UNIVERSITY OF CALIFORNIA SAN DIEGO

Two Histone Mutants Enhance the Transcriptional Silencing at rDNA Locus in *S. cerevisiae*

A Thesis submitted in partial satisfaction of the requirements for the degree
Master of Science

in

Biology

by

Wanying Tian

Committee in charge:

Professor Nan Hao, Chair
Professor Eric Allen, Co-Chair
Professor Stephanie Mel

2021

The Thesis of Wanying Tian is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

University of California San Diego

2021

EPIGRAPH

One must imagine Sisyphus happy.

Albert Camus.

TABLE OF CONTENTS

Thesis Approval Page…… iii

Epigraph……...…….... iv

Table of Contents……... v

List of Figures ……... vi

List of Tables ……..... vii

Acknowledgments.…… viii

Abstract of the Thesis ……..... x

Introduction…… 1

Materials and Methods.……..... 4

Results……...…… 6

Discussion……. 13

References……..... 18

LIST OF FIGURES

Figure 1. Overexpression of histone wild type or mutants has little effect on the euchromatin region.…… 6

Figure 2. Overexpression of histone mutant proteins enhanced the transcriptional silencing at the rDNA locus…… 8

Figure 3. The enhanced transcriptional silencing at the rDNA locus is Sir2 dependent…… 11

Figure 4. The enhanced transcriptional silencing at the rDNA locus caused by H2B E76K is Set1 dependent..……..... 12

Figure 5. Overexpression of histone mutant proteins do not enhance the transcriptional silencing at the telomeric and the silent mating loci…… 13

LIST OF TABLES

Table 1. Strains used or constructed in this study.…… 16

Table 2. Plasmids constructed in this study.……. 17

ACKNOWLEDGEMENTS

I would like to acknowledge Professor Nan Hao for giving me the wonderful opportunity to pursue my passion towards science under his guidance. Thank you so much for always being optimistic and supportive. Your encouragement mattered a lot especially when our experimental progress is not ideal.

I would like to acknowledge Dr. Peter D. Adams for initiating this project and sharing his insights with us. To Hiroshi Tanaka and Zong Ming Chua, thank you for providing the DNA sample of the histones and conducting the Western Blot experiment.

I would like to acknowledge Yutian Li and Yanfei Jiang for being my mentors in the lab. You taught me the basic protocol of yeast cloning and microfluidic technique. I cannot remember how many times you sat together with me to help me to troubleshoot the experiment and analyze the data. I would also like to acknowledge Zhen Zhou, for being my unofficial mentor and friend in the lab. You were always very patient and kind to my various questions and helped me figure out those scientific puzzles. Random chatting with you with micropipettes in our hands always sparks laughter and sometimes scientific meaningful thoughts. Also thank you for providing me the reporters of the silent mating and the telomeric loci for this project. To Beverly Naigles, I thank you for not only teaching me techniques, such as RT-PCR, but also supporting me to go through the graduate school application process. You have not only provided me countless draft revisions but helped me to reflect on myself and realize my inner passion. I will not be able to keep pursuing my scientific desire without your help. Also thank you for always keeping the lab organized and creating an interactive environment in the lab. To Julie Paxman, I thank you for inviting me to work with you on your excellent aggregation

project. It was a very enjoyable experience that I will always remember. I would also like to acknowledge Yang Li for his guidance and friendship. To Richard O'Laughlin and Quoc Tran, thank you for generously providing me the doxycycline inducible plasmid, which is indispensable for this project. Lastly, I would acknowledge Anusorn Mudla for taking care of the safety regulation and waste disposals and I would thank Sophia Ye for maintaining the stock of very single lab items to ensure the success of our experiments.

ABSTRACT OF THE THESIS

Two Histone Mutants Enhance the Transcriptional Silencing at rDNA Locus in *S. cerevisiae*

by

Wanying Tian

Master of Science in Biology

University of California San Diego, 2021

Professor Nan Hao, Chair
Professor Eric Allen, Co-Chair

Histones play an important role in packaging and organizing the genomes. These proteins regulate the accessibility and maintain the stability of the genome. Mutation of histone proteins would lead to alteration of histone core structure and change of transcriptional silencing status. In this study, two histone mutants originated from human cells were characterized in budding yeast. The first

histone mutant is known for promoting nucleosome stabilization. By contrast, the other histone mutant is shown to destabilize the nucleosome structure and is the most frequently occurring mutant in cancer cells. The physiological effect of doxycycline induced overexpression of the two histone mutants was observed by using GFP reporters at three major heterochromatin regions: the ribosomal DNA (rDNA), the silent mating, and the telomeric loci. We determined that both histone mutants enhanced the transcriptional silencing at the rDNA locus but not the silent mating and the telomeric loci. Additionally, in order to further elucidate the association between this enhanced silencing effect and posttranslational modification of the histones such as deacetylation and methylation, deletion strains of Sir2, a histone deacetylase, and Set1, a methyltransferase, were constructed. We found that the silencing enhancement of both histone mutants depended on Sir2 but only one of them is Set1-dependent.

Introduction

The genomic DNA of eukaryotes are organized by histone proteins to form the nucleosome unit and packaged into the chromosome structure within the nucleus. The histone proteins, H1, H2A, H2B, H3, and H4, play a critical role in the maintenance and regulation of genetic information. The presence and proper function of these histone proteins ensure genome stability during the various biological events happening throughout the cell cycle, such as transcription, DNA replication, recombination, and repair. Two copies of each of the four histone proteins (H2A, H2B, H3, and H4) form an octamer structure, which allows DNA to wrap around to become nucleosome, the basic repetitive units of eukaryotic chromatin. H1 protein binds to this nucleoprotein complex to organize them into more higher-order structures (Kornberg, 1977; McGhee and Felsenfeld, 1980).

Besides the canonical core histones that are incorporated during DNA synthesis, each core histone has corresponding histone variants that are incorporated throughout the cell cycle and substitute for their canonical counterparts to alter the chromatin structure and serve specialized functions in transcription, chromosome segregation, and DNA repair (Talbert and Henikoff, 2017). For instance, macroH2A is a vertebrate-specific H2A variant that preferentially localized in the inactive X chromosome of female mammals and centromeric heterochromatin (Chadwick et al., 2001; Chadwick and Willard, 2002; Costanzi and Pehrson, 1998; Pehrson and Fried, 1992). It has been found to promote transcriptional silencing and nucleosome stabilization (Chakravarthy et al., 2012; Kozlowski et al., 2018). Previous studies have revealed that macroH2A differs from canonical H2A at the L1 loop, where the H2A sequence is ^{38}NYAE^{41} and the macroH2A sequence is ^{38}HPKY^{41}. Net charge and hydrophobicity are increased in the

macroH2A variant. This variation was proven to be essential in the localization of macroH2A and its nucleosome stabilization function (Chadwick et al., 2001; Nusinow et al., 2007, p. 1).

Because of the principal role of histone proteins in shaping the chromosomal landscape and controlling gene expression, it is unsurprising that the mutation or dysfunction of histone proteins would cause severe diseases, including cancer. Previous studies have discovered many histone mutations that occur at high frequency and drive various carcinogenesis and cancer progression (Amatori et al., 2021; Mohammad and Helin, 2017; Qiu et al., 2018). For example, a glutamate-to-lysine point mutation of histone H2B at amino acid 76 (H2B-E76K) has been found to be the most frequently occurring mutant in cancer cells (Arimura et al., 2018; Bennett et al., 2019; Nacev et al., 2019). Studies have been done to characterize the structural alteration and biological effects of this mutation to illustrate how this single-amino-acid mutation in only one of the multiple copies of the histone H2B gene would head to oncogenesis. This H2B-E76K mutation was revealed to destabilize the nucleosome structure by disrupting the interaction between H2B and H4 and increasing chromatin accessibility, and also was proved to promote oncogenic colony formation in Hela cells and mammary epithelial cell line (Arimura et al., 2018; Bennett et al., 2019). Bennett et al. have found that the yeast strain with heterozygous mutant for E79K showed slow growth at high temperature and was more sensitive to MNase compared to wild-type yeast. Bennett et al also revealed that E79K disrupted nucleosome-mediated gene repression for the *PHO5* gene (Bennett et al., 2019). However, the effect of E79K on the heterochromatin region (the rDNA, the silent mating, and the telomeric loci) of yeast haven't been characterized in that study.

Phylogenetic study revealed that histone proteins are among the most conserved protein across eukaryotes (Malik and Henikoff, 2003). The similarity of H2A amino acid sequence

between human and *Saccharomyces cerevisiae* (*S. cerevisiae*) is around 70% and the similarity of H2B amino acid between human and *S. cerevisiae* is around 80%. The high similarity implied the possibility that the fundamental epigenetic principles found in yeast can also be applied to higher-order eukaryotes, including humans. Endowed by this simplest eukaryotic genome and well-developed genetic tools, many conserved epigenetic mechanisms have been discovered using *S. cerevisiae* (Grunstein and Gasser, 2013).

Giving the absence of DNA methylation, RNA interference (RNAi) machinery, and repressive histone H3K9 methylation, *S. cerevisiae* can serve as a reductive eukaryotic model organism to study histone modification patterns without the involvement of other epigenetic phenomenons (O'Kane and Hyland, 2019). There are three epigenetically repressed loci present in the yeast genome: the rDNA, the silent mating, and the telomeric loci (Grunstein and Gasser, 2013). In this study, the biological effects of H2A-^{38}HPKY^{41} and H2B-E76K on these three epigenetically repressed loci of *S. cerevisiae* and possible mechanisms behind them were investigated.

Materials and Methods

Plasmid and Yeast Strain construction

Standard methods for the growth, maintenance, and transformation of yeast and bacteria and for manipulation of DNA were used throughout. The yeast strains used in this study were generated from BY4741 (MAT a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) strain background.

Yeast strain with heterochromatin silencing reporter of the rDNA, the telomeric, and the silent mating loci was used as mother strain to integrate the histone plasmids. The construction of rDNA silencing reporter (RDN1_NTS1::pGPD_GFP-URA3) and euchromatin silencing reporter (TDH3-pRS306-pGPD-GFP) was described in a previous paper (Li et al., 2017), and the reporters of the telomeric (TEL12R::pTDH3-GFP) and the silent mating (HML-pTDH3-GFP) loci were made by Zhen Zhou.

The DNA of wild-type and mutant histone gene was manufactured by Integrated DNA Technologies (IDT). The plasmid with doxycycline rtta3 activator and Tet promoter and mRuby2 was provided and constructed by Richard O'Laughlin and Quoc Tran.

To make the plasmids with the histone gene and a doxycycline inducible system, histone genes and plasmid backbones with doxycycline inducible system were amplified by PCR and assembled by Gibson Assembly. The assembly was designed so that the H2A wild-type and mutant proteins were N-terminally tagged with mRuby2 while H2B wild-type and mutant proteins were C-terminally tagged with mRuby2. The plasmids were digested by NotI then integrated into the native Leu2 site in the genome of the mother strain with euchromatin and heterochromatin silencing reporter using. All transformations were conducted with the lithium acetate method (Gietz and Schiestl, 2007). Colony PCR was used to confirm plasmid integrations.

For strains with rDNA silencing reporter, after each plasmid integration, colonies with similar fluorescence levels as the mother strain were selected to avoid the occasional copy number changes, which are caused by the repetitious nature of the ribosomal DNA region during the transformation process.

Medium and solutions

Synthetic complete medium (SC, 2% dextrose) used in all the yeast cell culturing was made of 6.7g/L Difco Yeast Nitrogen Base without Amino Acids, 0.79g/L CSM (complete mixture), and 2% dextrose. Doxycycline hyclate power bought from Sigma-Aldrich was dissolved in SD and vacuum filtered to make the stock solution.

Live Cell Imaging and Image Analysis

All yeast cell images were taken using a Nikon Ti-E inverted fluorescence microscope with Perfect Focus, coupled with an EMCCD camera (Andor iXon X3 DU897). a CFI Plan Apochromat Lambda DM 60X Oil Immersion Objective (NA 1.40 WD 0.13MM) was used with Nikon Immersion Oil Type F with refractive index = 1.518, dispersion = 41, viscosity = 410 cst.

Yeast was first streaked on a YPD plate from -80°C stock and incubated at 30°C overnight. Then colonies from the plate were inoculated in 2 mL of synthetic complete medium (SC, 2% dextrose) medium and shack at 30°C overnight. The next day, 3 µl saturated overnight cultures were diluted in 20 ml SC medium and grown for 16 hrs until OD 0.05 was reached. The diluted cultures were then treated with doxycycline for 6 hrs until OD reached 0.5 to 0.6. The cells were then centrifuged and the supernatant was decanted. After 30s vortexing, 5 µl cells were pipetted on glass slides, covered with cover glasses, and then imaged using a microscope. Fisherbrand Frosted Microscope Slides; 3 x 1 in. (76 x 25.4mm) and Thermo Scientific Gold

Seal Cover Glasses; Rectangles; 24 x 50mm; Thickness 1.5 were used in all the live-cell imaging. Fluorescence intensity was quantified using the MATLAB code written by Zhen Zhou. The figures were generated using Prism 8 for MacOS, version 8.4.3.

Results

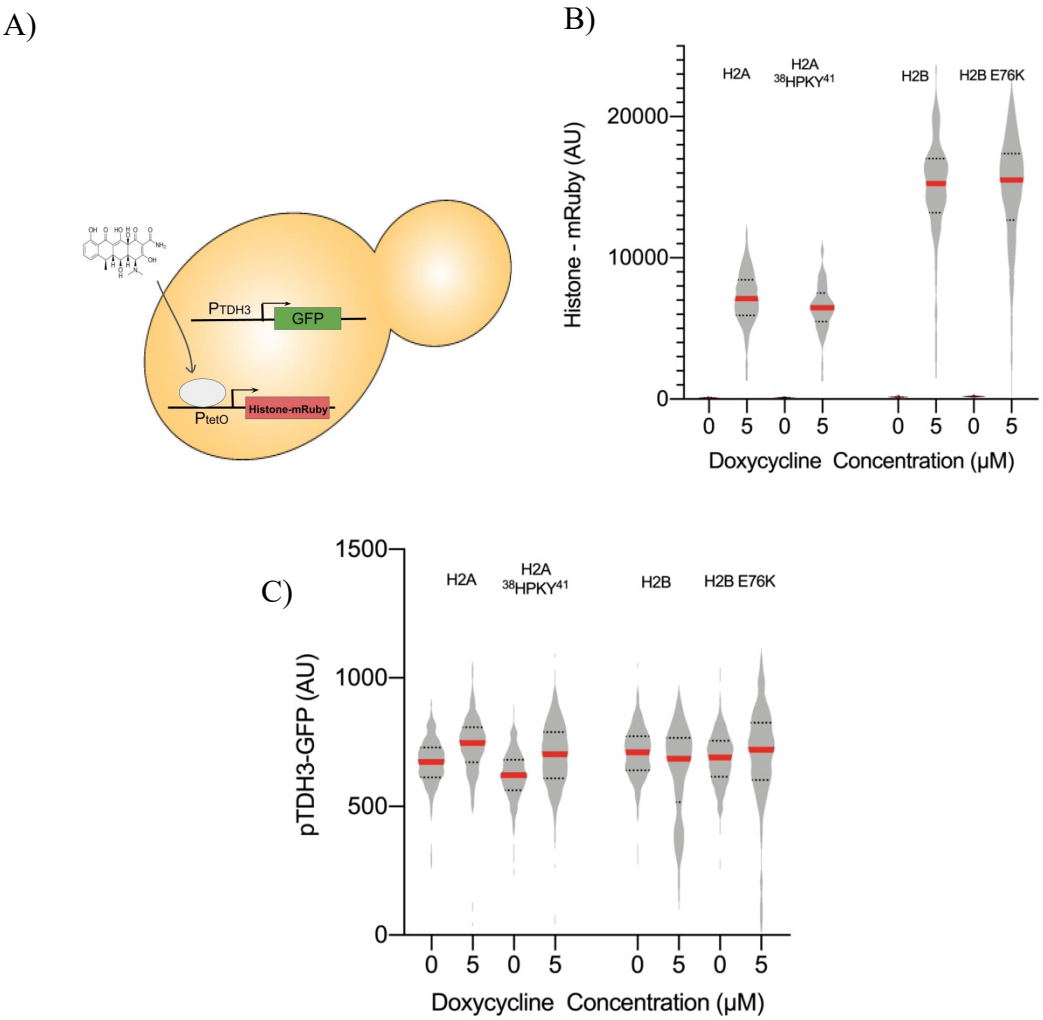


Figure 1. Overexpression of histone wild type or mutants has little effect on the euchromatin region. (A) Diagram shows the genomic construction of the yeast strain. (B) mRuby fluorescence intensity was quantified to reveal the relative abundance of histone proteins. Each red line marks the median value, and dash lines mark the quartiles. At least 300 cells were quantified in each experimental condition. (C) GFP fluorescence intensity was quantified to reveal the relative abundance of GFP reporter proteins, which represents the transcriptional silencing status of the euchromatin region. Each red line marks the median value, and dash lines mark the quartiles. At least300 cells were quantified in each experimental condition.

Overexpression of Histone protein have no impact on the euchromatin region

In order to test the effects of overexpressed H2A and H2B mutants on yeast cell physiology, plasmids containing inducible mRuby2 tagged H2A, H2A-^{38}HPKY^{41}, H2B, and H2B-E76K were integrated into a yeast strain with a GFP fluorescent protein driving by TDH3 promoter, as a reporter for euchromatin region (Figure 1A). The uninduced strains showed close to zero mRuby2 levels while high-level expression of mRuby2 was observed in the nucleus for the induced strains, which indicates this doxycycline inducible system worked successfully (Figure 1B). As expected, the expression levels of the pTDH3-GFP reporter only show limited changes between induced and uninduced strains (Figure 1C). This result confirms that the overexpression of H2A and H2B wild-type and mutant proteins have minimal impact on the euchromatin region of the yeast genome.

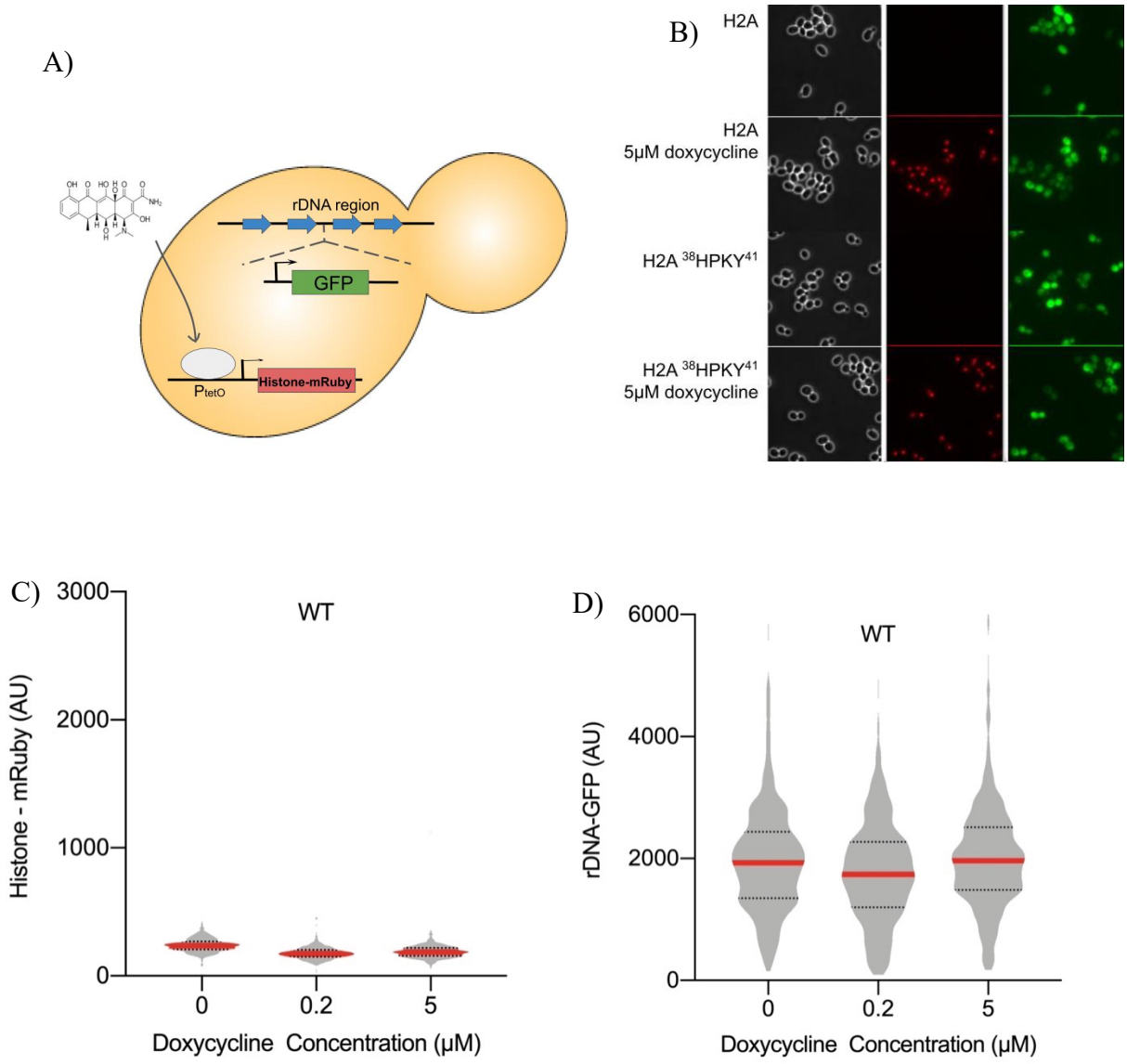
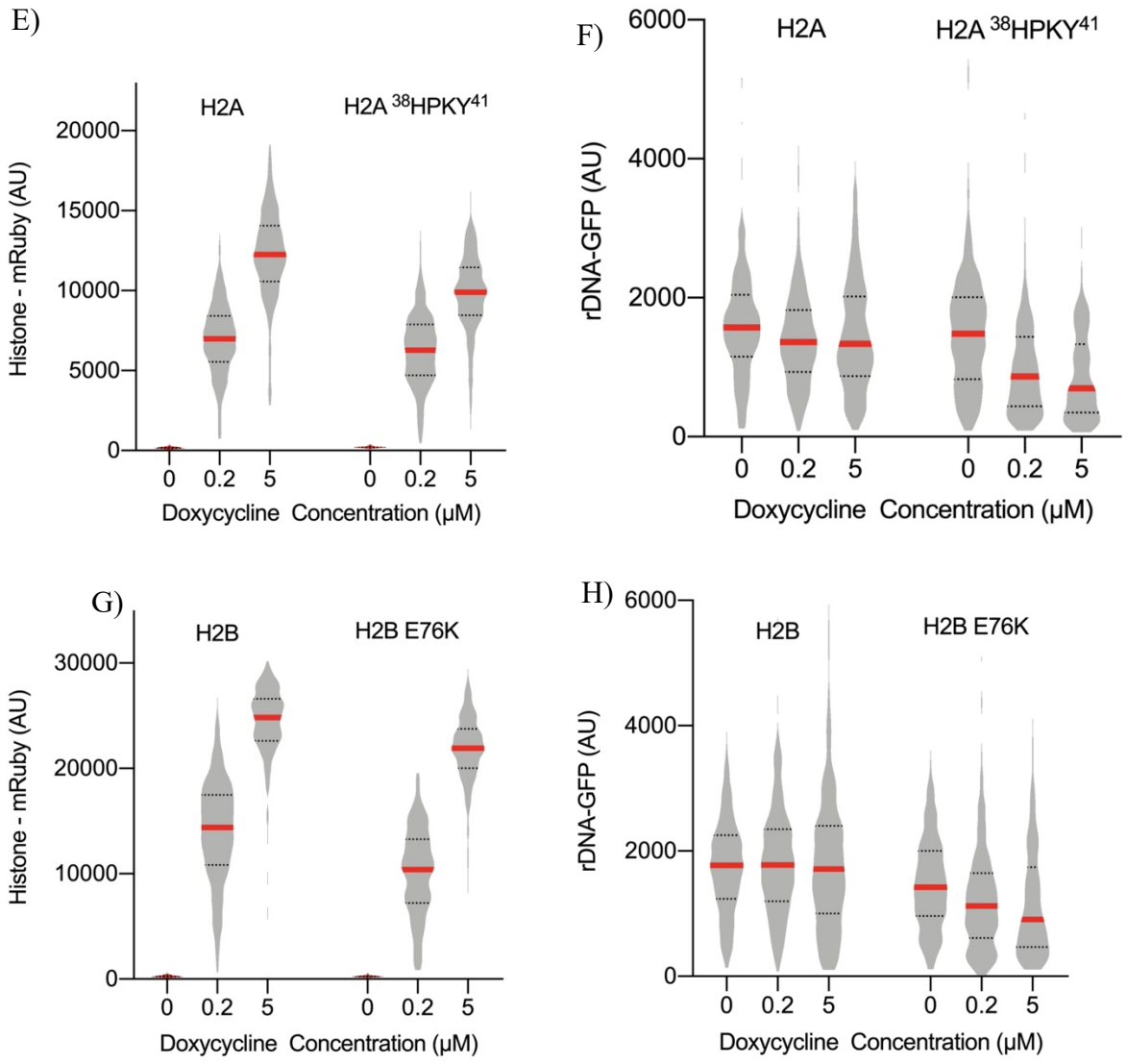


Figure 2. Overexpression of histone mutant proteins enhanced the transcriptional silencing at the rDNA locus. (A) Diagram shows the genomic construction of the yeast strain. (B) Representative photos showing yeast cells and fluorescence intensities obtained using phase, mRuby, and GFP channel. (C) mRuby fluorescence intensity of wild-type strain that does not contain the histone inducible plasmid. Each red line marks the median value, and dash lines mark the quartiles. (D) GFP fluorescence intensity of the wild-type strain that does not contain the histone inducible plasmid. Each red line marks the median value, and dash lines mark the quartiles. (E) mRuby fluorescence intensity was quantified to reveal the relative abundance of H2A wild-type or mutant proteins. Each red line marks the median value, and dash lines mark the quartiles. At least 300 cells were quantified in each experimental condition. GFP fluorescence intensity in H2A-^{38}HPKY^{41} (F) and H2B-E76K (H) was quantified to reveal the relative abundance of GFP reporter proteins, which represents the transcriptional silencing status of rDNA locus. Each red line marks the median value, and dash lines mark the quartiles. At least 300 cells were quantified in each experimental condition. (G) mRuby fluorescence intensity was quantified to reveal the relative abundance of H2A wild-type or mutant proteins. Each red line marks the median value, and dash lines mark the quartiles. At least 300 cells were quantified in each experimental condition.

Figure 2 continued



Overexpression of histone mutants enhanced transcriptional silencing at the ribosomal DNA locus

rDNA locus contains the genetic information for ribosome biogenesis. It is located on chromosome XII in the yeast nucleolus. This locus contains tandem repeats of 100–200 copies of

a 9.1 kb sequence, and half of these repeats remain silenced (Linskens and Huberman, 1988). Failure of maintaining the silent state cause accumulation of Extrachromosomal rDNA Circles, which is the major cause of yeast aging (Sinclair and Guarente, 1997). To investigate the effects of overexpressing H2A and H2B mutants on the rDNA locus, plasmids containing inducible mRuby2 tagged H2A, H2A-^{38}HPKY^{41}, H2B, and H2B-E76K were integrated into a yeast strain with a GFP silencing reporter of rDNA region (Figure 2A) as described in Li et al (Li et al., 2017). After 6-7 hours of doxycycline treatment, cell morphology and cell cycles of induced strains were similar to the uninduced control (Figure 2B). For the wild-type control strain without the doxycycline inducible plasmid, the mRuby intensity is close to zero, and the GFP intensity is unchanged after the addition of the doxycycline (Figure 2C and 2D). H2A and H2A-^{38}HPKY^{41} were induced as indicated by the level of mRuby (Figure 2E). The silencing states of rDNA locus were similar among H2A wild-type strains with 0 μM, 0.2 μM, or 5 μM doxycycline (Figure 2F). However, yeast cells with induced H2A-^{38}HPKY^{41} showed significant lower levels of GFP, indicating the silencing of rDNA locus was enhanced, and this effect is dosage-dependent (Figure 2F). Similar patterns were observed for the H2B wild-type and mutant strains, yeast cells with induced H2B-E76K showed significant lower levels of GFP while overexpression of wild-type H2B didn't affect the GFP level (Figure 2H). These results illustrated that overexpression of both histone mutants has enhanced the gene silencing at the rDNA locus, while the wild-type histone proteins didn't have this effect.

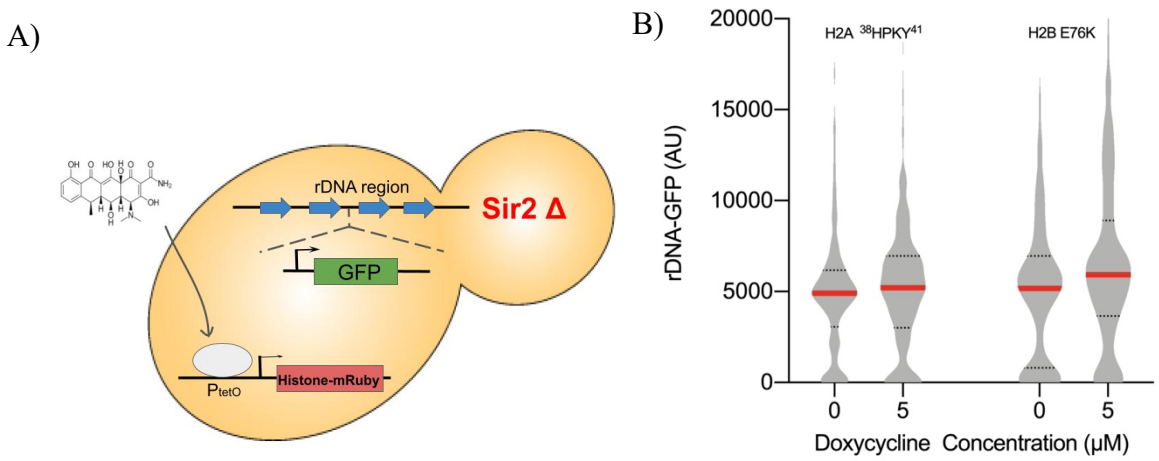


Figure 3. The enhanced transcriptional silencing at the rDNA locus is Sir2 dependent. (A) Diagram shows the genomic construction of the yeast strain. (B) GFP fluorescence intensity was quantified to reveal the relative abundance of GFP reporter proteins, which represents the transcriptional silencing status of the rDNA locus. Each red line marks the median value, and dash lines mark the quartiles. At least 300 cells were quantified in each experimental condition.

Next, we wanted to study if this enhancement of silencing at the rDNA locus is Sir2 dependent or not. Sir2 is a highly conserved NAD^+-dependent histone deacetylase (Blander and Guarente, 2004; Brachmann et al., 1995, p. 2). This protein plays an important role as a Silent Information Regulator to mediate the silencing at the rDNA tandem array, the silent mating-type loci HML and HMR, and the telomeric region of budding yeast (Gartenberg and Smith, 2016). Sir2 deletion strains containing the rDNA-GFP reporter and inducible H2A-^{38}HPKY^{41} and H2B-E76K were constructed to see if this protein is associated with the enhanced silencing effect (Figure 3A). Strikingly, the induced and uninduced Sir2 deletion cells showed similar levels of GFP (Figure3B), which means that both H2A-^{38}HPKY^{41} and H2B-E76K no longer have an impact on the silencing of rDNA when the Sir2 is absent. Thus, the enhanced silencing effects of both histone mutants are Sir2 dependent.

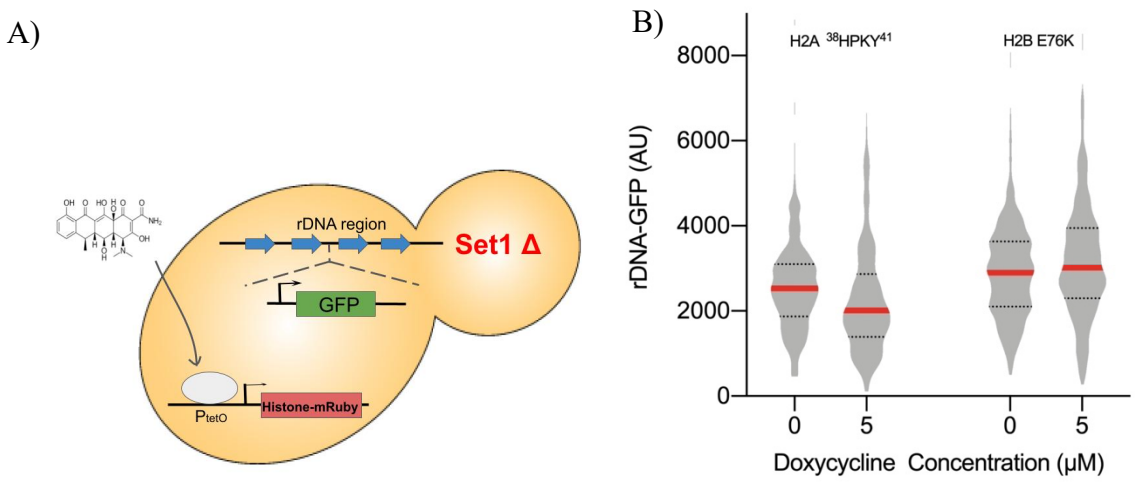


Figure 4. The enhanced transcriptional silencing at the rDNA locus caused by H2B E76K is Set1 dependent. (A) Diagram shows the genomic construction of the yeast strain. (B) GFP fluorescence intensity was quantified to reveal the relative abundance of GFP reporter proteins, which represents the transcriptional silencing status of the rDNA locus. Each red line marks the median value, and dash lines mark the quartiles. At least 300 cells were quantified in each experimental condition.

To order to probe the interaction of other posttranslational modifications and the enhanced silencing effect of histone mutants, Set1 deletion strains containing the rDNA-GFP reporter and inducible H2A-^{38}HPKY^{41} and H2B-E76K were constructed (Figure 4A). Set1 is a methyltransferase that belongs to the *trithorax* gene family. It has been found to regulate the transcriptional silencing at rDNA, the silent mating, and the telomeric loci (Briggs et al., 2001; Bryk et al., 2002; Nislow et al., 1997, p. 1). Interestingly, in the Set1 deleted strains, the cells with induced H2A-^{38}HPKY^{41} showed significantly lower of GFP intensities than the uninduced control (Figure 4B), which reveals that the absence of Set1 protein doesn't affect the enhanced silencing of rDNA locus by the H2A-^{38}HPKY^{41}. By contrast, the cells with induced H2B-E76K showed similar levels of GFP with the uninduced control in the Set1 deleted strains (Figure 4B), which indicates that the H2B-E76K is not able to enhance the rDNA silencing when Set1 is absent. In summary, enhanced silencing at the rDNA locus by H2A-^{38}HPKY^{41} is Set1-independent while the effect of H2B-E76K is dependent on Set1.

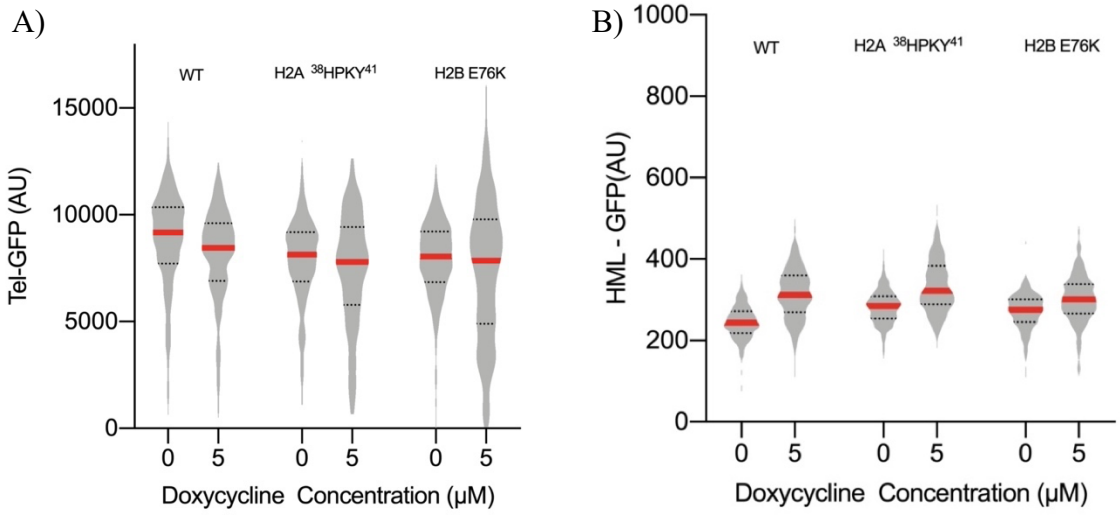


Figure 5. Overexpression of histone mutant proteins do not enhance the transcriptional silencing at the telomeric and the silent mating loci. (A) GFP fluorescence intensity was quantified to reveal the relative abundance of GFP reporter proteins, which represents the transcriptional silencing status of the telomeric locus. Each red line marks the median value, and dash lines mark the quartiles. At least 300 cells were quantified in each experimental condition. (B) GFP fluorescence intensity was quantified to reveal the relative abundance of GFP reporter proteins, which represents the transcriptional silencing status of the silent mating locus. Each red line marks the median value, and dash lines mark the quartiles. At least 300 cells were quantified in each experimental condition.

Overexpression of histone proteins have minimal impact on the silent mating and the telomeric loci

Besides the rDNA locus on chromosome XII, there are other heterochromatin-like regions in the yeast genome, such as the two silent mating loci located on chromosome III and the telomeric regions located on all chromosomes. Silencing of the two silent mating loci HML and HMR is not required for cell survival but is critical for the mating competence of the yeast, and the silencing at the telomeric region prevents the telomere from degradation and is maintained the chromosomal stability (Grunstein and Gasser, 2013). In order to explore the effects of overexpressed H2A and H2B mutants on the silent mating locus and telomere, plasmids containing inducible mRuby2 tagged H2A-^{38}HPKY^{41}and H2B-E76K were integrated into a yeast strain with a GFP silencing reporter of silent mating locus or telomere region. Surprisingly, the level of GFP, which represents the silencing status at the silent mating or the

telomeric loci, doesn't change significantly when either H2A-^{38}HPKY^{41} or H2B-E76K was induced by doxycycline (Figure 5A and 5B). This phenomenon suggests that the overexpression of neither H2A-^{38}HPKY^{41} nor H2B-E76K has an impact on the transcriptional silencing at the silent mating locus and telomere. The enhanced silencing effect is specific to the rDNA region.

Discussion

There are many questions inspired by the current findings. First, it is not clear why the enhanced silencing effects of H2A-^{38}HPKY^{41} and H2B-E76K are specific to the rDNA locus. One of possible explanation is that Sir2 interacted with different proteins at the rDNA locus comparing to the silent mating and the telomeric loci. Interestingly, the mechanism of the Sir2 silencing function is quite different in these three chromatin regions. Sir2 forms the RENT complex with Net1 and Cdc14 proteins to maintain the gene silencing at the rDNA silencing region (Shou et al., 1999; Straight et al., 1999). In contrast, at the silent mating and the telomeric DNA region, Sir2 assembles with Sir3, and Sir4 proteins to mediate the silencing (Moazed et al., 1997; Strahl-Bolsinger et al., 1997). Therefore, these non-histone proteins, such as Net1 and Cdc14, that are specifically involved in transcriptional silencing at the rDNA locus might be associated with changes caused by those histone mutants.

Second, since H2B-E76K is a destabilizing histone mutant, and the previous study has found that the expression level of the *PHO5* gene would increase due to the overexpression of H2B-E76K (Bennett et al., 2019), it is unexpected that this histone mutant can enhance the silencing at the rDNA locus rather than disrupting the silencing. It is possible that instead of directly silencing the rDNA locus, overexpression of H2B-E76K leads to the upregulation of other proteins by destabilizing the nucleosome structure and increasing chromatin accessibility, and then these proteins may play a role in rDNA silencing enhancement.

Third, all strains used in this project still contains the native two copies of the H2A and H2B proteins. It is unclear that how these native proteins expression level was regulated and how these proteins behaved under the circumstance that ectopic histone mutants were overexpressed. Western Blot needs to be performed to determine the relative abundance of the native and ectopic proteins. A previous study has found that the dosage compensation of histone proteins exists and the stoichiometric balance of the histone proteins is critical for maintaining genomic integrity (Libuda and Winston, 2006). Thus, it is very likely that the expression levels of native H2A and H2B histone proteins or even the level of H3 and H4 are changed by the overexpression of the mutant proteins.

Fourth, how these two histone mutants interacted with those non-histone proteins, such as Sir2 and Set1, to enhance the silencing at the rDNA locus remains unknown. Experiments such as CHIP or immunoprecipitation can be performed to test the interactions between histone mutants with specific loci and with different silencing responsible complexes.

Fifth, the silencing of the rDNA locus plays an imperative role during yeast replicative aging, and silencing loss leads to early cell death (Li et al., 2017). Consequently, because the overexpression of H2A-^{38}HPKY^{41} and H2B-E76K can enhance rDNA silencing even in a short period of time (6-7 hrs), it is highly possible that these mutant proteins can have an impact on the rDNA locus after longer time exposure and extend the life span of yeast cells. Aging experiments can be done to observe the long-term effect of those histone mutants and quantify the lifespan.

Table 1. Strains used or constructed in this study.

STRAIN NAME	DESCRIPTION
NH270	BY4741 (Meng), Nhp6a-iRFP:Kan RDN1_NTS1::pGPD_GFP-URA3
NH1441	BY4741 (Meng), Nhp6a-iRFP:Kan RDN1_NTS1::pGPD_GFP-URA3, pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1
NH1442	BY4741 (Meng), Nhp6a-iRFP:Kan RDN1_NTS1::pGPD_GFP-URA3, pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1 NYAQ->HPKY
NH1443	BY4741 (Meng), Nhp6a-iRFP:Kan RDN1_NTS1::pGPD_GFP-URA3, pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1_mRuby2
NH1444	BY4741 (Meng), Nhp6a-iRFP:Kan RDN1_NTS1::pGPD_GFP-URA3, pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1 E80K_mRuby2
NH1189	BY4741 (Meng), Nhp6a-iRFP:Kan; TDH3-pRS306-pGPD-GFP
NH1490	BY4741 (Meng), Nhp6a-iRFP:Kan; TDH3-pRS306-pGPD-GFP, pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1
NH1491	BY4741 (Meng), Nhp6a-iRFP:Kan; TDH3-pRS306-pGPD-GFP, pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1 NYAQ->HPKY
NH1492	BY4741 (Meng), Nhp6a-iRFP:Kan; TDH3-pRS306-pGPD-GFP, pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1_mRuby2
NH1493	BY4741 (Meng), Nhp6a-iRFP:Kan; TDH3-pRS306-pGPD-GFP, pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1 E80K_mRuby2
NH277	BY4741 (Meng), Nhp6a-iRFP:Kan NTS1::pGPD-GFP-URA3 sir2::HIS
NH1526	BY4741 (Meng), Nhp6a-iRFP:Kan NTS1::pGPD-GFP-URA3 sir2::HIS, pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1 NYAQ->HPKY
NH1527	BY4741 (Meng), Nhp6a-iRFP:Kan NTS1::pGPD-GFP-URA3 sir2::HIS, TetO7pLeu2_HTB1 E80K_mRuby2
NH1399	BY4741 (Meng), Nhp6a-iRFP:Kan, HML-pTDH3-GFP
NH1528	BY4741 (Meng), Nhp6a-iRFP:Kan, HML-pTDH3-GFP, pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1
NH1529	BY4741 (Meng), Nhp6a-iRFP:Kan, HML-pTDH3-GFP, pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1 NYAQ->HPKY
NH1530	BY4741 (Meng), Nhp6a-iRFP:Kan, HML-pTDH3-GFP, pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1
NH1531	BY4741 (Meng), Nhp6a-iRFP:Kan, HML-pTDH3-GFP, pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1 E80K_mRuby2
NH1196	BY4741 (Meng), Nhp6a-iRFP:Kan TEL12R::pTDH3-GFP;
NH1532	BY4741 (Meng), Nhp6a-iRFP:Kan TEL12R::pTDH3-GFP; pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1
NH1533	BY4741 (Meng), Nhp6a-iRFP:Kan TEL12R::pTDH3-GFP; pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1 NYAQ->HPKY
NH1534	BY4741 (Meng), Nhp6a-iRFP:Kan TEL12R::pTDH3-GFP; pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1
NH1535	BY4741 (Meng), Nhp6a-iRFP:Kan TEL12R::pTDH3-GFP; pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1, E80K_mRuby2
NH1561	BY4741 (Meng), Nhp6a-iRFP:Kan RDN1_NTS1::pGPD_GFP-URA3, pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1 E80K_mRuby2, Set1ΔHis
NH1566	BY4741 (Meng), Nhp6a-iRFP:Kan RDN1_NTS1::pGPD_GFP-URA3, pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1 NYAQ->HPKY, Set1ΔHis
NH1567	BY4741 (Meng), Nhp6a-iRFP:Kan RDN1_NTS1::pGPD_GFP-URA3, Set1ΔHis

Table 2. Plasmids constructed in this study.

PLASMID NAME	DESCRIPTION
NHB1105	pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1
NHB1106	pRPL18B_rtTA3_tADH1, TetO7pLeu2_mRuby2_HTA1 NYAQ->HPKY
NHB1107	pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1
NHB1108	pRPL18B_rtTA3_tADH1, TetO7pLeu2_HTB1 E80K_mRuby2

References

Amatori, S., Tavolaro, S., Gambardella, S., Fanelli, M., 2021. The dark side of histones: genomic organization and role of oncohistones in cancer. Clin. Epigenetics 13. https://doi.org/10.1186/s13148-021-01057-x

Arimura, Y., Ikura, M., Fujita, R., Noda, M., Kobayashi, W., Horikoshi, N., Sun, J., Shi, L., Kusakabe, M., Harata, M., Ohkawa, Y., Tashiro, S., Kimura, H., Ikura, T., Kurumizaka, H., 2018. Cancer-associated mutations of histones H2B, H3.1 and H2A.Z.1 affect the structure and stability of the nucleosome. Nucleic Acids Res. 46, 10007–10018. https://doi.org/10.1093/nar/gky661

Bennett, R.L., Bele, A., Small, E.C., Will, C.M., Nabet, B., Oyer, J.A., Huang, X., Ghosh, R.P., Grzybowski, A.T., Yu, T., Zhang, Q., Riva, A., Lele, T.P., Schatz, G.C., Kelleher, N.L., Ruthenburg, A.J., Liphardt, J., Licht, J.D., 2019. A Mutation in Histone H2B Represents A New Class Of Oncogenic Driver. Cancer Discov. 9, 1438–1451. https://doi.org/10.1158/2159-8290.CD-19-0393

Blander, G., Guarente, L., 2004. The Sir2 Family of Protein Deacetylases. Annu. Rev. Biochem. 73, 417–435. https://doi.org/10.1146/annurev.biochem.73.011303.073651

Brachmann, C.B., Sherman, J.M., Devine, S.E., Cameron, E.E., Pillus, L., Boeke, J.D., 1995. The SIR2 gene family, conserved from bacteria to humans, functions in silencing, cell cycle progression, and chromosome stability. Genes Dev. 9, 2888–2902. https://doi.org/10.1101/gad.9.23.2888

Briggs, S.D., Bryk, M., Strahl, B.D., Cheung, W.L., Davie, J.K., Dent, S.Y.R., Winston, F., Allis, C.D., 2001. 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. https://doi.org/10.1101/gad.940201

Bryk, M., Briggs, S.D., Strahl, B.D., Curcio, M.J., Allis, C.D., Winston, F., 2002. Evidence that Set1, a Factor Required for Methylation of Histone H3, Regulates rDNA Silencing in S. cerevisiae by a Sir2-Independent Mechanism. Curr. Biol. 12, 165–170. https://doi.org/10.1016/S0960-9822(01)00652-2

Chadwick, B.P., Valley, C.M., Willard, H.F., 2001. Histone variant macroH2A contains two distinct macrochromatin domains capable of directing macroH2A to the inactive X chromosome. Nucleic Acids Res. 29, 2699–2705. https://doi.org/10.1093/nar/29.13.2699

Chadwick, B.P., Willard, H.F., 2002. Cell cycle–dependent localization of macroH2A in chromatin of the inactive X chromosome. J. Cell Biol. 157, 1113–1123. https://doi.org/10.1083/jcb.200112074

Chakravarthy, S., Patel, A., Bowman, G.D., 2012. The basic linker of macroH2A stabilizes DNA at the entry/exit site of the nucleosome. Nucleic Acids Res. 40, 8285–8295. https://doi.org/10.1093/nar/gks645

Costanzi, C., Pehrson, J.R., 1998. Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals. Nature 393, 599–601. https://doi.org/10.1038/31275

Gartenberg, M.R., Smith, J.S., 2016. The Nuts and Bolts of Transcriptionally Silent Chromatin in Saccharomyces cerevisiae. Genetics 203, 1563–1599. https://doi.org/10.1534/genetics.112.145243

Gietz, R.D., Schiestl, R.H., 2007. Quick and easy yeast transformation using the LiAc/SS carrier DNA/PEG method. Nat. Protoc. 2, 35–37. https://doi.org/10.1038/nprot.2007.14

Grunstein, M., Gasser, S.M., 2013. Epigenetics in Saccharomyces cerevisiae. Cold Spring Harb. Perspect. Biol. 5. https://doi.org/10.1101/cshperspect.a017491

Kornberg, R.D., 1977. Structure of Chromatin. Annu. Rev. Biochem. 46, 931–954. https://doi.org/10.1146/annurev.bi.46.070177.004435

Kozlowski, M., Corujo, D., Hothorn, M., Guberovic, I., Mandemaker, I.K., Blessing, C., Sporn, J., Gutierrez-Triana, A., Smith, R., Portmann, T., Treier, M., Scheffzek, K., Huet, S., Timinszky, G., Buschbeck, M., Ladurner, A.G., 2018. MacroH2A histone variants limit chromatin plasticity through two distinct mechanisms. EMBO Rep. 19, e44445. https://doi.org/10.15252/embr.201744445

Li, Y., Jin, M., O'Laughlin, R., Bittihn, P., Tsimring, L.S., Pillus, L., Hasty, J., Hao, N., 2017. Multigenerational silencing dynamics control cell aging. Proc. Natl. Acad. Sci. 114, 11253–11258. https://doi.org/10.1073/pnas.1703379114

Libuda, D.E., Winston, F., 2006. Amplification of histone genes by circular chromosome formation in Saccharomyces cerevisiae. Nature 443, 1003–1007. https://doi.org/10.1038/nature05205

Linskens, M.H., Huberman, J.A., 1988. Organization of replication of ribosomal DNA in Saccharomyces cerevisiae. Mol. Cell. Biol. 8, 4927–4935. https://doi.org/10.1128/MCB.8.11.4927

Malik, H.S., Henikoff, S., 2003. Phylogenomics of the nucleosome. Nat. Struct. Mol. Biol. 10, 882–891. https://doi.org/10.1038/nsb996

McGhee, J.D., Felsenfeld, G., 1980. Nucleosome Structure. Annu. Rev. Biochem. 49, 1115–1156. https://doi.org/10.1146/annurev.bi.49.070180.005343

Moazed, D., Kistler, A., Axelrod, A., Rine, J., Johnson, A.D., 1997. Silent information regulator protein complexes in Saccharomyces cerevisiae: A SIR2/SIR4 complex and evidence for a

regulatory domain in SIR4 that inhibits its interaction with SIR3. Proc. Natl. Acad. Sci. 94, 2186–2191. https://doi.org/10.1073/pnas.94.6.2186

Mohammad, F., Helin, K., 2017. Oncohistones: drivers of pediatric cancers. Genes Dev. 31, 2313–2324. https://doi.org/10.1101/gad.309013.117

Nacev, B.A., Feng, L., Bagert, J.D., Lemiesz, A.E., Gao, J., Soshnev, A., Kundra, R., Schultz, N., Muir, T.W., Allis, C.D., 2019. The expanding landscape of 'oncohistone' mutations in human cancers. Nature 567, 473–478. https://doi.org/10.1038/s41586-019-1038-1

Nislow, C., Ray, E., Pillus, L., 1997. SET1, A Yeast Member of the Trithorax Family, Functions in Transcriptional Silencing and Diverse Cellular Processes. Mol. Biol. Cell 8, 2421–2436.

Nusinow, D.A., Sharp, J.A., Morris, A., Salas, S., Plath, K., Panning, B., 2007. The histone domain of macroH2A1 contains several dispersed elements that are each sufficient to direct enrichment on the inactive X chromosome. J. Mol. Biol. 371, 11–18. https://doi.org/10.1016/j.jmb.2007.05.063

O'Kane, C.J., Hyland, E.M., 2019. Yeast epigenetics: the inheritance of histone modification states. Biosci. Rep. 39. https://doi.org/10.1042/BSR20182006

Pehrson, J.R., Fried, V.A., 1992. MacroH2A, a Core Histone Containing a Large Nonhistone Region. Science 257, 1398–1400.

Qiu, L., Hu, X., Jing, Q., Zeng, X., Chan, K.-M., Han, J., 2018. Mechanism of cancer: Oncohistones in action. J. Genet. Genomics 45, 227–236. https://doi.org/10.1016/j.jgg.2018.04.004

Shou, W., Seol, J.H., Shevchenko, Anna, Baskerville, C., Moazed, D., Chen, Z.W.S., Jang, J., Shevchenko, Andrej, Charbonneau, H., Deshaies, R.J., 1999. Exit from Mitosis Is Triggered by Tem1-Dependent Release of the Protein Phosphatase Cdc14 from Nucleolar RENT Complex. Cell 97, 233–244. https://doi.org/10.1016/S0092-8674(00)80733-3

Sinclair, D.A., Guarente, L., 1997. Extrachromosomal rDNA Circles— A Cause of Aging in Yeast. Cell 91, 1033–1042. https://doi.org/10.1016/S0092-8674(00)80493-6

Strahl-Bolsinger, S., Hecht, A., Luo, K., Grunstein, M., 1997. SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. Genes Dev. 11, 83–93. https://doi.org/10.1101/gad.11.1.83

Straight, A.F., Shou, W., Dowd, G.J., Turck, C.W., Deshaies, R.J., Johnson, A.D., Moazed, D., 1999. Net1, a Sir2-Associated Nucleolar Protein Required for rDNA Silencing and Nucleolar Integrity. Cell 97, 245–256. https://doi.org/10.1016/S0092-8674(00)80734-5

Talbert, P.B., Henikoff, S., 2017. Histone variants on the move: substrates for chromatin dynamics. Nat. Rev. Mol. Cell Biol. 18, 115–126. https://doi.org/10.1038/nrm.2016.148