellular processes. All changes in chromatin that do not involve a change in DNA sequence (*i.e.* DNA methylation and histone tail modifications) have been defined as epigenetics.[36, 54] Most epigenetics researchers have focused on the

histone modifications as they provide the most variable and dynamic platform for the transmission of cellular information. A cell's identity is based on its epigenetic patterns of gene expression, and these patterns must be maintained through each cycle of division.[55] Epigenetics are now known to be a key to understanding cell differentiation and development, as histone modifications are both heritable and highly dynamic.

Histone proteins as a whole are invariant across species, but their tails are even more conserved: when comparing yeast and human histones, H3 and H4 N-termini are identical with the exception of one amino acid, and it is a conservative substitution.[56, 57] With more than 20 modifiable sites across the four histone tails, a 'histone code' has been proposed in which the information potential of DNA is extended, with histone modifications acting sequentially or in combination with one another to provide cellular information that is read by other proteins.[58, 59] There have been some experiments that support this hypothesis, such as the finding that phosphorylation of histone H3 on serine 10 controls the acetylation of lysine 9.[60] However, this 'histone code' has been controversial and has spurred a variety of debates and it is now well accepted that histone modifications do not act in a specific 'code.' A primary argument against the histone code has been its potential magnitude; with eight histone tails per nucleosome and several modifying sites per tail, there are more potential code patterns than there are genes in the yeast genome.[61] Data is beginning to emerge that demonstrates that, instead of a code in which specific modifications are made on specific residues at specific times, it is more likely that patterns across regions of nucleosomes generate messages along the chromatin.[62]

In fact, it has been show that many histone modification sites have redundant functions and that to show phenotypic effects, it is necessary to delete or mutate entire tails. For example, histone H2A and H2B have interchangeable tails and either, but not both, can be deleted without affecting cellular viability.[63] Also, deletion of either the H3 or the H4 tails allows proper cell growth and nucleosome assembly, but deletion of both causes cell death.[64]

Instead of a specific code, it is more likely that specific modifications serve to 1) alter the histone-histone and histone-DNA interactions, and 2) create epitopes for the binding of specific protein complexes.[65] While there are some proteins that only have the ability to interact with a specific modification at a specific location, most have bromodomains or chromodomains that allow a general binding to acetylated or phosphorylated residues, respectively. Within the cell, there is a constant interplay between varying chromatin remodeling complexes; histone modifications are very dynamic and this contributes to the mobility of histones along the DNA.[65]

The histone code hypothesis was presented as an explanation for the long known connection between histone tail acetylation and transcriptional activation. The link between histone modifications and transcription was first hypothesized in 1964 by V.G. Allfrey *et al.* when they observed that histone acetylation is reversible.[66] Chromatin has two main structural forms: euchromatin, a more open form that is generally considered to be transcriptionally active; and heterochromatin, a closed form that is transcriptionally silent. Acetylation of histone tails is the distinguishing feature between these two forms, euchromatin being hyperacetylated and heterochromatin being hypoacetylated. The open conformation of euchromatin allows easier access for transcription factors and the RNA

polymerases to bind DNA,[31] whereas the closed conformation of heterochromatin blocks access to these proteins. It is also thought that the neutralization of the positively charged lysine helps to loosen the connection between neighboring nucleosomes.[36, 67] The link between histone acetylation and transcription was further supported with the finding that deletion of the N-terminal tails does not alter nucleosome stability, as originally predicted, but does deleteriously affect gene expression and thus, cell cycle progression.[67] Many transcription factors have been found to have bromodomains and some even have more than one bromodomain.[68-70] Also, some transcription factors have been shown to recruit histone acetyltransferases, indicating that gene activation requires a more open chromatin conformation.[71]

Recent evidence is beginning to indicate that there is a very strong cross-talk between the histone modifications (reviewed by Latham and Dent[72]). Many experiments have shown that individual modifications are able to influence other modifications on the same histone, on a neighboring histone within the same nucleosome, and even on neighboring nucleosomes. The simplest form of this cross-talk is shown in examples where one modification chemically blocks another modification on the same residue; lysines can be either acetylated or methylated, but not both. Methylation of lysine 9 of histone H3 has been shown to prevent the acetylation of lysine 9, and thus opposes the gene regulation functions of that acetylated residue.[72, 73] Other examples have shown that a mutation resulting in the loss of one modification also results in increase or decrease of other modifications. In *Drosophila*, mutation to the phosphorylation site threonine 119 of H2A causes the loss of acetylation on H3 lysine 14 and H4 lysine 5, suggesting that these modifications are cross-regulated.[74]

More examples of histone cross-talk are discovered every day and their functions and regulatory mechanisms are very poorly understood. These instances started as isolated cases varying across species and have grown to become functional networks involving histone modifications across entire regions of chromatin.[72] The elucidation of the mechanisms controlling this cross-talk is currently limited by the resolution of site-specific antibody western blotting and immunoprecipitations, and mass spectrometry, which prevents the analysis of subtle changes across nucleosomes, or of changes taking place in only specific genomic loci. The development of methods that allow single nucleosome resolution of histone modifications will contribute greatly to our understanding of their regulation and cross-talk.[72]

Heterochromatin, or silent chromatin, is tightly regulated by specific classes of chromatin remodeling complexes and is primarily found at telomeres.[75] Heterochromatin is also found at the centromeres of most organisms.[76] It is known to be involved in X-chromosome inactivation in human cells,[77] and can be found at the mating-type genes in yeast.[75] Though heterochromatin is generally considered to be hypoacetylated, it is often found to be acetylated on lysine 12 of histone H4,[78, 79] however, acetylation of lysine 16 of H4 prevents the formation of heterochromatin.[31] This pattern of hypoacetylation and an occasional mark on H4 K12 has been shown to regulate silencing of the yeast mating type loci *HML* and *HMR*.[80] Also, there is a methylation mark on lysine 9 of H3 that is found in heterochromatin and thought to block further acetylation.[81] These patterns are tightly regulated in yeast by the silencing information regulator (*SIR*) genes.[79]

Saccharomyces cerevisiae has the most well characterized analysis of histone modifications.[82] To date, there are over 20 known histone acetyltransferases and

deacetylases in yeast, indicating their importance in cell viability.[83] Because of the ease of yeast genetic manipulations, it is the model organism for chromatin studies, due both to the availability of a wide range of histone mutations affecting a variety of cellular processes and the extensive characterization of the known chromatin modifying complexes.[84] Studies have been done both at very specific residues and promoters, as well as on global levels. One study showed that yeast nucleosomes carry an average of 13 acetylated lysines per nucleosome.[85] An analysis of the acetylation of the four lysines on H4 (K5, K8, K12, and K16) found that 12% of the total H4 is unacetylated, 36% is acetylated on one lysine, 28% on two, 13% on three lysines, and 12% on all four available lysines.[82] These numbers became more interesting with the finding that lysine 16 is the preferred site, with 80% of the total H4 acetylated here, and nearly all of the monoacetylated forms are at this residue. Lysine 16 of H4 is the only acetylation site on that histone that is known to have very specific and essential functions in transcription, the others seem to have interchangeable or redundant functions.[68]

Acetylation is the most studied modification overall because aberrant acetylation has been linked to several human diseases, including lupus, leukemia, and several cancers.[86] It has even been demonstrated that a proper stoichiometric balance between histone modifications is important to cellular processes. Edmondson *et al.* showed that H3 acetylation and phosphorylation must be balanced for normal cell cycle progression.[60] A study by Lin *et al.* showed that inhibiting acetyltransferases or deacetylases to cause hypoacetylation or hyperacetylation are equally deleterious to the cell, and that balanced acetylation is crucial.[83]

In addition to its role in transcriptional activation and gene silencing, histone acetylation also has major roles in other cellular processes, including nucleosome deposition after DNA synthesis and chromosome segregation. It is known that there are specific acetylation marks on histones signaling for their import into the nucleus after protein synthesis and deposition into newly synthesized DNA.[87] Once deposited onto DNA, the proteins are then rapidly deacetylated and converted into the necessary chromatin formation based on their deposition locus and the modification status of the surrounding nucleosomes from the original strand.[31] In yeast, lysines 5 and 12 of H4 are the known import marks, but they have some redundancy built in. Lysines 5, 8, and 12 must be mutated before nucleosome assembly is inhibited.[79] It has also been shown that the H3 and H4 tails are interchangeable, and nucleosome assembly will proceed if at least one of the two tails is present and intact.[79]

Histone acetylation is also known to play a pivotal role in the process of chromosome segregation, as I will discuss in more detail later. As stated above, most organisms have heterochromatic regions around their centromeres. This has never been demonstrated in *S. cerevisiae*, likely due to the small size of yeast centromeres, but the centromeric region is known to be transcriptionally silent.[88] However, this does not preclude the relevance of histone modifications at and around the centromere, nor their involvement in chromosome segregation. Though much of the cell cycle dependence on histone acetylation is related to the transcription of cell cycle genes, there is also growing evidence that specific nucleosome patterns at the centromere are also necessary for maintaining cell cycle function. Phosphorylation of serines 10 and 28 of H3 has been shown to be essential for mitotic progress, despite the fact that at the centromere H3 is

replaced by a specialized version of the histone; Cse4 in yeast, CENP-A in mammals, hereafter referred to as CenH3.[89] The reasoning for the requirement of the phosphorylation of H3 is that it is necessary to prevent acetylation, and thus allow chromosomal condensation during mitosis; the mark has been shown to initialize in the pericentromeric region (usually heterochromatic) and then spread outward across the chromosome.[67] Centromere and kinetochore formation have been shown to be regulated by a variety of epigenetic modifications (as reviewed by Gieni *et al.*[90]). It is thought that pericentromeric chromatin plays a role in centromere identity and in the formation of the kinetochore, as well as in the spindle assembly checkpoint, regardless of heterochromatic state.

E. *ARP4* and the NuA4 Complex

ARP4 encodes an actin related protein, and is also known as *ACT3*. Arp4 is the only actin related protein that is essential for cell viability.[91] It is a subunit of the chromatin remodeling complexes INO80 and SWR1, and the histone acetyltransferase (HAT) complex NuA4 and, as such, is able to interact with all four core histones,[92] suggesting that it plays a role in allowing these complexes access to chromatin.[93] INO80 is known to be involved in regulation of gene expression, DNA damage response, and the establishment of proper sister chromatid cohesion.[94, 95] It is involved in these processes by catalyzing the ATP-dependent sliding of nucleosomes.[96] SWR1 is also involved in transcription and DNA repair, but acts through the replacement of the H2A-H2B dimer for an H2AZ-H2B dimer[94] (H2Az is a variant of H2A that is found at double-strand break sites). NuA4 acts as a chromatin remodeler by acetylating lysines in the tails of histones H4 and H2A, thus opening chromatin for transcription.[97] There is growing evidence

suggesting that SWR1 and NuA4 work together in transcription.[94] These findings along with Arp4's known ability to interact with all four core histones has led to the conclusion that Arp4 is the subunit responsible for recruiting these complexes to chromatin.[98]

Arp4 is also known to interact with centromeres, suggesting a role for it or Arp4-containing complexes in kinetochore function.[27] In fact, INO80 has been found at centromeres and is thought to be involved in proper assembly of cohesin around the chromatids.[95] Arp4 has also been implicated in transcriptional regulation by recruiting chromatin remodeling complexes onto chromatin.[99] Mutations in *ARP4* are known to cause defects in transcriptional regulation, chromatin structure, and an arrest in G2/M as well as abolish interactions of Arp4 with some inner-kinetochore proteins.[27]

Arp4 interaction with H4 can be completely abolished by substituting all four lysines in the H4-tail with glutamine.[93] More recently, it has been shown that Arp4 recognizes H2A phosphorylated at serine 129 which allows it to recruit the NuA4 complex to DNA-double strand break sites where the complex acetylates histone H4, which signals double strand break repair.[100] In fact, Arp4 is required for NuA4 acetylation of histone H4, even though it is not the catalytic subunit.[101]

The catalytic subunit of NuA4 is the histone acetyltransferase Esa1.[102] Conditional mutants of *esa1* have shown that it is important is cell cycle progression, since mutants arrest in G2/M.[103] This suggests that Esa1 may play a role in chromosome segregation, though more work is necessary to demonstrate that.

The fission yeast homolog to Arp4, Alp5, has been shown to be required for histone H4 acetylation, kinetochore-spindle attachment, and centromeric gene silencing.[101] Alp5 was shown to be required for histone H4 acetylation at lysines 5, 8,

and 12. It was found to be required for transcriptional silencing at the core domain of the centromere, but not for transcriptional repression in the flanking heterochromatic regions.[101] This work demonstrated two main cellular functions for Alp5. One is its role in global histone H4 acetylation, and the other is a role in maintaining centromere/kinetochore integrity. It is not yet clear how these two roles are connected, or if they even are connected. Centromeric regions are known to be hypoacetylated, so it was thought that removing an acetyltransferase complex would have no affect on centromere function. However, this proved untrue when repression of Alp5 led to desilencing of the centromere. It is possible that the cause involves an overall imbalance of histone acetylation/deacetylation. It could also be that the cause is indirect, and Alp5 mutants actually disrupt the transcription of other centromere/kinetochore proteins. Or, it could be that Alp5 is a member of another complex that regulates centromere function. It is apparent that much more work is necessary to gain a full understanding of the role of this protein.[101] This evidence has strengthened our understanding of the interplay between histone modifications because an acetyltransferase is still important to a region of the genome known to be hypoacetylated.

Work by Ogiwara *et al.* has shown much more definitively that Arp4 is involved directly in kinetochore assembly, and that the observations of Minoda *et al*. were not a result of defects in the transcription of kinetochore components.[27]

F. Centromere

The *Saccharomyces cerevisiae* centromere (CEN) is defined by a nuclease resistant region originally hypothesized to be comprised of CEN DNA, histones, and the DNA-binding proteins of the kinetochore.[104] Extensive characterization has revealed that

the *S. cerevisiae* centromere is comprised of an essential 125-bp of DNA conserved across all 16 chromosomes. This region can be further divided into three conserved DNA elements (*CDEI, II,* and *III*). *CDEII* is an A-T rich element that is flanked by two shorter regions (*CDEI* and *III*). *CDEI* is a short 8-bp region and *CDEIII* is 25-bp and the least amendable to changes in sequence.[53] It is thought that the ~80-bp of *CDEII* represents one turn around a nucleosome, and that *CDEI* and *III* are brought into close contact on one surface of the CEN nucleosome. Studies have shown that the CEN is both necessary and sufficient for the formation of an intact kinetochore, and the removal of entire chromosomal arms still allows for functional kinetochores if the CEN remains intact.[105] Both *CDEI* and *CDEIII* incorporate palindromic sequences, suggesting their importance in DNA binding to centromere and kinetochore proteins.[106]

While *S. cerevisiae* has a very specific sequence at the centromere, no other organism does, a surprising realization since every chromosome in every organism has a very specific region that is always the centromere.[54] Centromeres of other organisms can range from the 50-100 kilobases in fission yeast *Schitzosaccharomyces pombe* or the several megabases in mammalian centromeres.[12] This further demonstrates the importance of epigenetics in centromere formation and identity. One explanation for the size differences of centromeres in different organisms can be found in the fact that *S. cerevisiae* kinetochores only need to bind one microtubule, whereas human kinetochores bind between 15 and 25 kinetochores.[107] Also, human centromeres contain large arrays of α-satellite repeats that seem to serve a structural function,[108] as well as the hypoacetylated pericentric heterochromatin.[76] Though budding yeast does not have centromeric heterochromatin, there is evidence for a chromatin structure variation at the

centromere.[109] More recent studies in human and *Droshophila* cells have found that though heterochromatin is apparent in centromeres, the modification pattern is different from that of traditional heterochromatin. One of the key marks of heterochromatin has been the dimethylation of lysine 9 on H3, a mark not found on CenH3 as it is only homologous to H3 in the C-terminal globular domain, and not in the N-terminal tail.[110, 111] Centromeric regions of human and *Drosophila* cells have also been hypothesized to have alternating CenH3 nucleosomes with traditional nucleosomes.[112]

By inhibiting histone deacetylases, Gilchrist *et al.* concluded that centromere localization is independent of histone acetylation.[113] However, all three subunits of a histone deacetylase complex were found as suppressors of a chromosome missegregation phenotype caused by an H2A mutant, suggesting a connection between histone deacetylation and centromere function.[2] This is consistent with the observation that pericentric heterochromatin consists of well-phased nucleosomes, which are characteristic of heterochromatin.[114] These data support the hypothesis that although budding yeast has no centromeric heterochromatin, it does have an altered form of chromatin at and around the centromere. Also in support of this hypothesis is the finding that yeast centromeres are resistant to DNA repair, the cells retaining DNA lesions in favor maintaining kinetochore structure.[115]

Centromere identity has been an intriguing problem, since specific sequence has been ruled out beyond budding yeast, and CenH3 is essential but not sufficient for centromere identity. One model suggests that centromere identity relies on kinetochore and centromere proteins remaining from the previous division cycle.[54, 90] A study following centromeres across several generations under conditions in which certain

kinetochore components were depleted shows that centromeres retain mitotic stability once established, supporting the idea that centromere identity is epigenetically regulated.[116]

The presence of a CenH3-containing nucleosome in all eukaryotes suggests that it, and not any specific sequence, is the primary identifier of centromere localization.[11, 112] All centromeres are responsible for marking the chromosomal location accessible to mitotic machinery and for providing a scaffold for kinetochore assembly[117]; they must also have the ability to withstand the physical force and constraints involved in separating the chromosomes.[118] Since centromeres require the presence of CenH3, and centromeric chromatin structure can be altered by reduction in the levels of H2B and H4, it has been proposed that CEN DNA must be wrapped around an altered nucleosome.[119] CenH3 is actually considered to be the earliest binding protein for kinetochore assembly.[120] However, CenH3 is not sufficient for kinetochore assembly. When it is relocated to a non-centromeric locus, it still recruits some kinetochore components, but not all of them, indicating that there are other CEN signals marking kinetochore assembly.[111]

It is known that CenH3 is deposited into the CEN during telophase and into early G1, as soon as chromosome pairs have segregated; this is in contrast to H3, which gets deposited during S phase.[121] Chromatin immunoprecipitation (ChIP) experiments have shown CenH3 to be localized in the center of CEN DNA and its presence diminishes rapidly upon moving away from the CEN.[122] In yeast, it has been found that CenH3 deposition is dependent on the localization of the kinetochore CBF3 complex, and while CenH3 is required for kinetochore assembly, very few kinetochore proteins require CenH3.[122, 123] Little else is known about CenH3 regulation.[124] While in budding yeast, it

appears that CenH3 localization is sequence directed, in higher eukaryotes its localization is directed by RNAi-induced heterochromatin.[125] Also, it has been shown that yeast CenH3 localization is aided by ubiquitin-mediated proteolysis.[124]

Mutations in *CSE4*, the budding yeast CenH3 gene, cause chromosome segregation phenotypes. Other histone mutants that cause the same phenotype are in H4[84] and H2A.[1] These mutations also cause altered chromatin structure at the centromere.[1, 126] Interestingly, an overexpression of *CSE4* can suppress the temperature sensitive phenotype of an H4 mutant.[84] Overexpressing H3 causes it to compete with CenH3 and results in defective kinetochore function.[49] Also, when kinetochore protein *SCM3* is overexpressed, it can suppress the chromosome missegregation phenotype of a *CSE4* mutant.[127] It has been shown that a CenH3/H4 tetramer is actually more compact than an H3/H4 tetramer, not due to size (CenH3 is bigger) but due to the fact that CenH3 is bound more tightly to H4 than H3.[54] The N-terminal domain of yeast CenH3 has been shown to be unnecessary for proper chromosome segregation, an interesting finding when combined with the knowledge that the N-terminus is the most divergent part of all CenH3s.[128]

The small CEN sequence (~125-bp) in yeast has presented a problem in explaining the presence of a centromeric nucleosome (traditional nucleosomes require ~146-bp of DNA). Some have proposed that the CEN nucleosome lacks H2A and H2B and others have proposed that it is similar to the 'hemisome' in *Drosophila* in which the nucleosome is a heteromeric tetramer instead of an octamer.[129] However, recent evidence has supported a new and more likely hypothesis. It was found that Scm3 displaces H2A and H2B from CEN nucleosomes,[130] leaving a heterohexamer with two copies each of

Cse4, H4, and Scm3.[118] These nucleosomes would be smaller and more able to accommodate the smaller sequence of the budding yeast CEN. Scm3 is an essential protein and is known to be required for CenH3 localization and chromosome segregation.[130] Though CenH3 is highly conserved, *SCM3* is not, and no obvious ortholog has been found outside of fungi,[130] suggesting that the 'hemisome' hypothesis in *Drosophila* may still be correct in metazoans. Also, this model is consistent with chromatin immunoprecipitation data indicating that Cse4 is bound to *CDEII*, but never *CDEIII*.[131]

G. Kinetochore

Kinetochore studies have typically been done in large eukaryotic cells where the mitotic machinery is visible microscopically. Despite the fact that this is not possible in yeast due to its small size, even with recent advances in microscopy, yeast remains a model organism for the system, not because it can be viewed, but because yeast have a wide array of biochemical and genetic tools available and because yeast are more tolerable to chromosome segregation defects and aneuploidy.[26] Also, kinetochores were originally hypothesized in yeast after failed attempts to identify a direct interaction between DNA and microtubules.[114] In addition, yeast is used as a model for the kinetochore problem because it presents a very simple version of a very complex cellular machine, whose complete molecular understanding is a daunting task.[16] What can be done microscopically in mammals can be done biochemically in yeast.[18]

Contributing to the functions of the centromere, the kinetochore serves to maintain cohesion between sister chromatins, to attach to spindle microtubules, and to signal the spindle checkpoint when proper attachment has not been achieved.[123] Once the

centromere is established, the kinetochore must be able to build on it. In order for accurate chromosome segregation, centromeres must be able to bind microtubule-binding proteins, cohesion factors, and check-point proteins.[132] These roles are accounted for in the formation of the megacomplex called the kinetochore. The centromere-kinetochore complex has even been called an organelle.[132]

CDEI binds a protein called Cbf1 that is thought to contribute to the structural integrity of the kinetochore.[108] As mentioned above *CDEII* is bound to CenH3 and is thought to be a spacial linker between *CDEI* and *III*. *CDEIII* is the binding site for the essential CBF3 complex (containing Ndc10, a protein commonly used in kinetochore function studies).[108] This complex is essential for chromosome segregation as well as for CenH3 recruitment.[133] It is thought that *CDEI* and *CDEIII* come in close contact on the face of the nucleosome, allowing Cbf1 to interact with CBF3.[134] Interestingly, both Cbf1 and CBF3 induce significant bending of the DNA they are bound to, suggesting a general remodeling of the centromere to accommodate the space needed by kinetochore proteins.[130] These DNA and protein components comprise the inner kinetochore.

Kinetochore assembly does not seem to be limited to a particular cell cycle stage,[122] consistent with the idea that it serves as an epigenetic mark for centromere localization in successive generations. The model proposed by Cheeseman *et al.*[16] divides the kinetochore into three regional domains. The first is the inner kinetochore, which binds to the centromere and consists primarily of the CEN nucleosome, the CBF3 complex, Cbf1, and Mif2 (a protein that binds to *CDEI* near Cbf1). The next region is the central kinetochore, which serves primarily as a linker between DNA-binding proteins and microtubule-binding proteins. The primary complex of this region is the

Ctf19 complex, which has been shown to bind both inner kinetochore components and outer kinetochore components.[135] Central kinetochore proteins also include the Ctf3 complex, the Ndc80 complex, as well as others not yet well described.

The final region is the outer kinetochore, which serves to connect chromosomes to microtubules, consisting of the Dam1 complex, Bik1, Bim1, MAPs (microtubule associated proteins), and several motor proteins. The motor proteins and microtubule associated proteins function together in the binding of microtubules.[20] There are also a few regulatory proteins which are not easily classified into one of these regions because they seem to move. These include the Mad and Bub proteins of the spindle assembly checkpoint, and the Ipl1/Sli15/Bir1 proteins that serve various regulatory functions throughout the kinetochore and have been found both near the microtubules and at the CEN.

Ipl1 is a kinase that serves to control kinetochore function by phosphorylating several proteins within the kinetochore, and is essential to chromosome segregation.[136] It has known interactions with Sli15, and Bir1, a chromosomal passenger protein[137] that is thought to carry Ipl1 between the centromere and the microtubule binding proteins. Bir1 has been found bound to the histone deacetylase complex Hda1, which is known to alter the acetylation state of histones H3 and H2B around the centromere.[138] That study found that Bir1 is able to associate with the centromere without the Hda1 complex. It was proposed that Bir1 is responsible for bringing Ipl1 to the centromere, where it phosphorylates Hda1, which then deacetylates pericentromeric nucleosomes.[138]

Figure 2 shows a simple depiction of a yeast kinetochore as modeled by Westermann *et al.*[139] with some more recent additions[122]. Unfortunately, this figure is

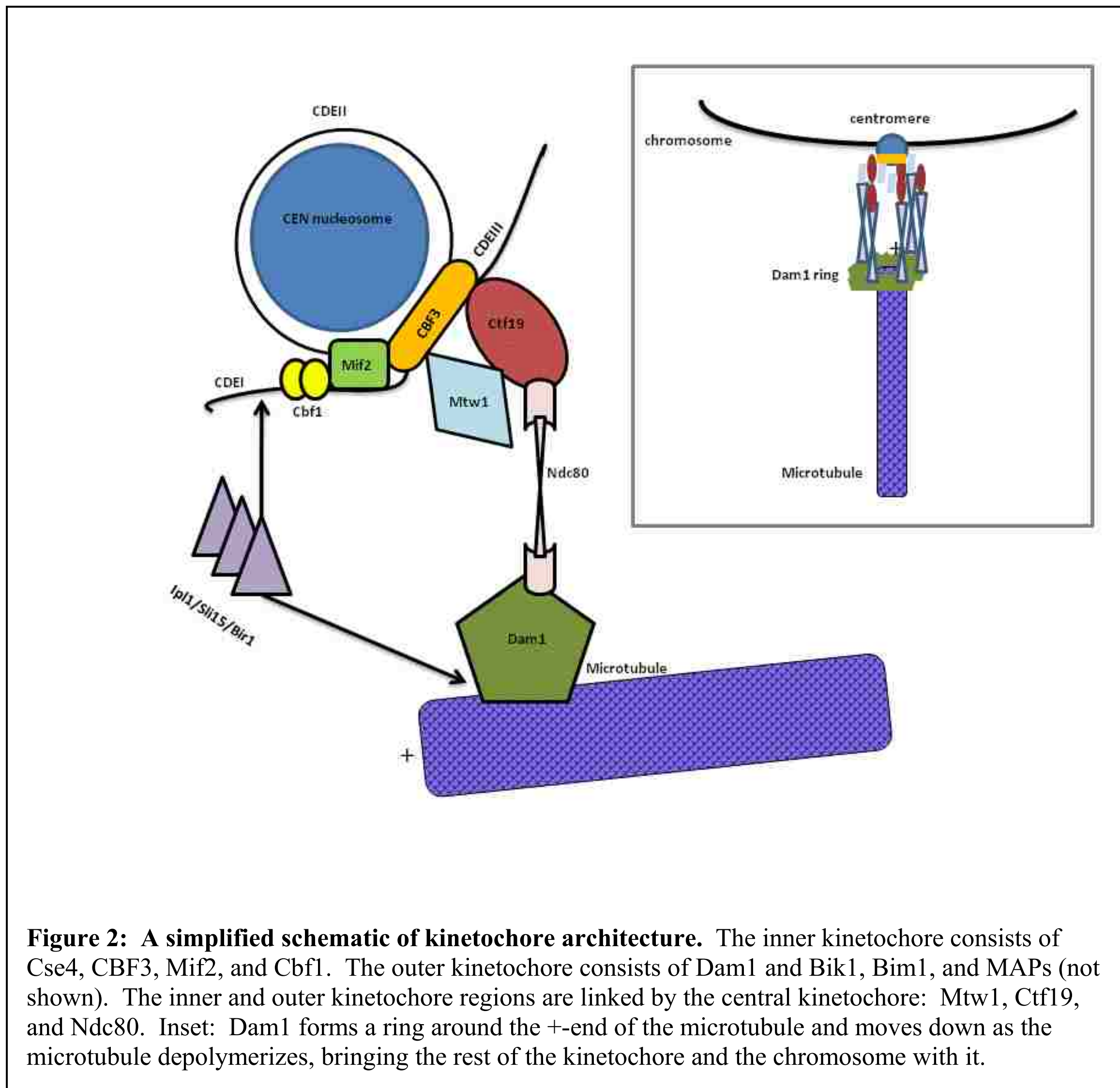


Figure 2: A simplified schematic of kinetochore architecture. The inner kinetochore consists of Cse4, CBF3, Mif2, and Cbf1. The outer kinetochore consists of Dam1 and Bik1, Bim1, and MAPs (not shown). The inner and outer kinetochore regions are linked by the central kinetochore: Mtw1, Ctf19, and Ndc80. Inset: Dam1 forms a ring around the +-end of the microtubule and moves down as the microtubule depolymerizes, bringing the rest of the kinetochore and the chromosome with it.

only a two-dimensional representation of a three-dimensional object, so it cannot be a perfect depiction of true kinetochore architecture, nor can it include all of the known proteins involved. Thus, this is a much simplified model based on known interactions. No simple linear model has been proposed that can explain the known interactions among kinetochore proteins.[120] It is likely that assembly isn't even linear, but is affected by multiple pathways working at the same time.[18]

Kinetochores are currently thought to attach to microtubules on the lateral side and then move toward the plus-end of the microtubule for end-on binding and

chromosome movement (as shown in **Figure 2**).[23] This is thought to be accomplished through the binding of the Ndc80 complex to the Dam 1 complex, which is thought to form a ring around the microtubule.[122] The figure only shows one copy of each complex, but it has been proposed that there are actually several copies of Mtw1, directing multiple copies of Ndc80 to the Dam1 ring.[122] These ideas stem from the fact that in higher organisms, microscopic measurements show kinetochores to be between 100 and 500 nm in diameter and are well organized, as well as biochemical data indicating the existence of several copies of some proteins per chromosome.[6, 140]

An electron micrograph of a *Drosophila* kinetochore shows it to have three primary domains: the inner centromere, the inner kinetochore, and the outer kinetochore.[19] The inner centromere is shown as a heterochromatic region that binds cohesion proteins and is regulated by passenger proteins. Outside of budding yeast, homologs to the CBF3 complex and other inner kinetochore proteins cannot be found; however, it is possible that the presence of heterochromatin serves the same structural function as these proteins. The *Drosophila* inner kinetochore is likely the functional homolog of the *Saccharomyces* central kinetochore, as it contains proteins that seem to serve as connectors between chromatin and the outer kinetochore microtubule binding and motor proteins.[19] Computational comparisons of known kinetochore components have identified inner kinetochore components that only exist in the point centromeres of budding yeast, but have also demonstrated that the basic structural features are well conserved from yeast to man.[141] It was even found that human proteins are equally similar in sequence to their yeast counterparts as to their *Drosophila* and *Caenorhabditis elegans* counterparts.[141] Though we don't yet know all the proteins involved, some

groups are already planning to reconstruct a kinetochore *in vitro*, thus allowing a complete understanding of all of the protein interactions involved.[26] Meraldi *et al.* have hypothesized that the kinetochores of all eukaryotes have a single ancestor and that the budding yeast point centromeres are a slight divergence from metazoans.[141] Interestingly, kinetochore proteins have been found to be among the most rapidly evolving, despite their highly conserved functions.[141] This may contribute to the difficulty in finding obvious orthologs across organisms based on sequence alone.

Biggins and Walczak have categorized yeast kinetochore proteins into four groups based on their mutant phenotypes.[18] The first class causes complete loss of microtubule attachment, leaving the entire DNA content in the mother cell; the spindle checkpoint does not get activated. Most kinetochore mutants fall into the second class in which the spindle checkpoint is activated and cells arrest in metaphase. In the third class, the checkpoint is sometimes activated and sometimes not and the chromosomes are segregated unequally. The final class constitutes the mutants that have only subtle chromosome loss phenotypes and it is assumed that these mutants represent proteins with redundant functions.[18] Based on this assessment it is clear that the kinetochore not only serves as a microtubule binding site, but also serves as a quality-control mechanism for cell division.[142]

Mutants of *NDC10*, a member of the CBF3 complex, completely abolish kinetochore assembly as well as checkpoint response so they are often used to determine if new proteins assemble to the kinetochore because their localizations almost always require Ndc10.[14, 120, 143] *CSE4* is also frequently used for the same purpose, though it too depends on Ndc10 for localization.

Mutants that lack components of the central kinetochore complex Ndc80 show loss of kinetochore-microtubule attachment without losing complete kinetochore assembly.[120] Members of the Dam1 complex are known to directly interact with microtubules and their mutants abolish attachment without affecting kinetochore assembly.[134]

H. Histone genetics

Histones are extraordinarily conserved and can be evolutionarily traced back to archeal origins.[144] More specifically, it has been found that the histone fold domain, or the histone interaction surfaces, is especially well conserved.[145] Yeast histone genes are arranged in pairs such that the H3 genes are divergently transcribed from the same promoter region as the H4 genes,[146] and H2A from the same promoter region as H2B (**Figure 3**, modeled from[147]). The pairs are called copy I and copy II as denoted by the number following their gene names. It is not clear why there are two copies of each gene, but due to the fact that the copy I and II promoter regions of *HHT/HHF* are highly

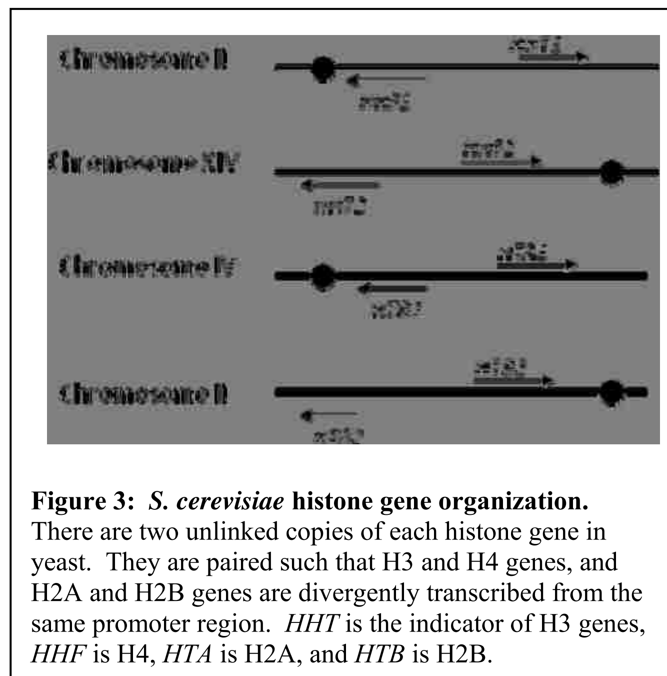


Figure 3: *S. cerevisiae* histone gene organization. There are two unlinked copies of each histone gene in yeast. They are paired such that H3 and H4 genes, and H2A and H2B genes are divergently transcribed from the same promoter region. *HHT* is the indicator of H3 genes, *HHF* is H4, *HTA* is H2A, and *HTB* is H2B.

divergent, it is likely that the gene duplication occurred early in the evolution of *S. cerevisiae*.[148] The divergent transcription of the histone genes is likely a regulatory mechanism for the maintenance of proper histone stoichiometry, as it has been demonstrated repeatedly that the ratio of H2A-H2B to H3-H4 is an important factor in chromosome integrity.[3] The two copies of H3 and H4 express identical proteins. However, the two H2A and H2B genes produce slight variants. These variations seem to be functionally exchangeable, but subtle phenotypic variations can be found between the two strains.[149] For all four histones, either gene can produce sufficient protein needed for cell viability.[150]

Histones are synthesized in late G1 and early S phases to allow for ample protein supply at the onset of DNA synthesis.[151] Further support linking histone synthesis with DNA synthesis was the finding that inhibiting DNA synthesis causes a rapid disappearance of histone mRNA.[151] Histone synthesis is controlled both transcriptionally at the promoters as well as posttranscriptionally by mRNA degradation.[152] This is demonstrated by experiments utilizing cells in which H2A and H2B genes were overexpressed that showed no increase in the amount of mRNA present; instead, the rate of mRNA turnover increased to compensate for the increased rate of transcription.[153] Histone regulation is also controlled posttranslationally by degradation signaled by the Rad53 kinase.[124]

H2A and H2B, but not H3 and H4, gene pairs are able to alter transcription to compensate for changes in gene dosage, as demonstrated by overexpression studies[153, 154] However, deletion of the H2A-H2B genes has shown that, in a diploid strain, copy I is responsible for ~60% of the transcripts produced, and a deletion (Δ) of *hta1-htb1* results

in a significantly reduced amount of protein.[155] Another study found that *HTA1-HTB1* is able to compensate for *(hta2-htb2)Δ*, but *HTA2-HTB2* cannot compensate for *(hta1-htb1)Δ*.[48] This observation explains why the phenotypes from an *hta1-htb1* deletion strain are more drastic than the phenotypes from a deletion of *hta2-htb2*, which is phenotypically indistinguishable from a WT strain.[48, 156] Thus, increasing the amounts of the H2A and H2B genes can be compensated for, but deleting them cannot.

H3 and H4 copy II genes expression rates are 5-7 times higher than that of copy I.[154] These genes do not demonstrate dosage compensation, despite the fact that either gene pair produces sufficient protein for viability, even in the absence of the other copy. Similar results were found in mouse histone gene pairs.[157] The lack of dosage compensation from these genes can be explained by assuming that histone gene expression is not the rate limiting step in histone protein synthesis.

Overexpression of any pair of histone genes has been shown to result in an increase in the rate of chromosome loss, further demonstrating a role for histones in chromosome segregation.[3, 158] However, if the stoichiometric balance is restored by increasing the copy-number of all four histones, the chromosome loss phenotype is suppressed.[3] Overexpressing single histone genes does not result in the chromosome loss phenotype. The chromosome loss phenotype was also observed in a deletion of *hht2-hhf2*, but not *hht1-hhf1*.[158] Au *et al.* have found that improper stoichiometry affects the localization of CenH3 in relation to H3 at the centromere, and can disrupt chromosome segregation.[159]

II. INTRODUCTION

The body of this dissertation will focus on the study of two specific mutations to the *HTA1* gene, *hta1-300* and *hta1-200*. Each represents a change to a single amino acid that confers phenotypes of increase-in-ploidy, increase in chromosome loss rates, and cold sensitivity. The chromosome segregation phenotypes are consistent with cell cycle mutants[160] and a class of mutants involving DNA damage and affecting chromosome segregation.[80] Cold sensitive phenotypes are typically associated with increased instability in protein complex formation. *hta1-300* represents a change at amino acid 30 from a glycine to an aspartate. *hta1-200* represents a change at amino acid 20 from a serine to a phenylalanine.[1]

Both mutants were isolated in a screen by Hirschhorn *et al.* that was performed primarily to identify histone mutants defective in transcription.[161] Random mutagenesis was performed by PCR on the *HTA1* gene. Sixteen mutant candidates were selected based on their inability to grow on raffinose (Raf-), a phenotype associated with a defect in *SUC2* expression. The mutants were then screened for defective transcription of other SWI/SNF-dependent genes (SWI/SNF is a chromatin remodeling complex known to be required for transcriptional activation of some genes), mating defects, cold sensitivity, and temperature sensitivity. All screens were done in a strain with a deletion of *hta2-htb2* so that the only available H2A was from the mutant gene; deletion of this locus causes no apparent growth phenotypes[162]. *hta1-300* and *-200* were found to be semi-dominant in their Raf- phenotype, indicating a gain of function rather than a loss of function. However, upon mRNA quantitation, it was found that both mutants decrease the level of *SUC2*-mRNA by up to 10-fold. However, it was found that not all SWI/SNF-

dependent genes are affected by these mutants. Thus, it was concluded that the Raf- phenotype is independent of SWI/SNF-related transcription. An analysis of the chromatin structure around the *SUC2* gene showed a looser conformation than WT in the *hta1-300* and *hta1-200* mutant strains, but not in the other *HTA1* mutants, indicating a defect in transcriptional activation at this promoter.[161]

The fact that these two *hta1* mutants stood out from the other isolates led Pinto and Winston to further characterize them.[1] It was in this study that it was discovered that these two mutants confer chromosome segregation defects in addition to the cold sensitivity and Raf- phenotypes discussed above. Based on crystallographic data it was found that both mutations reside on the nucleosome surface in close contact with each other and with the DNA. Upon difficulty with genetic analysis (poor spore germination when crossed with a haploid strain), it was concluded that these strains have an inability to maintain a haploid state and thus become diploid. This was confirmed by DNA-content analysis by flow cytometry. The mutants were also found to have a decreased viability consistent with a G2-M cell cycle delay. This delay is consistent with cells in which there is a defect in microtubule assembly[163-166] so spindle morphology was analyzed by staining with anti-tubulin. It was found that microtubules and spindle pole bodies are normal in these mutants, indicating that the chromosome segregation phenotypes are not caused by an indirect affect on transcription of tubulin genes.[1]

The chromosome segregation defects of *hta1-300* and *hta1-200* were further analyzed by determining the rate of chromosome loss in these strains. Diploid strains were screened for loss of chromosome III and it was found that *hta1-200* mutants exhibit a chromosome loss frequency of 24×10^{-6}, *hta1-300* mutants have a chromosome loss

frequency of $48x10^{-6}$, and WT strains show a loss rate of only $3.6x10^{-6}$.[1] It was hypothesized that the defect causing chromosome loss was also causing the increase in ploidy. This was tested by combining the *hta1* mutants with kinetochore mutants. This resulted in a drastic slow growth phenotype, leading to the conclusion that H2A interacts with kinetochore mutants and that the *hta1* mutants affect centromere function. This conclusion was supported by the finding that the *hta1* mutants have an altered chromatin structure at the centromere.[1] Altered chromatin structure at the centromere has also been found in mutants of H2B and H4.[53] Chromosome segregation phenotypes were also observed in an H2B mutant in *S. pombe*.[167]

To confirm that these phenotypes were not an indirect result of transcriptional defects, a high-copy suppressor screen was carried out on both the cold sensitive phenotype and the Raf- phenotype. Suppressors of both phenotypes were isolated, but none of them overlapped indicating that the phenotypes represent different functions of H2A. Interestingly, high-copy *ACT3/ARP4* was found to suppress the cold-sensitivity and was able to partially suppress the increase-in-ploidy, suggesting that these two phenotypes are linked to the same H2A function. Overall, it was concluded that histone H2A functions both in transcription and in centromere function.[1]

Our interest in Arp4 is in its role(s) at the kinetochore and centromere and in the role it plays as a high-copy suppressor of the H2A mutant phenotypes. The fact that high-copy *ARP4* is able to suppress both the cold sensitivity and the increase in ploidy of *hta1-300* suggests that the two H2A phenotypes are related to the same function.[1] Also, Arp4 is known to be a transient component of the kinetochore and is likely involved in

kinetochore assembly and regulation through chromatin modifications at the centromere.[27]

Kanta *et al.* carried out an even further analysis and characterization of the two *hta1* mutants by performing a genetic screen for suppressors of the increase-in-ploidy phenotype.[2] Five genes were identified: *HDA1, HDA2, HDA3, MKS1,* and *HHT1.* Mutants of all five of these genes were able to suppress the increase-in-ploidy phenotype of *hta1-300*.

All three *HDA* genes are members of a histone deacetylase (Hda1) complex, in fact, they are the only members. This data presented the first evidence for a role for the Hda1 complex in centromere function. To further characterize this possible role for the Hda1 complex, deletions of all three alleles were made. Interestingly, *hda1Δ* and *hda2Δ* showed no apparent phenotype, but *hda3Δ* was both slow growing and cold sensitive. The *hda3Δ* mutant was also found to have a G2-M delay and an increase in chromosome loss suggesting a distinct role for Hda3 in chromosome segregation outside of the Hda1 complex. This was supported by the finding that *HDA3* has genetic interactions with several kinetochore components. These interactions were distinct from those of *HTA1* suggesting that, while both genes have roles in kinetochore function, they operate in distinct pathways. Adding to this, *hda3Δ* did not have an altered chromatin structure at the centromere, but all three members of the Hda1 complex were found to associate with centromeric DNA.[2]

The role for the Hda1 complex in chromosome segregation was further characterized by Almutairi.[138] It was found that all three members of the Hda1 complex interact with the centromere and that mutations of these proteins cause the centromere

association to be lost. The complex was found to be responsible for the deacetylation of histone H3 at lysine 14 and histone H2B at lysine 16 in the pericentric chromatin, contributing to the hypothesis that yeast has an altered form of chromatin at the centromere. Interestingly, it was found that the *hta1-300* and *hta1-200* mutants prevent accurate deacetylation of H3 K14. Since *hda* mutants are known to suppress in increase-in-ploidy phenotype of the *hta1* mutants,[2] it was thought that the loss of deacetylation was responsible for the suppression. However, a combination of the two mutants showed the same acetylation pattern as the *hta1-300* mutant alone.[138]

Of the other genes found in the increase-in-ploidy suppressor screen,[2] *MKS1* encodes a protein implicated in lysine biosynthesis, the TOR kinase pathway, and several other regulation pathways. With no obvious connection between this gene and chromatin or centromere function, it was concluded that the suppression was an indirect affect of the allele and no further characterization has been done. However, *HHT1* encodes histone H3 and it will be further characterized as a suppressor in this document.

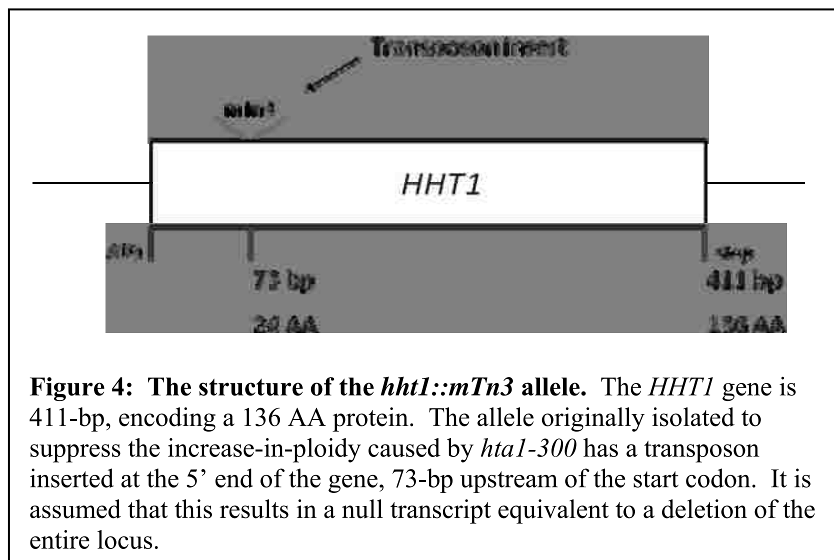


Figure 4: The structure of the *hht1::mTn3* allele. The *HHT1* gene is 411-bp, encoding a 136 AA protein. The allele originally isolated to suppress the increase-in-ploidy caused by *hta1-300* has a transposon inserted at the 5' end of the gene, 73-bp upstream of the start codon. It is assumed that this results in a null transcript equivalent to a deletion of the entire locus.

The mutant allele (*hht1::mTn3*) has a transposon inserted near the 5' end of the *HHT1* gene and was assumed to represent a null transcript (**Figure 4**).[2] A null transcript of this gene would result in reduced levels of H3, with *HHT2* still intact. In this light, I set out to expand the general understanding of the role of histones in chromosome segregation by studying this H2A mutant (*hta1-300*) in combination with alteration is histone gene dosage, with emphasis on *HHT1*.

III. METHODS

A. Yeast strains, genetic methods, and media

All yeast strains are isogenic to FY2, originally derived from S288C, unless otherwise indicated.[168] Strain genotypes are listed in **Table 1**. Lower case letters indicate mutant alleles and upper case letters indicate wild-type (WT) alleles. Genetic manipulations and strain construction were all carried out using standard methods.[169-171] Yeast transformation was done according to Gietz *et al*.[172] All yeast media, including yeast extract/peptone/dextrose (YPD), synthetic minimal (SD), synthetic complete (SC), omission media (SC-), media containing 5-fluoroorotic acid (5-FOA), and sporulation media were made as described previously.[171] Canavanine plates were made as described,[2] and contained 60 μg/mL of canavanine sulfate.

B. Bacterial strains

Plasmids were amplified and isolated from *Escherichia coli* strain *DH5α* (*F'80lacZ-M15-(lacZYA-argF) U169 endA1 recA1 hsdR17* ($r_{k-}m_{k+}$) *deoR thi1 supE44 gyrA96 relA1*), according to standard procedures.[173] *E. coli* was grown in LB or in LB containing 100 μg/mL of ampicillin as described.[174]

Table 1: Yeast Strains and Genotypes

Strain	Genotype
FY1333	*MATα leu2Δ0 ura3Δ0*
FY1331	*MATa trp1Δ63 ura3Δ0*
FY604	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1*
FY605	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1*
FY987	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 lys2-128δ (hta2-htb2)Δ::TRP1 hta1-200*
FY988	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 lys2-128δ (hta2-htb2)Δ::TRP1 hta1-300*
FY1819	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 lys2-128δ (hta2-htb2)Δ::TRP1 hta1-200*
IPY15	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 arp4Δ::HIS3 [pIP46]*
IPY60	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 lys2-128δ (hta2-htb2)Δ::TRP1 hta1-200*
IPY68	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hta1-300 [pSAB6]**
IPY69	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hta1-300 [pSAB6]**
IPY75	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1*
IPY136	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 HIS4/his4Δ::URA3*
IPY137	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 lys2-128δ/lys2-128δ (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hta1-200/hta1-200 HIS4/his4Δ::URA3*
IPY139	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hta1-300/hta1-300 HIS4/his4Δ::URA3*
IPY285	*MAT? his3Δ200 leu2Δ1 trp1Δ63 ura3-52 lys2-128δ ade2*Δ3::HIS3::ade2Δ5 *(hta2-htb2)Δ::TRP1 hta1-300 hht1::mTn3*
IPY308	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3*
IPY321	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht1Δ::HIS3*
IPY392	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf1Δ::HIS3*
IPY393	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht2-hhf2)Δ::HIS3*
IPY399	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf2Δ::HIS3*
IPY400	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht2Δ::HIS3*
IPY437	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf2Δ::HIS3 hta1-300*
IPY439	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf2Δ::HIS3*
IPY440	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hta1-300*
IPY444	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht2Δ::HIS3*

Table 1 continued:

Strain	Genotype
IPY451	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht2Δ::HIS3 hta1-300*
IPY501	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht1Δ::HIS3*
IPY502	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hta1-300 hht1Δ::HIS3*
IPY503	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht1Δ::HIS3*
IPY504	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hta1-300 hht1Δ::HIS3*
IPY552	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1*
IPY553	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3 hta1-300*
IPY555	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3*
IPY558	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3 hta1-300*
IPY559	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf1Δ::HIS3*
IPY561	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf1Δ::HIS3 hta1-300*
IPY563	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf1Δ::HIS3 hta1-300*
IPY569	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht2-hhf2)Δ::HIS3 hta1-300*
IPY687	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf2Δ::HIS3 hta1-200*
IPY668	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf1Δ::HIS3 hta1-200*
IPY669	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf1Δ::HIS3*
IPY670	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hta1-200*
IPY679	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht2Δ::HIS3 hta1-200*
IPY708	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht2-hhf2)Δ::HIS3 hta1-200*
IPY710	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht2-hhf2)Δ::HIS3*
IPY714	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht1Δ::HIS3 hta1-200*
IPY716	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht1Δ::HIS3*
IPY720	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht1Δ::HIS3 hta1-200*
IPY723	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3 hta1-200*
IPY724	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3*

Table 1 continued:

Strain	Genotype
IPY727	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3 hta1-200*
IPY812	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 arp4-26*
IPY822	*MATa his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hhf1Δ::HIS3 hta1-200*
IPY824	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hht1Δ::HIS3/hht1Δ::HIS3*
IPY825	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hta1-300/hta1-300 hht1Δ::HIS3/hht1Δ::HIS3*
IPY826	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hhf1Δ::HIS3/hhf1Δ::HIS3*
IPY829	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hhf1Δ::HIS3/hhf1Δ::HIS3 hta1-300/hta1-300*
IPY831/832	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hht1Δ::HIS3/hht1Δ::HIS3 HIS4/his4Δ::URA3*
IPY833/834	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hta1-300/hta1-300 hht1Δ::HIS3/hht1Δ::HIS3 HIS4/his4Δ::URA3*
IPY835	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3/(hht1-hhf1)Δ::HIS3*
IPY836	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3/(hht1-hhf1)Δ::HIS3 hta1-300/hta1-300*
IPY837	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hhf1Δ::HIS3/hhf1Δ::HIS3 HIS4/his4Δ::URA3*
IPY910	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hht1Δ::HIS3/hht1Δ::HIS3 hta1-200/hta1-200*
IPY911	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hhf1Δ::HIS3/hhf1Δ::HIS3 hta1-200/hta1-200*
IPY912	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3/(hht1-hhf1)Δ::HIS3 hta1-200/hta1-200*
IPY913	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hta1-300/hta1-300 hht1::mTn3/hht1::mTn3*
IPY914/915	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hhf1Δ::HIS3/hhf1Δ::HIS3 hta1-300/hta1-300 HIS4/his4Δ::URA3*

Table 1 continued:

Strain	Genotype
IPY916/917	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3/(hht1-hhf1)Δ::HIS3 HIS4/his4Δ::URA3*
IPY918/919	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3/(hht1-hhf1)Δ::HIS3 hta1-300/hta1-300 HIS4/his4Δ::URA3*
IPY926/927	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hht1Δ::HIS3/hht1Δ::HIS3 hta1-200/hta1-200 HIS4/his4Δ::URA3*
IPY928/929	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hhf1Δ::HIS3/hhf1Δ::HIS3 hta1-200/hta1-200 HIS4/his4Δ::URA3*
IPY930/931	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 (hht1-hhf1)Δ::HIS3/(hht1-hhf1)Δ::HIS3 hta1-200/hta1-200 HIS4/his4Δ::URA3*
IPY932/933	*MATa/MATα his3Δ200/his3Δ200 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63 ura3-52/ura3-52 (hta2-htb2)Δ::TRP1/(hta2-htb2)Δ::TRP1 hta1-300/hta1-300 hht1::mTn3/hht1::mTn3 HIS4/his4Δ::URA3*
IPY934	*MATa ura3- his3- leu2- trp1- (hta2-htb2)Δ::TRP1 hat1Δ::Kanx*
IPY935	*MATa ura3- his3- leu2- trp1- (hta2-htb2)Δ::TRP1 hat1Δ::Kanx hta1-300*
IPY941	*MATa ura3- his3- leu2- trp1- (hta2-htb2)Δ::TRP1 hat1Δ::Kanx hht1Δ::HIS3*
IPY942	*MATα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 (hta2-htb2)Δ::TRP1 hht1Δ::HIS3 hta1-300 arp4-26*

C. Construction of overexpression plasmids

All plasmids are listed in **Table 2**. Cloning was completed through the use of restriction enzymes purchased from Promega and used according to the manufacturer's recommendations. DNA fragments were isolated by separation on 0.8% agarose gel electrophoresis. Appropriate fragments were excised and purified using the GeneClean kit from MP Biomedical. DNA ligations were performed as previously described.[174] The histone H3 and H4 genes (*HHT1, HHT2, HHF1,* and *HHF2*) were cloned from PCR fragments in which the PCR primers contained a region homologous to the gene of interest attached to a region containing a restriction site convenient for cloning into the pRS vectors. The pRS vectors are an expression system designed by Sikorski and

Table 2: Plasmids

Plasmid Name	Genes, Markers, and Origin of Replication Type	Cloning Vector/Sites	Reference
pLG40	*his4-912δ'-URA3-lacZ-his4*	pUC118 *Sal*I	Pinto and Winston, 2000
pSAB6	*HTA1, URA3, ARS-CEN*		Pinto and Winston, 2000
pIP43	*ARP4, LEU2,* 2µ*m*	pRS425 *Nhe*I/*Eag*I	Pinto and Winston, 2000
pIP46	*ARP4, URA3, ARS-CEN*	pRS316 *Eag*I/*Xho*I	Pinto and Winston, 2000
pIP47	*ARP4, LEU2, ARS-CEN*	pRS415 *Eag*I/*Xho*I	Pinto and Winston, 2000
pIP48	*ARP4, LEU2, ARS-CEN*	pRS415 *Xba*I/*Xho*I	Pinto and Winston, 2000
pIP49	*ARP4, LEU2,* 2µ*m*	pRS425 *Spe*I/*Sal*I	Pinto and Winston, 2000
pIP90	*HHT1-HHF1, LEU2, 2µm*	pRS425 *BamH*I/*Hind*III	This study
pIP93	*HHT1, LEU2, 2µm*	pRS425 *BamH*I/*Hind*III	This study
pIP94	*HHF1, LEU2, 2µm*	pRS425 *BamH*I/*Hind*III	This study
pIP103	*HHT2-HHF2, LEU2, 2µm*	pRS425 *Xho*I/*Not*I	This study
pIP104	*HHT2, LEU2, 2µm*	pRS425 *Xho*I/*Not*I	This study
pIP105	*HHF2, LEU2, 2µm*	pRS425 *Xho*I/*BamH*I	This study
pIP117	*ARP4, URA3, ori*	pRS406 *Eag*I/*Xho*I	This study
pIP124	*arp4-26, LEU2, ARS-CEN*	pIP47 *Sph*I/*Bgl*II	This study
pIP125	*arp4-12, LEU2, ARS-CEN*	pIP47 *Bgl*II/*Nhe*I	This study
pIP126	*arp4-26, URA3, ori*	pRS406 *Eag*I/*Xho*I	This study
pIP129	*arp4-12, URA3, ori*	pRS406 *Eag*I/*Xho*I	This study

Hieter[175, 176] that allow for plasmid amplification in *E. coli* and gene expression in yeast using either the gene's own promoter (*ori*), an autonomous replicating sequence (*ARS-CEN*), or a 2µm promoter for high-copy number expression. All PCR primers are listed in **Table 3**.

HHT1 was cloned into a high-copy expression vector (pRS425) using PCR fragments cloned from genomic DNA with primers oIP128 and oIP158 and ligated into the vector using the *BamH*I/*Hind*III fragment (pIP93). Yeast colonies carrying the plasmid were selected by plating the cells on minimal media lacking leucine. *HHF1* was cloned into pRS425 using the PCR fragment from primers oIP127 and oIP158 digested with *BamH*I and *Hind*III (pIP94). A plasmid containing the entire copy I locus (*HHT1-*

HHF1, pIP90) was constructed from the PCR fragment of oIP127 and oIP128 digested with *BamH*I and *Hind*III. *HHT2* was cloned using primers oIP181 and oIP183 and the fragment was digested with *Xho*I and *Not*I (pIP104). The entire copy II locus (*HHT2-HHF2*) was cloned using primers oIP181 and oIP182 and digested with *Not*I and *Xho*I (pIP103). *HHF2* was cloned from the *HHT2-HHF2* fragment of pIP103 digested with *Xho*I and an endogenous *BamH*I site (pIP105).

Table 3: PCR Primers

Name	Sequence	Purpose
oIP8	5'GCCTCATCCAAAGGC3'	*HIS3* universal test oligo
oIP29	5'CAGCGCCAACACCTATGG3'	5' *CEN3* probe
oIP30	5'CCCGGGTGGGAAACTGAAGA3'	3' *CEN3* probe
oIP56	5'GTTAATAAGAAAAACATCTAACATAAATATAT AAACGCAAGATTGTACTGAGAGTGCAC3'	5' for deletion of *HHT1*
oIP57	5'TTTGTTCGTTTTTTACTAAAACTGATGACAATC AACAAACTGTGCGGTATTTCACACCG3'	3' for deletion of *HHT1*
oIP72	5'GTATTCTTCGGGGATACATCTC3'	5' confirmation of *hht1*Δ
oIP73	5'GTTAAAGAACCCAGTAAACCT3'	3' confirmation of *hht1*Δ
oIP74	5'GCAGTTGAATACGAATCCCAAATATTTGCTTG TTGTTACAGATTGTACTGAGAGTGCAC3'	3' for deletion of *HHT1-HHF1*
oIP77	5'TGGTTTCCGTCGCATTATTGTACTCTATAGTAC TAAAGCAGATTGTACTGAGAGTGCAC3'	5' for deletion of *HHF1*
oIP78	5'GCAGTTGAATACGAATCCCAAATATTTGCTTG TTGTTACCTGTGCGGTATTTCACACGC3'	3' for deletion of *HHF1*
oIP87	5'GACCAATTTGATGGATAAAT3'	5' confirmation of *hhf1*Δ
oIP88	5'TACACTCATATTTGTAGAAG3'	3' confirmation of *hhf1*Δ
oIP127	5'CTACGGATCCCTATTCCATGCAAGTTCGGT3'	5' cloning of *HHT1-HHF1,* creates *BamHI*
oIP128	5'GCGGAAAGCTTATATAACTGACTCATGAATG3'	3' cloning of *HHT1-HHF1,* creates *HindIII*
oIP142	5'GATCAGCGCCAAACAATATGG3'	5' *CEN3* core
oIP143	5'AACTTCCACCAGTAAACGTTTC3'	3' *CEN3* core
oIP146	5’GCAAAGGTTGAAGCCGTTATG3’	5’ *CEN16* core
oIP147	5’GCTTTGCCGATTTCGCTTTAG3’	3’ *CEN16* core

Table 3 continued:

Name	Sequence	Purpose
oIP148	5'CTCCACTTCAAGTAAGAGTTTGGG3'	5' *HMRa*
oIP149	5'CGCAGTAGAAAGACATATTTCTCTC3'	3' *HMRa*
oIP152	5'CAACCTTTAACGGGCACTCTC3'	5' *CEN16* -3 kb left
oIP153	5'GGTTATCAATAGGAACGTAAT3'	3' *CEN16* -3 kb left
oIP154	5'CCCAACAATTATCTCAACATT3'	5' *CEN16* +5 kb right
oIP155	5'GTCAAGAGATGTTCGAATTAG3'	3' *CEN16* +5 kb right
oIP157	5'CTATGGATCCTTTACCTCTACCGGACATAT3'	5' cloning of *HHT1*, creates *BamH*I
oIP158	5'CGACAAGCTTTGTTCTGGCCATTGTTTGCG3'	3' cloning of *HHF1*, creates *Hind*III
oIP172	5'CTCCTCATGTCGTTAAAAGCATTGCGAATAGA TAGATGAATAAGATTGTACTGAGAGTGCAC3'	5' for deletion of *HHT2-HHF2*
oIP173	5'CTATCTAAGACAGTTCGGAAACTAGTTCTTTT ATTGAGACTTCTGTGCGGTATTTCACACCG3'	3' for deletion of *HHT2-HHF2*
oIP174	5'GACCACTGTTTTGTGACTTCCACTTTGGCCCT TCCAACTGTTCTGTGCGGTATTTCACACCG3'	5' for deletion of *HHT2*
oIP175	5'CTACCTCGAGCTATTCCATGCAAGTTCGGT3'	3' cloning of *HHF1*, creates *Xho*I
oIP176	5'CTACAAGCTTTTTACCTCTACCGGACATAT3'	5' cloning of *HHT1*, creates *Hind*III
oIP177	5'GCGGCTCGAGATATAACTGACTCATGAATG3'	3' cloning of *HHT1*, creates *Xho*I
oIP180	5'GTTATATCATATATAAGTATATTAGGATGAGG CGGTGAAAGAGATTGTACTGAGAGTGCAC3'	5' for deletion of *HHF2*
oIP181	5'CTAGGCGGCCGCGTATACTATCTAAGCATCTA 3'	5' cloning of *HHT2-HHF2*, creates *NotI*
oIP182	5'CTAGCTCGAGCACATAAGGGAAGACTATCT3'	3' cloning of *HHT2-HHF2*, creates *Xho*I
oIP183	5'CTAGCTCGAGGCTACTCTTTTGAACAAGAT3'	3' cloning of *HHT2*, creates *Xho*I
oIP184	5'GCTTGATCAGCAGTTCATC3'	5' confirmation of (*hht2-hhf2)Δ*
oIP185	5'TCCTACTTAGCCAGTGACTC3'	3' confirmation of *(hht2-hhf2)Δ*
oIP192	5'AACGACCACAGTTGTCCGTT3'	3' confirmation of *hht2Δ*
oIP204	5'GCGAACCCTTCTCCATTTGGCAAT3'	5' *CEN3* -5 kb left
oIP205	5'CCTCGAAGGCCATCAAGTAGAAAA3'	3' *CEN3* -5 kb left
oIP206	5'CCGAAGGCTGGTATGTGATTTGTT3'	5' *CEN3* -2 kb left
oIP207	5'GATGGGCCAAAATACTGGAATATCG3'	3' *CEN3* -2 kb left
oIP208	5'ACTGCTATTAAGCGCCACTT3'	5' *CEN3* -1 kb left
oIP209	5'TTCTAACCACTGTGTCATCCGT3'	3' *CEN3* -1 kb left
oIP210	5'CCGTATCATGGACGATTTCCTT3'	5' *CEN3* -0.5 kb left
oIP211	5'TTGTCAAGTTGCTCACTGTGATTT3'	3' *CEN3* -0.5 kb left

Table 3 continued:

Name	Sequence	Purpose
oIP212	5'CCATCCAATACCTTGATGAACTTTTC3'	5' *CEN3* -0.25 kb left
oIP213	5'CGCCATGCCATGTTTATGAA3'	3' *CEN3* -0.25 kb left
oIP214	5'CGTTTACTGGTGGAAGTTTTGCTC3'	5' *CEN3* +0.25 kb right
oIP215	5'GGGGCGGAAATTCATTTGAA3'	3' *CEN3* +0.25 kb right
oIP216	5'CAAATGAATTTCCGCCCCAT3'	5' *CEN3* +0.5 kb right
oIP217	5'CAGTAGGTTTGTACTATAATGTGGGTG3'	3' *CEN3* +0.5 kb right
oIP218	5'ACGTGCATTAAATCTCACTGTCAC3'	5' *CEN3* +1 kb right
oIP219	5'TGCAGGTGCTATTTGACGACT3'	3' *CEN3* +1 kb right
oIP220	5'CGTCCAAACATGAAAGTGCTCCTT3'	5' *CEN3* +2 kb right
oIP221	5'CTGGCCTTCTTATCATACGTTGTC3'	3' *CEN3* +2 kb right
oIP222	5'GAAAACGCATACCGCTAAAGAAG3'	5' *CEN3* +5 kb right
oIP223	5'CCGCTCCTTGTATTCTACCATTG3'	3' *CEN3* +5 kb right
oIP260	5'GAAATTTTATTGCTAGGAAATTTATCAATCAC3'	megaprimer *arp4-26*
oIP261	5'GTTCGTTGTCAAAAACAATCT3'	*arp4-26* first flanking primer
oIP262	5'CTCCGGAATACCAGCTCTGTTAACTG3'	*arp4-26* second flanking primer
oIP263	5'CCAGTCATGGCTTAGCGGTAGTATAC3'	megaprimer *arp4-12*
oIP264	5'GTTGAAAACGCGCTTGCTTAACC3'	*arp4-12* first flanking primer
oIP265	5'CCGAGGGTTCTTTCAAGAGTGC3'	*arp4-12* second flanking primer

A WT strain (FY604), a strain carrying *hta1-300* (IPY69), and a strain carrying *hta1-200* (FY1819) were transformed with each of the plasmids. Transformants were streaked onto SC-Leu to select for only cells carrying the plasmid. These were then replica plated to media containing 5-FOA but lacking leucine (5-FOA-Leu) to select for cells that had lost pSAB6, the plasmid carrying WT *HTA1* which covers the *hta1* mutants so the strains remain haploid. At least 50 transformants were assayed from each plasmid in each strain.

D. Construction of histone gene deletions

Stains carrying deletions of the histone genes (*hht1Δ, hhf1Δ, (hht1-hhf1)Δ, hht2Δ, hhf2Δ, and (hht2-hhf2)Δ*) were constructed by PCR-mediated disruption as previously described.[169, 177] *HIS3* was amplified using primers homologous to *HIS3* flanked by sequences homologous to the target gene. This PCR product was used to transform FY604 such that *HIS3* replaced the target gene by homologous recombination. Transformants in which this recombination event occurred were selected by plating on minimal medium lacking histidine. The strains were then confirmed by PCR using primers that hybridize outside the sequences targeted for recombination.

Strains carrying multiple mutations were constructed using standard yeast genetic techniques in which strains carrying the individual mutations of interest were crossed, the resulting diploid sporulated and dissected, and the spores screened for phenotypes corresponding to the individual mutants.[169]

The strain carrying the *hat1Δ* allele was obtained from the yeast gene deletion library.[177]

E. Construction of *ARP4* temperature sensitive mutants

Mutations were made to *ARP4* by the megaprimer method of site-directed mutagenesis.[178] The two mutations are *arp4-26* (G187R) and *arp4-12* (G455S), as described.[99] For clarity, *arp4-26* will be referred to as ts26, and *arp4-12* as ts12. The DNA template was the *ARP4* clone from the *Eag*I/*Xho*I fragment of pIP47.[1] Mutagenic primers were developed that were 26 and 23 bp long for the ts26 and ts12 strains, respectively (oIP260 and oIP263). These primers were entirely homologous to the *ARP4* sequence except that one nucleotide was changed to confer the specified change in amino

acid. These were complemented by a limiting amount of a shorter first-flanking primer at the 3' end of a convenient clone site within the gene (oIP261 and oIP264, respectively). PCR was conducted using only 5 cycles. The resulting product was the 'megaprimer' to be used in a second round of PCR after purification. The megaprimer was purified by separating it from other PCR fragments on a 0.8% TAE agarose gel, excising the band, and cleaning it using the GeneClean kit from MP Biomedicals. The second round of PCR used the same template with the megaprimer (now the 3' end) and the second flanking primer (5' to the convenient clone site, oIP262 and oIP265). This PCR product was again separated on a 0.8% agarose gel, excised, and cleaned with the GeneClean kit. The mutations were confirmed by DNA sequencing at the University of Arkansas DNA resource center.

Once mutations were confirmed, the PCR products were cloned into an integrating plasmid (pIP117) using the *Sph*I/*Bgl*II sites for ts26 or the *Bgl*II/*Nhe*I sites for ts12. These plasmids were named pIP124 and pIP125 and were linearized with *Hpa*I (*Hpa*I site is 327bp downstream of *ARP4* start) and introduced into IPY15 (*arp4Δ* containing pIP46, a *CEN* plasmid containing a *URA3* marker and a WT *ARP4* gene). The resulting transformants were then grown on 5-FOA to select for the loss of pIP46 and then screened for temperature sensitivity.

F. Canavanine assay for ploidy

An assay was performed to measure gain in chromosome copy number based on papillation.[1] Chromosome V ploidy was assayed by monitoring the *CAN1* gene as described by Schild *et al*.[179] Since canavanine resistance is conferred by recessive mutations in the *CAN1* gene, the frequency of canavanine resistant mutants is much

greater among haploids than among diploids. Yeast strains were replica plated to plates with and without canavanine and mutagenized by UV irradiation (300 ergs/mm^2). After several days of incubation, papillae were observed in haploid strains, whereas diploid strains typically remained sensitive to the canavanine.

G. Flow Cytometry

DNA content of yeast cells was determined as described previously using a Becton Dickinson FACSCalibur instrument.[1] Briefly, cells were passed though several culture generations and collected in early log phase, fixed with 70% ethanol, treated with RNase, and stained with propidium iodide as described.[180]

H. Growth curves

To determine growth rates in strains carrying high-copy plasmids expressing histone genes, FY604, IPY69, and FY1819 were each transformed with pRS425, pIP90, pIP93, pIP94, pIP103, pIP104, and pIP105. The strains were then grown in rich medium (YPD) and replica plated to 5-F0A and grown for two days to select for the loss of pSAB6, which contains the WT *HTA1* allele. Representative 5-FOA^R colonies were then inoculated in liquid SC-Leu and grown to saturation. 300 µL of this culture were transferred to 10 mL of fresh SC-Leu. 250 µL of this culture were removed and placed in 50 µL of a 37.8% formaldehyde solution; this was marked as time=0 hours. In the same manner, a sample was taken from each culture every 2 hours for 8 hours. Samples preserved in formaldehyde were counted on a hemacytometer and plotted against time. This was repeated twice for each culture. Error bars were calculated by standard deviation of several independent counts of a representative sample for each culture.

I. Chromosome loss and recombination assay

Rates of chromosome loss were determined as described previously.[1, 2] Diploid strains homozygous for *HTA1, hta1-300, hta1-200, hht1Δ, hht1Δ hta1-300, hht1Δ hta1-200, hht1::mTn3 hta1-300, hhf1Δ, hhf1Δ hta1-300, hhf1Δ hta1-200, (hht1-hhf1)Δ, (hht1-hhf1)Δ hta1-300,* and *(hht1-hhf1)Δ hta1-200* were marked at the *HIS4* locus on the right arm of chromosome III by the integration of *URA3* into *HIS4* by transformation of the strains with the *Sal*I fragment of pLG40. The resulting strains are *HIS4/his4Δ10::URA3*. Construction of these alleles was confirmed by Southern blot analysis as described previously.[174] Each strain was grown overnight on SC-Ura, streaked for single colonies on YPD, and grown for 3 or 5 days (all strains carrying an *hta1* mutant are slow growers and required 5 days of growth, all other strains took only 3 days). 5-10 colonies were excised from each plate using a sterile scalpel and resuspended in 1 mL YPD. Usually 5 colonies were isolated from two isogenic strains obtained independently. The cells were counted on a hemacytometer and dilutions were plated on 5 plates containing 5-FOA each. These plates were again grown 3 or 5 days. 5-FOA is a toxic analog to a uracil precursor; cells containing a WT *URA3* gene are unable to grow on 5-FOA.[181] Thus, 5-FOA-resistant (5-FOA^R) colonies result from either the loss of chromosome III or a mitotic recombination event between the *CEN3* and *his4Δ10::URA3* alleles, losing the *URA3* gene. To distinguish between these two events, 5-FOA^R colonies were counted and then assayed for mating type (*MAT*). The yeast *MAT* locus is located on the left arm of chromosome III; thus, colonies in which chromosome III was lost would be either *MATa* or *MATα* and should therefore mate. However, mitotic recombinants would remain *MATa/MATα* and should not mate. Colonies that were *MATa* or *MATα* were

counted and chromosome loss and recombination rates were calculated by the method of the median.[182]

J. Mass spectrometry

All mass spectrometry experiments were performed in the laboratory of collaborator Alan J. Tackett using histones extracted in our lab by the procedure described below.

K. Histone purification

Histones were purified as described by Edmonson *et al*.[183] except that the concentration of sodium butyrate was increased to 50mM as suggested by Waterborg.[85] Briefly, 2L of yeast were grown in YPD to about $2x10^8$ cells/mL. Cells were centrifuged in 500-mL aliquots at 5000rpm for 5 minutes at 4°C in a Beckman J2-21 centrifuge using a JA-10 rotor. They were then pooled together into one bottle and washed in about 200mL sterile water. These cells were resuspended in 50mL 0.1mM Tris pH 9.4, 10mM DTT and incubated at 30°C for 15 minutes with gentle shaking. They were then centrifuged again and washed in SH buffer (1.2M sorbitol, 20mM HEPES pH7.4). After another centrifugation, cells were resuspended again in SH buffer with 2mL 10mg/mL Zymolyase and incubated at 30°C for 45-60 minutes with gentle shaking. After incubation, 100mL of ice-cold 1.2M sorbitol, 20mM PIPES, 1mM $MgCl_2$ pH 6.8 was added and the spheroplasts were centrifuged at 3500 rpm for 5 minutes at 4°C. The pellet was resuspended in 50mL ice-cold nuclei isolation buffer (NIB, 0.25M sucrose, 60mM KCl, 14mM $MgCl_2$, 1mM $CaCl_2$, 15mM MES pH6.6, 1mM PMSF, 0.8% TritonX-100) and incubated on ice for 20 minutes. The lysate was then centrifuged at 4000 rpm for 5 minutes at 4°C. The NIB wash was repeated twice. The nuclei were then resuspended in

50mL “A” wash (10mM Tris pH8.0, 0.5% NP-40, 75mM NaCl, 50mM NaButyrate, 1mM PMSF) and incubated on ice for 15 minutes. This was followed by centrifugation at 4000 rpm for 5 minutes at 4°C. The “A” wash was repeated twice, except on the final wash, the volume was decreased by half and the nuclei were only held on ice for 5 minutes. Nuclei were then resuspended in 50mL “B” wash (10mM Tris pH8.0, 0.4M NaCl, 50mM NaButyrate, 1mM PMSF) and incubated on ice for 10 minutes. After another centrifugation, the nuclei were resuspended in 25mL of “B” wash and immediately centrifuged again. Histones were extracted by resuspending the pellet in 10mL cold 0.4N H_2SO_4 and incubating it on ice for 30 minutes with occasional vortexing. This was followed by centrifugation at 10,000 rpm for 10 minutes at 4°C in the JA-10 rotor. The supernatant was transferred to 30-mL glass tubes and TCA was added to a final concentration of 20%. This was incubated on ice for 30 minutes and then centrifuged at 12,000 rpm for 30 minutes at 4°C in a JA-17 rotor. The pellet was then washed in acidified acetone (acetone + 1% HCl) and then in acetone and allowed to air dry. Histones were resuspended in 10mM Tris pH8.0 and stored at -20°C.

Acid-extracted proteins were separated on a 15% acrylamide gel with a 30:0.15 acrylamide:bisacrylamide ratio as described,[184, 185] except the pH of the running buffer was increased to pH8.8. These gels were either stained with Coomassie blue or transferred to nitrocellulose membranes for western blotting as described below.

L. Whole cell extracts and western blotting

Whole cell extracts for Western blots with antibodies against histone proteins were prepared as described previously.[186] Cells were grown to an OD_{600} of about 0.8 to 1.0, centrifuged and washed in sterile water. They were then frozen overnight at -

80°C. Cells were then defrosted and resuspended in breaking buffer (10mM Tris pH7.4, 300mM sorbitol, 600mM NaCl, 5mM $MgCl_2$, 5mM EDTA, 1µg/mL aprotinin, 1µg/mL leupeptin, 1µg/mL pepstatin A, 1mM PMSF, 50mM NaButyrate). Glass beads were added and cells were broken open by three 30 second pulses on a Mini-Beadbeater-8 (Biospec Products). Lysate was then separated from the beads and centrifuged in a refrigerated-microcentrifuge for 10 minutes at 4°C. About 15µL of a 1:10 dilution of the supernatant was electrophoresed on a 15% acrylamide gel and Western blotting was completed as described below.

Proteins were transferred from the acrylamide gels to 0.2µm nitrocellulose membranes on a BioRad Trans-Blot system at 4°C, at 30V, for 90 minutes in 25mM Tris, 190mM glycine, 20% methanol. The nitrocellulose membrane was stained using either Ponceau S[174] or MemCode Nitrocellulose Stain purchased from Pierce.

Western blotting was done as described below, a procedure modified from a protocol provided by Abcam.[187] The membrane was blocked in 5% BSA resuspended in TBST for 1 hour at room temperature (RT). The membrane was then incubated with the primary antibody (generally according to manufacturer's instructions) in 5% BSA/TBST for 1.5 hours at RT. The membrane was then washed twice in TBST for 5 minutes and twice for 10 minutes. It was then incubated with the secondary antibody (2µL anti-rabbit IgG from goat, Promega) in 1% BSA/TBST for 1 hour at RT. The membrane was then washed again as above and developed using the Millipore Immobilon Western Chemiluminescent HRP Substrate and imaged in an Alpha Innotech MultiImage Light Cabinet. Primary antibodies used include α-H2A pAb from Active Motif, α-H2B pAb from Active Motif, α-acetyl-H2B (Lys 16) from Upstate [α-H2BacK16], α-H3 pan from

Upstate, α-acetyl-H3 (Lys 14) from Upstate [αH3acK14], α-acetyl-H3 (Lys 16) from Upstate [α-H3acK16], α-acetyl-H3 (Lys 18) from Upstate [α-H3acK18], α-acetyl-H3 (Lys27) from Upstate [α-H3acK27], α-acetyl-Histone H4 (Lys5) from Upstate/Millipore [α-H4acK5], α-H4 pan from Millipore, and α-acetyl-H4 (Lys12) from Upstate/Millipore [α-H4acK12].[187]

M. Preparation of nuclei and indirect-end labeling analysis

Yeast chromatin structure was analyzed using the indirect-end labeling analysis procedure as described by Pinto and Winston[1] with modifications to the nuclei isolation by Sharp *et al*.[188]

For nuclei isolation, 1.5 L of cells were grown in YPD and centrifuged at 6000 rpm for 10 minutes at 4°C. The pellet was washed in sterile water and centrifuged again. Cells were washed in a cold DTT solution (10mM DTT, 20mM potassium phosphate pH7, 1M sorbitol) and then resuspended in cold S buffer containing 0.5mg/mL zymolyase (S buffer is 1.1M sorbitol, 20mM potassium phosphate pH7, 0.5mM $CaCl_2$, 0.5mM PMSF). The cells were allowed to spheroplast at 30°C with gentle shaking for about 35 minutes and then centrifuged at 5000 rpm for 5 minutes at 4°C. The supernatant was removed and 15mL of cold SPC buffer were added to the pellet (SPC buffer is 1M sorbitol, 20mM PIPES pH6.3, 0.1mM $CaCl_2$, 1.72µg/mL aprotinin, 0.1µg/mL chymostatin, 7.2µg/mL E-64, 1µg/mL pepstatin A, 100mM PMSF). The tubes were positioned in the centrifuge such that the pellet would pass through the buffer during centrifugation and were centrifuged at 5000 rpm for 5 minutes at 4°C. The pellet was then gently resuspended in 0.25mL/g cold SPC buffer and slowly added to 50-fold excess cold FL buffer (9% wt/wt Ficoll 400, 20mM PIPES pH6.3, 0.5mM $CaCl_2$, 1.72µg/mL

aprotinin, 0.1μg/mL chymostatin, 7.2μg/mL E-64, 1μg/mL pepstatin A, 100mM PMSF). This was then centrifuged at 2000 rpm for 2 minutes at 4°C. The upper layer of the supernatant was transferred to a new tube and centrifuged at 13,250 rpm for 20 minutes at 4°C. The pellet was then resuspended in 17mL SPC buffer using the centrifugation method as described above. The pellets were resuspended in MNase buffer (50% glycerol, 2mM $CaCl_2$, 50mM Tris pH9) and stored at -80°C.

Nuclei were digested with increasing concentrations of micrococcal nuclease as described previously.[2] After digestion, the DNA was isolated by phenol:chloroform extraction and ethanol precipitation. This product was then digested overnight with *BamH*I for detection of the centromere core and nucleosomes 5' to *CEN3*.[1] These digestions were separated on 1.8% TAE agarose gels and then transferred to GeneScreen nylon membranes by standard methods.[174] A Southern blot was performed on this membrane using a probe for *CEN3* made from the PCR product of primers oIP29 and oIP30. The membrane was then placed on a phosphor-imager screen and after exposure, the screen was imaged using a Typhoon scanner.

N. Chromatin immunoprecipitations

In vivo crosslinking and chromatin immunoprecipitations were done as described previously[189] with modifications from Almutairi[138] and Duina[190] as follows. Yeast cultures (100mL) were grown to an OD_{600} of 0.5-0.6 and crosslinked in a solution containing 1% formaldehyde with 10mM NaCl, 0.1mM EDTA, and 5mM HEPES pH7.6 at room temperature for 20 minutes. These cells were collected and washes twice in TBS buffer (20mM Tris pH7.6, 150mM NaCl). The pellet was then resuspended in 0.5 mL of breaking buffer (100mM Tris pH8.0, 20% glycerol, 1mM PMSF, 1x protease inhibitors

(Complete EDTA-free, Roche)) and glass beads were added. Cells were vortexed in a Biospec Mini Bead-beater three times for 2 minutes each, with 2 minute rests on ice between pulses. This lysate was washed in FA buffer (50mM HEPES PH7.6, 150mM NaCl, 1mM EDTA, 1% TritonX-100, 0.1% Na-deoxycholate, and 1x protease inhibitors) and resuspended in 1.5 mL of FA buffer. The lysate was sonicated on a Misonix Sonicator to an average chromatin size of approximately 500 bp. Immunoprecipitation was performed using 5µL αH4acK5 or αH4acK12 (both from Upstate/Millipore) bound to Protein A Dynabeads (from Dynal) with 450 µL sheared chromatin; 50 µL of sheared chromatin were saved as the input sample. After immunoprecipitation, chromatin was washed three times in FA buffer, twice in FA-HS buffer (FA buffer with 1% Na-deoxycholate), and twice in RIPA buffer (10mM Tris pH8.0, 250mM LiCl, 0.5% NP-40, 0.5% Na-deoxycholate, 1mM EDTA, and 1x protease inhibitors). The chromatin was then resuspended in TE and treated with RNaseA. Crosslinks were eluted in 25mM Tris pH7.6, 10mM EDTA, 0.5% SDS at 65°C overnight and then treated with 0.5mg/mL Proteinase K for 4-5 hours to remove the bound proteins. DNA was purified by phenol:chloroform extraction and precipitation in ethanol and sodium acetate. PCR was performed using primers for *CEN3, CEN3* ± 0.25 kb, *CEN3* ± 0.5 kb, *CEN3* ± 1 kb, *CEN3* ± 2 kb, *CEN3* ± 5 kb, *HMRa, CEN16, CEN16* -3 kb, and *CEN16* +5 kb as described previously and shown here in **Table 3**.[2, 132, 138] The PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and imaged on a UV light in an Alpha Innotech MultiImage Light cabinet.

IV. RESULTS

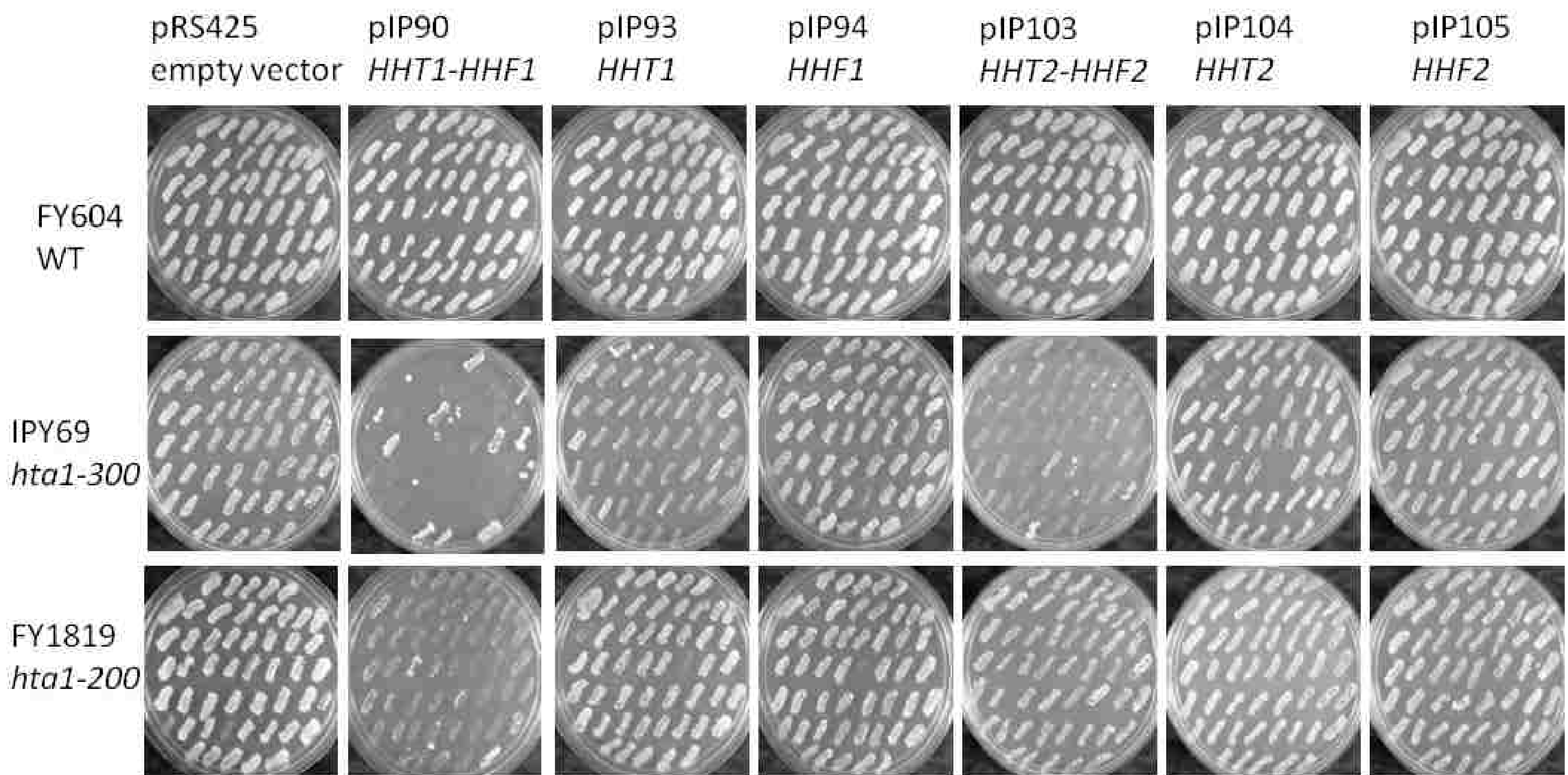
A. Overexpression of H3/H4 genes in *hta1* mutant strains causes synthetic dosage sickness and synthetic dosage lethal phenotypes

In order to determine whether the *hht1::mTn3* allele is able to suppress the *hta1* increase-in-ploidy phenotype because of an overall change in histone dosage or because of an effect specific to that allele, we made high-copy expression vectors containing each of the H3 and H4 gene pairs. It was predicted that if alteration in histone dosage was sufficient for suppression, then either deletion or overexpression of these genes would be able to suppress the phenotype. The genes were cloned using PCR-generated restriction sites and were placed into the 2μm pRS425 plasmid.[176] The vectors vary in expression copy number from 10-30 per haploid genome.[176]

Each vector was introduced into FY604 (WT), IPY69 (*hta1-300*), and FY1819 (*hta1-200*). 50 transformants from each vector were selected and streaked onto minimal media. These were then replica plated onto YPD (and incubated at 13°C to screen for cold sensitivity), SC-Arg and SC-Arg+Canavanine (both mutagenized by UV irradiation to screen for increase-in-ploidy as previously described[2]), and minimal media containing 5-FOA (to select for strains that lost the pSAB6 vector containing a WT *HTA1*).

It was found that there was no suppression of the cold-sensitive phenotype (data not shown). Growth on 5-FOA is shown in **Figure 5**. No additional growth phenotypes were apparent in the WT strain. In the strain containing *hta1-300*, 2μm-*HHT1-HHF1* resulted in a synthetic dosage lethal phenotype in which most transformants were unable

Figure 5: Plates showing effects of overexpression of H3 and H4 genes. No growth phenotypes are observed when 2µm H3 and H4 genes are added to WT strains. However, when added to *hta1-300* or *hta1-200*, dosage-dependant synthetic lethal or synthetic sickness phenotypes are observed.



to grow. However, some had minor resistance to the 5-FOA, indicating that the synthetic lethal phenotype is dosage dependant, and low doses of plasmid copy number resulted in a synthetic sickness phenotype. Synthetic lethal and synthetic sickness phenotypes indicate genetic interactions in which the genes of interest commonly function in the same essential pathway or in parallel nonessential pathways.[100] Both 2µm-*HHT1* and *HHF1* resulted in a dosage-dependent synthetic sickness phenotype; *HHT1* having a much more dramatic affect. The copy-II phenotypes are similar, but less drastic. 2µm-*HHT2-HHF2* resulted in a synthetic sickness phenotype, much more severe than the synthetic sickness phenotype exhibited by 2µm-*HHT2*. 2µm-*HHF2* had no apparent growth phenotype.

The phenotypes associated with the *hta1-200* allele were consistent with those of *hta1-300* except that they were weaker. 2µm-*HHT1-HHF1* is still dosage dependent synthetic lethal, and 2µm-*HHT1* is synthetic sick, but 2µm-*HHF1* had no apparent phenotype in combination with this mutant. 2µm-*HHT2-HHF2* has a synthetic sickness phenotype, whereas 2µm-*HHT2* or 2µm-*HHF2* had no apparent growth phenotype.

These data are supported by a growth curve in which representative transformants for each plasmid were grown on solid minimal medium containing 5-FOA, and then inoculated in liquid minimal medium selecting for the marker on the vector (SC-Leu). Samples were removed from the culture and preserved in formaldehyde every two hours for a total growth time of eight hours and cells were counted using a hemacytometer. A sample of growth curves for IPY69 (*hta1-300*) are shown in **Figure 6**. Due to the variation in growth rates caused by variation in histone gene expression, only the growth curves from representative transformants of each strain are shown. A strain containing

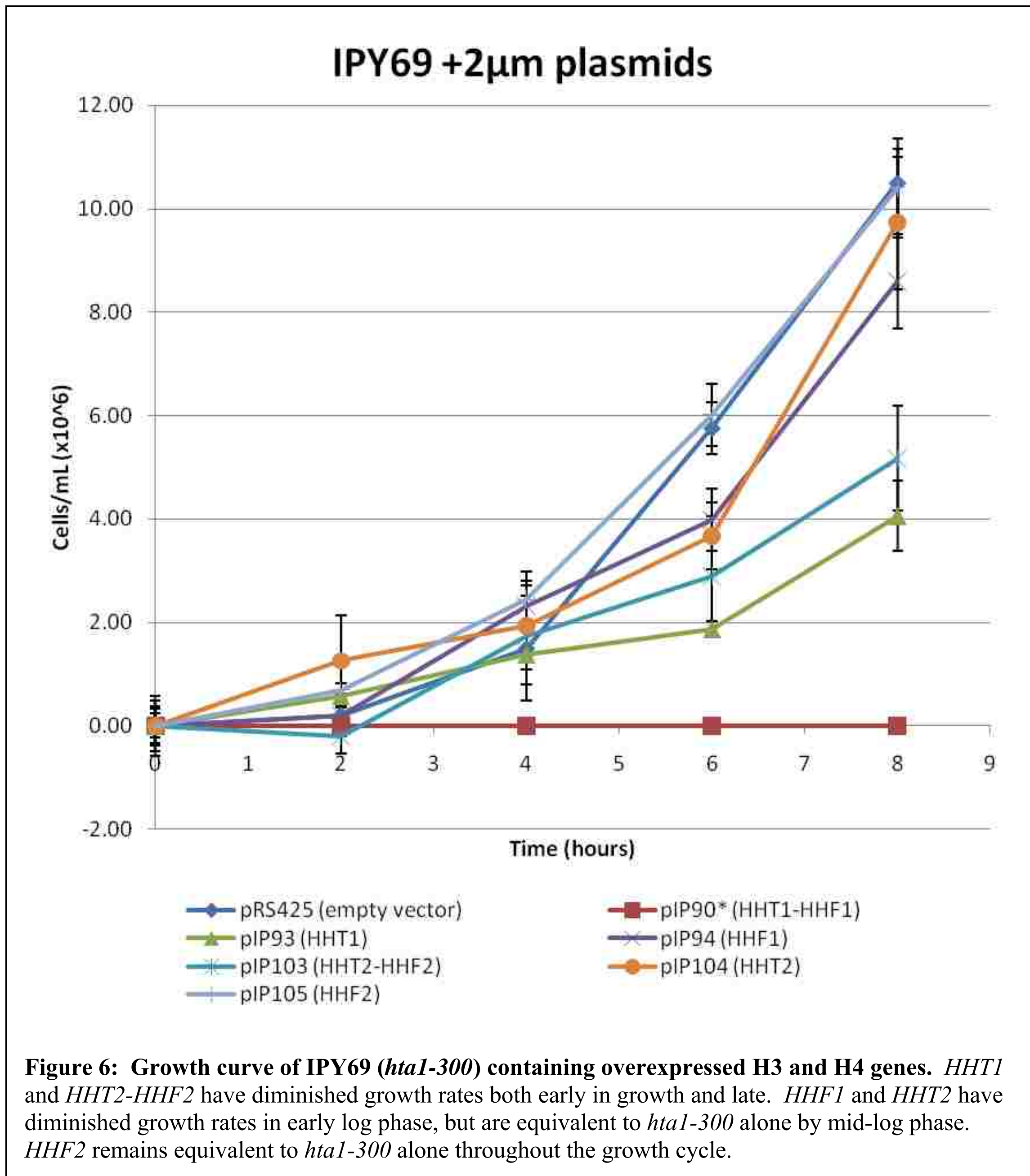


Figure 6: Growth curve of IPY69 (*hta1-300*) containing overexpressed H3 and H4 genes. *HHT1* and *HHT2-HHF2* have diminished growth rates both early in growth and late. *HHF1* and *HHT2* have diminished growth rates in early log phase, but are equivalent to *hta1-300* alone by mid-log phase. *HHF2* remains equivalent to *hta1-300* alone throughout the growth cycle.

pIP90 (*HHT1-HHF1*) was not included because representative transformants were all dead on selective medium. Growth rates from 4 to 6 hours (early-log phase) were compared with growth rates from 6 to 8 hours (mid-log phase) because by mid-log phase those cells with lower histone dosage became the dominant cells in the culture and growth rates could be compared with *hta1-300* alone. Culture doubling times were

calculated in log phase for each vector and are as follows: *hta1-300* + pRS425 (empty vector) is 1.43 hours, + pIP93 (*HHT1*) is 2.57 hours, + pIP94 (*HHF1*) is 2.11 hours, + pIP103 (*HHT2-HHF2*) is 2.53 hours, + pIP104 (*HHT2)* is 1.72 hours, and + pIP105 (*HHF2*) is 1.88 hours. The strains exhibiting the most drastic synthetic sickness phenotypes on agar (*HHT1* and *HHT2-HHF2*) had the most retarded growth rates in liquid medium as well. These strains grew more slowly throughout the growth period and had longer lag phases. Strains that exhibited less severe synthetic sickness phenotypes on agar (*HHF1* and *HHT2*) had reduced growth rates during early log phase, but by mid-log phase, were growing at rates equivalent to *hta1-300* alone. Consistent with the growth on agar, *HHF2* had no growth defect in liquid medium. These growth curve results are consistent with the observed growth on agar.

Ploidy was determined by the canavanine assay mentioned above, as well as by DNA-content analysis through flow cytometry. These data are shown in **Figure 7**. It was determined that overexpression of histone genes does not suppress the increase-in-ploidy phenotypes of the *hta1* mutants. In fact, overexpression of *HHT1* caused an increase in ploidy in the WT strain. WT strains with overexpressed H3 genes also demonstrated a marked delay in G1, as shown by the much higher G1 peak, a phenotype also apparent in the *hta1* mutant.

These data lead to the conclusion that the *hht1::mTn3* allele is not able to suppress the increase-in-ploidy phenotypes by alteration in histone gene stoichiometry. Increase in histone gene dosage actually resulted in synthetic lethal and synthetic sickness phenotypes indicating that a change in dosage is not responsible for the phenotype suppression, and that increased dosage is actually deleterious to these cells. These data

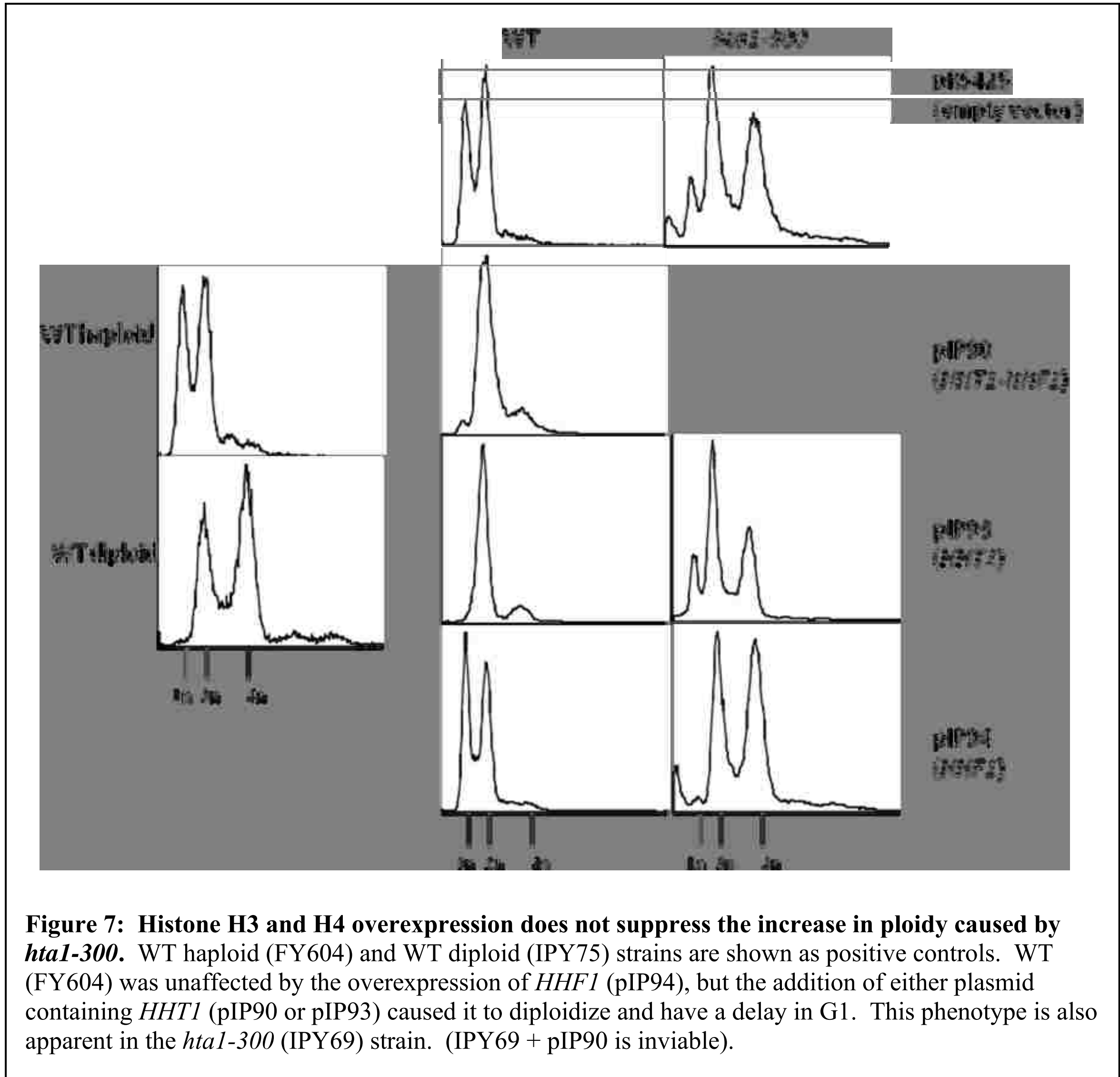


Figure 7: Histone H3 and H4 overexpression does not suppress the increase in ploidy caused by *hta1-300*. WT haploid (FY604) and WT diploid (IPY75) strains are shown as positive controls. WT (FY604) was unaffected by the overexpression of *HHF1* (pIP94), but the addition of either plasmid containing *HHT1* (pIP90 or pIP93) caused it to diploidize and have a delay in G1. This phenotype is also apparent in the *hta1-300* (IPY69) strain. (IPY69 + pIP90 is inviable).

do not rule out the possibility that reduced dosage, but not increased dosage, is responsible for the suppression. It is also interesting to note that overexpression of H3 results in increase in ploidy and a delay in G1, further supporting the need for proper dosage in cell cycle maintenance and progression.

B. Development of H3 and H4 gene deletions

In order to show that the *hht1::mTn3* allele suppresses the increase-in-ploidy phenotype because it results in a null-transcript, and not by interference from the expression of a small polypeptide, it was necessary to delete the entire *hht1* locus and

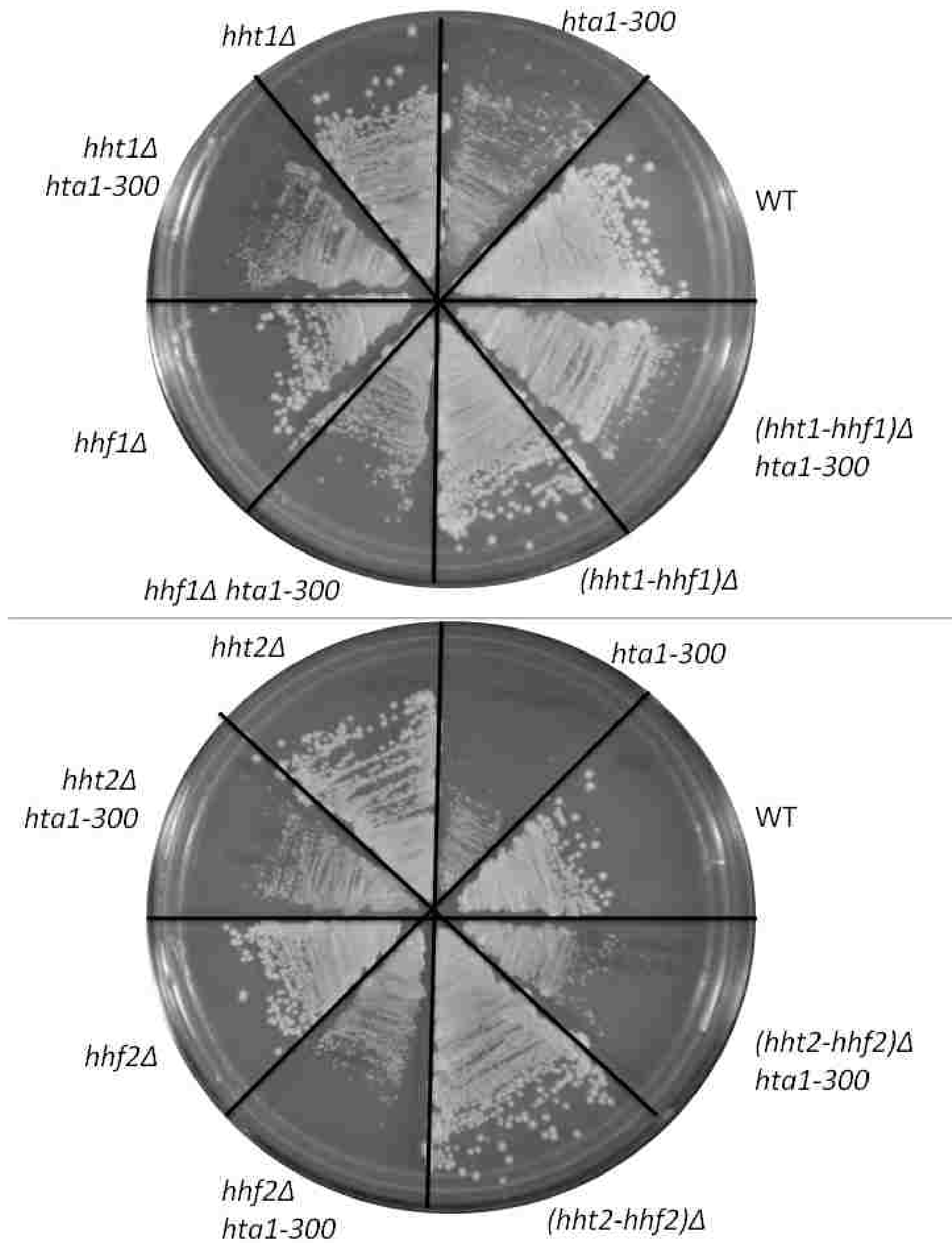


Figure 8a: Growth of deletion strains and double mutants. *hta1-300* mutants are slow growth and have heterogeneous colony sizes. H3 and H4 gene deletions do not alter this growth phenotype. Strain keys are depicted below photographs.

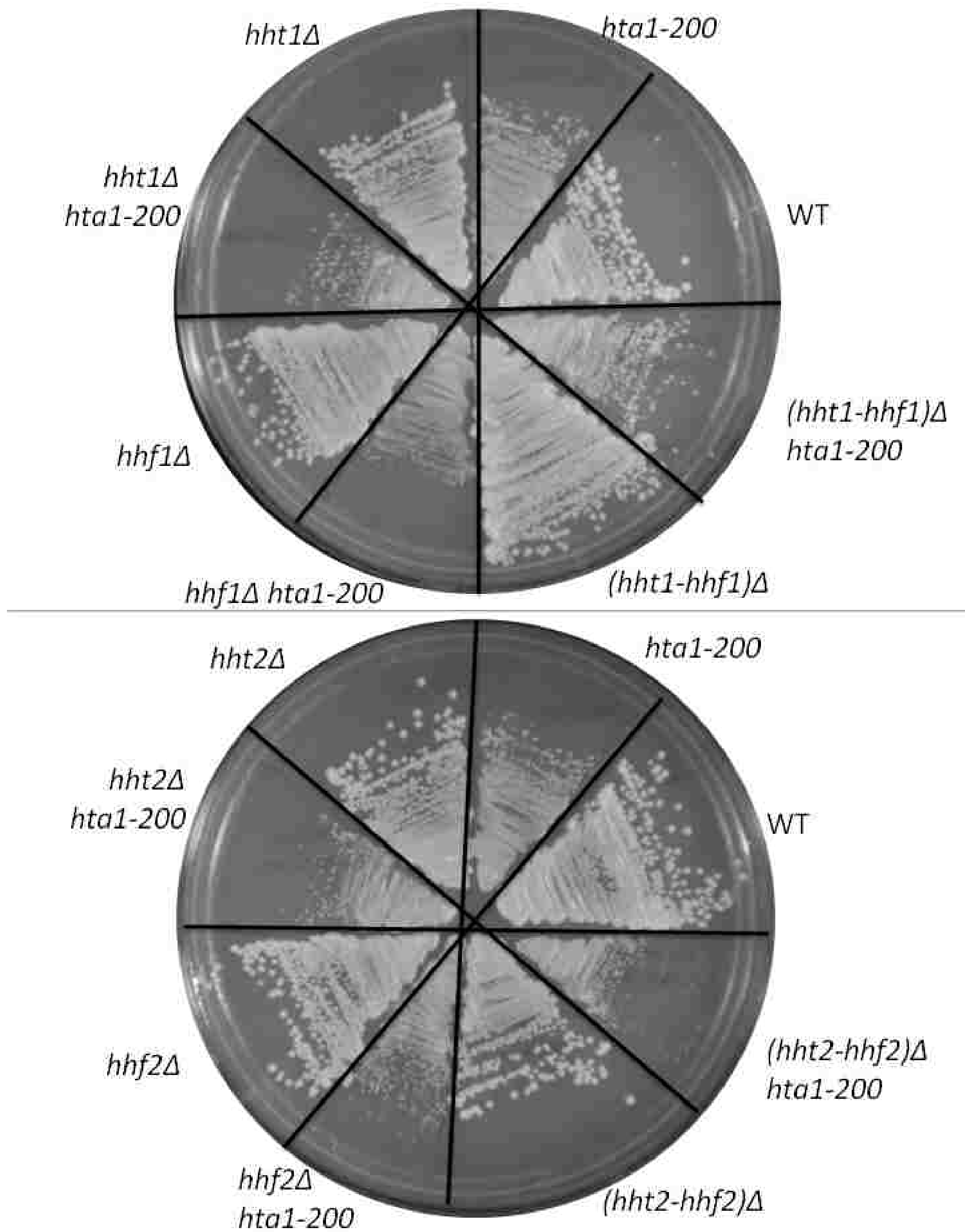


Figure 8b: Growth of deletion strains and double mutants. *hta1-200* mutants are slow growth and have heterogeneous colony sizes. H3 and H4 gene deletions do not alter this growth phenotype. Strain keys are depicted below photographs.

compare phenotypes. *hht1* was deleted by PCR-mediated disruption in which the gene was replaced by a *HIS3* marker in a strain carrying the *(hta2-htb2)Δ::TRP1* allele. This strain was then crossed with both *hta1* mutants to form double mutants. Similar deletions and crosses were also made at the *hhf1, (hht1-hhf1), hht2, hhf2,* and *(hht2-hhf2)* loci.

The *hta1* mutants cause slow growth with heterogeneous colony sizes, a phenotype commonly associated with chromosome segregation defects. None of the H3 or H4 deletions have a growth phenotype on their own, and they were unable to suppress the growth defects of the *hta1* mutants. Growth of these strains is shown in **Figure 8**.

C. H3 and H4 gene deletions suppress the increase-in-ploidy phenotype

All of the above mentioned mutant strains were tested for alterations in DNA content by flow cytometry. These data are shown in **Figure 9**.

It was found that, after many generations, most of the histone gene deletions were able suppress the increase in ploidy, as ascertained by the fact that strains containing *hta1* mutants were able to remain haploid. The only exception is that *hht2Δ* is only partially able to suppress the increase-in-ploidy of *hta1-200* as shown by the presence of both 1n and 4n peaks, indicating that the culture contained a mixed population of haploid and diploid cells. The *hta1-200* strain by itself has only 2n and 4n peaks after the same number of generations.

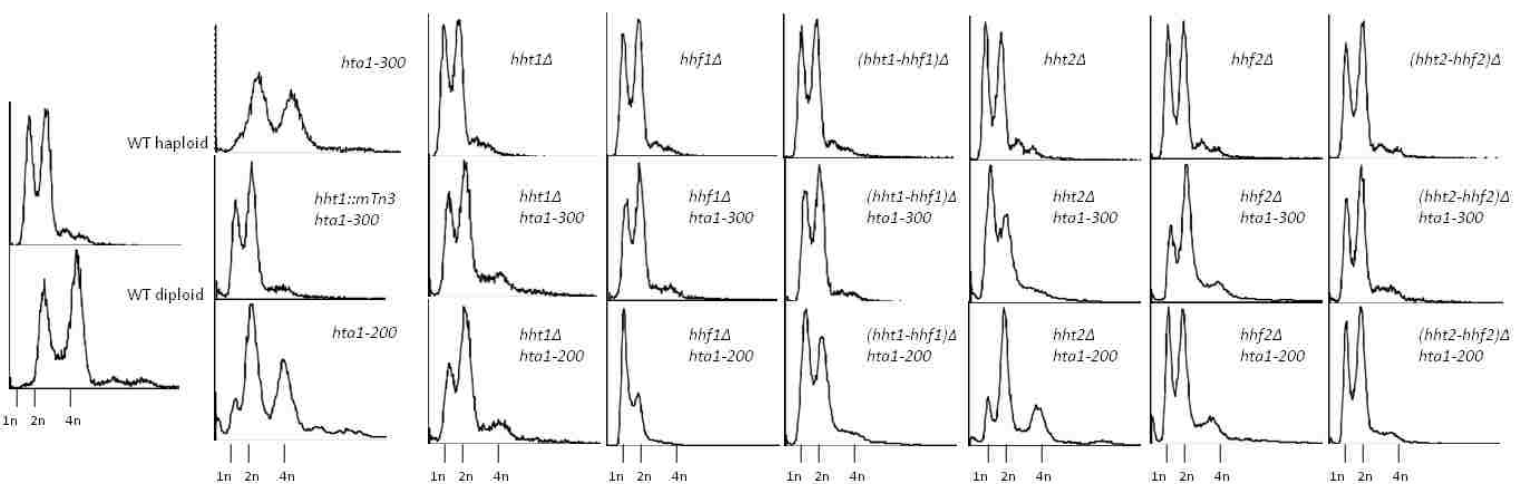


Figure 9: Deletions of H3 and H4 genes are able to suppress the increase in ploidy phenotype of *hta1* mutants. DNA content analysis of each strain shows that deletions of almost every H3 or H4 gene/gene set is able to suppress the increase-in-ploidy phenotype. The only exception is that *hht2Δ* is only partially able to suppress the increase in ploidy of *hta1-200*.

D. H3 and H4 gene deletions do not suppress the increased rate of chromosome loss

Since the ploidy phenotype is suppressed by the *hht1Δ* allele, we wanted to know if the increased rate of chromosome loss phenotype would also be suppressed. Chromosome loss and recombination assays were performed on all of the copy I deletion strains and the rates are shown in **Table 4**. These data show that strains carrying an *hta1* mutant still have an increased rate of chromosome loss, despite the alterations in histone gene dosage caused by H3 or H4 deletions. Interestingly, the *hhf1* deletion has an increased rate of chromosome loss on its own; a phenotype that is amplified upon addition of the *hta1-300* allele, but not the *hta1-200* allele. This is consistent with previous experiments showing that an *hhf1* mutant exhibits an increased rate of chromosome loss when it was the only copy of the H4 gene present.[84]

Table 4: Chromosome Loss and Recombination Rates

Strain	Genotype	Chromosome Loss Rate ($x10^{-6}$)	Recombination Rate ($x10^{-6}$)
IPY136	WT	4	8
IPY139	*hta1-300*	16	3
IPY831/IPY832	*hht1Δ*	3	2
IPY833/834	*hht1Δ hta1-300*	15	9
IPY932/933	*hht1::mTn3 hta1-300*	7	5
IPY837	*hhf1Δ*	11	4
IPY914/915	*hhf1Δ hta1-300*	45	35
IPY916/IPY917	*(hht1-hhf1)Δ*	6	2
IPY918/IPY919	*(hht1-hhf1)Δ hta1-300*	41	46
IPY137	*hta1-200*	7	6
IPY926/IPY927	*hht1Δ hta1-200*	29	26
IPY928/IPY929	*hhf1Δ hta1-200*	9	9
IPY930/931	*(hht1-hhf1)Δ hta1-200*	5	4

It was expected that if the alteration in histone dosage was sufficient for suppressing the increase-in-ploidy phenotype, then it would also suppress the chromosome loss phenotype. However, this has proven untrue. From these data, it was concluded that the alteration in histone gene dosage is not likely to be responsible for the suppression of the increase-in-ploidy phenotype, and that there must be some other aspect of the mutation that contributes to correcting one chromosome segregation phenotype, but not the other. It is also possible that the dosage change is responsible for the increase-in-ploidy suppression and that the chromosome loss phenotype is caused by a different mechanism unaffected by the change in histone gene dosage.

E. Chromatin structure remains altered at the centromere

It has been shown that strains carrying *hta1-300* have an altered chromatin structure at the centromere as demonstrated by indirect end-labeling analysis.[1] As shown in **Figure 10**, the altered structure at the centromere is not corrected by the *hht1* deletion. The phasing of nucleosomes seen in the WT strain (FY604) is altered in the *hta1-300* strain (FY988), with new digestion sites appearing in both the *hta1-300* strain and the double mutant (*hta1-300 hht1Δ*, IPY502). This was surprising since it was thought that the connection between the altered chromatin structure at the centromere and the chromosome segregation phenotypes associated with the *hta1-300* allele would enable a suppressor to affect both phenotypes. Since the *hht1Δ* allele was able to suppress the increase in ploidy phenotype, it was thought that the allele would either restore WT chromatin structure, or at least alter the structure differently than the *hta1-300* allele does. The fact that the *hht1Δ* allele is unable to suppress the chromatin structure phenotype of

hta1-300 indicates that the suppressor does not act by directly reversing the alteration caused by *hta1-300*.

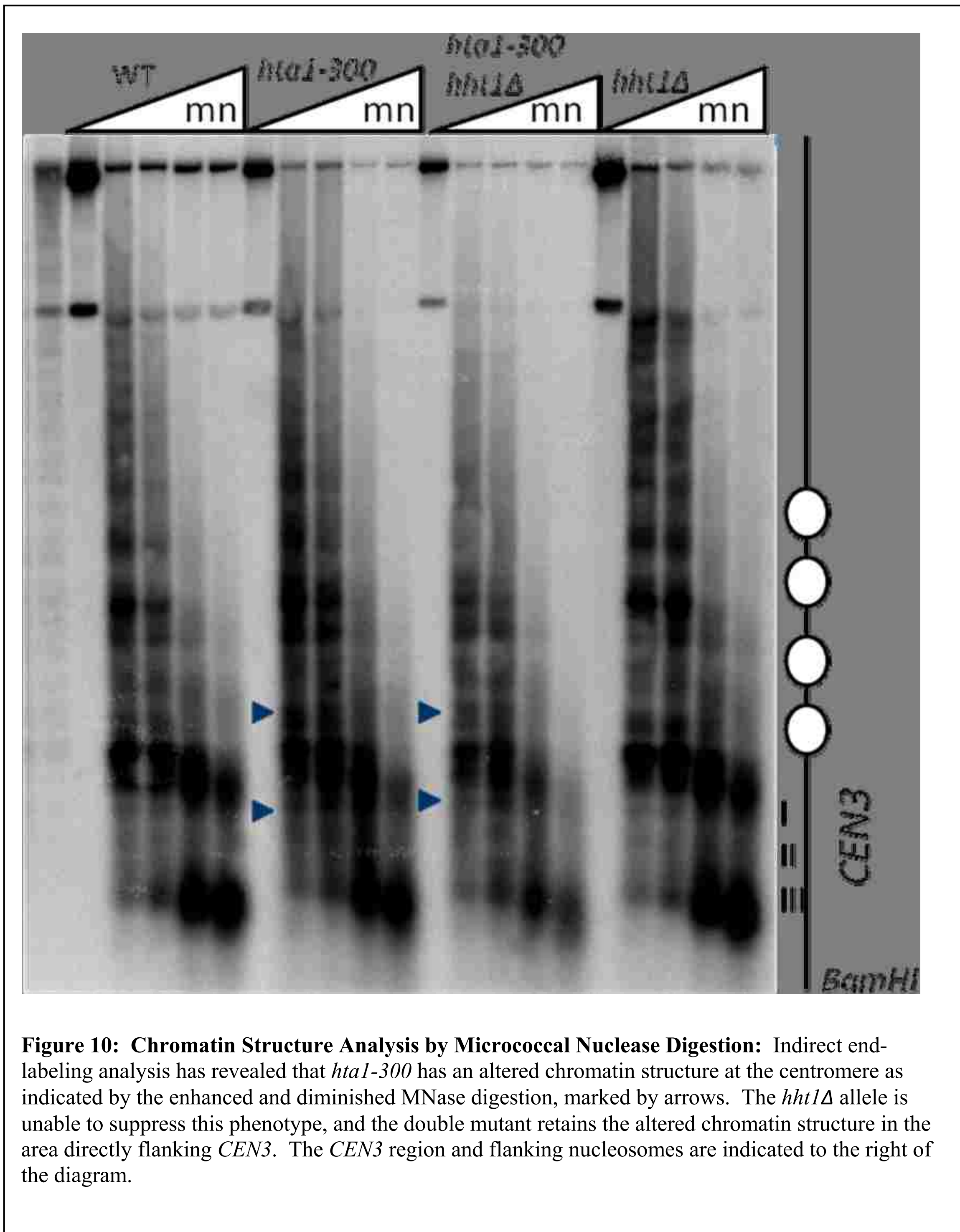


Figure 10: Chromatin Structure Analysis by Micrococcal Nuclease Digestion: Indirect end-labeling analysis has revealed that *hta1-300* has an altered chromatin structure at the centromere as indicated by the enhanced and diminished MNase digestion, marked by arrows. The *hht1Δ* allele is unable to suppress this phenotype, and the double mutant retains the altered chromatin structure in the area directly flanking *CEN3*. The *CEN3* region and flanking nucleosomes are indicated to the right of the diagram.

F. Whole histone protein amounts are not altered

To explore other aspects of these mutants in order to elucidate how the suppressor was functioning, histones were acid-extracted and separated on SDS-PAGE to determine if there were changes to histone protein amounts. As shown by a Coomassie Blue stain of acid-extracted histones separated on a 15% acrylamide gel (**Figure 11**), protein amounts remain constant in all strains, though do not appear to be stoichiometrically balanced. This is likely caused by the fact that the stain has a different affinity for differently charged proteins, as can be the case for differently modified histones, as Western blots of whole cell extracts using antibodies against histone proteins do not show these differences (**Figure 12**).

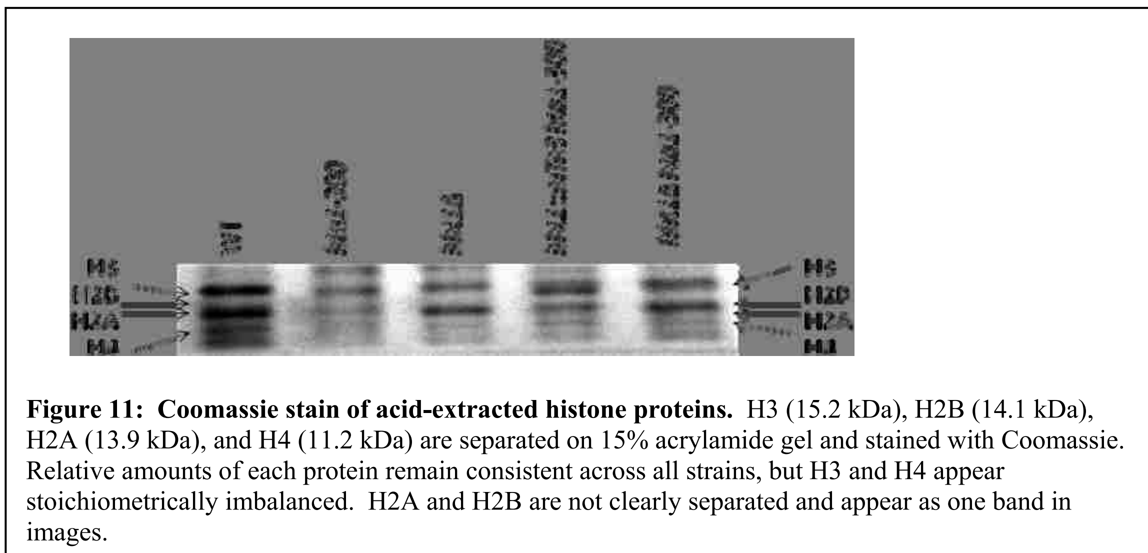


Figure 11: Coomassie stain of acid-extracted histone proteins. H3 (15.2 kDa), H2B (14.1 kDa), H2A (13.9 kDa), and H4 (11.2 kDa) are separated on 15% acrylamide gel and stained with Coomassie. Relative amounts of each protein remain consistent across all strains, but H3 and H4 appear stoichiometrically imbalanced. H2A and H2B are not clearly separated and appear as one band in images.

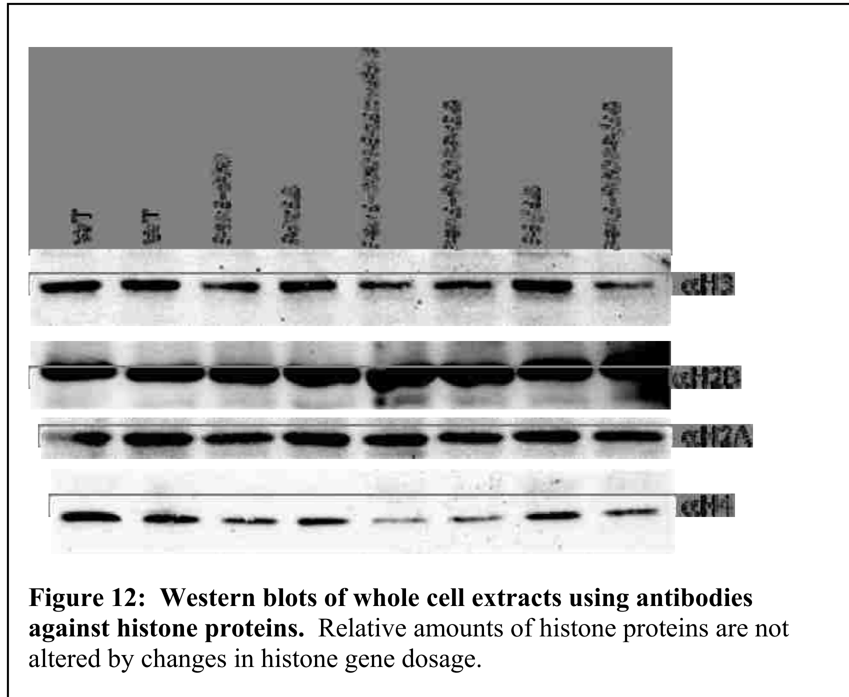


Figure 12: Western blots of whole cell extracts using antibodies against histone proteins. Relative amounts of histone proteins are not altered by changes in histone gene dosage.

G. Histone H4 has altered amounts of acetylation on lysines 5 and 12.

Mass spectrometry was performed (in collaboration with A. Tackett) on acid-extracted histones from WT (FY604) and *hta1-300* (FY988) strains to observe changes in

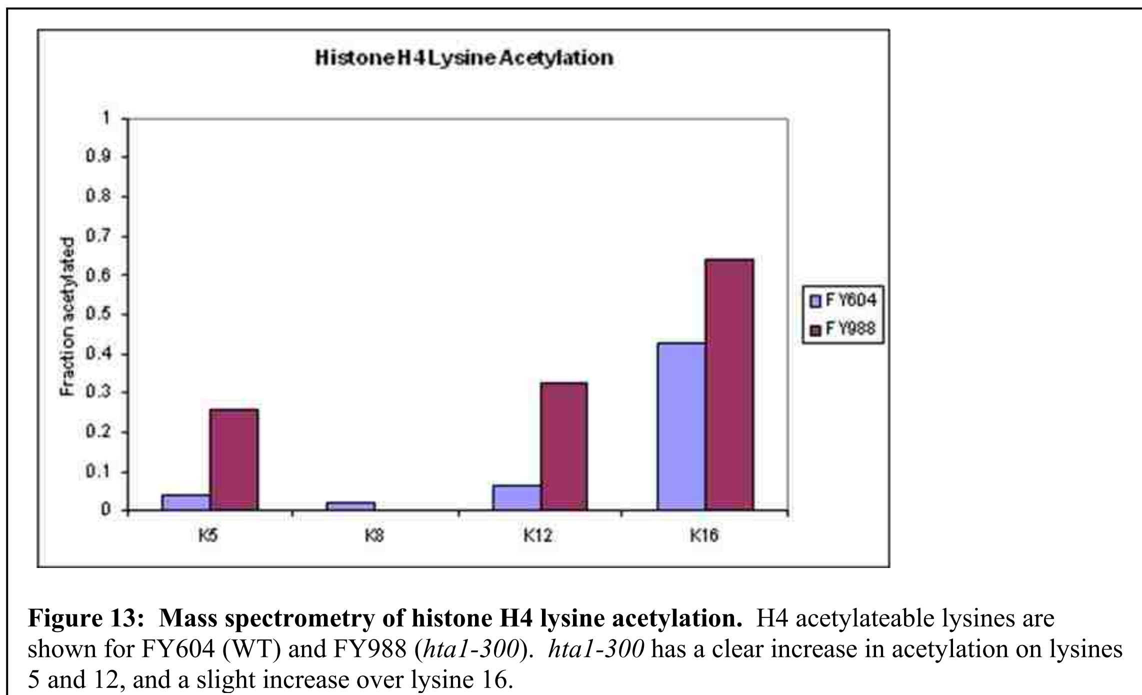


Figure 13: Mass spectrometry of histone H4 lysine acetylation. H4 acetylateable lysines are shown for FY604 (WT) and FY988 (*hta1-300*). *hta1-300* has a clear increase in acetylation on lysines 5 and 12, and a slight increase over lysine 16.

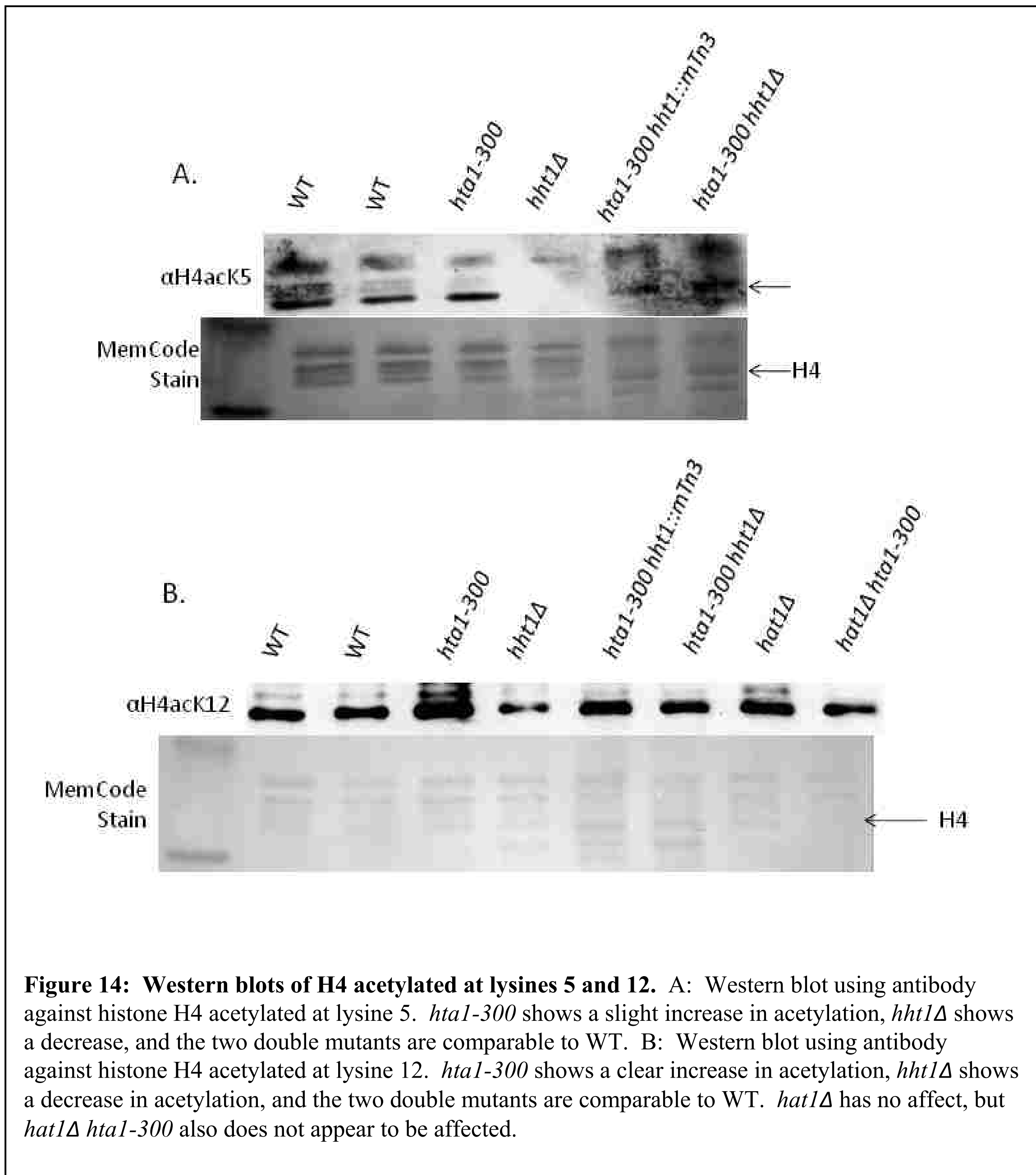


Figure 14: Western blots of H4 acetylated at lysines 5 and 12. A: Western blot using antibody against histone H4 acetylated at lysine 5. *hta1-300* shows a slight increase in acetylation, *hht1Δ* shows a decrease, and the two double mutants are comparable to WT. B: Western blot using antibody against histone H4 acetylated at lysine 12. *hta1-300* shows a clear increase in acetylation, *hht1Δ* shows a decrease in acetylation, and the two double mutants are comparable to WT. *hat1Δ* has no affect, but *hat1Δ hta1-300* also does not appear to be affected.

histone modifications. The only differences noticed were on lysines 5 and 12 of histone H4 (**Figure 13**). *hta1-300* has an increased amount of acetylation on both lysines 5 and 12, and a slight increase on lysine 16 as compared to the WT strain.

These data are supported by Western blots of acid-extracted histones using antibodies against H4 acetylated on lysines 5 and 12 (**Figure 14**). It was shown that both

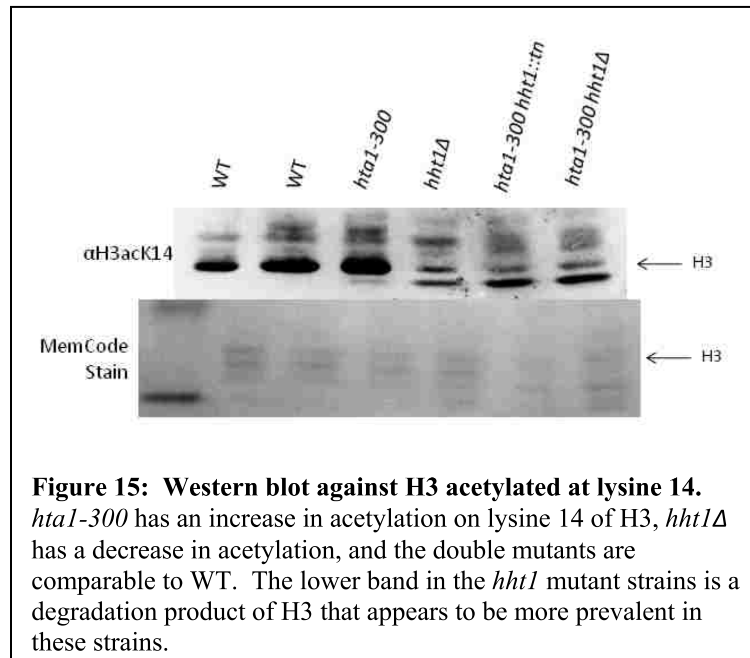


Figure 15: Western blot against H3 acetylated at lysine 14. *hta1-300* has an increase in acetylation on lysine 14 of H3, *hht1Δ* has a decrease in acetylation, and the double mutants are comparable to WT. The lower band in the *hht1* mutant strains is a degradation product of H3 that appears to be more prevalent in these strains.

lysines have an increased amount of acetylation in strains containing *hta1-300*. The *hht1Δ* strain has greatly decreased levels of acetylation at these lysines.

Since *hta1* mutants have been shown to have increased amounts of acetylation on lysine 14 of histone H3 at the centromere,[138] and that *hda1Δ* (another suppressor of the increase-in-ploidy phenotype) also exhibits this increase in acetylation, we tested our strains by western blot for acetylation of H3 on lysine 14 (**Figure 15**). The same pattern appeared as in the westerns with antibodies against H4acK5 and K12. Western blots using antibodies against acetylation at lysines 18 and 27 of H3, lysine 16 of H2B, and dimethylation of lysine 4 of H3 were also tested and no discernable differences were found (data not shown).

Interestingly, the H4acK12 data are not supported by the same western blots of whole cell extracts (**Figure 16**). However, this disparity between acid-extracted histones and whole cell extracts has been observed before. In 2008, Poveda *et al.*[191] found that their mutants had a difference in acetylation on lysine 12 of histone H4 on western blots

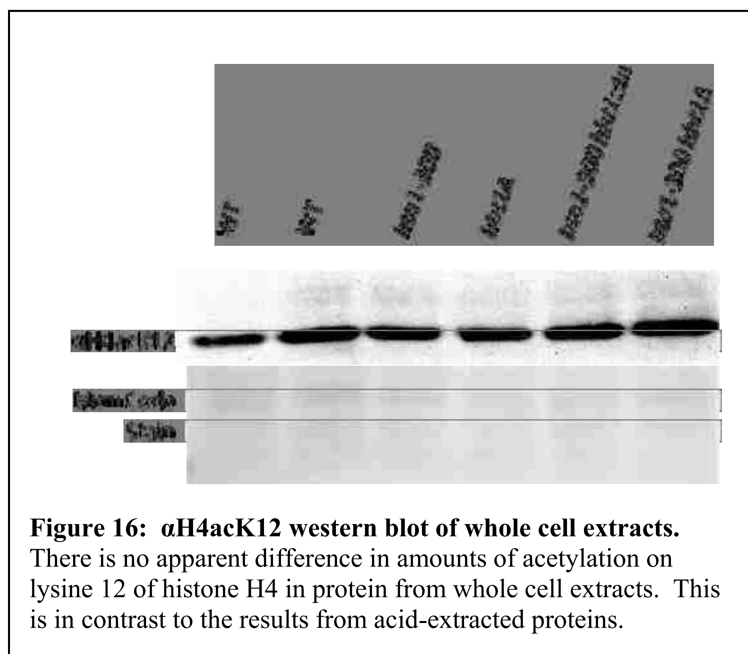


Figure 16: αH4acK12 western blot of whole cell extracts. There is no apparent difference in amounts of acetylation on lysine 12 of histone H4 in protein from whole cell extracts. This is in contrast to the results from acid-extracted proteins.

of whole cell extracts, but not acid-extracts. They reasoned that this disparity was due to the fact that they were observing Hat1-dependent acetylation on proteins outside of the nucleus; Hat1 is a histone acetyltransferase known to function outside the nucleus to acetylate histone H4 on lysines 5 and 12 to signal the protein for import into the nucleus.

This information would indicate that our acetylation differences are occurring on proteins that are already in the nucleus and associated with chromatin. To test this, a deletion of *hat1* (IPY934) was crossed with the *hta1-300* mutant (IPY69) and the resulting double mutant was compared to existing data by western blot (**Figure 14.B**). The *hat1Δ* strain had no affect on the acetylation of H4 lysine 12. From this, it was concluded that the increase in acetylation on lysines 5 and 12 of histone H4 is Hat1-independent and is due to an as yet unidentified histone acetyltransferase. Hat1 is the only histone acetyltransferase in yeast currently known to specifically modify lysines 5 and 12 *in vivo*, though Esa1, the catalytic subunit of NuA4 is able to acetylate all four lysines on the H4 tail *in vitro*. *ESA1* has also been shown to be important for cell cycle

progression, *esa1* temperature sensitive mutants arrest in G2/M,[103] and Esa1 has been shown to localize to the centromere along with other chromatin remodeling proteins.[27] Esa1 is an interesting candidate for study and more work will be necessary to determine if it has a role in this process, or if there is another acetyltransferase involved. A strain containing an *esa1-ts* mutant is currently being crossed with IPY69 (*hta1-300*).

H. Chromatin immunoprecipitation shows an overall increase in H4 acetylation at lysine 12 in *hta1-300* mutants

To further characterize the chromatin structure and acetylation state at the centromere, specific modifications were analyzed at the centromere and in pericentromeric regions by chromatin immunoprecipitations (**Figure 17**). Sheared chromatin was immunoprecipitated (IP) with an antibody against histone H4 acetylated at lysine 12. The resulting DNA was then amplified by PCR using oligos against the centromere and pericentromeric regions (*CEN3* and regions 0.25 kb, 0.5 kb, 1 kb, 2 kb, and 5 kb to either side of the centromere). A positive control was included using oligos against the *HMRa* locus.[80] Input (in) DNA was extracted from chromatin that had not been immunoprecipitated with the antibody. The relative intensities of the IP bands were compared to their respective input bands using FluorChem software. There were no obvious acetylation patterns across the region. However, the *hta1-300* strains had an overall higher amount of acetylation as compared to the WT strain. The ratios of IP:in intensities were calculated and are shown in the graph on **Figure 18**. Again, there is an overall increase in the amount of acetylation on the strains carrying the *hta1-300* allele. It is also important to note that the *hht1Δ* strain has levels of acetylation that are

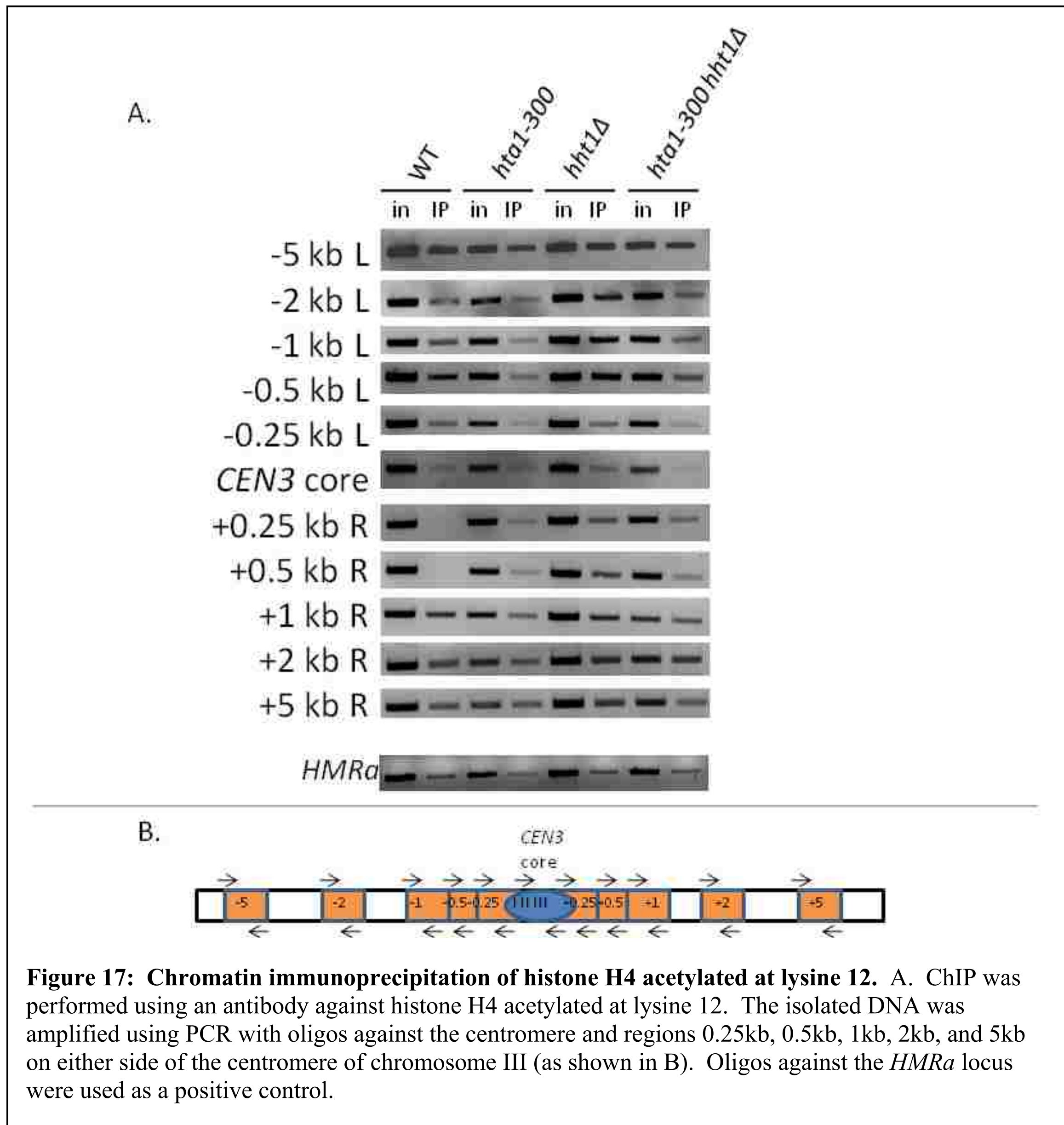


Figure 17: Chromatin immunoprecipitation of histone H4 acetylated at lysine 12. A. ChIP was performed using an antibody against histone H4 acetylated at lysine 12. The isolated DNA was amplified using PCR with oligos against the centromere and regions 0.25kb, 0.5kb, 1kb, 2kb, and 5kb on either side of the centromere of chromosome III (as shown in B). Oligos against the *HMRa* locus were used as a positive control.

comparable to WT, in contrast to the information we obtained on the western blots of chromatin from the entire cell.

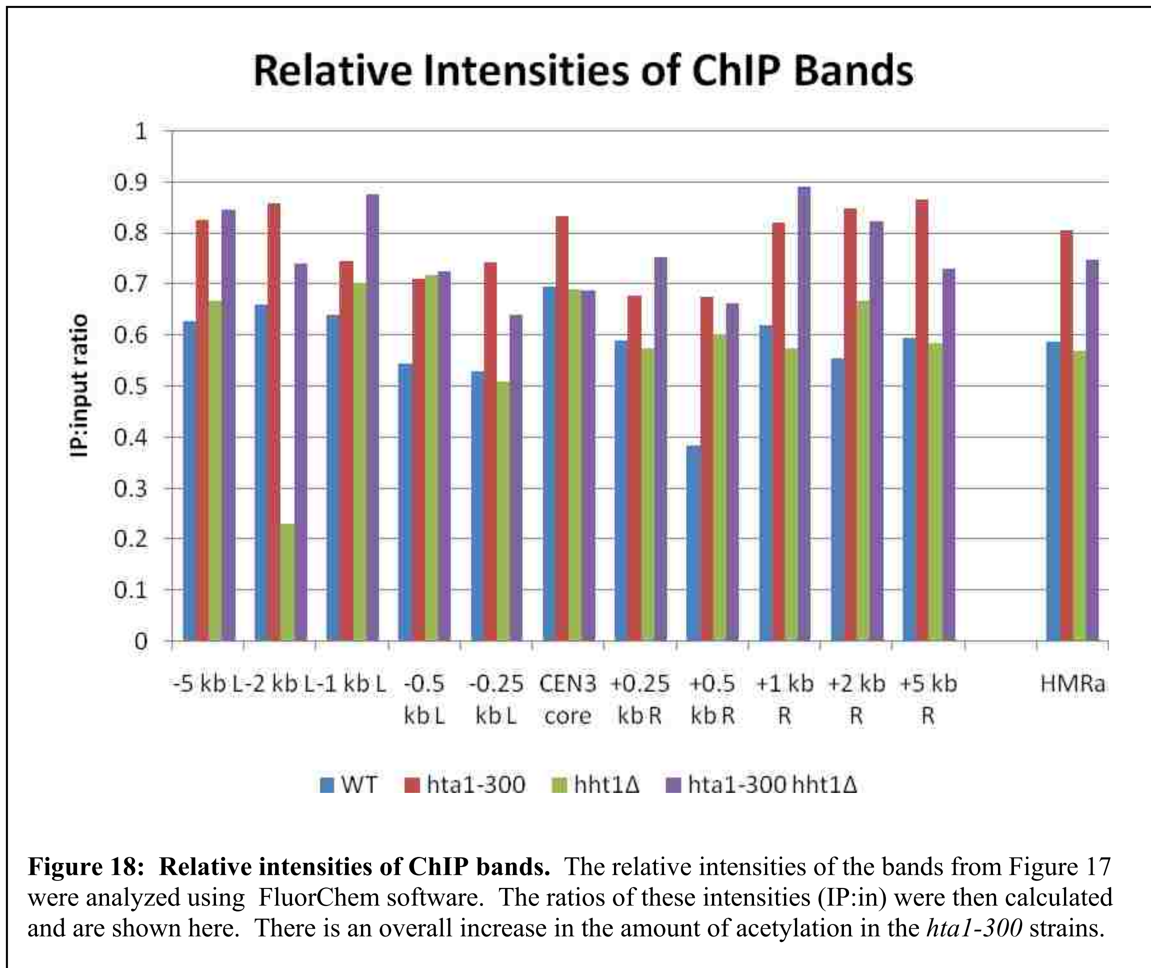


Figure 18: Relative intensities of ChIP bands. The relative intensities of the bands from Figure 17 were analyzed using FluorChem software. The ratios of these intensities (IP:in) were then calculated and are shown here. There is an overall increase in the amount of acetylation in the *hta1-300* strains.

To demonstrate that the results found at *CEN3* are not unique, ChIP was also done using primers at *CEN16*, as well as 3 kb to the left and 5 kb to the right (**Figure 19**). These data are consistent with the *CEN3* results. The WT strain (FY604), however, does show a sharp increase in H4K12 acetylation 5 kb to the right of *CEN16*. This is likely related to the transcription of a gene that is at that location (*RPC40*, an RNA polymerase subunit) and unrelated to chromosome segregation or centromere function.

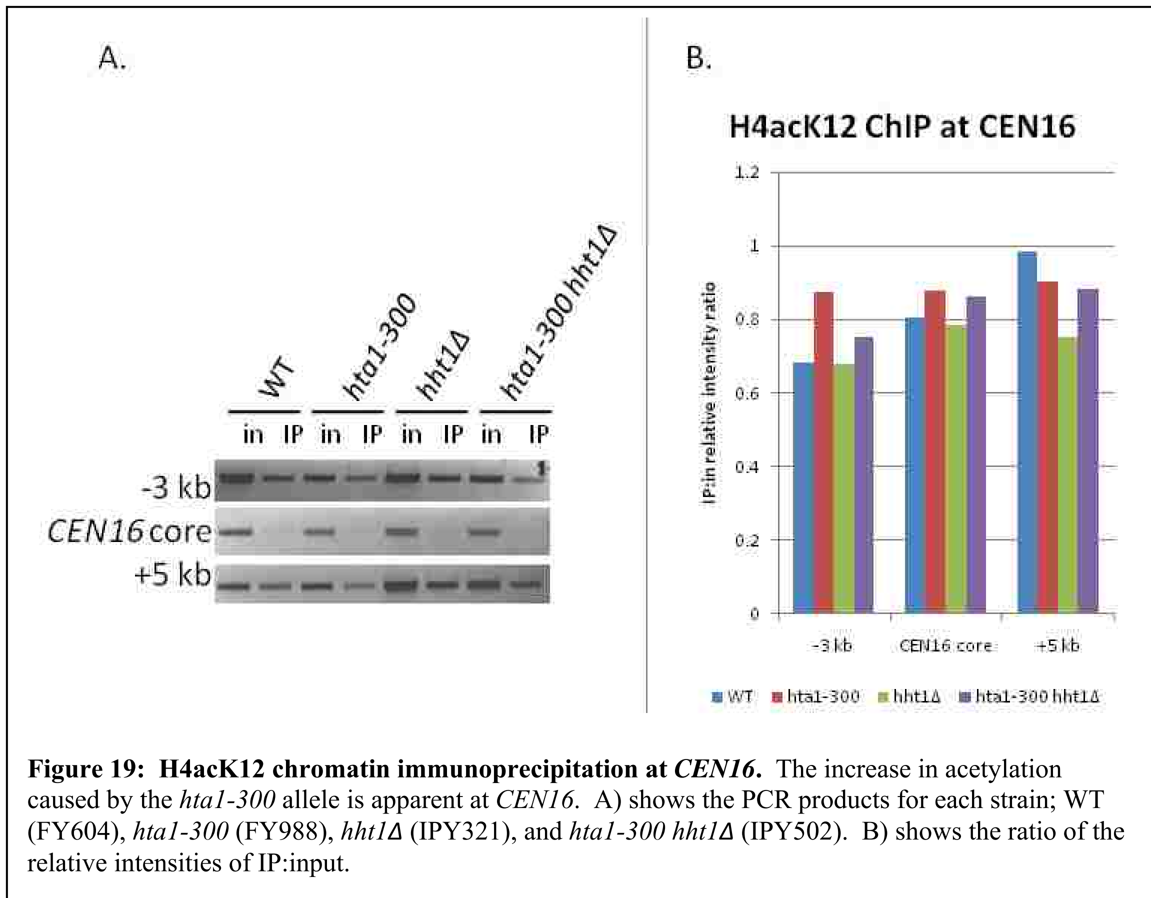


Figure 19: H4acK12 chromatin immunoprecipitation at *CEN16*. The increase in acetylation caused by the *hta1-300* allele is apparent at *CEN16*. A) shows the PCR products for each strain; WT (FY604), *hta1-300* (FY988), *hht1Δ* (IPY321), and *hta1-300 hht1Δ* (IPY502). B) shows the ratio of the relative intensities of IP:input.

I. Construction of *ARP4* temperature sensitive mutant

To investigate the role of *ARP4* (also known as *ACT3*) in chromosome segregation, it was first necessary to develop conditional mutants of the gene. Harata *et al.* characterized two temperature sensitive mutants of *ARP4*, *act3-26* and *act3-12,* which carried point mutations converting amino acid 187 from a glycine to an arginine (*act3-26,* G187R) or amino acid 455 from a glycine to a serine (*act3-12*, G455S). Both mutations were able to suppress the transcriptional defects caused by the insertion of a transposable element and caused an alteration in the chromatin structure of the promoter analyzed.[99] Since theses strains were developed in a yeast strain with a vastly different genetic background, we decided to generate these mutations in our strain background (S288C) to

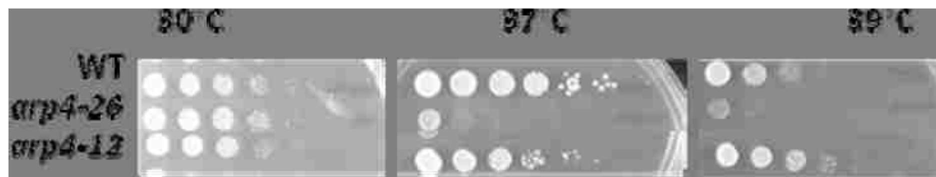


Figure 20: Temperature sensitivity of *arp4* mutants. *arp4-26* was slightly temperature sensitive at 37°C, and more so at 39°C. *arp4-12* was only not temperature sensitive at any temperature.

avoid the appearance of unrelated phenotypes. Mutations were made using the megaprimer method of site-directed mutagenesis.[178]

The *arp4-26* strain was slow growth at 37°C and weakly temperature sensitive at 39°C and the *arp4-12* strain was not temperature sensitive in our genetic background (**Figure 20**). However, the temperature sensitivity of *arp4-26* was leaky.

J. An *ARP4* mutant suppresses the cold sensitive and increase-in-ploidy phenotypes of *hta1-300*

To study the role of Arp4 in chromosome segregation, the *arp4-26* strain was crossed with a strain carrying *hta1-300*. Several rounds of crosses and dissections were necessary before a double mutant was obtained. It quickly became apparent that Arp4 plays an important role in cell function because spores containing both mutations were rare. In addition, the cold sensitivity of *hta1-300* was suppressed by *arp4-26* making the alleles difficult to distinguish. The increase-in-ploidy phenotype was suppressed as shown by the late development of papillae in the canavanine assay at permissive temperature (**Figure 21**). This suppression is also shown in **Figure 22** by DNA content analysis through flow cytometry. These results are consistent with a role for *ARP4* in the maintenance of ploidy.[1] It is interesting that both overexpression of and mutation to *ARP4* are able to suppress the increase-in-ploidy and the cold sensitivity of *hta1-300*.

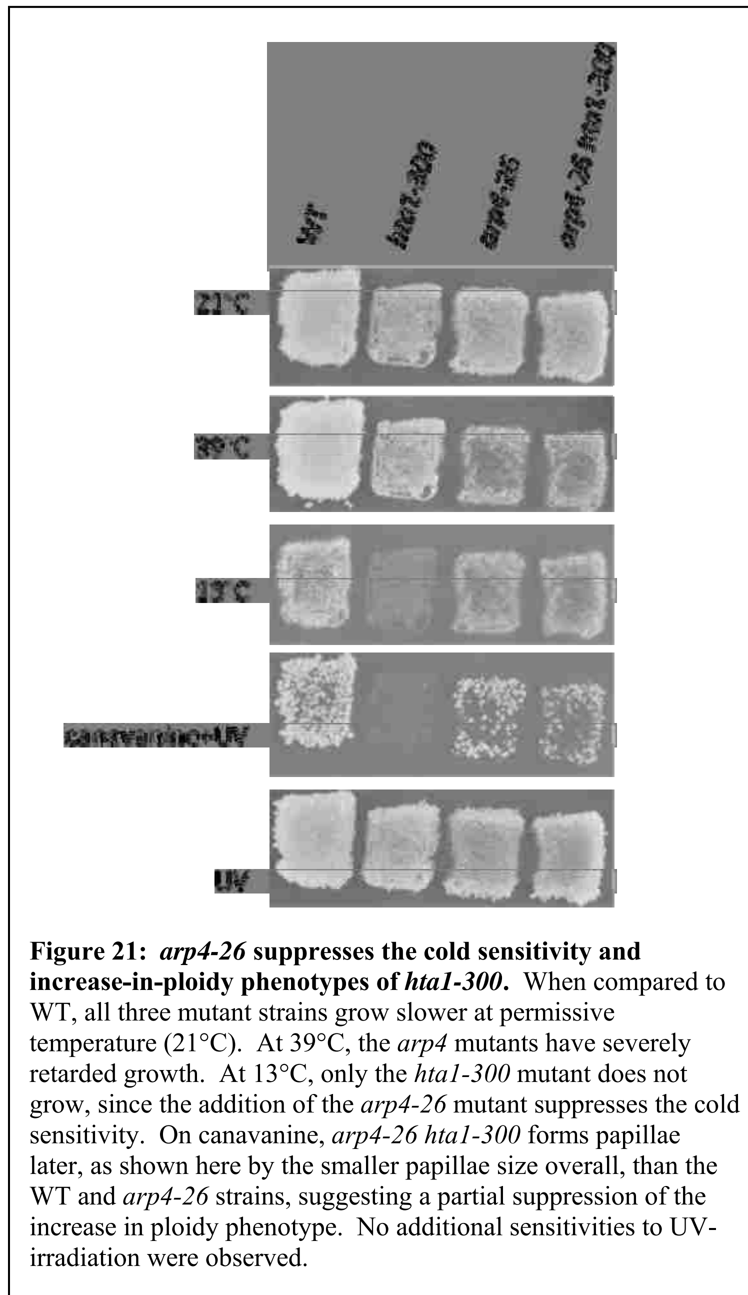


Figure 21: *arp4-26* suppresses the cold sensitivity and increase-in-ploidy phenotypes of *hta1-300*. When compared to WT, all three mutant strains grow slower at permissive temperature (21°C). At 39°C, the *arp4* mutants have severely retarded growth. At 13°C, only the *hta1-300* mutant does not grow, since the addition of the *arp4-26* mutant suppresses the cold sensitivity. On canavanine, *arp4-26 hta1-300* forms papillae later, as shown here by the smaller papillae size overall, than the WT and *arp4-26* strains, suggesting a partial suppression of the increase in ploidy phenotype. No additional sensitivities to UV-irradiation were observed.

These data suggest that Arp4's role in chromosome segregation involves its role in histone modification. It seems likely that increasing the expression of *ARP4* serves to correct the histone acetylation imbalance correlated with the *hta1-300* mutant and this further exemplifies the need for analysis of an *esa1* mutant.

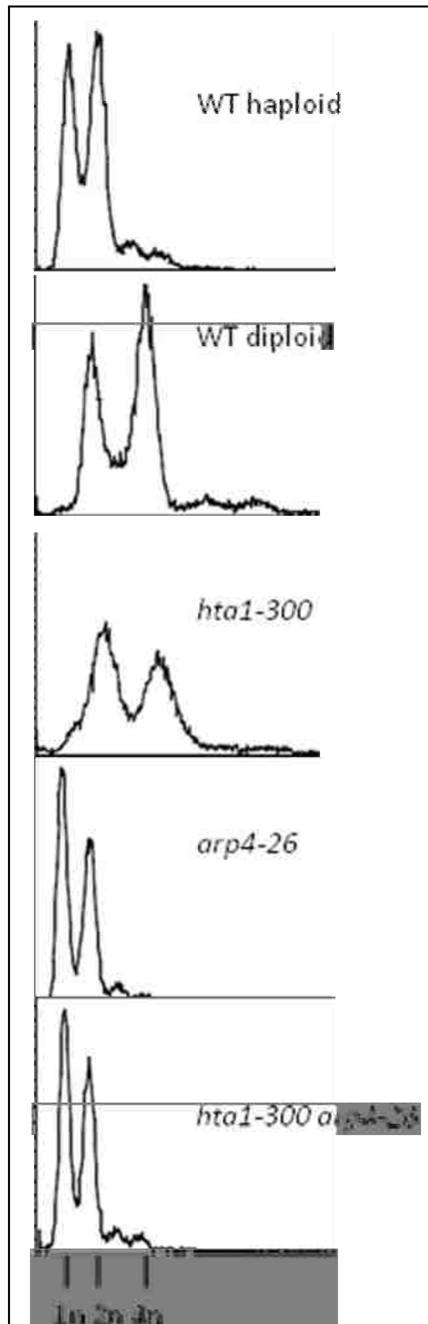


Figure 22: Flow cytometric analysis of DNA content. The increase-in-ploidy phenotype of *hta1-300* is suppressed by *arp4-26*.

V. DISCUSSION

The genetic interactions shown here indicate pericentromeric chromatin plays a role in chromosome segregation in *Saccharomyces cerevisiae*. These interactions also provide the first occurrence of experiments showing a change in dosage of one histone gene to affect the modifications on another histone.

The overexpression data shown here is consistent with previous data that shows that overexpression of a histone gene set (H2A and H2B or H3 and H4) results in increased rates of chromosome loss.[3] Overexpression of histone H3 has also been found to be dosage lethal in *cse4* mutants[192] and in kinetochore mutants.[159] It is thought that overexpression of H3 causes it to compete against CenH3 (Cse4) and thus leads to chromosome segregation phenotypes. It is conceivable that H3 overexpression here is also a result of H3 competing against CenH3 at the centromere, further perturbing the chromosome segregation machinery in a system already perturbed by *hta1-300*.

Combining the overexpression data with the deletion studies, it is apparent that not only are histone genes required to be stoichiometrically balanced, but histone modifications must also have a proper balance. While overexpression of histone genes in a *hta1-300* background worsens the growth phenotypes, deletion of the genes is able to partially suppress the chromosome segregation phenotypes. This indicates that proper histone stoichiometry is indeed important for cell function and proper chromosome segregation. Also, the mass spectrometry and western blot data demonstrates that stoichiometry plays a role in maintenance of histone modifications as demonstrated by the decrease in acetylation on H4K5 and K12 in the *hht1Δ* strain.

The fact that the deletions are able to suppress the increase in ploidy but not the chromosome loss or altered chromatin structure phenotypes suggests that the *hta1-300* allele is affecting more than one mechanism within the chromosome segregation machinery. These mechanisms are likely related, and possibly act in parallel. Details on these mechanisms will require further study.

It is interesting that, in respect to gene dosage, the most prominent phenotypes were observed when copy I of the H3 and H4 genes was altered. Copy I is expressed 5-7 times lower than copy II,[154] making this data all the more intriguing.

In light of the new evidence demonstrating that the *hta1-300* allele causes an increase in acetylation of the N-terminal tail of histone H4, we propose that this phenotype contributes to the increase-in-ploidy phenotype by causing improper kinetochore assembly (**Figure 23**). It is possible that the altered charges in pericentromeric chromatin are causing the kinetochore to assemble in a monopolar fashion instead of the bipolar assembly of a WT cell. This monopolar kinetochore assembly would lead to genome-wide chromosome gain, and thus, the increase-in-ploidy phenotype. Histone gene deletions that are able to suppress this phenotype likely do so by restoring a charge balance around the centromere, thus allowing the kinetochore to assemble correctly. We hypothesize that *hht1Δ* is able to suppress the increase-in-ploidy phenotype of *hta1-300* by restoring an acetylation balance on histone H4. Since it is known that *hda1Δ* can suppress the increase-in-ploidy phenotype of *hta1-300*, and that it results in increased acetylation on H2BK16 and H3K14, we propose that, when combined with the increase in acetylation of H4K5 and K12 of *hta1-300*, the proper stoichiometric

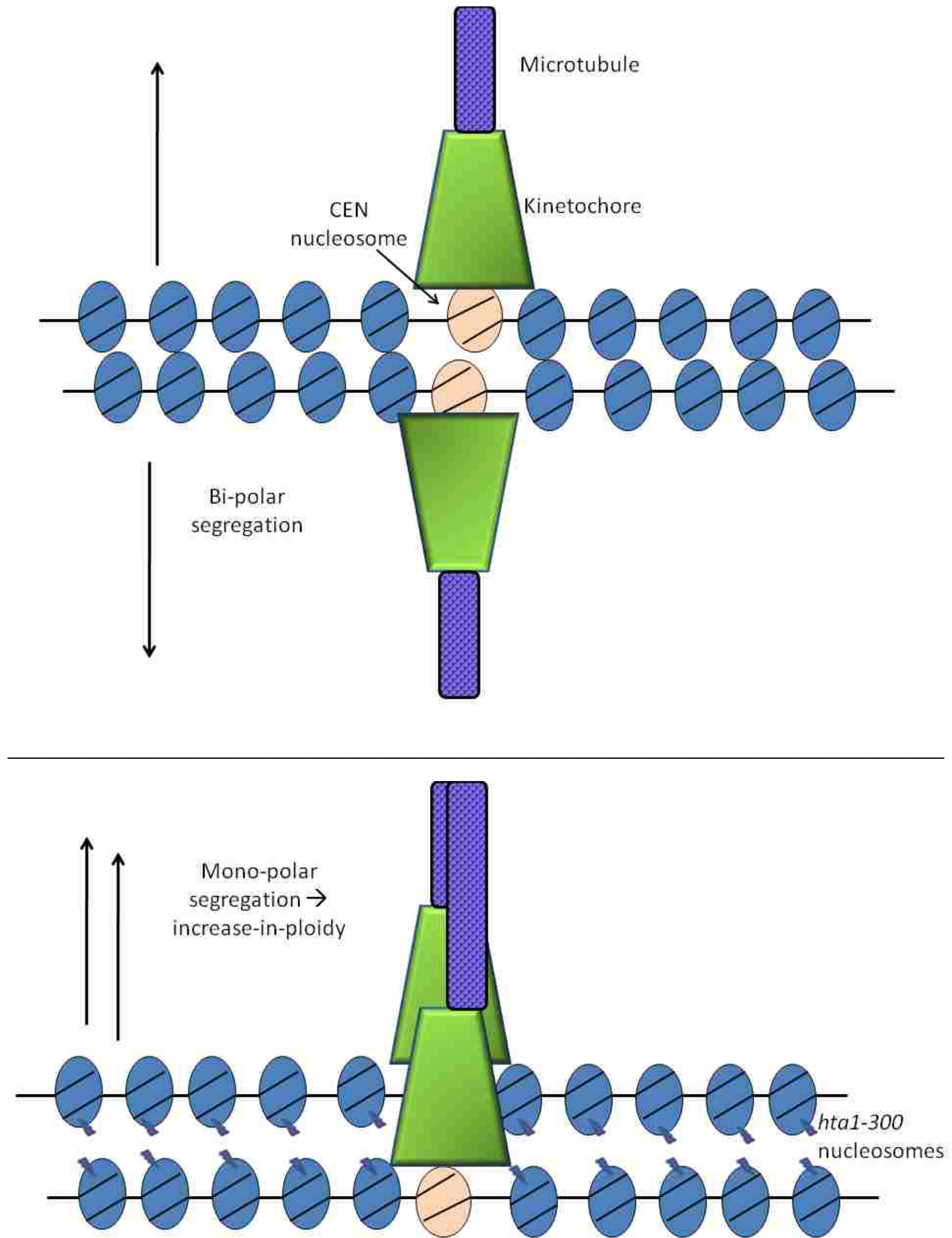


Figure 23: Model for increase-in-ploidy mechanism. Top: WT cells segregate chromosomes in a bipolar manner. Bottom: *hta1-300* cells appear to have a defect in kinetochore assembly that causes chromosomes to segregate in a monopolar manner.

balance of histone modifications is restored such that the ploidy phenotype is suppressed. In a similar manner, the decrease in H4K5 and K12 and H3K14 acetylation caused by *hht1Δ* may also be restoring the stoichiometric balance of nucleosome charges. However, the chromatin immunoprecipitation data does not support this hypothesis because *hht1Δ* behaves like a WT strain and *hta1-300 hht1Δ* behaves like the *hta1-300* mutant alone at the centromere. However, the decrease in acetylation on H3K14 caused by the *hht1Δ* allele may be restoring a nucleosome charge balance, thus leading to suppression of the increase-in-ploidy phenotype. Alternatively, other modifications that weren't studied here may be affected in a similar manner. It is important to note here that subtle changes to modifications around the centromere are beyond the detection level of our methods, but may have a profound effect on centromere function.

It is unclear why a point mutation on histone H2A would affect N-terminal tail modifications of histone H4. H2A and H4 are often paired together in analyses of their modifications and many histone modifying complexes act on both H2A and H4.[67, 193] The same link can be found between H3 and H2B. Microarray experiments in which modifications were compared across regions of the genome have grouped H4K5, H4K12, and H3K14, along with H2AK7, H3K9, and H3K18 together based on the fact that they are often modified together within the same regions of the genome.[62] Also, it is known that the tail of histone H4 interacts with histone H2A of neighboring nucleosomes and this interaction is involved in chromatin compaction.[44] This has led to the hypothesis that the H2A mutation disrupts the chromatin structure such that the acetylation on the neighboring H4 is affected. In the same manner, it is thought that the other modifications discussed here are affected (H4K5 and H3K14) and likely other modifications not

discussed here as well. It is still unclear whether this is a result of a lack of deacetylase activity, or of excess acetyltransferase activity, or a combination of the two.

Aside from the nuclear import signal,[29] little else is known about the biological role of lysines 5 and 12 of histone H4.[191] It has been found that H4acK12 functions as a memory mark for propagating the expression state of a telomeric gene during mitosis in HeLa cells.[78] Hat1 is the acetyltransferase responsible for acetylating H4K5 and K12 outside the nucleus. It has also been found to be required for telomeric silencing and to have a role in DNA damage repair.[194] Though the increase in acetylation shown here is Hat1-independent, it is possible that these marks are interfering with the normal functions of Hat1 or other chromatin-associated proteins. Since H4acK12 is linked to the recruitment of DNA repair machinery,[115] it is possible that the increase in acetylation on that mark in strains carrying *hta1-300* would result in the recruitment of DNA repair complexes to the centromeric region, thus disrupting kinetochore formation and thus, chromosome segregation. Alternatively, excess acetylation on H4K12 could disrupt the association of kinetochore and other chromosome segregation proteins with the centromeric region.

The link between histone acetylation and nucleosome deposition/chromatin assembly also leads to an interesting hypothesis. It is possible that the increases in acetylation caused by *hta1-300* lead to a high turn-over of chromatin, which results in unstable chromatin structure, and thus chromosome segregation defects. A clear understanding of histone deposition-related acetylation has been difficult to acquire because upon deposition, newly synthesized histones are rapidly remodeled to fulfill local transcriptional requirements.[195] In addition to this, biochemical characterizations have

been hampered by the findings that other lysines are redundantly able to compensate for missing or altered lysines that would normally be preferential.[195] These can be found either on H4 (Lys8) or on the tail of H3, as demonstrated by the fact that cells remain viable upon the deletion of either the H3 tail or the H4 tail, but not both.[196,197]

Rpd3 and Hda1 are both histone deacetylases known to act on lysines 5 and 12 of H4.[198] Deletions of either gene causes marked increases in the amounts of acetylation on those lysines.[199] However, Hda1 preferentially deacetylates H2B and H3.[193] The stoichiometric balance of histone modifications is clearly important, but mutations to histone modifying enzymes show surprisingly mild phenotypes.[103] These mild phenotypes support hypotheses that many of the histone modifying enzymes act in redundant fashions such that one enzyme can compensate for a mutation in another.

ARP4 is shown here to also be able to suppress the increase-in-ploidy phenotype of *hta1-300*. Both mutation to and overexpression of the *ARP4* gene show a genetic interaction with *hta1*. Arp4 is the subunit of the NuA4 histone acetyltransferase complex thought to be responsible for bringing that complex to chromatin.[200] Esa1 is the catalytic subunit of NuA4 and has been shown to be able to acetylate histone H4 at K5 and K12 *in vitro*.[103] Though more work is necessary to elucidate the mechanism of *ARP4*'s suppression, we hypothesize that Arp4 is bringing NuA4 to chromatin and mutations to Arp4 affect it such that the stoichiometric balance of histone modifications is altered though the action or misaction of Esa1. This could be the means by which *ARP4* is able to suppress the phenotypes of *hta1-300*. If *ARP4* mutants are unable to bring NuA4, and thus the histone acetyltransferase, to chromatin, then an overall decrease in acetylation on H4 would be expected. This decrease could restore the increase in acetylation caused by

hta1-300 back to a level acceptable for proper chromosome segregation. An *esa1 hta1-300* double mutant will contribute to the analysis of this hypothesis. Also, because *ARP4* is able to suppress the cold sensitivity of *hta1-300*, we propose that it is involved in a protein-protein interaction that is destabilized by the *hta1* mutant and leads to the cold sensitive phenotype. It is unlikely, however, that this is the only protein interaction contributing to this phenotype.

VI. REFERENCES

1. Pinto, I. & Winston, F. Histone H2A is required for normal centromere function in Saccharomyces cerevisiae. *EMBO J.* **19**, 1598-1612 (2000).

2. Kanta, H., Laprade, L., Almutairi, A. & Pinto, I. Suppressor analysis of a histone defect identifies a new function for the hda1 complex in chromosome segregation. *Genetics* **173**, 435-450 (2006).

3. Meeks-Wagner, D. & Hartwell, L. H. Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. *Cell* **44**, 43-52 (1986).

4. Skibbens, R. V. & Hieter, P. Kinetochores and the checkpoint mechanism that monitors for defects in the chromosome segregation machinery. *Annu. Rev. Genet.* **32**, 307-337 (1998).

5. Tanaka, T. U. Bi-orienting chromosomes on the mitotic spindle. *Curr. Opin. Cell Biol.* **14**, 365-371 (2002).

6. Hauf, S. & Watanabe, Y. Kinetochore orientation in mitosis and meiosis. *Cell* **119**, 317-327 (2004).

7. Galitski, T., Saldanha, A. J., Styles, C. A., Lander, E. S. & Fink, G. R. Ploidy regulation of gene expression. *Science* **285**, 251-254 (1999).

8. Uhlmann, F. Chromosome cohesion and separation: from men and molecules. *Curr. Biol.* **13**, R104-14 (2003).

9. Sherman, F. Getting started with yeast. *Methods Enzymol.* **350**, 3-41 (2002).

10. Goffeau, A. *et al*. Life with 6000 genes. *Science* **274**, 546, 563-7 (1996).

11. Stoler, S., Keith, K. C., Curnick, K. E. & Fitzgerald-Hayes, M. A mutation in CSE4, an essential gene encoding a novel chromatin-associated protein in yeast, causes chromosome nondisjunction and cell cycle arrest at mitosis. *Genes Dev.* **9**, 573-586 (1995).

12. Hyman, A. A. & Sorger, P. K. Structure and function of kinetochores in budding yeast. *Annu. Rev. Cell Dev. Biol.* **11**, 471-495 (1995).

13. Holy, T. E. & Leibler, S. Dynamic instability of microtubules as an efficient way to search in space. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 5682-5685 (1994).

14. McAinsh, A. D., Tytell, J. D. & Sorger, P. K. Structure, function, and regulation of budding yeast kinetochores. *Annu. Rev. Cell Dev. Biol.* **19**, 519-539 (2003).

15. Saunders, M. J., Yeh, E., Grunstein, M. & Bloom, K. Nucleosome depletion alters the chromatin structure of Saccharomyces cerevisiae centromeres. *Mol. Cell. Biol.* **10**, 5721-5727 (1990).

16. Cheeseman, I. M., Drubin, D. G. & Barnes, G. Simple centromere, complex kinetochore: linking spindle microtubules and centromeric DNA in budding yeast. *J. Cell Biol.* **157**, 199-203 (2002).

17. Uhlmann, F. Chromosome cohesion and segregation in mitosis and meiosis. *Curr. Opin. Cell Biol.* **13**, 754-761 (2001).

18. Biggins, S. & Walczak, C. E. Captivating capture: how microtubules attach to kinetochores. *Curr. Biol.* **13**, R449-60 (2003).

19. Cleveland, D. W., Mao, Y. & Sullivan, K. F. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. *Cell* **112**, 407-421 (2003).

20. He, X., Rines, D. R., Espelin, C. W. & Sorger, P. K. Molecular analysis of kinetochore-microtubule attachment in budding yeast. *Cell* **106**, 195-206 (2001).

21. Lengauer, C., Kinzler, K. W. & Vogelstein, B. Genetic instabilities in human cancers. *Nature* **396**, 643-649 (1998).

22. Draviam, V. M., Xie, S. & Sorger, P. K. Chromosome segregation and genomic stability. *Curr. Opin. Genet. Dev.* **14**, 120-125 (2004).

23. Wang, Y. Chromosome instability in yeast and its implications to the study of human cancer. *Front. Biosci.* **13**, 2091-2102 (2008).

24. Krause, S. *et al*. Examination of tumorigenesis of precursor lesions in bladder cancer by in situ hybridization. *Urol. Int.* **72**, 118-122 (2004).

25. Visscher, D. W., Wallis, T. L. & Crissman, J. D. Evaluation of chromosome aneuploidy in tissue sections of preinvasive breast carcinomas using interphase cytogenetics. *Cancer* **77**, 315-320 (1996).

26. Kitagawa, K. & Hieter, P. Evolutionary conservation between budding yeast and human kinetochores. *Nat. Rev. Mol. Cell Biol.* **2**, 678-687 (2001).

27. Ogiwara, H. *et al*. Actin-related protein Arp4 functions in kinetochore assembly. *Nucleic Acids Res.* (2007).

28. Pearson, C. G. *et al*. Stable kinetochore-microtubule attachment constrains centromere positioning in metaphase. *Curr. Biol.* **14**, 1962-1967 (2004).

29. Peterson, C. L. & Laniel, M. A. Histones and histone modifications. *Curr. Biol.* **14**, R546-51 (2004).

30. Oudet, P., Gross-Bellard, M. & Chambon, P. Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. *Cell* **4**, 281-300 (1975).

31. Shahbazian, M. D. & Grunstein, M. Functions of Site-Specific Histone Acetylation and Deacetylation. *Annu. Rev. Biochem.* (2007).

32. McGhee, J. D. & Felsenfeld, G. Nucleosome structure. *Annu. Rev. Biochem.* **49**, 1115-1156 (1980).

33. Chakravarthy, S., Park, Y. J., Chodaparambil, J., Edayathumangalam, R. S. & Luger, K. Structure and dynamic properties of nucleosome core particles. *FEBS Lett.* **579**, 895-898 (2005).

34. Funk, M., Hegemann, J. H. & Philippsen, P. Chromatin digestion with restriction endonucleases reveals 150-160 bp of protected DNA in the centromere of chromosome XIV in Saccharomyces cerevisiae. *Mol. Gen. Genet.* **219**, 153-160 (1989).

35. Workman, J. L. & Kingston, R. E. Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu. Rev. Biochem.* **67**, 545-579 (1998).

36. Verdone, L., Agricola, E., Caserta, M. & Di Mauro, E. Histone acetylation in gene regulation. *Brief Funct. Genomic Proteomic* **5**, 209-221 (2006).

37. Kornberg, R. D. & Lorch, Y. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* **98**, 285-294 (1999).

38. An, W. Histone acetylation and methylation: combinatorial players for transcriptional regulation. *Subcell. Biochem.* **41**, 351-369 (2007).

39. Lee, K. P., Baxter, H. J., Guillemette, J. G., Lawford, H. G. & Lewis, P. N. Structural studies on yeast nucleosomes. *Can. J. Biochem.* **60**, 379-388 (1982).

40. Kossel, A. Ueber die Bildung von Thymin aus Fischsperma, Zeit. physiol. *Chem* **22**, 176-177-187 (1896).

41. Kornberg, R. D. Chromatin structure: a repeating unit of histones and DNA. *Science* **184**, 868-871 (1974).

42. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**, 251-260 (1997).

43. Luger, K. Structure and dynamic behavior of nucleosomes. *Curr. Opin. Genet. Dev.* **13**, 127-135 (2003).

44. White, C. L., Suto, R. K. & Luger, K. Structure of the yeast nucleosome core particle reveals fundamental changes in internucleosome interactions. *EMBO J.* **20**, 5207-5218 (2001).

45. Han, M., Chang, M., Kim, U. J. & Grunstein, M. Histone H2B repression causes cell-cycle-specific arrest in yeast: effects on chromosomal segregation, replication, and transcription. *Cell* **48**, 589-597 (1987).

46. Kim, U. J., Han, M., Kayne, P. & Grunstein, M. Effects of histone H4 depletion on the cell cycle and transcription of Saccharomyces cerevisiae. *EMBO J.* **7**, 2211-2219 (1988).

47. Clark-Adams, C. D., Norris, D., Osley, M. A., Fassler, J. S. & Winston, F. Changes in histone gene dosage alter transcription in yeast. *Genes Dev.* **2**, 150-159 (1988).

48. Norris, D., Dunn, B. & Osley, M. A. The effect of histone gene deletions on chromatin structure in Saccharomyces cerevisiae. *Science* **242**, 759-761 (1988).

49. Castillo, A. G. *et al*. Plasticity of fission yeast CENP-A chromatin driven by relative levels of histone H3 and H4. *PLoS Genet.* **3**, e121 (2007).

50. Holmes, S. G. & Mitchell Smith, M. Replication of minichromosomes in Saccharomyces cerevisiae is sensitive to histone gene copy number and strain ploidy. *Yeast* **18**, 291-300 (2001).

51. Gunjan, A. & Verreault, A. A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in S. cerevisiae. *Cell* **115**, 537-549 (2003).

52. Prado, F. & Aguilera, A. Partial depletion of histone H4 increases homologous recombination-mediated genetic instability. *Mol. Cell. Biol.* **25**, 1526-1536 (2005).

53. Bloom, K., Hill, A., Kenna, M. & Saunders, M. The structure of a primitive kinetochore. *Trends Biochem. Sci.* **14**, 223-227 (1989).

54. Vos, L. J., Famulski, J. K. & Chan, G. K. How to build a centromere: from centromeric and pericentromeric chromatin to kinetochore assembly. *Biochem. Cell Biol.* **84**, 619-639 (2006).

55. Turner, B. M. Cellular memory and the histone code. *Cell* **111**, 285-291 (2002).

56. Hansen, J. C., Tse, C. & Wolffe, A. P. Structure and function of the core histone N-termini: more than meets the eye. *Biochemistry* **37**, 17637-17641 (1998).

57. Megee, P. C., Morgan, B. A., Mittman, B. A. & Smith, M. M. Genetic analysis of histone H4: essential role of lysines subject to reversible acetylation. *Science* **247**, 841-845 (1990).

58. Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* **403**, 41-45 (2000).

59. Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074-1080 (2001).

60. Edmondson, D. G. *et al*. Site-specific loss of acetylation upon phosphorylation of histone H3. *J. Biol. Chem.* **277**, 29496-29502 (2002).

61. Iizuka, M. & Smith, M. M. Functional consequences of histone modifications. *Curr. Opin. Genet. Dev.* **13**, 154-160 (2003).

62. Liu, C. L. *et al*. Single-nucleosome mapping of histone modifications in S. cerevisiae. *PLoS Biol.* **3**, e328 (2005).

63. Schuster, T., Han, M. & Grunstein, M. Yeast histone H2A and H2B amino termini have interchangeable functions. *Cell* **45**, 445-451 (1986).

64. Ma, X. J., Wu, J., Altheim, B. A., Schultz, M. C. & Grunstein, M. Deposition-related sites K5/K12 in histone H4 are not required for nucleosome deposition in yeast. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 6693-6698 (1998).

65. Agricola, E., Verdone, L., Di Mauro, E. & Caserta, M. H4 acetylation does not replace H3 acetylation in chromatin remodelling and transcription activation of Adr1-dependent genes. *Mol. Microbiol.* **62**, 1433-1446 (2006).

66. ALLFREY, V. G., FAULKNER, R. & MIRSKY, A. E. Acetylation and Methylation of Histones and their Possible Role in the Regulation of Rna Synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **51**, 786-794 (1964).

67. Cheung, P., Allis, C. D. & Sassone-Corsi, P. Signaling to chromatin through histone modifications. *Cell* **103**, 263-271 (2000).

68. Dion, M. F., Altschuler, S. J., Wu, L. F. & Rando, O. J. Genomic characterization reveals a simple histone H4 acetylation code. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 5501-5506 (2005).

69. Nicolas, R. H. & Goodwin, G. H. Molecular cloning of polybromo, a nuclear protein containing multiple domains including five bromodomains, a truncated HMG-box, and two repeats of a novel domain. *Gene* **175**, 233-240 (1996).

70. Lin, Y. J. *et al*. Solution structure of the extraterminal domain of the bromodomain-containing protein BRD4. *Protein Sci.* **17**, 2174-2179 (2008).

71. Hassan, A. H., Neely, K. E. & Workman, J. L. Histone acetyltransferase complexes stabilize swi/snf binding to promoter nucleosomes. *Cell* **104**, 817-827 (2001).

72. Latham, J. A. & Dent, S. Y. Cross-regulation of histone modifications. *Nat. Struct. Mol. Biol.* **14**, 1017-1024 (2007).

73. Nakayama, J., Rice, J. C., Strahl, B. D., Allis, C. D. & Grewal, S. I. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* **292**, 110-113 (2001).

74. Ivanovska, I., Khandan, T., Ito, T. & Orr-Weaver, T. L. A histone code in meiosis: the histone kinase, NHK-1, is required for proper chromosomal architecture in Drosophila oocytes. *Genes Dev.* **19**, 2571-2582 (2005).

75. Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S. M. & Grunstein, M. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. *Cell* **80**, 583-592 (1995).

76. Ekwall, K., Olsson, T., Turner, B. M., Cranston, G. & Allshire, R. C. Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. *Cell* **91**, 1021-1032 (1997).

77. Keohane, A. M., O'neill, L. P., Belyaev, N. D., Lavender, J. S. & Turner, B. M. X-Inactivation and histone H4 acetylation in embryonic stem cells. *Dev. Biol.* **180**, 618-630 (1996).

78. Valls, E., Sanchez-Molina, S. & Martinez-Balbas, M. A. Role of histone modifications in marking and activating genes through mitosis. *J. Biol. Chem.* **280**, 42592-42600 (2005).

79. Grunstein, M. Histone acetylation in chromatin structure and transcription. *Nature* **389**, 349-352 (1997).

80. Morgan, B. A., Mittman, B. A. & Smith, M. M. The highly conserved N-terminal domains of histones H3 and H4 are required for normal cell cycle progression. *Mol. Cell. Biol.* **11**, 4111-4120 (1991).

81. Durand-Dubief, M. & Ekwall, K. Heterochromatin tells CENP-A where to go. *Bioessays* **30**, 526-529 (2008).

82. Millar, C. B. & Grunstein, M. Genome-wide patterns of histone modifications in yeast. *Nat. Rev. Mol. Cell Biol.* **7**, 657-666 (2006).

83. Lin, Y. Y. *et al*. A comprehensive synthetic genetic interaction network governing yeast histone acetylation and deacetylation. *Genes Dev.* **22**, 2062-2074 (2008).

84. Smith, M. M. *et al*. A novel histone H4 mutant defective in nuclear division and mitotic chromosome transmission. *Mol. Cell. Biol.* **16**, 1017-1026 (1996).

85. Waterborg, J. H. Steady-state levels of histone acetylation in Saccharomyces cerevisiae. *J. Biol. Chem.* **275**, 13007-13011 (2000).

86. Su, X. *et al*. Histone H4 acetylation dynamics determined by stable isotope labeling with amino acids in cell culture and mass spectrometry. *Anal. Biochem.* **363**, 22-34 (2007).

87. Lucchini, R. & Sogo, J. M. Replication of transcriptionally active chromatin. *Nature* **374**, 276-280 (1995).

88. Clarke, L. & Carbon, J. The structure and function of yeast centromeres. *Annu. Rev. Genet.* **19**, 29-55 (1985).

89. Khorasanizadeh, S. The nucleosome: from genomic organization to genomic regulation. *Cell* **116**, 259-272 (2004).

90. Gieni, R. S., Chan, G. K. & Hendzel, M. J. Epigenetics regulate centromere formation and kinetochore function. *J. Cell. Biochem.* **104**, 2027-2039 (2008).

91. Harata, M., Karwan, A. & Wintersberger, U. An essential gene of Saccharomyces cerevisiae coding for an actin-related protein. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 8258-8262 (1994).

92. Harata, M. *et al*. The nuclear actin-related protein of Saccharomyces cerevisiae, Act3p/Arp4, interacts with core histones. *Mol. Biol. Cell* **10**, 2595-2605 (1999).

93. Galarneau, L. *et al*. Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. *Mol. Cell* **5**, 927-937 (2000).

94. Bao, Y. & Shen, X. INO80 subfamily of chromatin remodeling complexes. *Mutat. Res.* **618**, 18-29 (2007).

95. Ogiwara, H., Enomoto, T. & Seki, M. The INO80 chromatin remodeling complex functions in sister chromatid cohesion. *Cell. Cycle* **6**, 1090-1095 (2007).

96. Conaway, R. C. & Conaway, J. W. The INO80 chromatin remodeling complex in transcription, replication and repair. *Trends Biochem. Sci.* **34**, 71-77 (2009).

97. Durant, M. & Pugh, B. F. NuA4-directed chromatin transactions throughout the Saccharomyces cerevisiae genome. *Mol. Cell. Biol.* **27**, 5327-5335 (2007).

98. Georgieva, M., Harata, M. & Miloshev, G. The nuclear actin-related protein Act3p/Arp4 influences yeast cell shape and bulk chromatin organization. *J. Cell. Biochem.* **104**, 59-67 (2008).

99. Harata, M. *et al*. Correlation between chromatin association and transcriptional regulation for the Act3p/Arp4 nuclear actin-related protein of Saccharomyces cerevisiae. *Nucleic Acids Res.* **30**, 1743-1750 (2002).

100. Mitchell, L. *et al*. Functional dissection of the NuA4 histone acetyltransferase reveals its role as a genetic hub and that Eaf1 is essential for complex integrity. *Mol. Cell. Biol.* **28**, 2244-2256 (2008).

101. Minoda, A., Saitoh, S., Takahashi, K. & Toda, T. BAF53/Arp4 homolog Alp5 in fission yeast is required for histone H4 acetylation, kinetochore-spindle attachment, and gene silencing at centromere. *Mol. Biol. Cell* **16**, 316-327 (2005).

102. Allard, S. *et al*. NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *EMBO J.* **18**, 5108-5119 (1999).

103. Clarke, A. S., Lowell, J. E., Jacobson, S. J. & Pillus, L. Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol. Cell. Biol.* **19**, 2515-2526 (1999).

104. Saunders, M., Fitzgerald-Hayes, M. & Bloom, K. Chromatin structure of altered yeast centromeres. *Proc. Natl. Acad. Sci. U. S. A.* **85**, 175-179 (1988).

105. Brinkley, B. R. *et al*. Movement and segregation of kinetochores experimentally detached from mammalian chromosomes. *Nature* **336**, 251-254 (1988).

106. Hegemann, J. H. & Fleig, U. N. The centromere of budding yeast. *Bioessays* **15**, 451-460 (1993).

107. Ciferri, C., Musacchio, A. & Petrovic, A. The Ndc80 complex: hub of kinetochore activity. *FEBS Lett.* **581**, 2862-2869 (2007).

108. Sharp, J. A. & Kaufman, P. D. Chromatin proteins are determinants of centromere function. *Curr. Top. Microbiol. Immunol.* **274**, 23-52 (2003).

109. Pidoux, A. L. & Allshire, R. C. The role of heterochromatin in centromere function. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **360**, 569-579 (2005).

110. Sullivan, B. A. & Karpen, G. H. Centromeric chromatin exhibits a histone modification pattern that is distinct from both euchromatin and heterochromatin. *Nat. Struct. Mol. Biol.* **11**, 1076-1083 (2004).

111. Van Hooser, A. A. *et al*. Specification of kinetochore-forming chromatin by the histone H3 variant CENP-A. *J. Cell. Sci.* **114**, 3529-3542 (2001).

112. Furuyama, S. & Biggins, S. Centromere identity is specified by a single centromeric nucleosome in budding yeast. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 14706-14711 (2007).

113. Gilchrist, S., Gilbert, N., Perry, P. & Bickmore, W. A. Nuclear organization of centromeric domains is not perturbed by inhibition of histone deacetylases. *Chromosome Res.* **12**, 505-516 (2004).

114. Bloom, K. S. & Carbon, J. Yeast centromere DNA is in a unique and highly ordered structure in chromosomes and small circular minichromosomes. *Cell* **29**, 305-317 (1982).

115. Capiaghi, C., Ho, T. V. & Thoma, F. Kinetochores prevent repair of UV damage in Saccharomyces cerevisiae centromeres. *Mol. Cell. Biol.* **24**, 6907-6918 (2004).

116. Mythreye, K. & Bloom, K. S. Differential kinetochore protein requirements for establishment versus propagation of centromere activity in Saccharomyces cerevisiae. *J. Cell Biol.* **160**, 833-843 (2003).

117. Schulman, I. & Bloom, K. S. Centromeres: an integrated protein/DNA complex required for chromosome movement. *Annu. Rev. Cell Biol.* **7**, 311-336 (1991).

118. Black, B. E. & Bassett, E. A. The histone variant CENP-A and centromere specification. *Curr. Opin. Cell Biol.* **20**, 91-100 (2008).

119. Pluta, A. F., Mackay, A. M., Ainsztein, A. M., Goldberg, I. G. & Earnshaw, W. C. The centromere: hub of chromosomal activities. *Science* **270**, 1591-1594 (1995).

120. Maiato, H., DeLuca, J., Salmon, E. D. & Earnshaw, W. C. The dynamic kinetochore-microtubule interface. *J. Cell. Sci.* **117**, 5461-5477 (2004).

121. Carroll, C. W. & Straight, A. F. Centromeric chromatin gets loaded. *J. Cell Biol.* **176**, 735-736 (2007).

122. Westermann, S., Drubin, D. G. & Barnes, G. Structures and functions of yeast kinetochore complexes. *Annu. Rev. Biochem.* **76**, 563-591 (2007).

123. Smith, M. M. Centromeres and variant histones: what, where, when and why? *Curr. Opin. Cell Biol.* **14**, 279-285 (2002).

124. Collins, K. A., Furuyama, S. & Biggins, S. Proteolysis contributes to the exclusive centromere localization of the yeast Cse4/CENP-A histone H3 variant. *Curr. Biol.* **14**, 1968-1972 (2004).

125. Folco, H. D., Pidoux, A. L., Urano, T. & Allshire, R. C. Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres. *Science* **319**, 94-97 (2008).

126. Meluh, P. B., Yang, P., Glowczewski, L., Koshland, D. & Smith, M. M. Cse4p is a component of the core centromere of Saccharomyces cerevisiae. *Cell* **94**, 607-613 (1998).

127. Stoler, S. *et al*. Scm3, an essential Saccharomyces cerevisiae centromere protein required for G2/M progression and Cse4 localization. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 10571-10576 (2007).

128. Morey, L., Barnes, K., Chen, Y., Fitzgerald-Hayes, M. & Baker, R. E. The histone fold domain of Cse4 is sufficient for CEN targeting and propagation of active centromeres in budding yeast. *Eukaryot. Cell.* **3**, 1533-1543 (2004).

129. Dalal, Y., Furuyama, T., Vermaak, D. & Henikoff, S. Structure, dynamics, and evolution of centromeric nucleosomes. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 15974-15981 (2007).

130. Mizuguchi, G., Xiao, H., Wisniewski, J., Smith, M. M. & Wu, C. Nonhistone Scm3 and histones CenH3-H4 assemble the core of centromere-specific nucleosomes. *Cell* **129**, 1153-1164 (2007).

131. Keith, K. C. & Fitzgerald-Hayes, M. CSE4 genetically interacts with the Saccharomyces cerevisiae centromere DNA elements CDE I and CDE II but not CDE III. Implications for the path of the centromere dna around a cse4p variant nucleosome. *Genetics* **156**, 973-981 (2000).

132. Meluh, P. B. & Koshland, D. Budding yeast centromere composition and assembly as revealed by in vivo cross-linking. *Genes Dev.* **11**, 3401-3412 (1997).

133. Chen, Y. *et al*. The N terminus of the centromere H3-like protein Cse4p performs an essential function distinct from that of the histone fold domain. *Mol. Cell. Biol.* **20**, 7037-7048 (2000).

134. Cheeseman, I. M. *et al*. Implication of a novel multiprotein Dam1p complex in outer kinetochore function. *J. Cell Biol.* **155**, 1137-1145 (2001).

135. Kroll, E. S., Hyland, K. M., Hieter, P. & Li, J. J. Establishing genetic interactions by a synthetic dosage lethality phenotype. *Genetics* **143**, 95-102 (1996).

136. Zhang, K. *et al*. The Set1 methyltransferase opposes Ipl1 aurora kinase functions in chromosome segregation. *Cell* **122**, 723-734 (2005).

137. Amor, D. J., Kalitsis, P., Sumer, H. & Choo, K. H. Building the centromere: from foundation proteins to 3D organization. *Trends Cell Biol.* **14**, 359-368 (2004).

138. Almutairi, A. Molecular interactions that affect centromere function during chromosome segregation. (2008).

139. Westermann, S. *et al*. Architecture of the budding yeast kinetochore reveals a conserved molecular core. *J. Cell Biol.* **163**, 215-222 (2003).

140. Emanuele, M. J., McCleland, M. L., Satinover, D. L. & Stukenberg, P. T. Measuring the stoichiometry and physical interactions between components elucidates the architecture of the vertebrate kinetochore. *Mol. Biol. Cell* **16**, 4882-4892 (2005).

141. Meraldi, P., McAinsh, A. D., Rheinbay, E. & Sorger, P. K. Phylogenetic and structural analysis of centromeric DNA and kinetochore proteins. *Genome Biol.* **7**, R23 (2006).

142. Chan, G. K., Liu, S. T. & Yen, T. J. Kinetochore structure and function. *Trends Cell Biol.* **15**, 589-598 (2005).

143. Sorger, P. K., Severin, F. F. & Hyman, A. A. Factors required for the binding of reassembled yeast kinetochores to microtubules in vitro. *J. Cell Biol.* **127**, 995-1008 (1994).

144. Malik, H. S. & Henikoff, S. Phylogenomics of the nucleosome. *Nat. Struct. Biol.* **10**, 882-891 (2003).

145. Mardian, J. K. & Isenberg, I. Yeast inner histones and the evolutionary conservation of histone-histone interactions. *Biochemistry* **17**, 3825-3833 (1978).

146. Smith, M. M. & Andresson, O. S. DNA sequences of yeast H3 and H4 histone genes from two non-allelic gene sets encode identical H3 and H4 proteins. *J. Mol. Biol.* **169**, 663-690 (1983).

147. Jacobson, S. J., Laurenson, P. M. & Pillus, L. Functional analyses of chromatin modifications in yeast. *Methods Enzymol.* **377**, 3-55 (2004).

148. Freeman, K. B., Karns, L. R., Lutz, K. A. & Smith, M. M. Histone H3 transcription in Saccharomyces cerevisiae is controlled by multiple cell cycle activation sites and a constitutive negative regulatory element. *Mol. Cell. Biol.* **12**, 5455-5463 (1992).

149. Smith, M. M. & Santisteban, M. S. Genetic dissection of histone function. *Methods* **15**, 269-281 (1998).

150. Kolodrubetz, D., Rykowski, M. C. & Grunstein, M. Histone H2A subtypes associate interchangeably in vivo with histone H2B subtypes. *Proc. Natl. Acad. Sci. U. S. A.* **79**, 7814-7818 (1982).

151. Hereford, L. M., Osley, M. A., Ludwig, T. R.,2nd & McLaughlin, C. S. Cell-cycle regulation of yeast histone mRNA. *Cell* **24**, 367-375 (1981).

152. Xu, H., Kim, U. J., Schuster, T. & Grunstein, M. Identification of a new set of cell cycle-regulatory genes that regulate S-phase transcription of histone genes in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **12**, 5249-5259 (1992).

153. Osley, M. A. & Hereford, L. M. Yeast histone genes show dosage compensation. *Cell* **24**, 377-384 (1981).

154. Cross, S. L. & Smith, M. M. Comparison of the structure and cell cycle expression of mRNAs encoded by two histone H3-H4 loci in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **8**, 945-954 (1988).

155. Hanlon, S. E., Norris, D. N. & Vershon, A. K. Depletion of H2A-H2B dimers in Saccharomyces cerevisiae triggers meiotic arrest by reducing IME1 expression and activating the BUB2-dependent branch of the spindle checkpoint. *Genetics* **164**, 1333-1344 (2003).

156. Moran, L., Norris, D. & Osley, M. A. A yeast H2A-H2B promoter can be regulated by changes in histone gene copy number. *Genes Dev.* **4**, 752-763 (1990).

157. Graves, R. A., Wellman, S. E., Chiu, I. M. & Marzluff, W. F. Differential expression of two clusters of mouse histone genes. *J. Mol. Biol.* **183**, 179-194 (1985).

158. Smith, M. M. & Stirling, V. B. Histone H3 and H4 gene deletions in Saccharomyces cerevisiae. *J. Cell Biol.* **106**, 557-566 (1988).

159. Au, W. C., Crisp, M. J., DeLuca, S. Z., Rando, O. J. & Basrai, M. A. Altered dosage and mislocalization of histone H3 and Cse4p lead to chromosome loss in Saccharomyces cerevisiae. *Genetics* **179**, 263-275 (2008).

160. McGrew, J. T., Xiao, Z. X. & Fitzgerald-Hayes, M. Saccharomyces cerevisiae mutants defective in chromosome segregation. *Yeast* **5**, 271-284 (1989).

161. Hirschhorn, J. N., Bortvin, A. L., Ricupero-Hovasse, S. L. & Winston, F. A new class of histone H2A mutations in Saccharomyces cerevisiae causes specific transcriptional defects in vivo. *Mol. Cell. Biol.* **15**, 1999-2009 (1995).

162. Norris, D. & Osley, M. A. The two gene pairs encoding H2A and H2B play different roles in the Saccharomyces cerevisiae life cycle. *Mol. Cell. Biol.* **7**, 3473-3481 (1987).

163. Huffaker, T. C., Thomas, J. H. & Botstein, D. Diverse effects of beta-tubulin mutations on microtubule formation and function. *J. Cell Biol.* **106**, 1997-2010 (1988).

164. Rose, M. D. & Fink, G. R. KAR1, a gene required for function of both intranuclear and extranuclear microtubules in yeast. *Cell* **48**, 1047-1060 (1987).

165. Schatz, P. J., Solomon, F. & Botstein, D. Isolation and characterization of conditional-lethal mutations in the TUB1 alpha-tubulin gene of the yeast Saccharomyces cerevisiae. *Genetics* **120**, 681-695 (1988).

166. Thomas, J. H. & Botstein, D. A gene required for the separation of chromosomes on the spindle apparatus in yeast. *Cell* **44**, 65-76 (1986).

167. Maruyama, T., Nakamura, T., Hayashi, T. & Yanagida, M. Histone H2B mutations in inner region affect ubiquitination, centromere function, silencing and chromosome segregation. *EMBO J.* **25**, 2420-2431 (2006).

168. Winston, F., Dollard, C. & Ricupero-Hovasse, S. L. Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. *Yeast* **11**, 53-55 (1995).

169. Guthrie, C. & Fink, G. R. Guide to yeast genetics and molecular biology. **194** (1991).

170. Guthrie, C. Guide to Yeast Genetics and Molecular Cell Biology. **350** (2002).

171. Rose, M. D., Winston, F. & Hieter, P. in *Methods in Yeast Genetics: A Laboratory Course Manual* (Cold Spring Harbor Press, Cold Spring Harobr, NY, 1990).

172. Gietz, R. D., Schiestl, R. H., Willems, A. R. & Woods, R. A. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. *Yeast* **11**, 355-360 (1995).

173. Ausubel, F. M. *et al*. Current Protocols in Molecular Biology. (1991).

174. Sambrook, J., Fritsch, E. F. & Maniatis, T. in *Molecular cloning: a laboratory manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

175. Sikorski, R. S. & Hieter, P. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* **122**, 19-27 (1989).

176. Christianson, T. W., Sikorski, R. S., Dante, M., Shero, J. H. & Hieter, P. Multifunctional yeast high-copy-number shuttle vectors. *Gene* **110**, 119-122 (1992).

177. Brachmann, C. B. *et al*. Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14**, 115-132 (1998).

178. Tyagi, R., Lai, R. & Duggleby, R. G. A new approach to 'megaprimer' polymerase chain reaction mutagenesis without an intermediate gel purification step. *BMC Biotechnol.* **4**, 2 (2004).

179. Schild, D., Ananthaswamy, H. N. & Mortimer, R. K. An endomitotic effect of a cell cycle mutation of Saccharomyces cerevisiae. *Genetics* **97**, 551-562 (1981).

180. Lew, D. J., Marini, N. J. & Reed, S. I. Different G1 cyclins control the timing of cell cycle commitment in mother and daughter cells of the budding yeast S. cerevisiae. *Cell* **69**, 317-327 (1992).

181. Boeke, J. D., Trueheart, J., Natsoulis, G. & Fink, G. R. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. *Methods Enzymol.* **154**, 164-175 (1987).

182. Lea, D. E. & Coulson, C. A. The distribution of number and mutants in bacterial populations. *J. Genet.* **49**, 264-265-284 (1949).

183. Edmondson, D. G., Smith, M. M. & Roth, S. Y. Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. *Genes Dev.* **10**, 1247-1259 (1996).

184. Hames, B. D. & Rickwood, D. Gel Electrophoresis of Proteins: A Practical Approach. (1990).

185. Thomas, J. O. & Kornberg, R. D. An octamer of histones in chromatin and free in solution. *Proc. Natl. Acad. Sci. U. S. A.* **72**, 2626-2630 (1975).

186. Briggs, S. D. *et al*. Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in Saccharomyces cerevisiae. *Genes Dev.* **15**, 3286-3295 (2001).

187. http://www.abcam.com/ps/pdf/protocols/Histone%20western%20blotting.pdf.

188. Sharp, J. A., Franco, A. A., Osley, M. A. & Kaufman, P. D. Chromatin assembly factor I and Hir proteins contribute to building functional kinetochores in S. cerevisiae. *Genes Dev.* **16**, 85-100 (2002).

189. Crotti, L. B. & Basrai, M. A. Functional roles for evolutionarily conserved Spt4p at centromeres and heterochromatin in Saccharomyces cerevisiae. *EMBO J.* **23**, 1804-1814 (2004).

190. Duina, A. A. & Winston, F. Analysis of a mutant histone H3 that perturbs the association of Swi/Snf with chromatin. *Mol. Cell. Biol.* **24**, 561-572 (2004).

191. Poveda, A. & Sendra, R. Site specificity of yeast histone acetyltransferase B complex in vivo. *FEBS J.* **275**, 2122-2136 (2008).

192. Glowczewski, L., Yang, P., Kalashnikova, T., Santisteban, M. S. & Smith, M. M. Histone-histone interactions and centromere function. *Mol. Cell. Biol.* **20**, 5700-5711 (2000).

193. Wu, J., Suka, N., Carlson, M. & Grunstein, M. TUP1 utilizes histone H3/H2B-specific HDA1 deacetylase to repress gene activity in yeast. *Mol. Cell* **7**, 117-126 (2001).

194. Qin, S. & Parthun, M. R. Histone H3 and the histone acetyltransferase Hat1p contribute to DNA double-strand break repair. *Mol. Cell. Biol.* **22**, 8353-8365 (2002).

195. Sobel, R. E., Cook, R. G., Perry, C. A., Annunziato, A. T. & Allis, C. D. Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 1237-1241 (1995).

196. Benson, L. J. *et al*. Modifications of H3 and H4 during chromatin replication, nucleosome assembly, and histone exchange. *J. Biol. Chem.* **281**, 9287-9296 (2006).

197. Verreault, A. De novo nucleosome assembly: new pieces in an old puzzle. *Genes Dev.* **14**, 1430-1438 (2000).

198. Struhl, K. Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev.* **12**, 599-606 (1998).

199. Rundlett, S. E. *et al*. HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 14503-14508 (1996).

200. Downs, J. A. *et al*. Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Mol. Cell* **16**, 979-990 (2004).