DECIPHERING THE FUNCTION OF CHROMATIN MODIFIERS IN GENOME REGULATION AND MAINTENANCE IN *SACCHAROMYCES CEREVISIAE*

by

Nancy Lévesque

M. Sc., Université de Sherbrooke, 2007

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

in

THE FACULTY OF GRADUATE STUDIES

(Genetics)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

October 2012

Abstract

Chromatin is a dynamic structure that facilitates DNA compaction inside the cell nucleus and contributes nuclear process regulation such as transcription, DNA repair and DNA replication. Chromatin structure can be modified by several mechanisms, including the incorporation of histone variants, histone sliding or removal by ATP-dependent remodelling enzymes, addition of post-translational modifications, and addition of methylation marks on DNA. This dissertation aims to comprehend better the role of chromatin modifiers like histone acetyltransferases (HATs) in cellular processes such as genome regulation and maintenance in yeast *Saccharomyces cerevisiae*.

HATs play crucial roles in cells, as they are involved in transcription regulation and DNA damage response. This dissertation investigates the precise nature of the relationship between the histone acetyltransferase complexes NuA4 and picNuA4. I discovered that the smaller NuA4 counterpart picNuA4 is partially able to replace the function of the larger NuA4 when the latter is defective. Additionally, dissection of the NuA4 complex scaffolding subunit Eaf1 revealed that its C-terminus is largely required for NuA4-dependent function.

While NuA4 shares subunits with SWR1-C, an ATP-dependent chromatin-remodelling complex that replaces histone H2A for the histone variant H2A.Z, the role of shared modules in chromatin remodelling complexes remains unclear. Here I explored the role of shared modules through investigation of Swc4 and Yaf9, two members of the NuA4 and SWR1-C. Biochemical, genetic, and gene expression assays demonstrated that both Yaf9 and Swc4 similarly contributed to the shared module functions and generally behaved more like SWR1-

C, but also had distinct roles. For instance, large-scale genetic interaction profiles exposed noticeable differences between Yaf9 and Swc4, where Yaf9 behaved more similarly to SWR1-C NuA4, Swc4 to NuA4, in some cases.

Upon DNA damage, several chromatin modifications occur, such as acetylation of histone H4 tails by NuA4 or methylation of H3K79 by Dot1. Herein I propose a model explaining the role of H3K79 trimethylation in the DNA damage response in the context where cells are deficient for components of the DNA damage response. I suggest that, directly or indirectly, H3K79 trimethylation inhibits the translesion synthesis pathway, an alternative repair pathway.

Preface

In this dissertation, Chapter 2 is based on a manuscript submitted to Journal of Biological Chemistry (JBC). I conducted all of the experiments described in this chapter except for the E-MAP screen that was performed by Maria Aristizabal in collaboration with N. Krogan laboratory (UCSF) and mRNA expression profile array preformed by members of F. Holstege laboratory (U of Utrecht). However, I constructed the strains for these assays and performed all the data analysis. For this manuscript in preparation, I performed all data analysis, and created all the figures.

In Chapter 3, I conducted all of the experiments except for the E-MAP screen and mRNA expression profile array that have been performed by members of N. Krogan laboratory (UCSF) and F. Holstege laboratory (U of Utrecht), respectively. I performed data analysis and created all the figures.

A version of Chapter 4 was published in JBC in November 2010. This project was initiated by Alexandra Fok and became a major focus during my PhD. Alexandra Fok contributed to Figures 4.1, 4.3, 4.8. As equal first author, I was involved in developing research questions, research design, data analysis, and manuscript writing. I contributed to the experiments for Figures 4.1, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9. Finally, Grace Leung, joined the project and contributed to Figures 4.1, 4.2, 4.4, 4.6, 4.7.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Arp	Actin related protein
ATM	Ataxia telangiectasia mutated
ATP	Adenosine triphosphate
ATR	ATM related
CDK	Cell cycle cyclin-dependent kinase
ChIP	Chromatin immunoprécipitation
DDC	DNA-damage checkpoint,
DNA	Deoxyribose nucleic acid
E-MAP	Epistatic miniarray profiling
FRET	Fluorescence Resonance Energy Transfer
HA	Human influenza hemagglutinin
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
GNAT	Gcn5 N acetyltransferase related
HBO1	Histone acetyltransferase Bound to ORC
HSA	Helicase SANT associated
HU	Hydroxyurea
INO80	Inositol 80
ISWI	Imitation SWI/SNF
MLL	Mixed-lineage leukemia

MMS	Methyl methane sulfonate
MOF	Male on the first
MOZ	Monocytic leukemia zinc finger
MORF	Monocytic leukemia zinc finger protein-related factor
MYST	MOZ, Ybf2/Sas3, Sas2 and TIP60
N-Cor	Nuclear receptor corepressor
NuA4	Nucleosome acetyltransferase of H4
ORF	Open reading frame
PCAF	p300/CBP associated factor
PicNuA4	Piccolo NuA4
PCR	polymerase chain reaction
RES	pre-mRNA retention and splicing complex
RNA	Ribonucleic acid
RSC	Remodel the structure of chromatin
SAGA	Spt, Ada, Gcn5 et acetyltransferase
SANT	Swi3, Ada2, N-Cor et TFIIIB
SRCAP	Snf2-related CBP activator protein
SWI	Mating type switching
SWR1-C	Swi2/Snf2-related ATPase 1 - complex
TAP	Tandem affinity purification
TEV	Tobacco etch virus
TIP60	Tat interacting protein (60kDa)
Ub	Ubiquitination

Acknowledgements

I would like to thank first my supervisor Dr. Michael S. Kobor. I am grateful for the experience I acquired in his laboratory. During my Ph.D., I certainly valued his ideas for my different projects, support, excitement for science, and 'aggressivity' for 'pushing' science projects forward. His attention to details and the quality of the science ongoing in his lab surely contribute to student's development aspiring to become better researchers.

I would also like to thank my supervisory committee members: Michel Roberge, Peter Lansdorp, and LeAnn Howe. I truly appreciated their helpful comments, support, and input for my projects. Also thanks to Nevan Krogan, Frank Holstege, and Jacques Côté for collaboration, comments on manuscripts, and scientific discussions. This Ph.D. would not have possible as well without the help and support from Hugh Brock.

A special thanks to the incredible Kobor lab members. This amazing journey will not have been the same without their help, support, scientific discussions, and entertainment! They are responsible for several good memories created during this Ph.D. A particular thanks to Tanya Erb who has been a remarkable help. Thanks to the CMMT staff and students for the scientific discussions, interactions, and fun times.

I would also thank my family members and friends from all over the country for their love and support. They encouraged me during the hard times and gave me the motivation to persevere. Finally, I would like to thank Jean-François Schmouth who has been truly amazing. He has been an incredible support, help, and source of motivation. Without him, this Ph.D. would not have been possible. JF thanks for being such a good partner in crime, for following me in my crazy plans, and for your love.

"Genius is one per cent inspiration, ninety-nine per cent perspiration."
-Thomas A. Edison

Chapter 1

Introduction

DNA contains the genetic instructions for the development and function of all living organisms. In eukaryotes, genomic DNA is tightly packed and ordered in the nucleus. This highly organised structure includes the wrapping of nucleic acids around specific proteins resulting in the compaction of eukaryotic DNA into a nucleus that is only a few microns in diameter. This extremely condensed nucleoprotein complex called chromatin is not just a static template important for DNA compaction, but is an exceptional structure that contributes to regulation of numerous cellular processes by allowing DNA access to regulatory factors (Ehrenhofer-Murray, 2004; Rando et al., 2009). Initial investigations into chromatin focused on the structural composition of chromatin, but eventually studies revealed a more comprehensive picture of the biology of chromatin and its distinct roles in processes including transcription, DNA replication, repair, and recombination (Collins et al., 2007). The diverse arrangements of the chromatin structure can either fluctuate or become stable and even heritable, which is a hallmark of epigenetics. In biology, epigenetics (Greek: epi-επί: over, above, outer - and genetics) is defined as the study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than modifications in the DNA sequence.

1.1 Introduction to chromatin

The word chromatin was first used by W. Flemming more than a century ago (Henikoff and Grosveld 2008). Using staining techniques, he visualized coloured fibers in the nucleus that he named chromatin (Greek: *chroma*: color). Characterization of chromatin components, such as DNA and histones, was performed by F. Miescher, A. Kossel, and E. Hoppe-Seyle using a biochemical approach (Kossel, 1911; Kossel, 1928; Miescher, 1871). In 1974, progress in electron microscopy allowed the visualization for the first time of the chromatin fibers, revealing the appearance of "beads on a string" (Olins et al., 1974). The existence of the nucleosome, the basic unit of chromatin, was proposed (Oudet et al., 1975) and eventually its structure was solved by x-ray crystallography (Luger et al., 1997). Discovery of the nucleosome structure completely transformed the chromatin field. DNA was not coated with histones as previously thought but wrapped around the globular histone core, rendering DNA still accessible to other binding proteins involved in cellular processes such as transcription, replication, and repair (Oudet et al., 1975). Since then, the nucleosome has been considered the central unit responsible for changes happening in chromatin function.

After the discovery of the nucleosome, further investigations on chromatin structure and function demonstrated that chromatin is not only a static scaffold necessary for compaction of DNA. Chromatin is also a very dynamic template where interactions with other proteins can take place and where the addition and removal of chemical modifications allow structural regulation of this central entity (Ehrenhofer-Murray, 2004; Kouzarides, 2007; Rando, 2007).

1.2 Chromatin structure

Mammalian genomes are organised in chromosomes consisting of packed DNA that exhibit a high degree of plasticity. Chromatin organization is divided in three different levels: 1) compaction of DNA coiled around histones, forming the nucleosome; 2) the higher-order level of nucleosome compaction; and 3) the spatial arrangement of chromosomes inside the nucleus (Misteli, 2007).

The first level of compaction consists of 146 base pairs of DNA wrapped around an octamer composed of two molecules of each canonical histones (H2A, H2B, H3, and H4) (Fig. 1.1) (Arents et al., 1991; Luger et al., 1997). The second level of compaction comprises the 10 nm structure called "beads on a string", leading to the 30 nm fibers, which are transcriptionally inactive (Allis et al., 2007). Packaging of 10 nm to 30 nm fibers is achieved with incorporation of the histone linker H1 during interphase (Allis et al., 2007). However, the 30 nm structure is very dynamic and can be decondensed to a transcriptionally active 10 nm structure (Allis et al., 2007). The loose chromatin conformation where the DNA can still be accessible by enzymes is called euchromatin,

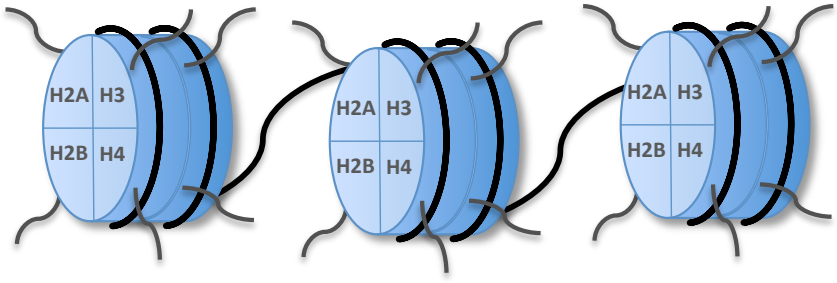


Figure 1.1 Structure of the nucleosome. Nucleosomes are composed of 146 base pairs of DNA wrapped around two molecules of each canonical histones H2A, H2B, H3, and H4.

while heterochromatin refers to the highly condensed structure that prevents DNA from being accessed. Higher compaction of the 30 nm structure leads to 60 nm, then 130 nm, and finally reaching the highest order of compaction of 1400 nm, resulting in the mitotic chromosome structure as we know it (Misteli, 2007). Another way that chromatin is regulated is by formation of chromatin loops that contribute to a higher level of compaction and also to gene expression regulation through interaction of DNA regions separated by large distances. Chromatin loops diverge widely, ranging from kilobases to giant loops of hundreds of kilobases (Fraser, 2006; van Driel et al., 2003). Shorter loops bring together the upstream regulatory region and the promoter-proximal element creating a highly active transcriptional environment, while larger loops might contribute to gene placement at distinct nuclear positions (Ansari et al., 2005; M. Martin et al., 2005; O'Sullivan et al., 2004).

The last level of chromatin organization is the arrangement of the genome in the cell nucleus. This type of organization is obtained with the formation of chromatin loops in combination with the stochastic self-organization of nuclear processes. For instance, DNA replication and repair occur at nuclear sites where the proper machinery is recruited. Upon DNA damage, key factors are recruited to specific sites forming repair foci, which can operate on several types of DNA damage (Essers et al., 2006; Lisby et al., 2003). For various periods of time, these repair centers can remain associated depending on their function, and finally disperse after completing the repair (Houtsmuller et al., 1999; Politi et al., 2005). In order to achieve the repair of damaged sites, the chromatin structure needs to be altered by numerous specialized factors and chromatin

modifications, such as phosphorylation of histone H2A.X and histone H4 acetylation (van Attikum et al., 2009).

1.3 Modifications of chromatin

As previously mentioned, chromatin is not only required for compaction of DNA in order to fit into the nucleus, but is also a highly dynamic template varying with changes in the environment. Alteration of the chromatin structure is crucial for regulation of several cellular processes such as DNA replication, transcription, and repair (Misteli, 2007). Chromatin can be altered by several mechanisms, such as ATP-dependent chromatin-remodelling complexes, which can slide or eject nucleosomes (Eberharter et al., 2004; Hargreaves et al., 2011; Lusser et al., 2003). Moreover, replacement of canonical histones with histone variants creates distinct chromatin neighbourhood (Sarma et al., 2005; Zlatanova et al., 2008). The H3 and H2A family variants, including H3.3, CenH3, H2A.Z, H2A.X, H2A-Bbd, and macroH2A are largely conserved through evolution, suggesting that histone variants are functionally very important in chromatin biology (Talbert et al., 2010; Zlatanova et al., 2008). It is now established that most histone variants are expressed throughout the cell cycle, unlike the canonical histones that are only synthesised during S phase. This mechanism allows the cells to favour the incorporation of histone variants into chromatin in a DNA-replication-independent manner (Jin et al., 2005). Finally, various post-translational modifications can be added on histones.

Research throughout the past decade has revealed that many different PTMs can be added on histone amino acids, including acetylation, methylation, phosphorylation, ADP-ribosylation, ubiquitilation, sumoylation, and biotinylation (Bannister et al., 2011). The x-ray structure resolution of the nucleosome indicates that the highly basic histone amino (N)-terminal tails project out of the nucleosome, rendering contacts with other nucleosomes possible and facilitating the access of tails for addition of post-translational modifications (PTMs) (Luger et al., 1997). The first post-translational histone modification reported was acetylation of the lysine residue (Allfrey et al., 1964). Acetylation of histone lysines is a transient modification that can be either added or removed by two different types of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs) respectively. Using acetyl CoA as a cofactor, HATs transfer an acetyl group to the lysine ε-amino group. This modification leads to neutralization of the positive charge of lysines, thereby weakening the interaction between histones and the negatively charged DNA (Bannister et al., 2011; Grunstein, 1997). In Chapter 2 of this dissertation, I present work that contributes to the understanding of histone acetylation by a specific HAT complex in the yeast *Saccharomyces cerevisiae*.

Various PTMs are involved in regulation of several cellular processes. For instance, histone acetylation is largely associated with transcription regulation and DNA damage response (Yang et al., 2007). Acetylation of H4K5, H4K8, H4K12, H3K9, H3K14, and H3K18 are a few examples of acetylated residues that are potentially involved with active transcription (Kouzarides, 2007). Except for H3K9, these acetylated residues are enriched

at enhancer elements and promoters of genes, enabling DNA access to transcription factors (Wang et al., 2008). Marks other than acetylation are also linked to transcriptional activation, such as H3K4 and H3K36 methylation. While H3K4me3 is enriched at transcription start sites, H3K36me3 spreads throughout coding regions (Bannister et al., 2005; Barski et al., 2007). Additionally, H3K9 methylation is linked to repressive chromatin, as it is located at heterochromatin such as subtelomeric regions (Trojer et al., 2007; Krishnan et al., 2011). In yeast, heterochromatin-euchromatin boundaries are maintained by histone modifications such as acetylation of the histone variant H2A.Z by NuA4 and methylation of H3K79 by Dot1 (Altaf et al., 2007; Babiarz et al., 2006; Katan-Khaykovich et al., 2005; Norris et al., 2010). Comparable to transcription, the DNA damage response is regulated by addition of specific marks on histones. For instance, H2A.X phosphorylation, H2B ubiquitination, and H3K79 methylation play a role in sensing and transmitting the DNA damage signal, while H4 acetylation enables the "opening" of chromatin by facilitating the access of the repair machinery to DNA (Nguyen et al., 2011; van Attikum et al., 2009).

Generating another layer of complexity, several different types of PTMs can occur on the same nucleosome (Lee et al., 2010), and crosstalk can occur between these different histone PTMs (Lee et al., 2010). This cross-regulation of histone modifications is a nuanced language that is considered as a "code" which contributes to the regulation of cellular processes, such as transcription or DNA repair, through the chromatin signalling pathway (Lee et al., 2010; van Attikum et al., 2009). Interestingly, crosstalk can also

occur with non-histone proteins, thus contributing to an additional layer of cellular regulation (Latham et al., 2007).

PTMs on chromatin do not only affect its structure by modifying the global charges, but they also recruit specific domain-containing factors that are able to recognize those modifications. These factors can be subunits of protein complexes having enzymatic activity that slide or exchange nucleosomes on the DNA template (Clapier et al., 2009; Eberharter et al., 2004; Lusser et al., 2003). These chromatin remodelers are required to allow accessibility to DNA for critical processes such as DNA replication, where nucleosomes need to be properly deposited and spaced genome-wide, and DNA repair, where nucleosomes must be moved or ejected, providing prompt access for repair factors. Transcription is another critical process requiring the action of chromatin remodelers, allowing the ejection of nucleosomes for the passage of RNA polymerase through the DNA template. Furthermore, chromatin remodelers are also involved in replication-independent assembly of chromatin by inserting nucleosomes in regions where they have been removed (Clapier et al., 2009). Finally, chromatin remodelers are responsible for incorporating histone variants into nucleosomes, whose primary amino acid sequence resembles canonical histones but are much less abundant (Talbert et al., 2010).

1.4 SWR1-C and histone variant H2A.Z

In the yeast *Saccharomyces cerevisiae*, H2A.Z is deposited into chromatin by the SWR1 complex (SWR1-C), an ATP-dependent remodeling complex found to specifically exchange H2A for H2A.Z in yeast (Kobor et al., 2004a; Krogan et al., 2004; Mizuguchi

et al., 2004). SWR1-C acquired its name from its catalytic subunit Swr1, which contains an ATPase/helicase domain, making it part of the Swi2/Snf2 chromatin-remodeling family. In yeast, the histone variant H2A.Z is acetylated by the HATs NuA4 and SAGA (Babiarz et al., 2006; Keogh, Mennella et al., 2006; Millar et al., 2006). Additionally, SWR1-C and NuA4 share four subunits in yeast while in higher organisms such as mammals, they are found as one large complex called TIP60 (Doyon et al., 2004; PLu et al., 2009). Although the main function of TIP60 is to acetylate histones, in vitro studies demonstrate that it is also able to exchange H2A with H2A.Z (Cai et al., 2003; Doyon et al., 2004; Gevry et al., 2007; Kusch et al., 2004; Martinato et al., 2008; Svotelis et al., 2009). There is another functional and structural counterpart of SWR1-C in mammals: the SRCAP (SNF2-related CREB-binding protein activator protein) complex (Table 1.1) (Cai et al., 2005; Eissenberg et al., 2005; Ruhl et al., 2006). Interestingly, these two complexes responsible for H2A.Z deposition into chromatin appear to regulate distinct processes. In fact, while p400, member of the TIP60 complex, is found at inactive promoters, the SRCAP subunit (SRCAP complex) is associated with active promoters (Gevry et al., 2007; Martinato et al., 2008; Wong et al., 2007). The existence of two complexes might have arisen through evolution in order to adapt to the more complex genomes of higher eukaryotes (Doyon et al., 2004; P. Y. Lu et al., 2009).

Table 1.1 Homologous subunits of the yeast NuA4 and SWR1-C in human (Adapted from (P. Y. Lu et al., 2009)) * Potential p400 homologues.

	S. cerevisiae	Human TIP60	Human SRCAP
SWR1-C	Swr1*	p400	SRCAP
	Bdf1	Brd8	
	Swc2	YL1	YL1
	Swc3		
	Swc5		
	Swc6		ZnF-HIT1
	Swc7		
	Arp6		Arp6
	Rvb1	TIP49a	TIP49a
	Rvb2	TIP49b	TIP49b
	Htz1	H2A.Z	H2A.Z
	Yaf9	GAS41	GAS41
	Swc4	DMAP1	DMAP1
	Arp4	BAF53a	BAF53a
	Act1	Actin	Actin
NuA4	Esa1	Tip60	
	Yng2	ING3	
	Epl1	EPC1	
	Tra1	TRRAP	
	Eaf1*	p400	
	Eaf3	MRG15	
	Eaf5		
	Eaf6	hEaf6	
	Eaf7	MRGBP	

Moreover, the histone variant H2A.Z is very conserved through evolution and is found to be essential in organisms as diverse as *Tetrahymena thermophila* (X. Liu et al., 1996), *Drosophila melanogaster* (Clarkson et al., 1999; van Daal et al., 1992), *Xenopus leavis* (Iouzalen et al., 1996; Ridgway et al., 2004), *Mus musculus* (Faast et al., 2001), and *Homo sapiens*, with sequence conservation of ~90% (Hatch et al., 1990). However, H2A.Z shares only ~60% identity with the canonical H2A, suggesting that the histone variant might contribute to an important and distinct function in the cell (Jackson et al., 2000; Thatcher et al., 1994). In yeast, genome-wide mapping of H2A.Z in yeast revealed

enrichment at promoters, 5'ends of genes at euchromatic regions, and subtelomeric regions, and is implicated in various biological processes, such as gene regulation, genome stability, DNA repair, cell cycle progression, and heterochromatin-euchromatin boundary establishment (Guillemette et al., 2005a; Kobor et al., 2004a; Krogan et al., 2004; Li, et al., 2005a; Meneghini et al., 2003; Raisner et al., 2005; Santisteban et al., 2000; Zanton et al., 2004; Zhang et al., 2004; Zhang et al., 2005b; Albert et al., 2007). Additionally, acetylation of H2A.Z by NuA4 or SAGA is required to block spreading of heterochromatin at subtelomeric regions (Babiarz et al., 2006).

The role of H2A.Z in gene regulation has been extensively studied over the past decades. Work in *Tetrahymena* provided the first evidence for a role of H2A.Z in transcription as the histone variant was only observed in the transcriptionally active macronucleus and absent from the inactive micronucleus (Allis et al., 1980). This role of H2A.Z in transcription regulation was further supported by several studies in yeast (Santisteban et al., 2000; Adam et al., 2001; Larochelle and Gaudreau, 2003; Farris et al., 2005). Other studies suggested that H2A.Z might be involved in negative transcription regulation as well. For instance, two different groups showed that H2A.Z was associated with lowly transcribed genes as well as genes induced under specific conditions (Guillemette et al., 2005; Li et al., 2005). Likewise, studies demonstrated that H2A.Z deletion leads to gene activation but also repression (Meneghini et al., 2003). Furthermore, a high-resolution nucleosome mapping assay revealed that removal of H2A.Z leads to nucleosome displacement at the *GAL1* promoter, suggesting a role in nucleosome positioning at inactive genes to poise them for transcriptional activation (Guillemette et al. 2005).

Although biochemical and physical properties of nucleosomes containing H2A.Z diverge from the ones including H2A, their structures remain similar (Suto et al., 2000; Dryhurst et al., 2004). However, controversial observations raised a debate on the stability of the H2A.Z-containing nucleosomes. While studies demonstrated reduced stability of nucleosomes containing H2A.Z in high ionic-strength buffer (Abbott et al. 2001), analysis of the salt-dependent stability of nucleosome core particles by Fluorescence Resonance Energy Transfer (FRET) revealed an increased stabilization of histone octamers in presence of H2A.Z compared to H2A (Park et al., 2004). These inconsistent results could be explained by disparities in the techniques used. For instance, several studies took advantage of *in vitro* recombinant histones to create nucleosomes, which were demonstrated to have dissimilar properties compared to native nucleosomes (Park et al. 2004; Ishibashi et al., 2009; Tolstorukov et al., 2009).

1.5 HATs and histone acetylation

The first PTMs were discovered almost 50 years ago by Allfrey and colleagues, who revealed the presence of acetyl and methyl groups on histones (Allfrey et al., 1964). The first protein with HAT activity was extracted from the larvae of brine shrimp (Cano et al., 1979). HAT enzymes facilitate the transfer of an acetyl group onto the lysine ε-amino group using acetyl CoA. In *Saccharomyces cerevisiae*, the first isolated HATs were Hat1a and Gcn5 (Brownell et al., 1996; Kleff et al., 1995), followed shortly after by the isolation of the first multisubunit complex, the SAGA complex (Spt-Ada-Gcn5-acetyltranferase) (Grant et al., 1997a). Increasing studies on HATs over the last decade

contributed to molecular and biochemical characterization of these enzymes. Isolation of novel HATs in numerous organisms revealed that these enzymes are highly conserved through evolution (Carrozza et al., 2003).

As mentioned earlier, addition of acetylation marks on histones is an actively reversible process. Not only is the addition of acetyl residues on histones important for regulation of cellular processes, but the balanced level of histone acetylation controlled by the coordination of different HATs and HDACs is also essential (Ekwall, 2005). HATs are commonly found as multisubunit complexes and their subunit composition confers on the complex its function and ability to bind to their substrates (Kimura et al., 2005; Utley et al., 2003). For instance, various protein domains within these subunits are able to recognize and bind specific histone modifications, leading to recruitment of the whole complex and consequent histone acetylation. Further characterization of HAT complexes led to their categorization into five different families based on their catalytic domain: the GNAT (Gcn5-related N-acetyltransferase) family including Gcn5/PCAF, the MYST family (MOZ, Ybf2/Sas3, Sas2 and Tip60), the TAFII250 family, the CBP/p300 family, the SRC family, and the HAT1 family (Yang, 2004) (Table 1.2).

One of the best characterized families is the MYST family that includes the yeast NuA4 HAT complex (Pokholok et al., 2005; Wang et al., 2008). Even though histone acetylation is generally linked to active transcription based on the presence of acetylation at the promoter and 5' end of transcriptionally active genes, enzymes from the MYST family can also be associated with gene silencing and dosage compensation (Carrozza et

al., 2003; Utley et al., 2003). However, HATs are not only involved in transcription regulation, but they play an important role in DNA repair, by "opening" the chromatin and also contributing to establishment of silencing boundary at subtelomeric regions through H4 and H2A.Z acetylation (Altaf et al., 2007; Babiarz et al., 2006; Downs et al., 2004a; Zhang et al., 2004).

Table 1.2 Histone acetyltransferases of the GNAT and MYST family and their substrates.

HAT complexes (GNAT & MYST family)	**Catalytic subunit**	**Histones modified**
Yeast		
SAGA	Gcn5	H2B, H2A.Z, H3, H4
SLIK		H2B, H3, H4
ADA		H3
HAT-A2		H3
HATB	Hat1	H2A, H4
Elongator	Elp3	H3
Hpa2	Hpa2	H3/H4
NuA4	Esa1	H2A, H2A.Z, H4
picNuA4		H2A, H2A.Z, H4
NuA3	Sas3	H3
SAS	Sas2	H4
Human		
TIP60	Tip60	H2A, H4
HBO1	HBO1	H3, H4
MOZ/MORF	MOZ/MORF	H3

1.5.1 NuA4 complex structure and function

The catalytic activity of the multisubunit NuA4 complex is performed by the essential component Esa1 (essential SAS2-related acetyltransferase) that was first identified based on its homology to Sas2 and Sas3 (Doyon et al., 2004). Esa1 is responsible for

acetylation of histones H4, H2A, and H2A.Z (Allard et al., 1999; Babiarz et al., 2006; Doyon et al., 2004; Keogh et al., 2006; Millar et al., 2006). Interestingly, more recent investigations suggest that some HATs, including Esa1, not only acetylate histones but also non-histone substrates (Babiarz et al., 2006; Doyon et al., 2004; Jeong et al., 2011; Keogh et al., 2006; Lin et al., 2008; Y. Y. Lin et al., 2009; Lu et al., 2011; Mitchell et al., 2011).

Like most HATs, the NuA4 complex is highly conserved from yeast to human with 12 of its 13 subunits conserved in human, making the budding yeast an attractive model to study the function of this HAT (Fig. 1.1A) (Cai et al., 2003; Doyon et al., 2004). On the basis of sequence homology, the human homologue of Esa1 is Tip60 (Tat interactive protein, 60kDa). Tip60 was identified as an interacting partner of the HIV1-tat protein (Kamine et al., 1996). Like Esa1, Tip60 acetylates histones H4, H2A and H2A.Z and is also found in a large multiprotein complex that participates in the regulation of transcription factors such as androgen receptors, Myc, NF-κB, E2F1, and p53 (Sapountzi

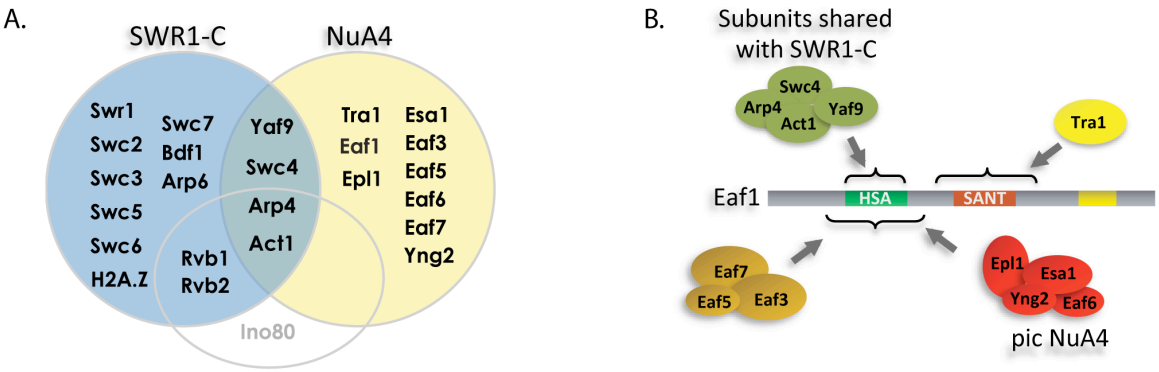


Figure 1.1 (A) Venn diagram of overlapping subunits of the NuA4, SWR1-C, and INO80 complexes (Adapted from (Kobor et al., 2004a)). (B) Scheme of NuA4 submodules associating to Eaf1 regions (Adapted from (Auger et al., 2008)).

et al., 2006). TIP60 is involved in many cellular processes such as regulation of the immune response, tumour-suppression (Gorrini et al., 2007) and amyloid-β precursors protein signalling (Sapountzi et al., 2006).

In yeast, NuA4 can be found in two different forms: the well-characterized large NuA4 complex containing 13 subunits and a smaller complex called piccolo NuA4 (picNuA4). In contrast to the NuA4 complex, which acetylates histones at specific loci, picNuA4, composed of the 4 subunits Esa1, Yng2, Eaf6, and Epl1, is involved in global acetylation (Auger et al., 2008; Boudreault et al., 2003). Deletion of the Epl1 C-terminus leads to formation of only picNuA4 (Boudreault et al., 2003). Nevertheless, picNuA4 is found in wild-type cells, which display an activity ratio of 2:1 between NuA4 and picNuA4 (Boudreault et al., 2003). However, the precise nature of picNuA4 is not clearly understood and in chapter 2 of this dissertation I investigate the potential roles of picNuA4 in *Saccharomyces cerevisiae*.

1.5.2 NuA4 submodules

NuA4 is composed of several submodules and Eaf1 is proposed to act as a scaffold for NuA4 assembly as its deletion leads to complex dissociation (Auger et al., 2008; Mitchell et al., 2008). However, previous data demonstrated that in the absence of Eaf1, other NuA4 submodules can still be recruited to chromatin (Ginsburg et al., 2009). Different Eaf1 regions are required for the interaction with distinct components of NuA4. For instance, the N-terminal region of Eaf1 is responsible for its association with picNuA4. In

cells containing an Epl1 C-terminal truncated mutant, Eaf1 is still able to bind the remaining submodules (Fig. 1.1B) (Auger et al., 2008; Chittuluru et al., 2011). The Eaf1 N-terminal region also binds the Eaf5/7/3 sub-module (Auger et al., 2008). Interestingly, the Eaf3 subunit is also part of the Rpd3S histone deacetylase (HDAC) complex (Auger et al., 2008).

Additionally, Eaf1 contains two protein domains that are important for its function. The SANT (SWI3-ADA2-N-CoR-TFIIIB) domain of Eaf1 binds Tra1 (Auger et al., 2008), a subunit involved in NuA4 recruitment to specific promoters through its interaction with transcription activators such as Gcn4, Hap4, and Gal4 (Brown et al., 2001). The HSA (helicase-SANT-associated) domain interacts with a module containing the four subunits Swc4, Arp4, Yaf9, and Act1, which are shared with SWR1-C, the H2A.Z deposition complex (Fig. 1.1A) (Allard et al., 1999; Auger et al., 2008; Szerlong et al., 2008).
These shared subunits are suggested to facilitate and regulate functions of NuA4 and SWR1-C on H2A.Z. It is proposed that H4 acetylation by NuA4 leads to the shared module recruiting and activating SWR1-C, which then replaces H2A with H2A.Z at promoter regions. Successively, NuA4 acetylates the newly deposited H2A.Z with the help of the shared module that is still anchored on chromatin (Altaf et al., 2010; Babiarz et al., 2006; Keogh et al., 2006; Wu et al., 2009; Zhou et al., 2010).

SWC4 is an essential gene encoding a shared subunit that also contains an N-terminal SANT (SWI3, ADA2, N-CoR, TFIIIB) domain, which is predicted to bind DNA and has been shown to interact with histones in other protein complexes (Aasland et al., 1996;

Boyer et al., 2004; Galarneau et al., 2000; Harata et al., 1999; Sterner et al., 2002; Sunada et al., 2005). The Swc4 C-terminus is capable of binding Yaf9, another shared subunit (Bittner et al., 2004). Moreover, Swc4 is required for proper DNA damage response, passage through G2/M, DNA replication, and chromosome segregation (Auger et al., 2008; Micialkiewicz et al., 2008). Yaf9, encoded by the only non-essential gene of the shared module *YAF9*, contains a YEATS domain, which is required for proper H2A.Z deposition and acetylation but whose exact molecular function is still unclear (Wang et al., 2009a). Yaf9 not only has a role in telomere silencing, cellular response to spindle stress, and chromosome segregation, but is also involved in acetylation of histone H4 at telomeres and H2A.Z deposition into chromatin (Le Masson et al., 2003; Zhang et al., 2004).

The Act1 and Arp4 subunits play a crucial role in the shared submodule, as they are responsible for the interaction between the shared module and both SWR1-C and NuA4 complexes through the HSA domains of Eaf1 in NuA4, and Swr1 in SWR1-C (Szerlong et al., 2008; Wu et al., 2009). *ACT1* is the gene encoding actin, which is an ATP-binding protein mainly located in the cytosol, where it plays a role in cell polarity, endocytosis, trafficking, and organelle organization (Pruyne et al., 2000). In the nucleus, actin contributes to transcription, mRNA processing, chromatin remodelling, and nuclear matrix association. However, actin's precise role in NuA4 and SWR1-C is still unclear (Bettinger et al., 2004; Bohnsack et al., 2006; Grummt, 2006; Miralles et al., 2006). Arp4, an actin-related protein, physically interacts with actin (Farrants, 2008; Szerlong et al., 2008). It is suggested that the ATP-binding pocket of Arp4 participates in the

regulation of picNuA4 formation (Sunada et al., 2005). Arp4 is involved in cell cycle regulation, DNA repair, replication, transcriptional regulation, and chromatin organization (Georgieva et al., 2008; Gorzer et al., 2003; Jiang et al., 1996; Ogiwara et al., 2007). Overall, the different chromatin-binding domains present in the shared subunits are proposed to facilitate the anchoring of SWR1-C and NuA4 to chromatin.

In yeast, other than NuA4 and SWR1-C, several chromatin-modifying complexes share subunits, such as SWI/SNF and RSC, SWR1-C and INO80. This proves that there is a substantial amount of cross-talk amongst chromatin remodelling complexes (Lu et al., 2009; Morrison et al., 2009; Vignali et al., 2000). However, the role of these shared modules is still not well known. For instance, are the shared subunits responsible for the same role in both complexes? Do they have their own specific function, or do they work as a single unit? In chapter 3 of this dissertation, I address the role of the shared submodule between NuA4 and SWR1-C in yeast *Saccharomyces cerevisiae*.

1.6 Role of chromatin in DNA damage

Other than transcription regulation, one of the most important tasks of the eukaryotic cell is to preserve its genomic integrity. In fact, genomic DNA is incessantly exposed to different sources of damage compromising the proper transmission of genetic information from one generation to the next. In order to counteract the accumulation of DNA lesions, the cell has developed a highly sophisticated and conserved DNA damage response, which in turn activates diversified and finely tuned repair pathways. Upon DNA repair, a multitude of factors are recruited to chromatin in order to fix the DNA lesion. However,

in order to facilitate DNA repair, the chromatin is required to be in an "open" state, which subsequently allows repair factors access to DNA. The "opening" of the chromatin structure is possible through the action of previously described ATP-dependent chromatin remodelling complexes that disrupt, evict or slide nucleosomes on DNA. The chromatin structure can also be altered by deposition of histone variants into the nucleosome, addition of PTMs, and also through the action of histone chaperones which help regulate nucleosome assembly/disassembly (van Attikum et al., 2009). Finally, the DNA damage response is composed of several steps including sensing the DNA lesion, accessing the DNA in order to repair the lesion, and reconstituting the initial state of chromatin.

1.6.1 DNA damage response and regulation

Upon DNA damage, cells first detect the lesions using sensors. Subsequently, a signal is propagated to activate the DNA damage checkpoint. One of the first chromatin modifications that occurs during the response is phosphorylation of the histone variant H2A.X (Redon et al., 2010). This modification happens a few minutes after the break and spreads for more than 50kb on either side of the double-strand breaks (DSBs) in yeast, or 1Mb in human cells (Downs et al., 2004a; Rogakou et al., 1998; Rogakou et al., 1999; Shroff et al., 2004b; Unal et al., 2004).

H2A.X is phosphorylated by the kinase proteins Mec1 and Tel1 in yeast (on serine 129), and by ATR (ATM and Rad3-related) and ATM (ataxia-telangiectasia mutated) in human (on serine 139) (Abraham, 2001; Shiloh, 2003; Shroff et al., 2004a). It has been suggested that changes in DNA conformation activate these kinases at the lesion site and

initiate the signalling cascade. Moreover, it is also proposed that activation of kinases is triggered by DNA damage detection through the MRN repair complex (Mre11, Rad50, Nbs1) (Lee et al., 2007). Interestingly, after DNA damage, H2A.X foci accumulate differently on distinct chromatin neighbourhoods. For instance, phosphorylated H2A.X tends to cluster more efficiently in euchromatin than heterochromatin. This can be due to fewer lesions in heterochromatin or to a more difficult spread of the chromatin modification on a compact chromatin structure (Cowell et al., 2007; Kim et al., 2007).

1.6.2 Role of chromatin modifiers during DNA damage response

H2A.X phosphorylation is one of the first and most significant chromatin modifications that occurs upon DNA damage. However, the modification itself does not cause chromatin reorganization but rather leads to recruitment of repair factors and chromatin remodelers at the lesion (Fink et al., 2007). Nevertheless, other data suggests that H2A.X phosphorylation might lead to chromatin relaxation (Downs et al., 2000), although it could also be an indirect effect of chromatin remodelers being recruited. Therefore, the complex regulation of the DNA damage response by signalling cascades is followed by numerous changes in chromatin structures that are crucial for regulating access to DNA during the repair process. Several chromatin remodelers, such as NuA4 and SWR1-C, are involved in altering the chromatin neighbourhood upon DNA damage.

1.6.2.1 Role of NuA4 complex in DNA damage

Interestingly, Arp4, which is shared between NuA4 and SWR1-C, recruits these two complexes to sites of DNA damage through interaction with phosphorylated H2A.X (Downs et al., 2004b; Morrison et al., 2004; Papamichos-Chronakis et al., 2006; van Attikum et al., 2007). The NuA4 complex located at DSBs is responsible for acetylating histone H4, which in turn recruits components of the DNA repair machinery and various chromatin-modifying complexes including SWR1-C (Bird et al., 2002; Downs et al., 2004b; Sapountzi et al., 2006; Tamburini et al., 2005).

Recruitment of SWR1-C leads to H2A.Z deposition into chromatin at DSBs shortly after DNA damage (Kalocsay et al., 2009). Subsequently, during DNA damage repair, both H2A.Z and H2A.X are removed from DSBs, which is consistent with general nucleosome eviction during DNA damage repair (van Attikum et al., 2007).

1.6.2.2 Role of Dot1 in DNA damage

Another example of a chromatin modifier playing a role in DNA damage response is the methylation of H3 K79 in its globular domain by the yeast methylase Dot1 (Feng et al., 2002; Lacoste et al., 2002; Ng, Xu et al., 2002; van Leeuwen et al., 2002). Dot1 has the capacity to catalyze mono-, di-, and trimethylation in a non-processive manner (Frederiks et al., 2008; Min et al., 2003). Dot1-dependent H3K79 methylation plays two distinct roles in the Rad9-mediated DNA damage response: activation of the G1 and S checkpoints that are regulated by the upstream Bre1-dependent H2B K123 ubiquitination, and repair of DNA damage in late G2 phase (Dover et al., 2002; Giannattasio et al., 2005; Grenon et al., 2007; Shilatifard, 2006; Wood et al., 2003; Wysocki et al., 2005). Dot1

also contributes to nucleotide excision repair, sister chromatid recombination, and repair of ionizing radiation damage (Chaudhuri et al., 2009; Conde et al., 2009; Game et al., 2005). Finally, upon failure of the base excision repair (BER) pathway to repair damages, cell cycle progression is completed by the translesion synthesis (TLS) pathway that uses error-prone polymerases to bypass DNA lesions. Dot1 negatively regulates TLS, thereby bypassing DNA replication blocks (Conde et al., 2008; Conde et al., 2010; Levesque et al., 2010). In this dissertation, I present evidence that loss of Dot1 activity leads to suppression of Rtt107-dependent DNA damage sensitivity through the TLS pathway. Rtt107 is one of the Mec1 targets and is required for reinitiating replication after DNA damage (Roberts et al., 2006; Rouse, 2004).

In Chapter 2 of this dissertation, I study the relationship between NuA4 and picNuA4. Investigations show that picNuA4 is partially able to replace the function of NuA4 when the latter is defective. Additionally, I study the structure and function of NuA4 through dissection of the scaffold subunit Eaf1. This work demonstrates that the Eaf1 C-terminal region is required for NuA4 function in acetylating histones.

In Chapter 3, I investigate the role of shared modules in chromatin remodelling complexes. More specifically, I study the function of Swc4 and Yaf9, two unique members of NuA4 and SWR1-C. This analysis reveals specific roles of the shared module in each complex for particular cellular processes, but also similarities and differences between the two shared subunits Swc4 and Yaf9. Additionally, I show that *swc4* and *yaf9* mutants exhibit the same defects in histone acetylation and histone variant

deposition into chromatin, therefore it is surprising to observe that a *swc4* mutant suppresses the DNA damage sensitivity and defect in maintenance of heterochromatin boundary of the *yaf9* mutant.

In Chapter 4, I investigate the role of a specific histone modification in DNA damage. I propose a model explaining the effect of H3K79 trimethylation during DNA damage in cells lacking components of the DNA damage response. I suggest that H3K79 trimethylation leads to inhibition of the TLS pathway. However, the precise mechanism by which Dot1-mediated H3K79 trimethylation impedes the TLS pathway still needs to be investigated.

Chapter 2

On the relationship between NuA4 and picNuA4 in histone acetylation, gene expression and cellular function[1]

2.1 Introduction

Chromatin structure facilitates DNA compaction inside the cell nucleus and contributes to the regulation of nuclear processes such as transcription, DNA repair and DNA replication (Ehrenhofer-Murray, 2004; Rando et al., 2009). In eukaryotes, the basic building block of chromatin is the nucleosome, which is composed of 146 bp of DNA wrapped around an octamer containing two molecules of each of the four histones H2A, H2B, H3 and H4 (Khorasanizadeh, 2004; Luger et al., 1997). The structure of chromatin can be modified by several fundamental mechanisms, often involving specialized and dedicated protein complexes. For instance, ATP-dependent remodelling enzymes use energy derived from hydrolysis of ATP to slide or exchange nucleosomes on the DNA template (Clapier et al., 2009; Eberharter et al., 2004). Additionally, the composition of nucleosomes can be altered by incorporation of histone variants, who differ from their canonical cousins in primary amino acid sequence and generally are less abundant (Sarma et al., 2005; Zlatanova et al., 2008). Finally, both canonical histones and their

[1] Manuscript submitted to Journal of Biological Chemistry (JBC). Nancy Lévesque, Maria J. Aristizabal, Joris Benschop, Nevan J. Krogan, Frank C. P. Holstege[1], and Michael S. Kobor.

variants are subjected to numerous post-translational chemical modifications, including acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation (Bannister et al., 2011).

Budding yeast *Saccharomyces cerevisiae* contains several histone acetyltransferase (HAT) complexes, including NuA4 (Nucleosome acetyltransferase of H4). NuA4, the only essential HAT in yeast, is composed of 13 subunits and not only acetylates the N-terminal tails of histones H4, H2A, and H2A.Z but also several non-histone substrates (Babiarz et al., 2006; Doyon et al., 2004; Jeong et al., 2011; Keogh, Mennella et al., 2006; Lin et al., 2008; Lin et al., 2009; Lu et al., 2011; Mitchell et al., 2011). NuA4 is required for a diverse set of cellular functions, including cell cycle progression, DNA double-strand break repair, establishment of heterochromatin-euchromatin boundaries at subtelomeric regions, and transcription of ribosomal genes (Bird et al., 2002; Boudreault et al., 2003; Clarke et al., 1999; Reid et al., 2000; Smith et al., 1998; Zhang et al., 2004). Its catalytic activity resides in the Esa1 subunit, while other subunits coordinate specific functions of NuA4 or contribute to its structural stability. NuA4 is a modular complex that assembles on the Eaf1 subunit and as such different regions of the Eaf1 protein mediate the association of distinct components of NuA4. For example, the N-terminal region of Eaf1 is responsible for the association of the Eaf5/7/3 sub-module, while its SANT (SWI3-ADA2-N-CoR-TFIIIB) domain binds Tra1 (Auger et al., 2008), a subunit involved in NuA4 recruitment to specific promoters through its interaction with acidic transcription activators such as Gcn4, Hap4, and Gal4 (Brown et al., 2001). In addition, the HSA (helicase-SANT-associated) domain of Eaf1 interacts with a module containing Swc4, Arp4, Yaf9, and Act1 (Auger et al., 2008; Szerlong et al., 2008). Consistent with a

broad requirement of Eaf1 for NuA4 assembly, cells lacking *EAF1* have strongly reduced global levels of H4 tetra-acetylation (Kobor et al., 2004a).

The Swc4/Arp4/Act1/Yaf9 module provides an intriguing link to the H2A.Z histone variant as these four proteins are also components of SWR1-C, an ATP-dependent chromatin-remodelling complex that deposits H2A.Z into chromatin (Kobor et al., 2004a; Krogan et al., 2004; Mizuguchi et al., 2004; Zhang et al., 2004). These shared subunits likely facilitate the converging and coordinated activities of NuA4 and SWR1-C on H2A.Z. Specifically, H4 acetylation by NuA4 likely contributes to recruitment of SWR1-C to chromatin and stimulates SWR1-C to replace H2A with H2A.Z at nucleosome located at promoter regions. Subsequently- and perhaps mediated by the shared module staying anchored at sites of H2A.Z deposition - NuA4 acetylates the newly deposited H2A.Z (Altaf et al., 2010; Babiarz et al., 2006; Keogh et al., 2006; Wu et al., 2009; Zhou et al., 2010). In support of this model, cells lacking either *EAF1* or the genes encoding the shared subunits Yaf9 and Swc4 have defects in H2A.Z chromatin association and H2A.Z acetylation, the latter being critical for its role as a boundary element between heterochromatin and euchromatin (Auger et al., 2008; Babiarz et al., 2006; Raisner et al., 2005; A. Y. Wang et al., 2011; Zhang et al., 2004; Zhou et al., 2010). Genetic analysis further supports the close functional relationship between NuA4 and SWR1-C, as combining a deletion of the non-essential *EAF1* gene with deletions of non-essential genes encoding various SWR1-C subunits or its substrate, H2A.Z, result in lethal phenotypes, suggesting that these two complexes share an essential function (Kobor et al., 2004a; Krogan et al., 2004).

Previous work has shown that removal of the C-terminal region of Epl1, another NuA4 subunit, results in the dissociation of the piccolo NuA4 (picNuA4) submodule, composed of Esa1, Yng2, Epl1, and Eaf6 (Auger et al., 2008; Boudreault et al., 2003). In contrast to the locus-specific recruitment, acetylation, and activation of NuA4 (Nourani et al., 2004; Reid et al., 2000), picNuA4 is thought to catalyze non-targeted global histone acetylation and is more active *in vitro* in acetylating H4 and H2A than its larger cousin (Boudreault et al., 2003; Friis, Wu et al., 2009; Selleck et al., 2005). Although rooted in the discovery of picNuA4, untargeted histone acetylation is emerging as an important, yet poorly understood, property of HAT complexes (Friis et al., 2009). Increased cellular amounts of picNuA4 are also detected when the non-essential gene encoding the Eaf1 subunit is deleted (Auger et al., 2008). As described in the previous paragraph, additional distinct submodules can be biochemically purified from *eaf1*Δ mutants. However, somewhat contradictory work has shown that deletion of Eaf1 results only in loss of the Tra1 subunit from NuA4, leaving picNuA4 associated with the remainder of the NuA4 complex (Ginsburg et al., 2009).

Here we study the two scenarios represented by loss of the entire Eaf1 protein and the loss of the Epl1 C-terminus to better understand the precise nature of the relationship between NuA4 and picNuA4, especially in regards to their shared versus distinct *in vivo* functions. Somewhat surprisingly, large-scale genetic and gene expression analysis not only revealed the expected commonalities between *eaf1*Δ and *epl1* mutants, but also uncovered some distinct properties, suggesting that both genetic alterations actually lead to distinct functional consequences. Furthermore, while loss of Eaf1 caused a strong

reduction in bulk H4 acetylation, loss of the Epl1 C-terminus resulted in H4 acetylation levels that were higher than in wild-type cells. Removal of the Epl1 C-terminus could suppress the bulk H4 and H2A.Z acetylation defects associated with lack of Eaf1, again suggesting that the entities liberated in the two scenarios were likely quite different. However, other defects caused by loss of Eaf1 were not rescued by removal of the Epl1 C-terminus, including decreased promoter specific H4 acetylation and expression of ribosomal genes. Lastly, to better understand which parts of Eaf1 mediated its role in NuA4, we performed structure-function studies and identified its C-terminus as being broadly required for NuA4-dependent function.

2.2 Results

2.2.1 Distinct and overlapping cellular roles for NuA4 and picNuA4.

Built on previous studies demonstrating that a complete deletion of Eaf1 or truncation of the Epl1 C-terminus result in the liberation of picNuA4 from NuA4, we decided to compare these two scenarios in genome-wide assays to learn more about the relationship between NuA4 and picNuA4. This was further motivated by the fact that in the absence of Eaf1, NuA4 function is broadly compromised (Auger et al., 2008; Babiarz et al., 2006; Kobor et al., 2004b; Mitchell et al., 2008). For these studies we used either a complete deletion of the

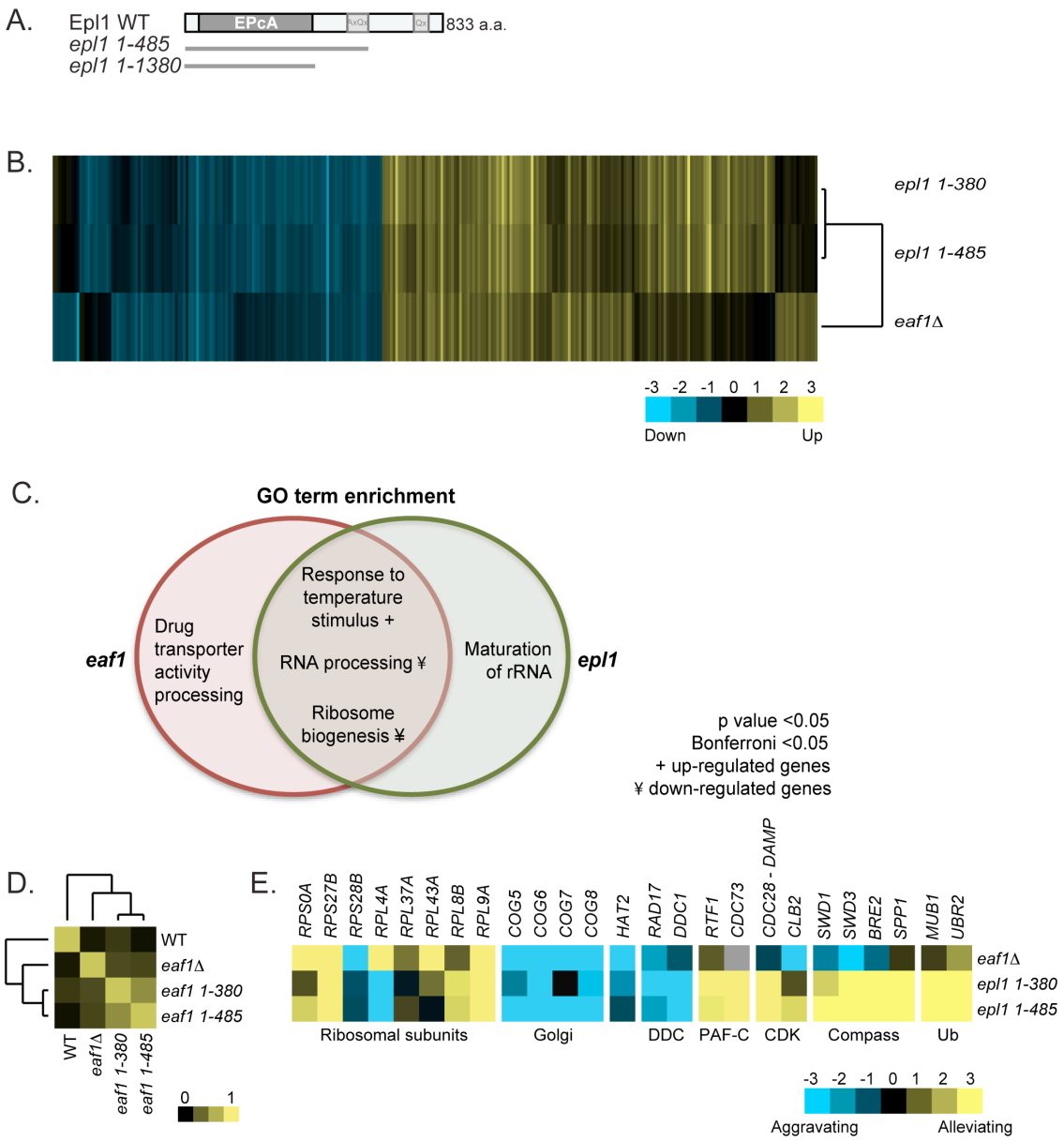


Figure 2.1 Genetic and gene expression analysis revealed similarities and distinctions between *eaf1* and *epl1* mutants. (A) Schematic representation of *epl1* C-terminal truncations. (B) Unsupervised hierarchical cluster matrix of *eaf1* and *epl1* alleles with their respective up- and downregulated genes. Yellow indicates upregulated genes, and blue represents downregulated genes. (C) Venn diagram showing the enrichment for Gene Ontology Fat terms for *eaf1* and *epl1* mutants using significant values ($p < 0.05$, $FC > 1.7$). (D) Unsupervised hierarchical cluster matrix of *eaf1* and *epl1* truncated alleles with their respective positive and negative genetic interactions. (E) Genetic interaction profiles of *eaf1* and *epl1* truncated alleles with indicated complexes. Blue indicates aggravating interactions, yellow represents alleviating interactions, and gray denotes missing data. DDC: DNA-damage checkpoint, CDK: cell cycle cyclin-dependent kinase, Ub: Ubiquitination.

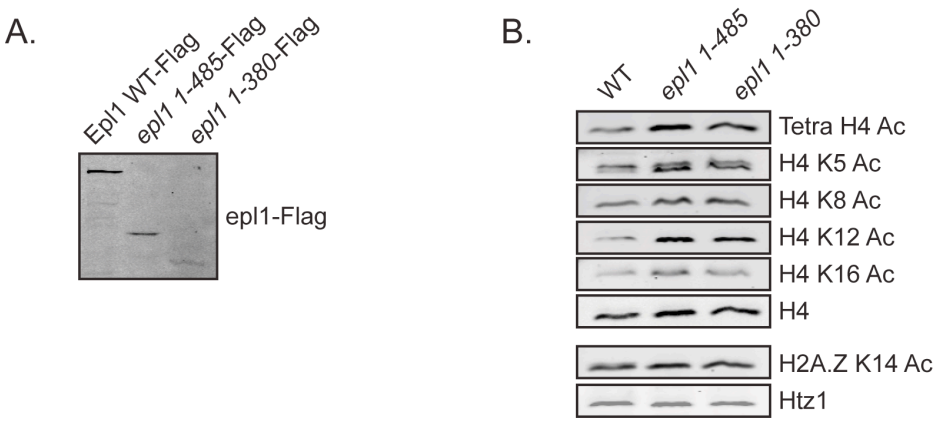


Figure 2.2 picNuA4 displayed a slight increase of H4 but not H2A.Z acetylation, as compared to wild-type. (A) Expression levels of indicated *epl1* mutants. Whole cell extracts of indicated strains were analyzed by protein blotting with anti-Flag antibodies. (B) Whole cell extracts of indicated strains were analyzed by protein blotting with anti-H4 tetra-, H4 K5, H4 K8, H4 K12, H4 K16, or H2A.Z K14 acetylation antibodies. Antibodies against H4 and H2A.Z were used as loading controls.

non-essential *EAF1* gene, or two previously published alleles encoding C-terminal truncations of the essential *EPL1* gene (Fig. 2.1A, 2.2A) (Boudreault et al., 2003). As determined by mRNA expression array studies, the *epl1 1-380* and *epl1 1-485* mutants had a similar gene expression profiles to the *eaf1*Δ mutant, although a few differences were noticeable, thus resulting in distinct clustering. This work suggested that despite both genetic manipulations being previously shown to lead to increased picNuA4 formation, the liberated complex might differ in structure and function (Fig. 2.1B). Although NuA4 has been primarily linked to gene activation, we observed approximately similar numbers of genes up-regulated and down-regulated in the 3 mutant strains. Gene ontology (GO) analysis, revealed that genes that required either Eaf1 or Epl1 for proper expression were involved in "Response to temperature stimulus", "RNA processing" and "Ribosome biogenesis". Genes that required only Epl1 were involved in "Maturation of

rRNA" while genes that required only Eaf1 were involved in "Drug transporter activity" (Fig. 2.1B) (Hoke et al., 2008; Lindstrom et al., 2006; Mitchell et al., 2011; Natsume-Kitatani et al., 2011).

Next, we interrogated our mutant strains carrying *eaf1*Δ or *epl1* derivatives by epistatic miniarray profiling (E-MAP), an assay that enables quantitative measurements of aggravating and alleviating genetic interactions against a library of 1,536 mutant genes involved in transcription, RNA processing, and chromatin biology (Schuldiner et al., 2006a). Similar to the expression data, correlation analysis of the genetic profiles revealed that the two *epl1* mutants (*epl1 1-380, 1-485*) clustered closer together than to the *eaf1*Δ mutant (Fig. 2.1D). Furthermore, reminiscent of the mRNA profiles, the E-MAP analysis revealed similarities and differences between *eaf1*Δ and *epl1* mutants. For instance, both *eaf1* and *epl1* mutants had generally alleviating interactions with ribosomal subunits encoding genes, suggesting that *EAF1* and *EPL1* both contribute to ribosomal function, a conclusion that agreed with the gene expression profiles which identified genes in "Ribosome biogenesis" as significantly misregulated in *epl1* and *eaf1* cells (Fig. 2.1C). Consistent with previous work, we also observed negative genetic interactions for both sets of mutants with genes involved in Golgi transport, DNA damage, and the histone acetyl transferase, HAT2 (Krogan et al., 2004; Mitchell et al., 2008). In contrast, *epl1* and *eaf1* mutants differed in their interactions with genes encoding for the PAF complex, cell cycle cyclin-dependent kinases, COMPASS, and the *MUB1-UBR2* ubiquitination complex (Fig. 2.1E). Lastly, while both *eaf1* and *epl1* mutants have known defects in cell cycle progression (Auger et al., 2008; Boudreault et al., 2003), our E-MAP analysis suggested a more nuanced involvement as we observed different genetic

interactions with *clb2* and *cdc28*, two key regulators of the cell cycle. Collectively, our large-scale genetic and gene expression analyses demonstrated that *EAF1* and *EPL1* have both overlapping yet also divergent functions, which is not easy to reconcile with these proteins acting similarly in regulating picNuA4 levels or activity.

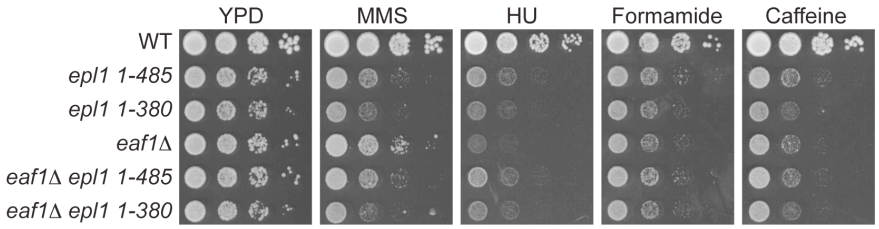
2.2.2 Balance of global histone acetylation levels by NuA4 and picNuA4.

Motivated by the differences in gene expression and E-MAP profiles, we wanted to examine the relationship between Eaf1 and Epl1 more closely. We reasoned that since both mutations lead to slightly different phenotypes, combining the single mutants would result in more pronounced defects typically associated with loss of NuA4 function. These include sensitivity to genotoxic agents such as methyl-methanesulfonate (MMS), hydroxyurea (HU), formamide, and caffeine, defects in H4 and H2A.Z acetylation, and gene expression (Auger et al., 2008; Babiarz et al., 2006; Boudreault et al., 2003; Kobor et al., 2004b). Interestingly, when combining a deletion of *EAF1* with either *epl1 1-380* or *epl1 1-485* alleles, we observed that the DNA damage sensitivity phenotype of the *eaf1 epl1* double mutant was not more severe than that of the individual single mutants (Fig. 2.3A). While this result was consistent with Eaf1 and Epl1 being integral parts of NuA4 and acting on the same pathway to confer resistance to DNA damage, it did not explain the differences observed in the two functional genomic assays. Given that loss of Eaf1 has been shown to reduce the level of bulk H4 and H2A.Z acetylation (Babiarz et al., 2006; Kobor et al., 2004b), we next evaluated the effect of removing the C-terminus of Epl1 on these core NuA4 activities. In striking contrast to the reduction observed in the *eaf1*Δ mutant, removal of the Epl1 C-terminus increased the bulk level of H4 tetra-

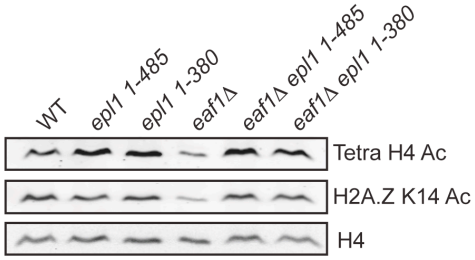
acetylation as determined by immunoblotting of whole cell extracts (Fig. 2.3B, 2.2B). This was in agreement with the increased HAT activity of picNuA4 *in* vitro (Boudreault et al., 2003; Selleck et al., 2005). Interestingly, the H4 acetylation levels in the *eaf1 epl1* double mutants were similar to that in the *epl1* mutants, suggesting that removing the C-terminus of Epl1 can rescue the acetylation defects caused by loss of Eaf1. This appeared to be true also for H2A.Z acetylation, although the *epl1* mutants alone did not cause an increase in H2A.Z acetylation (Fig. 2.3B, 2.2B).

Based on the ability of the *epl1* mutants to rescue the defects in bulk H4 acetylation caused by loss of Eaf1, we tested whether this translated to changes in H4 acetylation at specific genomic regions normally occupied by NuA4, such as promoters of ribosomal protein (RP) genes (Reid et al., 2000). In stark contrast to the bulk increase in H4 tetra-acetylation, we observed that *epl1 1-380* and *eaf1*Δ single mutants both led to decrease of H4 tetra-acetylation at promoters of *RPL19B*, *RPS11B*, and *RPS3*, using chromatin immunoprecipitation (ChIP) followed by qPCR (Fig. 2.3C). Not surprisingly, the *eaf1*Δ *epl1 1-380* double mutant also showed a decrease in H4 tetra-acetylation at these loci, suggesting that picNuA4 does not have an affinity for acetylating H4 at these specific loci (Fig. 2.3C). Consistently, the H4 acetylation pattern closely mirrored the mRNA expression patterns of these genes. As assayed by quantitative reverse transcriptase (qRT) PCR, expression of *RPL19B*, *RPS11B*, and *RPS3* was reduced in both single mutants, as well as the double mutant (Fig. 2.3D). Thus these results suggested that despite a seemingly

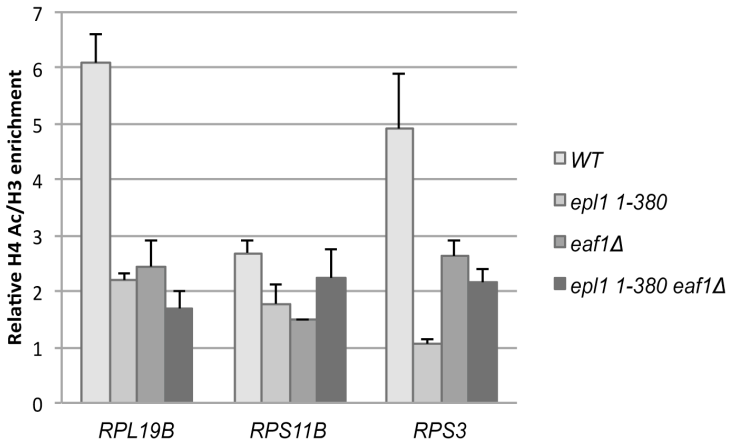
A.


YPD
MMS
HU
Formamide
Caffeine
WT
epl1 1-485
epl1 1-380
eaf1Δ
eaf1Δ epl1 1-485
eaf1Δ epl1 1-380

B.


WT
epl1 1-485
epl1 1-380
eaf1Δ
eaf1Δ epl1 1-485
eaf1Δ epl1 1-380
Tetra H4 Ac
H2A.Z K14 Ac
H4

C.


Relative H4 Ac/H3 enrichment
0
1
2
3
4
5
6
7
RPL19B
RPS11B
RPS3
WT
epl1 1-380
eaf1Δ
epl1 1-380 eaf1Δ

D.

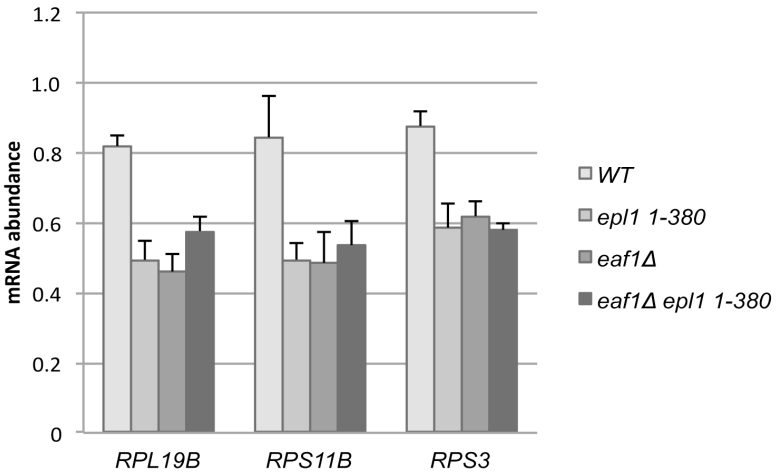

mRNA abundance
0
0.2
0.4
0.6
0.8
1.0
1.2
RPL19B
RPS11B
RPS3
WT
epl1 1-380
eaf1Δ
eaf1Δ epl1 1-380

Figure 2.3 Increased picNuA4 suppressed *eaf1* mutant histone H4 and H2A.Z global acetylation defects but not at specific loci. (A) Ten-fold serial dilutions of the indicated strains were plated onto media containing 0.005% MMS, 50mM HU, 1% Formamide, or 2mM Caffeine. (C, D) picNuA4 did not restore histone H4 acetylation levels at promoter regions or proper gene transcription at ribosomal protein genes in absence of NuA4 (*eaf1*Δ). (B) Whole cell extracts of indicated strains were analyzed by protein blotting with anti-H4 tetra- or H2A.Z K14 acetylation antibodies. Antibodies against H4 were used as loading controls. (C) ChIP of H4 tetra-acetylation at promoters of *RPL19B, RPS11B*, and *RPS3* in cells carrying wild-type, *epl1 1-380* allele, *eaf1*Δ, and *epl1eaf1* double mutants. Enrichment of H4 tetra-acetylation was normalized to H3 density. Error bars represent standard errors of the means for three independent experiments. Values greater than 1 indicate enrichment at the indicated locus. (D) Total cellular mRNA was prepared and measured by RT-qPCR. *RPL119B*, *RPS11B*, and *RPS3* mRNA levels were normalized to that of *ACT1*. Results are represented relative to untagged wild-type set as 1.0. Error bars represented standard errors of the means for three independent experiments.

global ability of increased picNuA4 to suppress NuA4 function, the suppression did not extend to the oberved specific loci regulated by NuA4.

2.2.3 The C-terminus of Eaf1 was important for NuA4 function.

Prompted by the marked differences of *eaf1* and *epl1* on some NuA4 dependent phenotypes, we sought to further understand the role of Eaf1 in NuA4 function. To determine which parts of Eaf1 might be responsible for mediating its broad requirement for NuA4 function, we created a set of *EAF1* alleles progressively truncated at the C-terminus by insertion of a 3xHA epitope tag at its genomic locus (Fig. 2.4A). The majority of the truncated *eaf1* alleles produced a protein product, with the exception of *eaf1 1-713* and *eaf1 1-644* which were expressed at lower levels, and the *eaf1 1-345* which yielded an undetectable protein product (Fig. 2.5A). When tested for growth on genotoxic drugs, cells containing shorter *eaf1* alleles (*eaf1 1-713, 1-644*, and *1-345*) had sensitivities similar to the *eaf1*Δ mutant, while cells containing longer *eaf1* alleles (*eaf1 1-939* to *1-815*) grew similar to wild-type cells (Fig. 2.4B). Interestingly, the *eaf1 1-420*

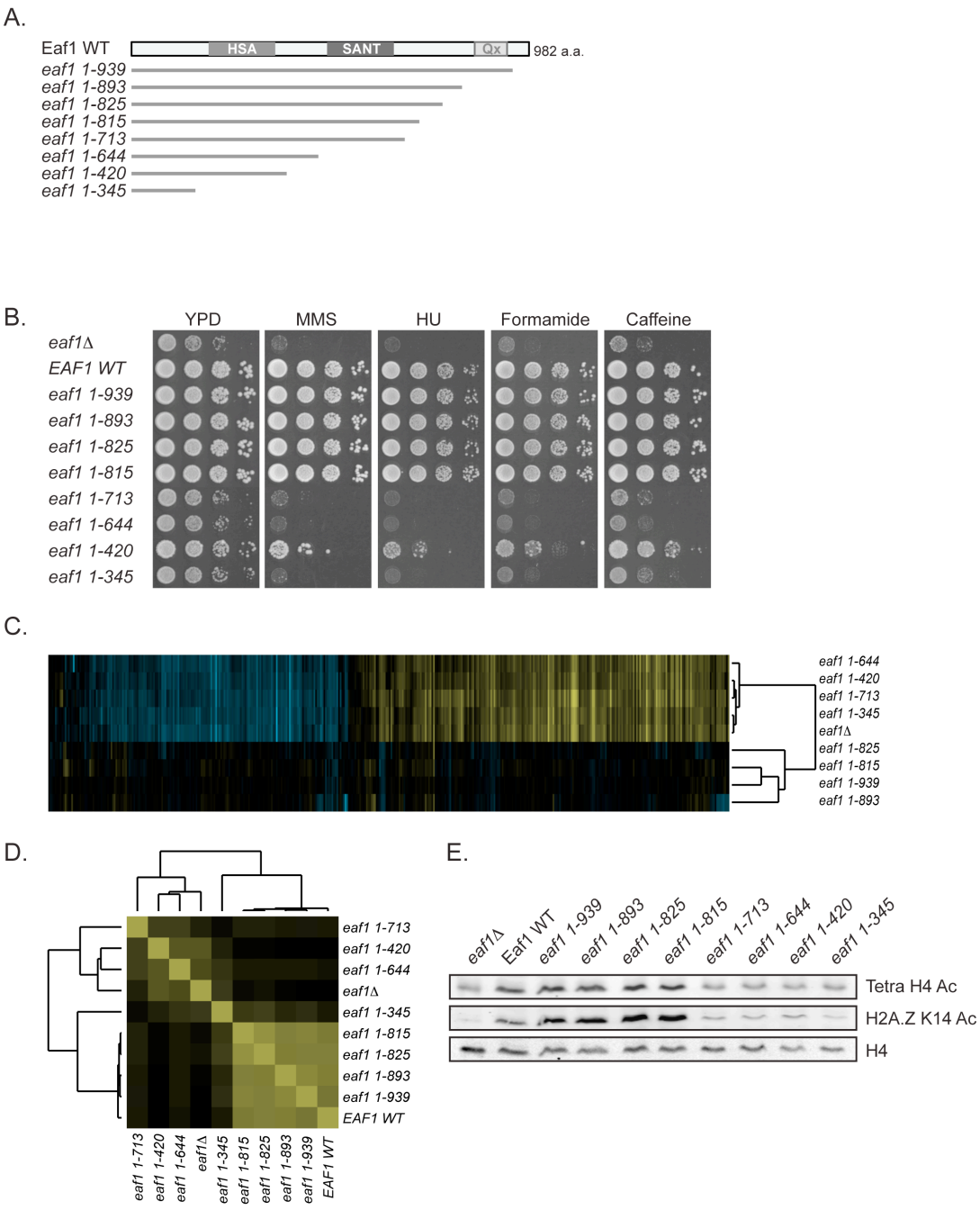


Figure 2.4 Eaf1 C-terminus was important for both histone H4 and H2A.Z acetylation. (A) Schematic representation of *eaf1* in C-terminal truncations. (B) Ten-fold serial dilutions of the indicated strains were plated onto media containing 0.005% MMS, 50mM HU, 1% Formamide, or 2mM Caffeine. (B) Unsupervised hierarchical cluster matrix of *eaf1* alleles with their respective up- and downregulated genes. Yellow indicates upregulated genes, and blue represents downregulated genes. (C) Unsupervised hierarchical cluster matrix of *eaf1* and *epl1* truncated alleles. (D) Whole cell extracts of indicated strains were analyzed by protein blotting with anti-H4 tetra-acetylation or anti-H2A.Z K14 acetylation antibodies. Antibodies against H4 were used as a loading control.

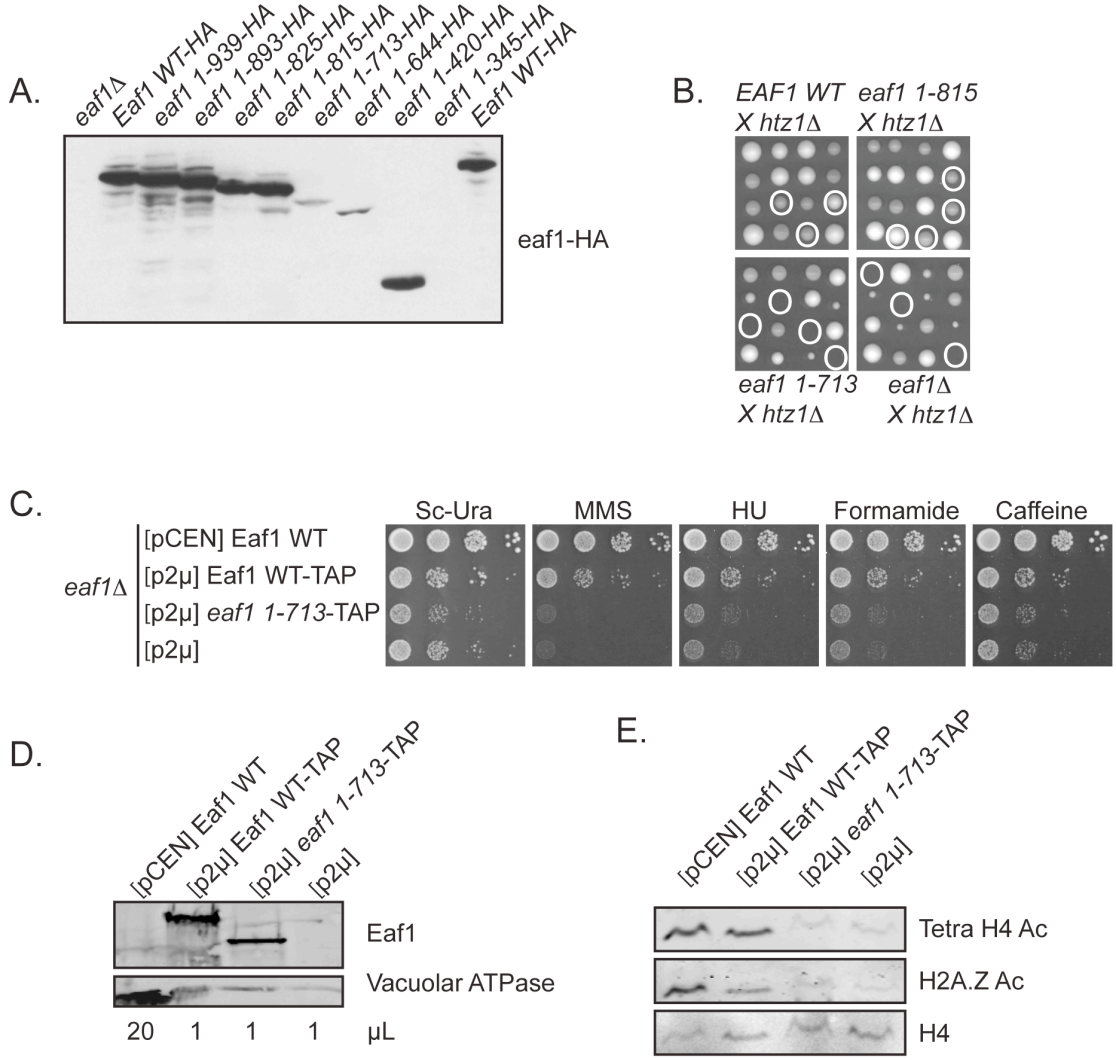


Figure 2.5 Overexpression of *eaf1 1-713* allele did not suppress defects observed in *eaf1* mutants. (A) Whole cell extracts of indicated strains were analyzed by protein blotting with anti-HA antibodies. No *eaf1 1-345* truncated protein was observed by immunoblotting. (B) Eaf1 C-terminus genetically interacted with the SWR1-C component H2A.Z (*HTZ1*). Genetic analysis of viable spores resulting from mating *eaf1* mutants and *htz1*Δ demonstrated that cells carrying *eaf1 1-713* alleles and *htz1*Δ were inviable as compared to cells containing *EAF1, eaf1 1-939* or *eaf1 1-893* alleles. *eaf1 htz1*Δ double mutants are circled in white. (C) Whole cell extracts of overexpressed wild-type Eaf1-TAP and Eaf1 1-713-TAP mutant were analyzed by protein blotting with anti-Eaf1 antibodies. (D) Ten-fold serial dilutions of overexpressed wild-type Eaf1-TAP and Eaf1 1-713-TAP mutant were plated onto media containing 0.005% MMS, 50mM HU, 1% Formamide, or 2mM Caffeine. (E) Whole cell extracts of overexpressed wild-type Eaf1-TAP and Eaf1 1-713-TAP mutant were analyzed by protein blotting with anti-H4 tetra-acetylation or anti-H2A.Z K14 acetylation antibodies. Antibodies against H4 were used as a loading control.

mutant grew more robustly than the *eaf1 1-713, 1-644,* and *1-345* mutants, consistent with the greater amount of Eaf1 protein produced by this allele. However *eaf1 1-420* mutant did still have decreased growth when compared to the wild-type and strains carrying longer *eaf1* alleles (Fig. 2.5A).

To learn more about the structure-function relationship of the different *eaf1* mutants, we examined their global genetic and gene expression profiles. mRNA profiles exposed a marked effect of *eaf1 1-713* mutant and shorter on regulation of gene expression compared to *eaf1 1-815* mutant and longer (Fig. 2.4C). Moreover, both the expression and E-MAP profiles of *eaf1* mutants agreed closely with the growth phenotypes and showed that strains carrying mutants *eaf1 1-825* or longer clustered separately from strains carrying *eaf1 1-713* and shorter, with the latter being tightly correlated to cells lacking Eaf1 entirely (Fig. 2.4C, D). In agreement with our growth assays, the fact that the *eaf1 1-420* mutants showed similar mRNA expression and genetic interaction profiles as the rest of the short mutants agreed with the hypotheses that defects other than protein levels were generating these phenotypes.

To analyze the role of the Eaf1 C-terminus on NuA4 function, we measured the acetylation levels of H4 and H2A.Z. Similar to *eaf1*Δ mutants, levels of H4 tetra-acetylation and H2A.Z K14 acetylation were decreased in all strains carrying alleles shorter than *eaf1 1-713*, whereas strains with longer alleles had no defects in acetylation of both NuA4 histone substrates (Fig. 2.4E). Furthermore, we analysed the requirement of the Eaf1 C-terminus in the shared function between NuA4 and SWR1-C by determining if the various Eaf1 C-terminal truncations were required to support growth in the absence of the histone variant H2A.Z (encoded by the *HTZ1* gene). Genetic analysis

by tetrad dissection showed that similar to a complete loss of *EAF1*, the *eaf1 1-713* allele was essential in the absence of *HTZ1*, whereas *eaf1 1-815 htz1*Δ double mutants were viable (Fig. 2.5B). Overall, this work showed that the Eaf1 C-terminus is required for the function of NuA4 complex.

Despite strong yet indirect evidence suggesting that decreased protein abundance in the short Eaf1 truncations was not the most crucial defect leading to the observed phenotypes, we decided to test this carefully. To do this, we cloned the *eaf1-713* and wild-type alleles in a 2μ plasmid and introduced it in an *eaf1*Δ mutant. Growth assays and histone acetylation measurements revealed that overexpression of the full-length *EAF1* caused a minor negative effect on cell fitness and H2A.Z acetylation, however the overexpressed *eaf1 1-713* allele remained just as defective in H2A.Z and H4 acetylation as the *eaf1*Δ*,* agreeing with the hypothesis that the defects in *eaf1* mutants were not solely a result of decreased protein levels in these mutants (Fig. 2.5C,D,E). However, we did not rule out the possibility that the observed growth phenotype can be due to protein fusion to TAP tag.

2.2.4 The Eaf1 C-terminus was required for efficient NuA4 recruitment to chromatin, histone H4 acetylation at promoter regions, and proper transcription of ribosomal protein genes.

Since Eaf1 C-terminal truncation mutants showed phenotypes consistent with impaired NuA4 function, we tested if these were a result of defects in NuA4 association with chromatin. Using a centrifugation-based fractionation assay that separates chromatin-

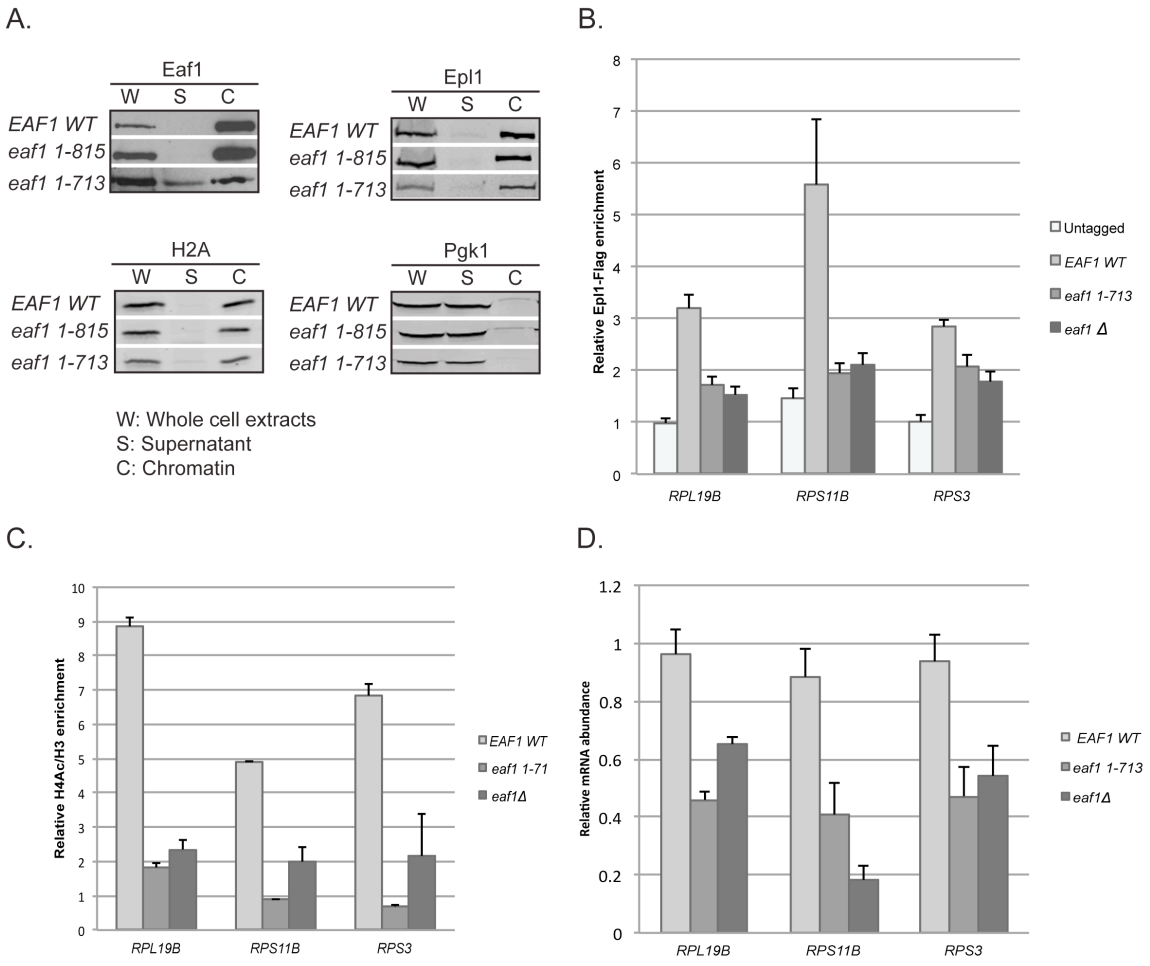


Figure 2.6 Eaf1 C-terminus was required for efficient NuA4 recruitment to chromatin, histone H4 acetylation at promoter regions, and proper transcription of ribosomal protein genes. (A) *eaf1* C-terminal truncation mutants had decreased histone deposition into chromatin. W, whole cell extract; S, supernatant; C, chromatin pellet. The relative amount of Eaf1-HA and Epl1 in each fraction was determined by immunoblotting in the different strains. Antibodies against histone H2A and Pgk1 were used as loading controls for chromatin pellet and supernatant, respectively. (B) Eaf1 C-terminus was important for the recruitment of NuA4 to promoters of RP genes. ChIP of Epl1-FLAG at promoters of *RPL19B, RPS11B*, and *RPS3* were performed in cells carrying *EAF1* wild-type, *eaf1 1-713* allele, and *eaf1*Δ. Strain containing untagged Epl1 was used as a negative control. Epl1-FLAG enrichment was normalized to a region that has no H4 acetylation. Error bars represent standard errors of the means for three independent experiments. Values greater than 1 indicate enrichment at the indicated locus. (C) Eaf1 C-terminus was required for H4 tetra-acetylation at RP genes promoters. ChIP of H4 tetra-acetylation at promoters of *RPL19B, RPS11B*, and *RPS3* was performed in cells carrying *EAF1* wild-type, *eaf1 1-713* allele, and *eaf1*Δ. Enrichment of H4 tetra-acetylation was normalized to H3 density. Error bars represent standard errors of the means for three independent experiments. Values greater than 1 indicate H4 tetra-acetylation enrichment at the indicated locus. (D) Eaf1 C-terminus was required for RP gene expression. Total cellular mRNA was prepared and measured by RT-qPCR.

bound proteins (pellet fraction) from the rest of the proteins (supernatant fraction), we found that Eaf1 and Eaf1 1-815 resided predominantly in the chromatin fraction whereas the Eaf1 1-713 mutant was partially lost from chromatin (Fig. 2.6A). Unexpectedly, in the *eaf1 1-713* mutant, the chromatin association of the NuA4 subunit Epl1 did not change when compared to *EAF1* or *eaf1 1-815* strains (Fig. 2.6A). In each case the fractionation efficiency was judged by the levels of the control proteins H2A and Pgk1 found in the chromatin fraction and the supernatant fraction, respectively. This result indicated that Eaf1 C-terminus might not influence global Epl1 recruitment to chromatin. Since general association with chromatin was normal for Epl1 in Eaf1 C-terminal mutants, we tested if this was also the case at the promoter of RP genes, which are typically regulated by NuA4. Using ChIP-qPCR, we found that Epl1-Flag was associated with the *RPL19B*, *RPS11B*, and *RPS3* promoters in *EAF1* cells, but had significantly decreased promoter association in *eaf1-713* mutants and *eaf1*Δ cells (Fig. 2.6B). The loss of NuA4 at RP gene promoters was accompanied by a decrease in H4 tetra-acetylation as determined by ChIP-qPCR (Fig. 2.6C), and a 2- to 3-fold reduction of *RPL19B*, *RPS11B*, and *RPS3* mRNA levels (Fig. 2.6D).

Given that truncating the Eaf1 C-terminus resulted in normal Epl1 bulk chromatin association but impaired Eaf1 association, it prompted us to examine NuA4 complex stability in *eaf1* mutants. To do this, we immunoprecipitated the Epl1-TAP subunit and determined if derivatives encoded by various Eaf1 alleles co-purified. We observed that the levels of Eaf1 bound to Epl1 decreased in cells containing the *eaf1 1-713* allele or shorter versions, as compared to longer *eaf1* alleles (Fig. 2.7A). Consistently, large-scale affinity purification of NuA4 via the Epl1-TAP tag revealed significantly decreased

levels of complete NuA4 complex in cells harbouring Eaf1 1-713 when compared to wild-type cells (Fig. 2.7B), suggesting that Eaf1 C-terminus plays a scaffolding role in NuA4 complex and therefore is important for complex stability. Although, the lower protein levels observed for Eaf1 1-713 could also be in part responsible for decreased NuA4 association.

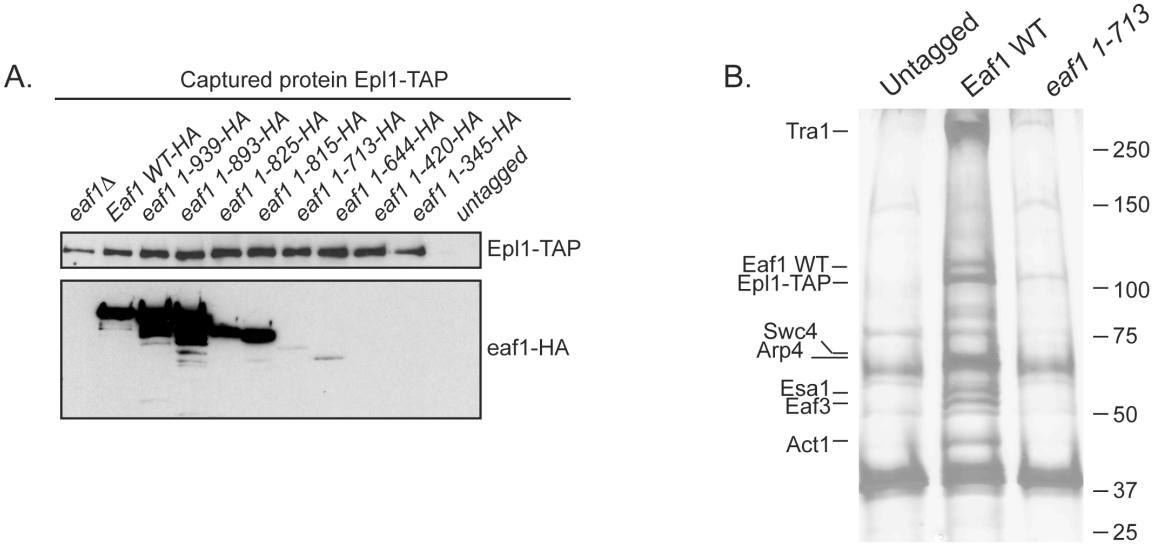


Figure 2.7 Cells lacking the Eaf1 C-terminal showed a defect in forming a complete NuA4 complex. (A) Eaf1 lacking its C-terminus (*eaf1 1-713* and *1-644*) co-immunoprecipitated with NuA4. The indicated *eaf1* mutant proteins were pulled-down to NuA4 using Epl1-TAP. Purified *eaf1* mutant proteins were analyzed by protein blotting with anti-HA antibodies. Untagged Epl1 was used as a negative control. The level of Epl1-TAP in each strain was visualized using IgG antibodies. (B) TAP purification of NuA4 using Epl1-TAP demonstrated dissociation of NuA4 subunits in *eaf1 1-713* mutant. Purified material from the indicated strains was loaded onto a 4-20% gradient SDS-PAGE gel and visualized by silver staining. Bands corresponding to NuA4 subunits are indicated on the left. Untagged Epl1 was used as a negative control.

2.2.5 The HSA domain of Eaf1 was important for NuA4 function.

Given the drastic loss of function caused by the *eaf1 1-713* mutant as compared to the *eaf1 1-815* mutant, we specifically deleted the region encoding amino acids from 713-815 while leaving the remainder of the protein intact (Fig. 2.8A). Surprisingly, cells harbouring an Eaf1 protein lacking these amino acids were not sensitive to genotoxic stress (Fig. 2.8B) nor did they have reduced levels of H4 or H2A.Z acetylation (Fig. 2.8C). To further test whether function of the Eaf1 protein was impaired when other internal regions were removed, we tested whether the HSA and SANT domains were required for function. Immunoblotting of whole cell extracts indicated that all three Eaf1 derivatives lacking these internal regions were expressed similarly to the full length Eaf1 protein (Fig. 2.9A). Interestingly, while cells lacking the Eaf1 HSA domain had phenotypes similar to deletion of *EAF1*, including defects in both H4 and H2A.Z acetylation and sensitivity to genotoxic stress, cells lacking the Eaf1 SANT domain had histone acetylation levels and growth comparable to wild-type (Fig. 2.8B, C). Similar to *eaf1*Δ, H4 and H2A.Z acetylation defects observed in cells lacking Eaf1 HSA domain were suppressed upon removal of the Epl1 C-terminus (Fig. 2.9B). Overall, these data suggested that Eaf1 HSA domain and possibly the shared subunits, Yaf9, Swc4, Act1 and Arp4, known to associate with this domain of the protein, were critical for NuA4 activity (Szerlong et al., 2008).

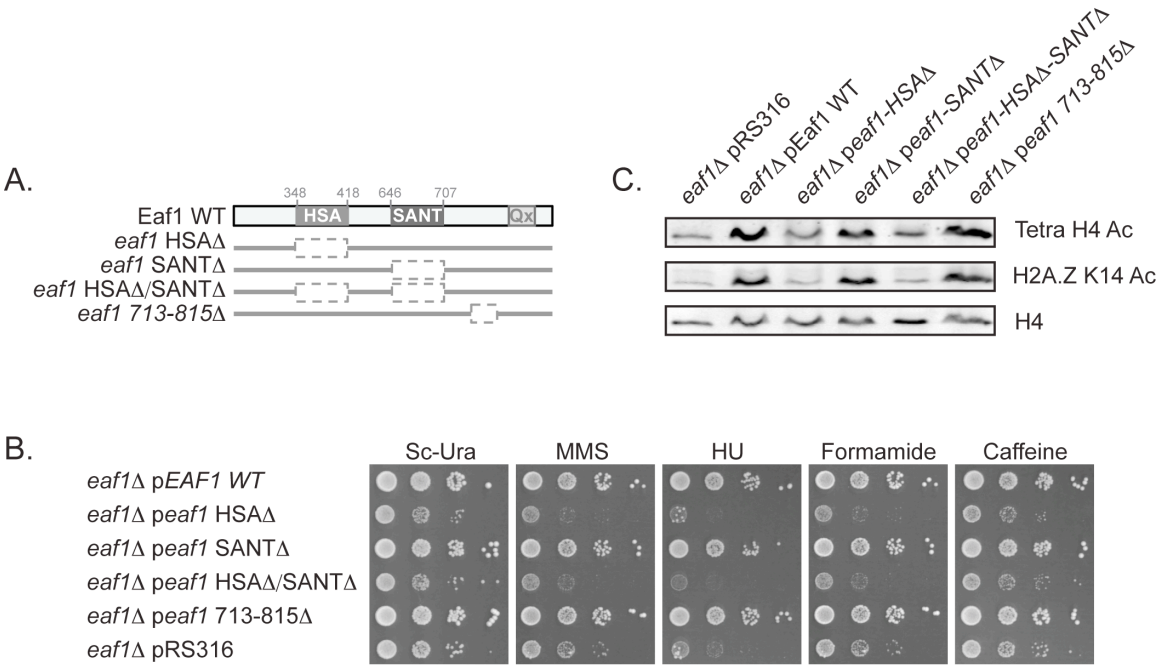


Figure 2.8 Eaf1 HSA domain was required for histone H4 and H2A.Z acetylation. (A) Schematic representation of *EAF1* internal region deletions: HSAΔ, SANTΔ, HSAΔ/SANTΔ, and 713-815Δ regions. (B) Ten-fold serial dilutions of the indicated strains were plated onto media containing 0.005% MMS, 50mM HU, 1% Formamide, or 2mM Caffeine. (C) Whole cell extracts of indicated strains were analyzed by protein blotting with anti-H4 tetra-acetylation or anti-H2A.Z K14 acetylation antibodies. Antibodies against H4 were used as a loading control.

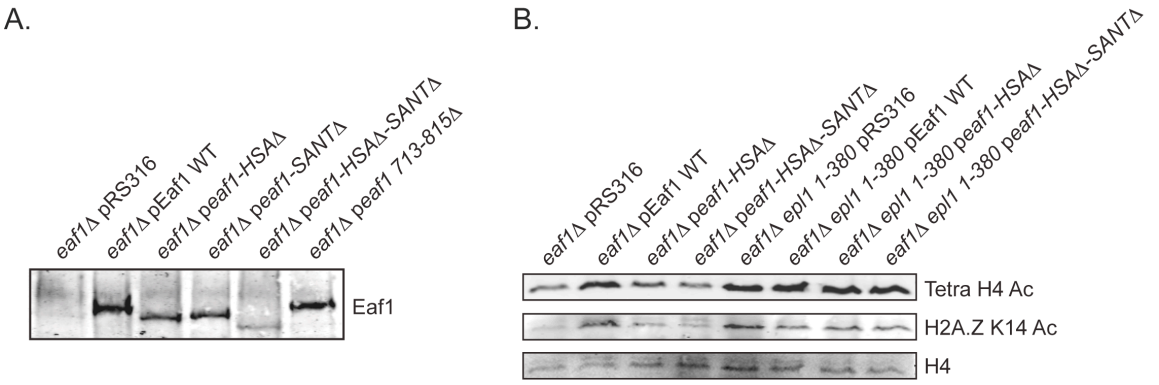


Figure 2.9 Increased picNuA4 suppressed histone H4 and H2A.Z acetylation defects in cells lacking Eaf1 HSA domain. (A) Expression levels of indicated *eaf1* mutants. Whole cell extracts of indicated strains were analyzed by protein blotting with anti-Eaf1 antibodies. (B) Whole cell extracts of indicated strains were analyzed by protein blotting with anti-H4 tetra- or H2A.Z K14 acetylation antibodies. Antibodies against H4 were used as loading controls.

2.3 Discussion

In this study, we used two different approaches derived from the literature to liberate picNuA4 in order to learn more about the relationship between NuA4 and picNuA4. Focusing our attempts to dissect NuA4 function on the contributions of the entire Eaf1 and the Epl1 C-terminus, the data revealed an expected set of common requirements, but perhaps more surprisingly also clearly distinct phenotypic and functional consequences. Primary support of the nuanced and condition-specific relationship between Eaf1 and the C-terminus of Epl1 was provided by the two genome-wide approaches we utilized. Secondary support was provided by the clearly distinct effects of the different single mutants on bulk H4 and H2A.Z acetylation levels, which contrasted with similar effects on gene-specific H4 acetylation and RP gene expression. Lastly, structure-function studies on Eaf1 revealed that specifically its C-terminus was required for NuA4-dependent function.

Gene expression array analysis identified both overlapping and distinct gene expression clusters between the mutants. Similarly, E-MAP uncovered genes required to support growth of both the *eaf1*Δ and *epl1 1-380* mutant, whereas other genes were required only in one or the other mutant. The validity of these data was supported by the proposed role of Epl1, but not Eaf1, in transcription elongation (Ginsburg et al., 2009). As such, *epl1 1-380* mutants had positive genetic interactions with genes encoding the transcription elongation complex PAF, which was not required for growth in the *eaf1*Δ mutants.

Genetic epistasis analysis further emphasized the complex and condition-specific relationship between the two key NuA4 components Eaf1 and Epl1, and by extension

between NuA4 and picNuA4. Specifically, *eaf1 epl1* double mutants had similar defects to *epl1* single mutant in conferring resistance to genotoxic stress and in supporting expression and H4 acetylation of the three RP genes tested, neither of which was rescued by the double mutants. In contrast, loss of Eaf1 or the Epl1 C-terminus had opposing effects on H4 acetylation levels, with the former causing a decrease and the latter causing an increase as would be expected from picNuA4's enhanced catalytic activity *in vitro*. Most surprisingly, the bulk H4 acetylation defects observed in *eaf1*Δ single mutant were rescued in the *eaf1*Δ *epl1 1-380* double mutant.

Collectively, these data are impossible to reconcile with the simple model by which both loss of Eaf1 or loss of the Epl1 C-terminus equally liberate picNuA4. Instead, our work suggested that the respective liberated picNuA4 might have significantly different modes of regulation and activity, or different protein composition. In fact, we favour the latter to best reflect the situation in *eaf1*Δ mutants, as our data discussed above are consistent with picNuA4 not being retained on its own but within a slightly smaller NuA4 subcomplex along with the Eaf3/5/7 trimer and the shared subunit module. Thus, this large picNuA4 effectively only lacks Eaf1 and Tra1, whose loss results in the complex not being efficiently targeted by transcriptional activators to specific promoters (Ginsburg et al., 2009). The strong decrease of H4 and H2A.Z acetylation in *eaf1*Δ mutants would suggest that "picNuA4" in this conformation has compromised HAT activity through a yet unidentified mechanism, potentially linked to its remaining attachment to other NuA4 subunits (Fig. 2.10). For instance, previous studies speculated that the binding of Arp4 to ATP leads to a change of conformation, facilitating picNuA4 dissociation from NuA4

(Sunada et al., 2005). The untargeted histone acetylation activity of picNuA4 is liberated from NuA4 by deletion of the Epl1 C-terminus (Boudreault et al., 2003), which probably also relieves the inhibition on the picNuA4 catalytic activity exerted by the remaining interaction partners. This model provides an explanation for the restoration of bulk H4 acetylation levels found in *eaf1*Δ *epl1 1-380* double mutant, which in contrast to the *eaf1*Δ mutant contains fully active picNuA4.

The second possible explanation is based on the slightly distinct forms of picNuA4 liberated depending on the method used. Specifically, picNuA4 in *eaf1*Δ mutants contains full-length Epl1 whereas the other contains Epl1 lacking its C-terminus. In this model, the C-terminus of Epl1 rather than an unidentified component of the other subcomplexes inhibits Esa1 function (Fig. 2.10). The former would be inhibited in its catalytic activity – in fact below the levels of the intact complex - whereas the latter would be catalytically active. This model is consistent with data showing that removal of Eaf1 causes loss of overall complex assembly while retaining picNuA4 as a separate entity (Auger et al., 2008). However, this model is harder to reconcile with the documented co-existence of picNuA4 and NuA4 in wild-type strains, and it would also require the assumption that Esa1 is more active in the context of NuA4 as compared to picNuA4 even though both contain full-length Epl1. Regardless, our data are consistent with a complex relationship between activities directed at gene-specific versus non-targeted histone acetylation (Friis et al., 2009).

While both of these models explain the opposing effects on global and promoter specific H4 acetylation, neither fully explains the opposing effects of deleting Eaf1 or Epl1 on

H2A.Z acetylation. One possible scenario to explain these results is that retention of the remaining NuA4 at H2A.Z acetylation loci sterically blocks the ability of picNuA4 to hyperacetylate H2A.Z specifically at promoter regions, in which H2A.Z is highly enriched (Albert et al., 2007; Guillemette, Bataille, Gevry, Adam, Blanchette, Robert, and Gaudreau, 2005b; B. Li, Pattenden, Lee, Gutierrez, Chen, Seidel, Gerton, and Workman, 2005b; Zhang et al., 2005b). Removal of Eaf1 could reduce NuA4 anchoring to chromatin, thus letting access to picNuA4 for untargeted H2A.Z acetylation.

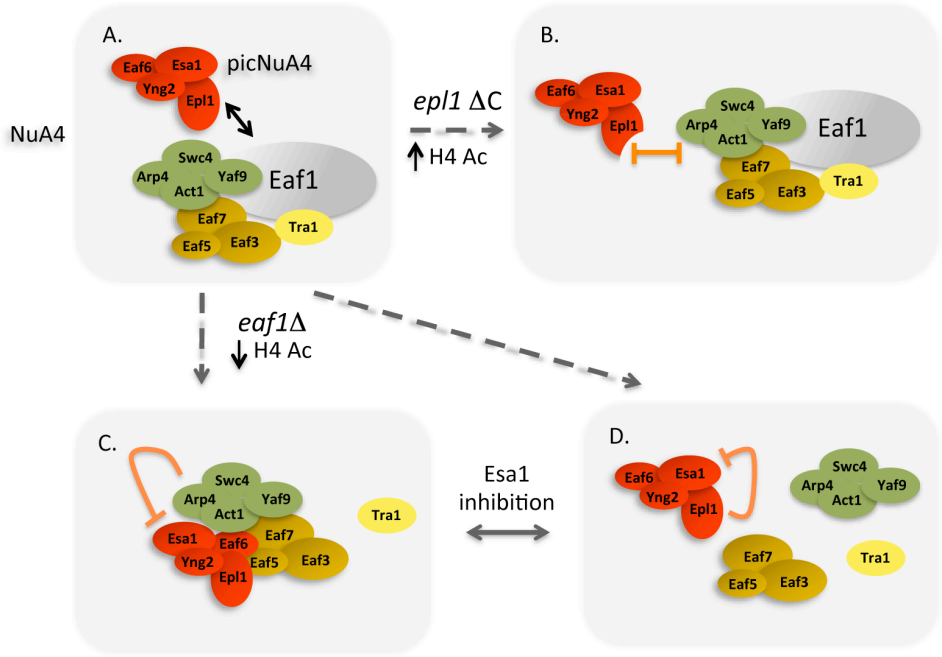


Figure 2.10 Model for repression of Esa1's catalytic activity in *eaf1*Δ and Epl1 C-terminus mutants. (A) NuA4 and picNuA4 co-exist in wild-type strain. (B) Removal of the C-terminus part of Epl1 leads to elevated levels of picNuA4 submodule resulting in increase of H4 acetylation. (C,D) Deletion of Eaf1 results in decrease of histone H4 acetylation. This could be explained by inhibition of Esa1 by either (C) the remaining NuA4 submodules, or (D) Epl1 C-terminal region.

The function of the Eaf1 in facilitating chromatin binding of NuA4 was derived in part from our dissection of Eaf1 regions that conferred protein function. Using structure-function relationship, this work identified two regions within the Eaf1 protein that were required for the function of the NuA4. First, we found that the Eaf1 C-terminus distal to amino acid 713 was needed for essentially all the functions associated with NuA4. The most trivial explanation for our results is that removal of the Eaf1 C-terminus simply reduced the level of the remaining protein below what is necessary to support assembly of NuA4. While we cannot fully exclude this possibility, we think it is unlikely given that overexpression of the Eaf1 1-713 derivative caused a very similar reduction in function. Regardless of the protein level, we discovered that Eaf1 1-713 was less tightly bound to chromatin compared to the full-length Eaf1 protein, suggesting that the C-terminus facilitates chromatin anchoring. As such, it is tempting to speculate that this region of the Eaf1 protein is involved in retention of NuA4 at specific gene promoters targeted by transcriptional activators whose principal contact in NuA4 is the Tra1 subunit. Our results can be reconciled with existing data in a model where the Eaf1 C-terminus confers a general chromatin affinity function to NuA4, which when lost would lead to a reduction of NuA4 from these specific promoters (Allard et al., 1999; Brown et al., 2001; Fishburn et al., 2005; Knutson et al., 2011). Second, and consistent with different domains of Eaf1 dividing the labour, the HSA domain of Eaf1 was required for H4 and H2A.Z acetylation and resistance to genotoxic stress. Our work extends previous mapping efforts focused on conserved domains of Eaf1, which identified the HSA and its adjacent N-terminal region as being required for the scaffolding of the distinct submodules into the complete NuA4 complex (Auger et al., 2008). The HSA domain and its adjacent N-terminal region

mediate the interaction with the Swc4, Arp4, Yaf9, Act1 submodule, perhaps through a direct interaction with Act1 and Arp4 (Szerlong et al., 2008). Thus, loss of the HSA domain might interfere with the interplay of NuA4 and SWR1-C at loci marked for H2A.Z deposition and acetylation. In addition, this region of the Eaf1 protein might also be necessary for association of the Eaf3/5/7 trimer and picNuA4, and as such, removal of this domain allows picNuA4 release but also liberation of other submodules whose associations with picNuA4 leads to a non functional unit likely explaining the observed decrease in H4 acetylation as opposed to the increase characteristic of picNuA4.

2.4 Experimental Procedures

2.4.1 Yeast strains

All yeast strains used in this study are listed in Table 2.1 and were created using standard yeast genetic techniques (Ausubel, 1987). Complete gene deletions and integration of the epitope tags (3xHA, TAP or 3xFLAG) at the 3' end of genes (Gelbart et al., 2001) were achieved using one-step gene integration of PCR-amplified modules (Longtine et al., 1998). The *EAF1* gene was PCR-amplified from genomic DNA and cloned into the centromeric vector pRS316 (*URA3*) containing a 3xHA epitope tag. Internal deletions of *EAF1* were performed by adapting the Quick Change site-directed mutagenesis method (Stratagene) and following the manufacturer's protocol (primers sequences available upon request). All mutations were confirmed by DNA sequencing. Overexpressed *EAF1* wild-type and *eaf1 1-713* alleles were generated by cloning the PCR-amplified DNA sequence into Gateway Entry vector (Invitrogen). The constructs were subsequently

transposed into the Gateway destination vector pAG426GPD, encoding a C-terminal TAP-tag (Alberti et al., 2007).

Table 2.1 Yeast strains

Strain	Relevant Genotype
MKY5	W303*, MATα ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 LYS2*
MKY6	W303*, MATA ADE2 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 lys2Δ*
MKY9	W303*, MATA/MATα ADE2/ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 LYS2/ lys2Δ*
MKY1378	MKY9, *EAF1-3XHA::NATMX6 htz1Δ::HYGMX6*
MKY1379	MKY9, *eaf1 1-815-3X HA::NATMX6 htz1Δ::HYGMX6*
MKY1380	MKY9, *eaf1 1-713-3X HA::NATMX6 htz1Δ::HYGMX6*
MKY1381	MKY9, *eaf1Δ-3X HA::NATMX6 htz1Δ::HYGMX6*
MKY1382	MKY6, *EAF1-3XHA::NATMX6*
MKY1383	MKY6, *eaf1 1-939-3XHA::NATMX6*
MKY1384	MKY6, *eaf1 1-893-3XHA::NATMX6*
MKY1385	MKY6, *eaf1 1-825-3XHA::NATMX6*
MKY1386	MKY6, *eaf1 1-815-3XHA::NATMX6*
MKY1387	MKY6, *eaf1 1-713-3XHA::NATMX6*
MKY1388	MKY6, *eaf1 1-644-3XHA::NATMX6*
MKY1389	MKY6, *eaf1 1-420-3XHA::NATMX6*
MKY1390	MKY6, *eaf1 1-345-3XHA::NATMX6*
MKY1391	MKY6, *eaf1Δ-3X HA::NATMX6*
MKY1392	MKY6, *eaf1Δ-3X HA::NATMX6 [pRS316]*
MKY1393	MKY6, *eaf1Δ-3X HA::NATMX6 [pRS316, EAF1]*
MKY1394	MKY6, *eaf1Δ-3X HA::NATMX6 [pRS316, eaf1 HSAΔ]*
MKY1395	MKY6, *eaf1Δ-3X HA::NATMX6 [pRS316, eaf1 SANTΔ]*
MKY1396	MKY6, *eaf1Δ-3X HA::NATMX6 [pRS316, eaf1 HSAΔ– SANTΔ]*
MKY1397	MKY6, *eaf1Δ-3X HA::NATMX6 [pRS316, eaf1 713-815Δ]*

Strain	Relevant Genotype
MKY1398	MKY5, *EPL1-3XFLAG:: KANMX6*
MKY1399	MKY5, *epl1 1-485-3XFLAG:: KANMX6*
MKY1400	MKY5, *epl1 1-380-3XFLAG:: KANMX6*
MKY1401	MKY6, *EAF1-3XHA::NATMX6 EPL1-3XFLAG:: KANMX6*
MKY1402	MKY6, *EAF1-3XHA::NATMX6 epl1 1-380-3XFLAG:: KANMX6*
MKY1403	MKY6, *eaf1Δ-3XHA::NATMX6 EPL1-3XFLAG:: KANMX6*
MKY1404	MKY6, *eaf1Δ3XHA::NATMX6 epl1 1-380-3XFLAG:: KANMX6*
MKY1405	MKY6, *eaf1 1-713-3XHA::NATMX6 EPL1-3XFLAG:: KANMX6*
MKY1406	MKY6, *EAF1-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1407	MKY6, *eaf1 1-939-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1408	MKY6, *eaf1 1-893-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1409	MKY6, *eaf1 1-825-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1410	MKY6, *eaf1 1-815-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1411	MKY6, *eaf1 1-713-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1412	MKY6, *eaf1 1-644-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1413	MKY6, *eaf1 1-420-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1414	MKY6, *eaf1 1-345-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1415	MKY6, *eaf1Δ-3XHA::NATMX6 EPL1 TAP::TRP1*
MKY1416	MKY6, *eaf1Δ-3XHA::NATMX6 [pAG426GPD]*
MKY1417	MKY6, *eaf1Δ-3XHA::NATMX6 [pAG426GPD, EAF1]*
MKY1418	MKY6, *eaf1Δ-3XHA::NATMX6 [pAG426GPD, eaf1 1-713]*
MKY583	BY4741*, MATα his3Δ1 leu2Δ0 LYS2 ura3Δ0 can1Δ::MATαPr-HIS3 lyp1Δ::MATαPr-LEU2*
MKY1419	MKY583*, eaf1 1-982-3XHA::NATMX6*
MKY1420	MKY583*, eaf1 1-939-3XHA::NATMX6*
MKY1421	MKY583*, eaf1 1-893-3XHA::NATMX6*
MKY1422	MKY583*, eaf1 1-825-3XHA::NATMX6*
MKY1423	MKY583*, eaf1 1-815-3XHA::NATMX6*
MKY1424	MKY583*, eaf1 1-713-3XHA::NATMX6*
MKY1425	MKY583*, eaf1 1-644-3XHA::NATMX6*

Strain	Relevant Genotype
MKY1426	MKY583*, eaf1 1-420-3XHA::NATMX6*
MKY1427	MKY583*, eaf1 1-345-3XHA::NATMX6*
MKY1428	MKY583*, eaf1*Δ*-3XHA::NATMX6*
MKY1429	MKY583, *EPL1-3XFLAG:: KANMX6*
MKY1430	MKY583, *epl1 1-485-3XFLAG:: KANMX6*
MKY1431	MKY583, *epl1 1-380-3XFLAG:: KANMX6*
MKY1432	BY4742*, MATa his3*Δ*1, leu2*Δ*0, lys2*Δ*0, ura3*Δ*0*
MKY1433	MKY1432*, eaf1 1-982-3XHA::NATMX6*
MKY1434	MKY1432*, eaf1 1-939-3XHA::NATMX6*
MKY1435	MKY1432*, eaf1 1-893-3XHA::NATMX6*
MKY1436	MKY1432*, eaf1 1-825-3XHA::NATMX6*
MKY1437	MKY1432*, eaf1 1-815-3XHA::NATMX6*
MKY1438	MKY1432*, eaf1 1-713-3XHA::NATMX6*
MKY1439	MKY1432*, eaf1 1-644-3XHA::NATMX6*
MKY1440	MKY1432*, eaf1 1-420-3XHA::NATMX6*
MKY1441	MKY1432*, eaf1 1-345-3XHA::NATMX6*
MKY1442	MKY1432*, eaf1*Δ*-3XHA::NATMX6*
MKY1443	MKY1432, *EPL1-3XFLAG:: KANMX6*
MKY1444	MKY1432, *epl1 1-485-3XFLAG:: KANMX6*
MKY1445	MKY1432, *epl1 1-380-3XFLAG:: KANMX6*
MKY1455	MKY6, *eaf1*Δ*-3X HA::NATMX6 epl1 1-380-3XFLAG:: KANMX6 [pRS316]*
MKY1456	MKY6, *eaf1*Δ*-3X HA::NATMX6 epl1 1-380-3XFLAG:: KANMX6 [pRS316, EAF1]*
MKY1457	MKY6, *eaf1*Δ*-3X HA::NATMX6 epl1 1-380-3XFLAG:: KANMX6 [pRS316, eaf1 HSA*Δ*]*
MKY1458	MKY6, *eaf1*Δ*-3X HA::NATMX6 epl1 1-380-3XFLAG:: KANMX6 [pRS316, eaf1 HSA*Δ*– SANT*Δ*]*

2.4.2 Drug sensitivity measurements

Overnight cultures grown in YPD or SC-Ura at 30°C were diluted to 0.3 $O.D_{600}$. The cells were tenfold serially diluted and spotted onto solid YPD plates or SC-Ura plates with 0.005% MMS (Sigma), 50 mM Hydroxyurea (HU) (Sigma), 1% Formamide (Sigma) or 2 mM Caffeine (Sigma). The plates were then incubated at 30°C for 2-3 days.

2.4.3 Analytical-scale affinity and large-scale affinity purifications

Co-immunoprecipitation assays were performed as described previously (Kobor et al., 2004b). Briefly, yeast cells were harvested, and lysed in TAP-IP Buffer (50 mM Tris [pH 7.8], 150 mM NaCl, 1.5 mM MgAc, 0.15% Nonidet P-40, 1 mM DTT, 10 mM NaPPi, 5 mM EGTA, 5 mM EDTA, 0.1 mM Na_3VO_4, 5 mM NaF, CompleteTM Protease inhibitor cocktail (Roche)) using acid-washed glass beads and mechanically disrupted using a bead beater (BioSpec Products). TAP-tagged fusion proteins were captured using IgG Sepharose beads (Amersham Biosciences) and subsequently washed in TAP-IP buffer. Captured material was analyzed by immunoblotting and copurifying proteins were detected using anti-HA-Peroxidase (Roche) and SuperSignal enhanced chemiluminescence (Pierce Chemical).

Purifications of native protein complexes were performed using extracts from strains encoding the TAP tag fused in-frame to the 3′ end of genes (Rigaut et al., 1999). Purifications were performed from extracts obtained from 2 L cultures that were harvested in late logarithmic phase. Large-scale purification was adapted from protocols previously described with minor modifications (Allard et al., 1999; Cote et al., 1994; Grant et al., 1997b). Briefly, cells were disrupted with a coffee grinder in the presence of

dry ice pellets and resuspended in 0.8 volumes/weight of Esa1-B1 (40 mM Hepes [pH 7.3], 350 mM NaCl, 0.1% Tween 20, 10% Glycerol, 5 mM β-mercaptoethanol, Complete Protease inhibitor cocktail (Roche)). Crude extracts were prepared by centrifugation in a SS34 rotor for 20 min at 14,000 rpm. These were then further clarified by ultracentrifugation (Ti70 rotor, 33,500 rpm for 60 min). The extract was incubated with 200 µl of IgG Sepharose beads (Amersham Biosciences) for 2 h at 4°C. The beads were then washed with 800 µl of Esa1-B2 (40 mM Hepes [pH 7.3], 200 mM NaCl, 0.1% Tween 20, 10% Glycerol, 5 mM β-mercaptoethanol). After washing, the TAP tag was cleaved by adding 10 µl of TEV protease (GIBCO) in 200 µl of Esa1-B2 buffer to the beads and incubating overnight at 4°C. Cleaved protein complexes were eluted with an additional 200 µl of Esa1-B2 + 2 mM $CaCl_2$. The material eluted by the TEV protease cleavage was washed with 200 ul of Esa1-B2 + 4 mM $CaCl_2$ and incubated with 200 µl of Calmodulin beads (Stratagene) for 2 h at 4 °C. Beads were washed with 4 X 100 µl of Esa1-B2 + 2 mM $CaCl_2$ followed by 2X 100 µl of Esa1-B2 + 0.5 mM $CaCl_2$. Finally, the proteins were eluted twice by adding 300 µl of Esa1-EB (50 mM Tris-Cl [pH 8.0], 100 mM NaCl, 5 mM EGTA, 10% Glycerol, 1 mM DTT) to the beads and incubating for 30 min at 4°C. The eluates were pooled and precipitated with trichloroacetic acid.

2.4.4 Chromatin association and histone acetylation assays

The chromatin association assay was adapted from a previously described protocol (Liang et al., 1997). Yeast cells were first incubated in Pre-Spheroblast Buffer (100 mM Pipes/KOH [pH 9.4], 10 mM DTT, 0.1% sodium azide) for 10 min at room temperature. Spheroblasts were produced by addition of 20 mg/ml Zymolyase-100T (Seikagaku

Corporation) in Spheroblast Buffer (50 mM KPO4 [pH 7.5], 0.6 M Sorbitol, 10 mM DTT) and incubated at 37°C for 15 min. Spheroblasts were washed with Wash Buffer (50 mM Hepes/KOH [pH 7.5], 100 mM KCl, 2.5 mM $MgCl_2$, 0.4M sorbitol), resuspended in equal volume of EB (50 mM Hepes/KOH [pH 7.5], 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, and Complete Protease inhibitor cocktail (Roche)), and lysed with 1% Triton X-100. Whole cell extracts (WCE) were collected, and the remaining lysate was separated into chromatin pellet (Pellet) and supernatant (SUP) fractions by centrifugation through EBSX (EB + 0.25% Triton X-100 and 40% sucrose). WCE, Pellet, and SUP were subjected to SDS/ PAGE and immunoblotted with anti-HA-Peroxidase (Roche), anti- H2A (Upstate) and anti-Pgk (Molecular Probes) antibodies. Immunoblots were scanned with the Odyssey Infrared Imaging System (Licor) or visualized with SuperSignal enhanced chemiluminescence (Pierce Chemical).

To determine the relative amounts of H4 and H2A.Z K14 acetylation, whole cell extracts were subjected to SDS/PAGE, immunoblotted with anti-H2A.Z K14ac (Upstate), anti-H4 tetra-ac (Upstate), anti-H4 K5ac (Upstate), anti-H4 K8ac (Abcam), anti-H4 K12ac (Abcam), anti-H4 K16ac (Upstate), and anti-H4 (Abcam) antibodies, and visualized with the Odyssey Infrared Imaging System (Licor).

2.4.5 Chromatin immunoprecipitation (ChIP)

ChIP experiments were performed as described previously (Schulze et al., 2009). In brief, yeast cells (500 ml) were grown in a rich medium to an OD_{600} of 0.5-0.6 and crosslinked with 1% formaldehyde (for 20 min when using anti-H4 tetra-acetylation antibody or 1 h for anti-FLAG antibody) before chromatin was extracted. The chromatin was sonicated (Bioruptor, Diagenode; Sparta, NJ: 10 cycles, 30 s on/off, high setting) to yield an average DNA fragment of 500 bp. Anti-FLAG antibody (Sigma, 4 µl), anti-H4 tetra-ac (Upstate, 4 ul), or anti-H3 (GenScript, Rabbit polyclonal raised to antigen CKDIKLARRLRGERS, order ID: 56737) was coupled to 60 µl of protein A magnetic beads (Invitrogen) per sample. After reversal of the crosslinking and DNA purification, the immunoprecipitated DNA and input DNA were analyzed by quantitative PCR (qPCR). Samples were analyzed in triplicate from three independent ChIP experiments. Epl1-FLAG enrichment was normalized to a region that has no H4 acetylation. H4 acetylation enrichment was normalized for H3 occupancy. Primers used are listed in Table 2.2.

Table 2.2 RT and ChIP-qPCR primers

Name	Method	Primer sequence (5'-3')
RPL19B Prom F	ChIP	CTCATCGCTATGGGAATTGG
RPL19B Prom R	ChIP	TGGTGACTTTTTATTCGATTTGG
RPS11B Prom F	ChIP	CATGTTTCCCGCTTTGTTTT
RPS11B Prom R	ChIP	GATTTTCAACAGACGCAGCA
RPS3 Prom F	ChIP	TTTGTTTCCGTAACATCCATACC
RPS3 Prom R	ChIP	AAGAGCTGCATTGATTTGGAA
Inter. reg. Ctrl F	ChIP	ACCAGTTCGCTCCACTTTGT

Name	Method	Primer sequence (5'-3')
Inter. reg. Ctrl R	ChIP	CTTACGACTCAGGCATGCAA
RPL19B ORF F	RT-qPCR	TGCAAGCGTTAATTCGATTG
RPL19B ORF R	RT-qPCR	ACAACAGAAGCGGCAAGTCT
RPS11B ORF F	RT-qPCR	GAAAAGCAGGACCTCGAATG
RPS11B ORF R	RT-qPCR	TGGCATTAGTCGGGTGAAAT
RPS3 ORF F	RT-qPCR	CACTCCAACCAAGACCGAAG
RPS3 ORF R	RT-qPCR	TCTGACGACACCGTAAGCAG
YIR042C Prom F	ChIP	TACGCCACTCGCTGAATTTG
YIR042C Prom R	ChIP	TTGTAAGCCCAGTAAACAGCTTC
RDS1 Prom F	ChIP	GTCCTCTTAAAGCACTTCAA
RDS1 Prom R	ChIP	GAATCCATCAGAGCATTTCA
GIT1 Prom F	ChIP	TTCATGAATTTCCTTACTGGAC
GIT1 Prom R	ChIP	GTTGACTAGTCACAAGAAACAG
Ctrl H4Ac F	ChIP	ACCAGTTCGCTCCACTTTGT
Ctrl H4Ac R	ChIP	CTTACGACTCAGGCATGCAA
YIR042C ORF F	RT-qPCR	TGCGGGACCATTCACTAAC
YIR042C ORF R	RT-qPCR	AGTGAACCGTTGGCTTCATC
RDS1 ORF F	RT-qPCR	AAGCCGTGAGATTGAAATGG
RDS1 ORF R	RT-qPCR	CTCCATCTGGCACAACAGAA
GIT1 ORF F	RT-qPCR	ATCGGTTCTGTAGTAGGCG
GIT1 ORF R	RT-qPCR	TTACCAGTCCAGCCATTGG
ACT1 ORF F	RT-qPCR	TGTCCTTGTACTCTTCCGGT
ACT1 ORF R	RT-qPCR	CCGGCCAAATCGATTCTCAA

2.4.6 Reverse transcriptase PCR (RT-PCR)

Cells were grown in YPD to an OD_{600} of 0.5. 10 OD_{600} units of cells were harvested for RNA extraction and purification using the Qiagen RNeasy Mini Kit as per the manufacturer's protocol. Genomic DNA was digested with RNase-free DNase I

(Qiagen). cDNA was synthesized using SuperScript III First-Strand Synthesis System for RT–PCR and oligo(dT) (Qiagen). cDNA was analyzed using the Rotor-Gene 6000 (Corbett Research) and PerfeCTa SYBR Green FastMix (Quanta Biosciences). *RPL19B*, *RPS11B*, and *RPS3* mRNA were normalized to *ACT1* mRNA levels. Results are represented relative to untagged wild-type set as 1.0. Samples were analyzed in triplicate from three independent RNA preparations. Primers used are listed in Supplementary Table 2.

2.4.7 E-MAP

E-MAP assay was performed as described previously (Schuldiner et al., 2006a). Briefly, using a Singer robot, *EAF1* and *EPL1* alleles were crossed to a library of 1,536 mutants representing a number of processes, including transcription, RNA processing, and chromatin biology. All strains were screened three to four times, and scores were calculated as previously described (Collins et al., 2010; Schuldiner et al., 2006a).

2.4.8 mRNA expression profile

Expression profiling was performed as described previously (van de Peppel et al., 2003). Briefly, *eaf1* and *epl1* mutants were processed four times from two independently inoculated cultures. Also, sets of mutants were grown together with additional wild-type cultures in order to correct for other sources of variations. Dual-channel 70-mer oligonucleotide arrays were used with a common reference wild-type RNA. After RNA isolation, all steps were operated using robotic liquid handlers. Scores were calculated as previously described (van Bakel et al., 2004; van de Peppel et al., 2003). Differentially

regulated genes were determined by p value < 0.05, Fold change (FC) +/- > 1.7). Enrichment for Gene Ontology Fat terms was performed using the DAVID service (http://david.abcc.ncifcrf.gov/summary.jsp) (Huang da et al., 2009a; Huang da et al., 2009b; Sherman et al., 2007).

Chapter 3

Which complex do you work for? – the role of the module shared between NuA4 and SWR1-C

3.1 Introduction

Reminiscent of nuclear RNA polymerases, several chromatin-modifying complexes contain identical subunits, suggesting some degree of relationship or common mechanistic features. In budding yeast, there are several examples of chromatin modifying complexes containing in part the same subunits that execute distinct functions, including SWI/SNF and RSC, SWR1-C and INO80, and SWR1-C and NuA4 (P. Y. Lu et al., 2009; Morrison et al., 2009; Vignali et al., 2000). Beyond the physical association of these shared subunits, their specific roles are not well defined and many questions remain unanswered. For instance, do the shared subunits perform the same role in both complexes? Are they equally important for the function of both complexes? Do they behave as a single unit or does every component of a shared subunit module have its own unique function?

The histone acetyltransferase (HAT) NuA4 shares subunits with SWR1-C, an ATP dependent chromatin-remodelling complex that replaces canonical histone H2A with the histone variant H2A.Z primarily in gene promoter regions and at heterochromatin boundaries (Guillemette et al., 2005a; Kobor et al., 2004a; Krogan et al., 2004; Li et al., 2005a; Mizuguchi et al., 2004; Raisner et al., 2005; Zhang et al., 2005b). As determined

by synthetic lethal genetic interactions, these two complexes share not only subunits but also an essential function (Kobor et al., 2004a). Moreover, cells require these two complexes for resistance to DNA damage and genotoxic stress (Kobor et al., 2004a). In the yeast *Saccharomyces cerevisiae,* NuA4 is the only essential HAT and is responsible for acetylation of histones H4, H2A, and H2A.Z, as well as several non-histone substrates (Babiarz et al., 2006; Doyon et al., 2004; Jeong et al., 2011; Keogh et al., 2006; Y. Y. Lin et al., 2008; Lin et al., 2009; Lu, Lin, Sheu et al., 2011; Millar et al., 2006; Mitchell et al., 2011). The catalytic subunit of NuA4, Esa1, and several other subunits are also found in a smaller entity called picNuA4, which in contrast to the locus-specific acetylation by NuA4, is involved in untargeted acetylation (Allard et al., 1999; Auger et al., 2008; Boudreault et al., 2003; Nourani et al., 2004; Reid et al., 2000; Selleck et al., 2005). While present on its own in a fraction distinct from NuA4, large amounts of picNuA4 can be liberated from NuA4 by removal of either the C-terminus of the Epl1 subunit or removal of the entire scaffolding subunit Eaf1 (Auger et al., 2008; Boudreault et al., 2003; Mitchell et al., 2008; Selleck et al., 2005). In the latter scenario, additional distinct associated groups of NuA4 subunits can be isolated, including the module shared with SWR1-C composed of Swc4, Arp4, Yaf9, and Act1 (Auger et al., 2008; Szerlong et al., 2008). Of these, Yaf9 and Swc4 are exclusively present in SWR1-C and NuA4, whereas Arp4 and Act1 are also found in the chromatin remodelling complex INO80 (Doyon et al., 2004).

Here, to deduce the specific contributions of the shared module, we focused on the two unique components Yaf9 and Swc4. While these two proteins physically interact through

their C-terminus (Bittner et al., 2004), suggesting a close cooperation, they are fundamentally different in that *YAF9* is a non-essential gene whereas with the exception of one report (Zhou et al., 2010), *SWC4* is an essential gene. Regardless, mutant yeast strains lacking *YAF9* or containing hypomorphic alleles of *SWC4* share common sensitivities to DNA damaging and genotoxic agents such as benomyl, methyl-methanesulfonate (MMS), and hydroxyurea (HU). This is generally consistent with phenotypes associated with SWR1-C and NuA4, and might directly or indirectly point to a role of these complexes in chromosome segregation, DNA replication, and DNA repair (Auger et al., 2008; Auger et al., 2008; Bittner et al., 2004; Kobor et al., 2004a; Krogan et al., 2003; Le Masson et al., 2003; Micialkiewicz et al., 2008; Zhang et al., 2004). Perhaps not surprisingly given their presence in NuA4 and SWR1-C, both Swc4 and Yaf9 are involved in histone H4 acetylation and H2A.Z deposition at heterochromatin boundary regions (Wang et al., 2009a; Zhang et al., 2004; Zhou et al., 2010). At least in part, the function of H2A.Z in restricting the spread of heterochromatin is mediated by acetylation at lysine residues in its amino-terminal tail (Babiarz et al., 2006; Meneghini et al., 2003; Zhang et al., 2004). While H2A.Z acetylation requires Yaf9, and specifically its YEATS domain, it has not been determined whether Swc4 is also involved (Wang et al., 2009a). Mechanistically, it is likely that the shared module exerts its role at least in part through histone or nucleosome binding. Specifically, Yaf9 can bind H3 and H4 *in vitro*, Arp4 binds to nucleosomes *in vitro*, and Swc4 contains a SANT domain, which in other proteins has been shown to bind histones and DNA (Aasland et al., 1996; Baker et al., 2009; Boyer et al., 2004; Downs et al., 2004b; Galarneau et al., 2000; P. Y. Lu et al., 2009; Park et al., 2010; Ryan et al., 2011; Sterner et al., 2002; Wang et al., 2009b; Yu et

al., 2003). Thus, it is tempting to speculate that the shared module contributes to chromatin docking of both NuA4 and SWR1-C, and perhaps even facilitates their exchange during H2A.Z deposition and acetylation. An attractive model evokes sequential action of these two enzymes, with NuA4 first acetylating histone H4 to facilitate recruitment of SWR1-C, which then replaces H2A with H2A.Z at specific promoter nucleosomes (Wu et al., 2009; Lu et al., 2009). Finally, mediated by the resident shared subunit module, NuA4 is solicited one more time to acetylate the newly deposited H2A.Z (Altaf et al., 2010; Babiarz et al., 2006; Keogh et al., 2006; Wu et al., 2009; Zhou et al., 2010).

In this chapter, we took advantage of the opportunity offered by the properties of Yaf9 and Swc4, as unique components of the shared module, to decipher its specific contribution as an entity to SWR1-C or NuA4. Furthermore, we tested whether Yaf9 and Swc4 equally contributed to the identity of the shared module or had distinct functions. To address these questions, we utilized a combination of genetic, biochemical, and functional genomic assays to compare the genes encoding subunits of the shared module to genes encoding specific components of either SWR1-C or NuA4. Strains lacking Yaf9 or the C-terminus of Swc4 had slightly different defects when grown on genotoxic drugs, but both showed compromised H2A.Z incorporation into chromatin and H2A.Z acetylation. Furthermore, Yaf9 and Swc4 tended to behave more like SWR1-C when assayed for global genetic interaction patterns and gene expression changes. The latter was particularly pronounced in subtelomeric regions, consistent with the established role of H2A.Z in forming heterochromatin boundaries. However, while the shared-module

level analysis pointed to a stronger association of this moiety with SWR1-C as compared to NuA4, large-scale genetic interaction analysis also revealed clear differences between Yaf9 and Swc4, with the latter more closely aligning in these cases with NuA4. Consistent with a more complex relationship existing within the shared module, the sensitivity of the *ya9f*Δ mutant to genotoxic agents and its defects in forming heterochromatin boundaries were partly rescued when combined with an allele encoding a C-terminal truncation of Swc4.

3.2 Results

3.2.1 The shared module contributed to cellular functions associated with both NuA4 and SWR1-C

To evaluate the contribution of the shared module to NuA4 and SWR1-C function, we compared different alleles representing each complex or submodule in standard assays for functional deficits associated with these moieties. These included resistance to genotoxic stress, H4 and H2A.Z acetylation, and H2A.Z chromatin association. Specifically, we used strains lacking either *YAF9* or the *swc4 1-400* allele encoding a carboxy-terminal truncation of Swc4, which likely abolishes the binding to Yaf9 (Bittner et al., 2004). To represent loss of NuA4 activity or increased picNuA4, respectively, we included a complete deletion of *EAF1* and an allele encoding a C-terminal truncation of Epl1 (*epl1 1-380*) (see Chapter 2) (Auger et al., 2008; Boudreault et al., 2003; Mitchell et al., 2008; Selleck et al., 2005). Lastly, a complete deletion of *SWR1* was used to represent loss of SWR1-C function and thus H2A.Z deposition. Growing these different mutants on

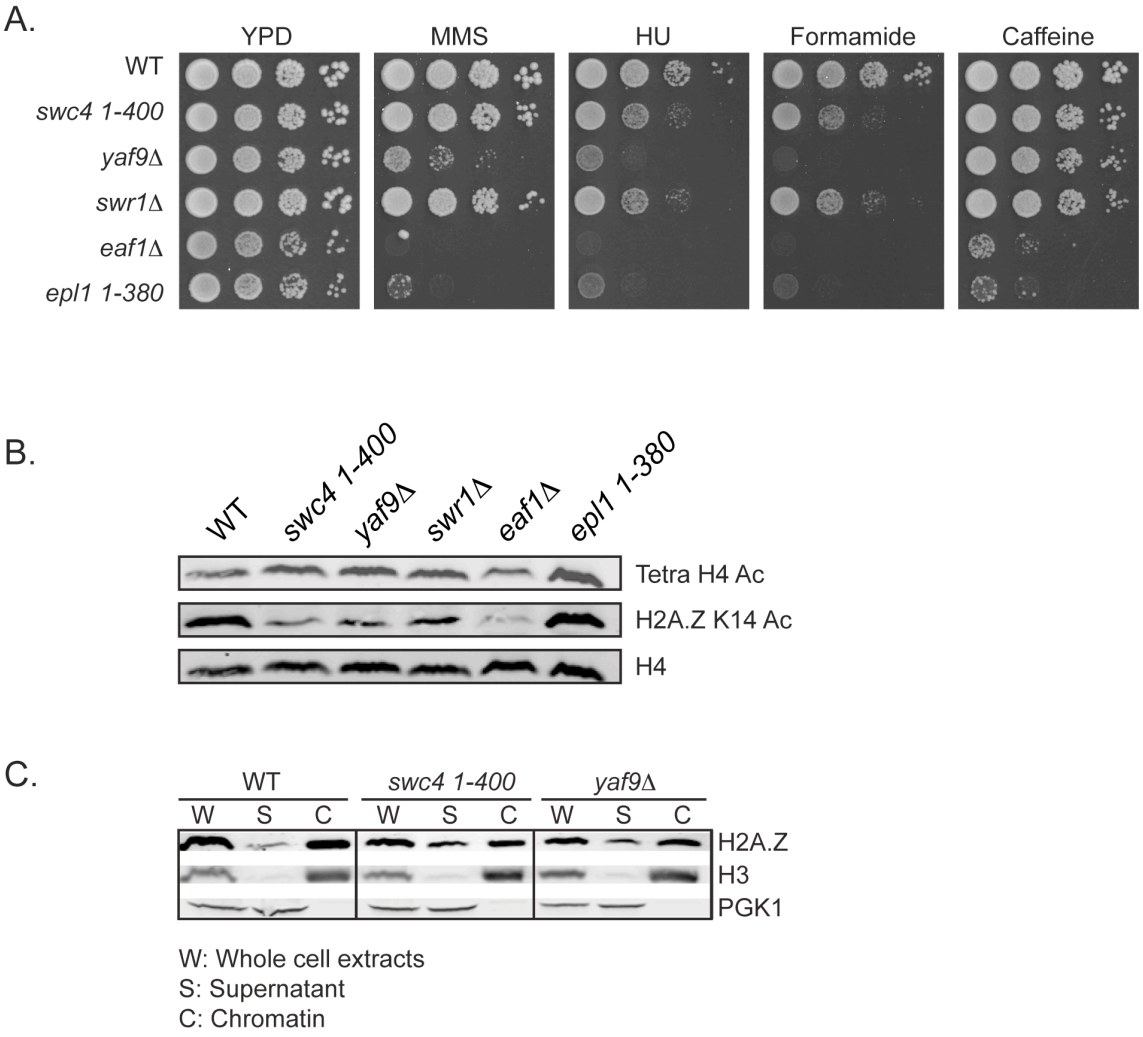


Figure 3.1 The shared module contributed to cellular functions associated with both NuA4 and SWR1-C. (A) Ten-fold serial dilutions of the indicated strains were plated onto media containing 0.015% MMS, 100mM HU, 2% Formamide, or 3mM Caffeine. (B) Whole cell extracts of the indicated strains were analyzed by protein blotting with anti-H4 tetra-acetylation or H2A.Z K14 acetylation antibodies. Antibodies against H4 were used as a loading control. (C) *swc4 1-400* and *yaf9*Δ mutants were defective H2A.Z chromatin deposition. W, whole cell extract; S, supernatant; C, chromatin pellet. The relative amount of Htz1 in each fraction was determined by immunoblotting in the different strains. Antibodies against histone H2A and Pgk1 were used as loading controls for chromatin pellet and supernatant, respectively.

relatively low levels of genotoxic agents such as MMS, HU, formamide, and caffeine to carefully compare their phenotypes, we observed general overlap yet distinct quantitative differences (Fig. 3.1A). Specifically, the *yaf9*Δ mutant was more sensitive than *swc4 1-400* mutant to HU, formamide, and MMS, with the latter not having an appreciable effect on the *swc4 1-400* mutant at the concentration used. The growth of the *swr1*Δ mutant closely resembled that of the *swc4 1-400* mutant, thus being less severely affected than the *yaf9*Δ mutant. Suggestive of a more pronounced requirement for NuA4 or picNuA4, the *eaf1*Δ and *epl1 1-380* mutants had the strongest phenotypes across the spectrum of genotoxic agents used here (Fig. 3.1A).

Given that the activities of SWR1-C and NuA4 converge most closely upon acetylation of H2A.Z, we next tested the contribution of the shared subunits in this process. As determined by immunoblotting of whole cell extracts, strains containing the *swc4 1-400* allele had greatly reduced levels of H2A.Z acetylation, similar to the *yaf9*Δ and *swr1*Δ mutants (Fig. 3.1B) (Wang et al., 2009a). As reported previously, loss of core NuA4 subunit Eaf1 also affected H2A.Z acetylation levels (Babiarz et al., 2006), whereas the loss of the Epl1 C-terminus did not cause any reduction (see also Chapter 2). The H2A.Z acetylation defects of the *yaf9*Δ and *swc4 1-400* mutants were not likely due to a reduction of overall HAT activity, as these strains did not have decreased bulk acetylation of histone H4, which is the major NuA4 catalytic substrate (Fig. 3.1B) (Le Masson et al., 2003; Kobor et al., 2004a; Zhou et al., 2010). Next, we tested the requirement of the shared subunits for H2A.Z deposition into chromatin by SWR1-C using a cellular

fractionation assay (Fig. 3.1C). Similar to the acetylation profile, separating chromatin-bound proteins (pellet fraction) from the rest of the cell (supernatant fraction) showed that H2A.Z association with chromatin was equally impaired in the *yaf9*Δ and *swc4 1-400* mutants (Fig. 3.1C). Thus, perturbation of the shared module via deletion of *YAF9* or removal of the Swc4 C-terminus revealed varying requirement for these subunits when cells are exposed to genotoxic agents. In contrast, bulk measures of histone H4 and H2A.Z acetylation as well as H2A.Z deposition suggested a common role more akin to SWR1-C.

3.2.2 Global genetic and gene expression analysis revealed distinct functional requirements of the shared module

To further dissect the requirement of the shared module for either SWR1-C or NuA4 function, we generated genetic interaction profiles using epistatic miniarray profiling (E-MAP). This assay quantitatively measures aggravating and alleviating genetic interactions between our mutant strains and a library of 1,536 mutant genes involved in transcription, RNA processing, and chromatin biology (Schuldiner, Collins, Weissman, and Krogan, 2006a). Overall correlation analysis of the genetic interaction profiles showed that the *yaf9*Δ and *swc4 1-400* clustered more closely with the *swr1*Δ mutant as opposed to the NuA4-specific mutants *eaf1*Δ and *epl1 1-380* (Fig. 3.2A). Upon closer inspection, the E-MAP analysis revealed clear similarities and differences between the shared module and its resident complexes. The most striking differences were seen in cases where genes encoding the NuA4-specific subunits Eaf1 and Epl1 had an opposite genetic interaction profile to *swr1*Δ, with *yaf9*Δ and *swc4 1-400* tending to behave

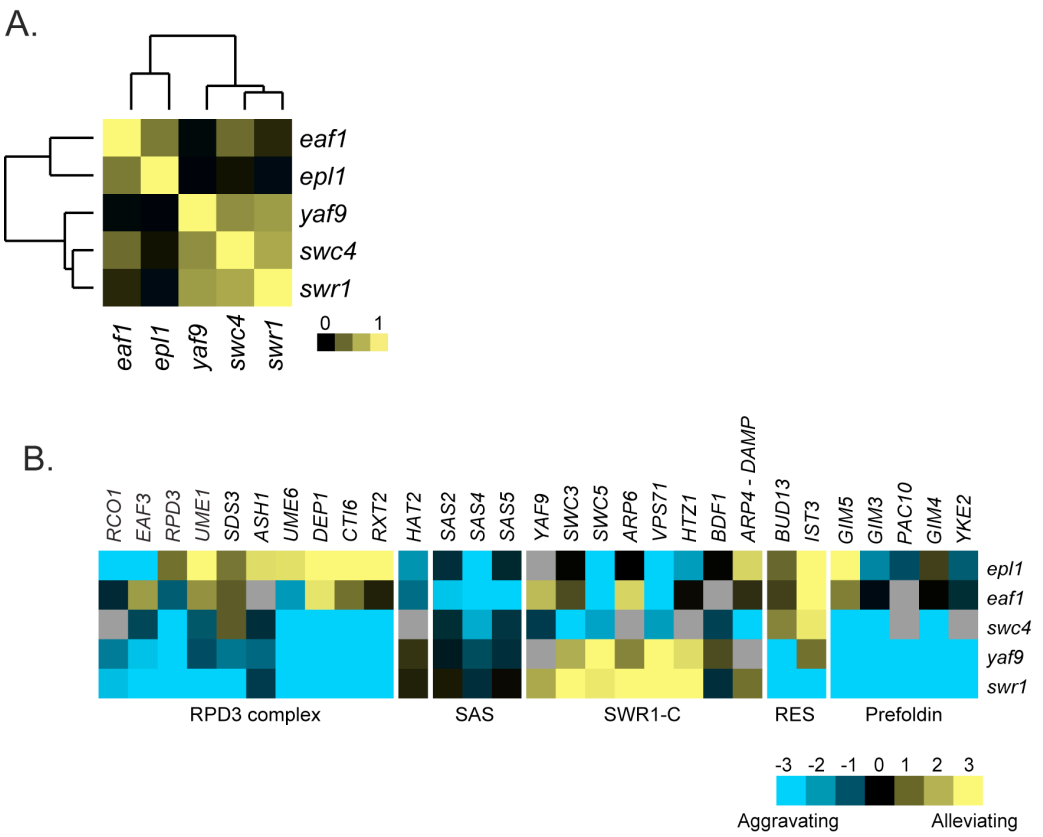


Figure 3.2 Genetic analysis revealed similarities and distinctions between NuA4 and SWR1-C shared module mutants. (A) Unsupervised hierarchical cluster matrix of *eaf1*Δ, *epl1 1-380*, *swc4 1-400*, *yaf9*Δ, and *swr1*Δ alleles. (B) Genetic interaction profiles of *eaf1*Δ, *epl1 1-380*, *swc4 1-400*, *yaf9*Δ, and *swr1*Δ alleles with indicated complexes. Blue indicates aggravating interactions, yellow represents alleviating interactions, and gray denotes missing data. RES: pre-mRNA retention and splicing complex.

similarly to *swr1*Δ. This was exemplified by the interaction profile with genes encoding members of the Rpd3L complex, which had alleviating interactions with the NuA4 node and aggravating interactions with the SWR1-C node, with latter including yaf9 and *swc4 1-400*. Similarly, clearly distinct interaction profiles with several HAT complexes such as HAT2 and SAS were evident, as these had aggravating interactions with *eaf1*Δ and *epl1 1-380,* while not interacting genetically with *yaf9*Δ, *swc4 1-400*, and *swr1*Δ. In an opposite example of this general category, *yaf9*Δ, *swc4 1-400*, and *swr1*Δ alleles negatively interacted with mutants of the prefoldin complex, while *eaf1*Δ and *epl1 1-380* did not exhibit any strong interactions (Fig. 3.2B). In addition to the scenarios above in which both *yaf9*Δ and *swc4 1-400* behaved similarly, we also observed instances where *swc4 1-400* and *yaf9*Δ mutants themselves had different genetic interaction profiles, thus separating individual components within the shared module. In these cases, *yaf9*Δ generally behaved more like the *swr1*Δ mutant while *swc4 1-400* resembled more closely the *eaf1*Δ and *epl1 1-380* mutants. One striking example for this was the positive interaction profile of *yaf9*Δ with genes encoding subunits of SWR1-C itself, a situation often observed within members of the same protein complex (Schuldiner, Collins, Weissman, and Krogan, 2006b). This contrasted with the negative interaction profile of *swc4 1-400*, *eaf1*Δ and *epl1 1-380* with genes encoding SWR1-C subunits. The interaction pattern with genes encoding the RES complex, which is involved in pre-mRNA retention and splicing, constituted another similar example of opposing behaviour between *yaf9*Δ and *swc4 1-400* (Fig. 3.2B).

To complement the E-MAP, we also used DNA microarray technology to determine gene expression profiles for the same set of mutants. Consistent with the E-MAP profiles, expression profiles of *swc4 1-400* and *yaf9*Δ mutants were more correlated to the *swr1*Δ mutant than the *eaf1*Δ and *epl1 1-380* mutants (Fig. 3.3A). Interestingly, the correlation pattern was dependent on the direction of the expression change observed. Specifically, when only including the 52 genes that were down-regulated in the shared module mutants, the *swc4 1-400* and *yaf9*Δ mutants clustered closer to the *swr1*Δ mutant (Fig. 3.3B). In contrast, the *swc4 1-400* and *yaf9*Δ mutants clustered closer to the NuA4-specific mutants *eaf1*Δ and *epl1 1-380* when the analysis was limited only to the 63 genes that were up-regulated in mutants of the shared module (Fig. 3.3C).

Close examination of the expression changes revealed that subsets of co-expressed genes drove the overall clustering. Specifically, and similar in principle to the E-MAP analysis, we observed distinct groups of genes whose expression changes in the *yaf9*Δ and *swc4 1-400* mutants were similar to the ones measured in the *swr1*Δ mutant, while other groups were more similar to the changes observed in the mutants encoding NuA4-specific subunits. The biological specificity of these changes was reflected in distinct Gene Ontology (GO) enrichment terms associated with the significantly up or down-regulated genes (Fig. 3.3D). For instance, groups of down-regulated genes in *swc4 1-400*, *yaf9*Δ and *swr1*Δ mutants were enriched for "Amino acid and vitamin metabolic processes" and "Sporulation" while Eaf1 and Epl1-dependent genes were enriched for "Cell wall organization" and "RNA processing" (Fig. 3.3D). In contrast, groups of genes that were

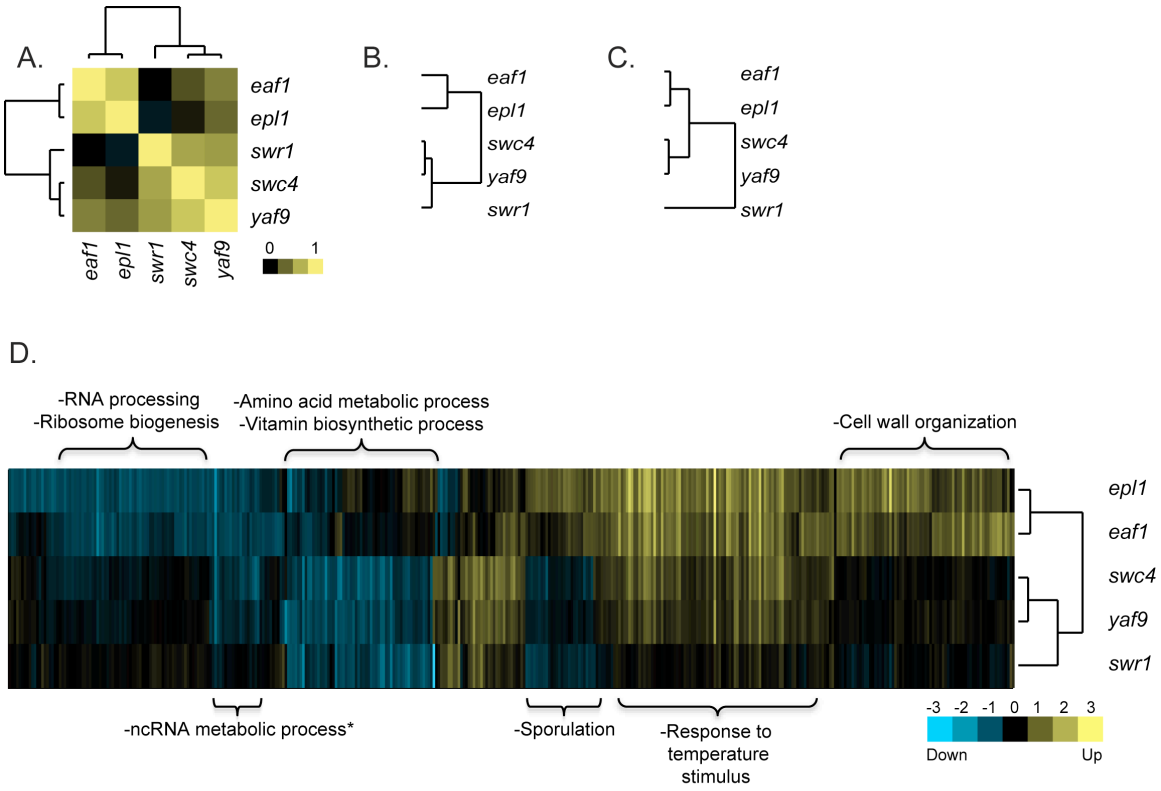


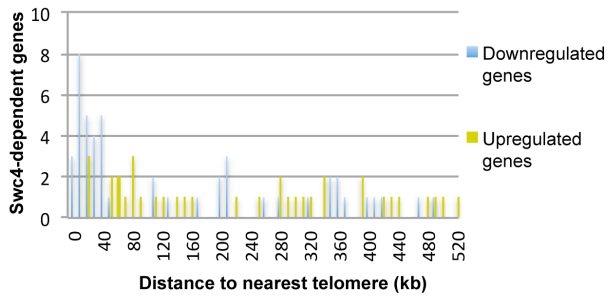
Figure 3.3 Gene expression analysis revealed similarities and distinctions between NuA4 and SWR1-C shared module mutants. (A) Unsupervised hierarchical cluster matrix of *eaf1*Δ*, epl1 1-380*, *swc4 1-400*, *yaf9*Δ, and *swr1*Δ alleles with their respective up- and down-regulated genes. Unsupervised hierarchical cluster matrix of *eaf1*Δ*, epl1 1-380*, *swc4 1-400*, *yaf9*Δ, and *swr1*Δ alleles restricted to genes that are only (B) down- or (C) up-regulated in *swc4 1-400* and *yaf9*Δ mutants. Yellow indicates up-regulated genes, and blue represents down-regulated genes. (D) Gene expression profile showing the enrichment for Gene Ontology Fat terms for *eaf1*Δ*, epl1 1-380*, *swc4 1-400*, *yaf9*Δ, and *swr1*Δ mutants using significant values ($p < 0.05$, $FC > 1.7$) Indicated significant GO term enrichment clusters: $p < 0.05$, * non-significant enrichment.

up- or down-regulated in *swc4 1-400*, *yaf9*Δ, *eaf1*Δ, and *epl1 1-380* mutants were significantly enriched for "Response to temperature stimulus" and tended to be enriched for "ncRNA metabolic process" (Fig. 3.3D).

3.2.3. Shared subunits contributed to SWR1-C dependent regulation of gene expression at subtelomeric regions

Given the established function of SWR1-C and H2A.Z of regulating gene expression at heterochromatin boundaries (Babiarz et al., 2006; Meneghini et al., 2003; Zhang et al., 2004), we next asked whether Swc4 contributed to this role by determining the chromosomal location of genes whose expression was altered in the *swc4 1-400* strain (Fig. 3.4A). Genes whose expression was down-regulated in *swc4 1-400* mutant had a highly significant enrichment for location within 20 kb of chromosomal ends ($p=1.4X10^{-5}$ as determined by hypergeometric distribution), while up-regulated genes were found randomly distributed across the chromosomes (Fig. 3.4B). As expected, and consistent with the existing literature, the same result was found for genes down-regulated in *yaf9*Δ and *swr1*Δ mutants (Babiarz et al., 2006; Zhang et al., 2004), but not for *eaf1*Δ and *epl1 1-380* mutants (data not shown) (Fig. 3.4C). Thus, these data suggested that Swc4 similar to Yaf9, contributed to SWR1-C function at subtelomeric euchromatin-heterochromatin boundary.

A.



B.

Bin	Number of Swc4-dependent downregulated genes	Total number of genes	p-value
0-10 kb	3	138	0.075
10-20 kb	8	143	1.40E-05
20-30 kb	5	142	0.0045
30-40 kb	4	146	0.023
> 40 kb	30	5631	3.80E-09
Total	50	6200	

C.

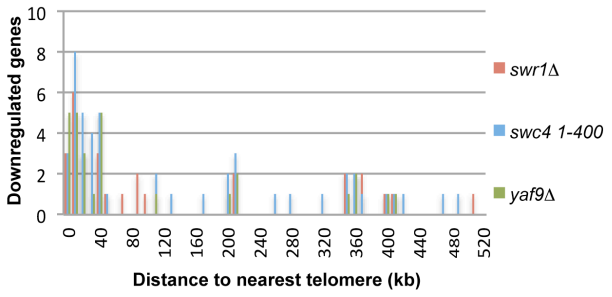


Figure 3.4 Shared subunits played a role in SWR1-C function in regulating gene expression at subtelomeric regions. (A) Histogram showing the number of Swc4-dependent genes with their distance to the nearest telomere end. (B) Statistical significance of the enrichment of *swc4* down-regulated genes with their distance to the nearest chromosome end, which was determined using the hypergeometric function (Tavazoie et al. 1999). (C) Histogram showing the number of down-regulated genes in *swc4 1-400*, *yaf9*Δ, and *swr1*Δ with their distance to the nearest telomere end.

3.2.4 Deletion of the Swc4 C-terminus could partially rescue defects caused by loss of Yaf9

Our large-scale genomic assays not only revealed similarities but also nuanced qualitative differences between *YAF9* and *SWC4*, suggesting that there might be a separation of function within the shared module. To more formally explore the relationship between these two components, we used genetic epistasis analysis. As shown in Fig. 3.1A, a *swc4*

mutant was generally less sensitive than a *yaf9* mutant when exposed to genotoxic agents. Surprisingly, the *swc4 1-400 yaf9*Δ double mutant grew similar to the *swc4 1-400* single mutant, suggesting that Swc4 C-terminus was in part responsible for detrimental phenotype observed in the *yaf9*Δ mutant (Fig. 3.5A). Next, we tested whether this surprising genetic relationship was further reflected in a more complicated mechanistic interaction. However, the impaired H2A.Z association with chromatin observed in the *yaf9*Δ and *swc4 1-400* mutants was not rescued in the *yaf9*Δ *swc4 1-400* double mutant (Fig. 3.5B) nor was the H2A.Z acetylation defect (Fig. 3.5C).

Next, we extended this analysis to the relationship of Yaf9 and Swc4 at subtelomeric euchromatin-heterochromatin boundaries. Guided by our global gene expression profiles, we selected for quantitative analysis a subtelomeric gene (*YCR099C*) that was down-regulated in both the *yaf9*Δ and *swc4 1-400* mutants, along with two neighbouring genes (*YCR101C* and *YCR094C*), whose expression was not significantly down-regulated in the DNA microarray studies. Using quantitative reverse transcriptase (qRT) PCR, we quantified the mRNA levels of *YCR101C*, *YCR099C*, and *YCR094W* in wild-type, *swc4 1-400* and *yaf9*Δ single mutants, and the *swc4 1-400 yaf9*Δ double mutant. Consistent with Yaf9's function in forming the subtelomeric heterochromatin boundary, mRNA levels of *YCR101C*, *YCR099C*, and *YCR094W* were significantly lower in the *yaf9*Δ mutant (Fig. 3.5D). The expression of *YCR099C* and *YCR094W* was decreased in the *swc4 1-400* mutant while *YCR101C* was increased. However, for *YCR099C* and *YCR101C*, the expression levels in the *swc4 1-400 yaf9*Δ double mutant were

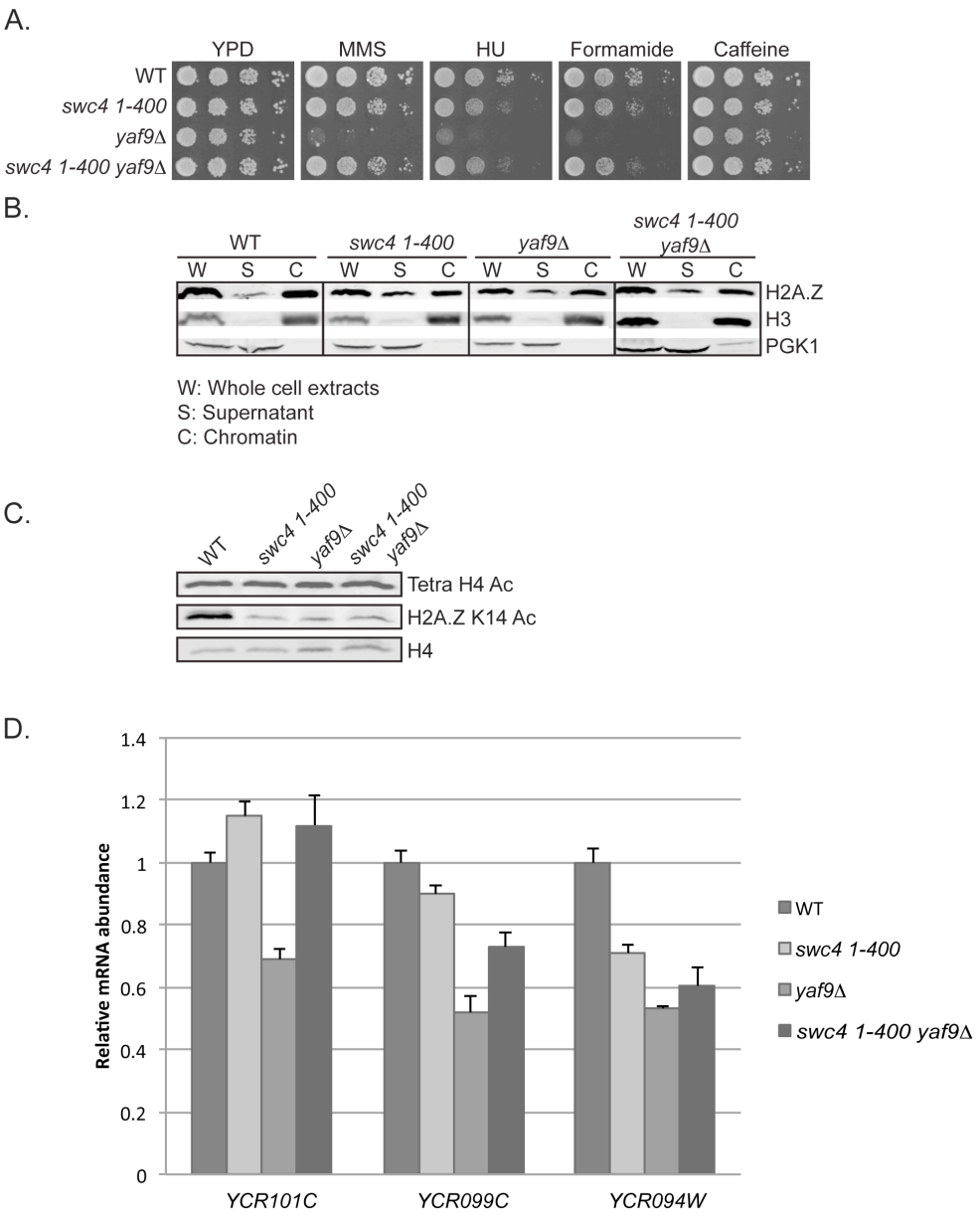


Figure 3.5 Partial rescue of defects caused by loss of Yaf9 by removal of the Swc4 C-terminus. (A) Ten-fold serial dilutions of the indicated strains were plated onto media containing 0.015% MMS, 100mM HU, 2% Formamide, or 3mM Caffeine. (B) *swc4 1-400*, *yaf9*Δ single mutants and *swc4 1-400 yaf9*Δ double mutants showed decreased H2A.Z deposition into chromatin. W, whole cell extract; S, supernatant; C, chromatin pellet. The relative amount of Htz1 in each fraction was determined by immunoblotting in the different strains. Antibodies against histone H2A and Pgk1 were used as loading controls for chromatin pellet and supernatant, respectively. (C) Whole cell extracts of indicated strains were analyzed by protein blotting with anti-H4 tetra- or H2A.Z K14 acetylation antibodies. Antibodies against H4 were used as loading controls. (D) *swc4 1-400* mutant suppressed a silencing defect observed in *yaf9*Δ mutant. Total cellular mRNA was prepared and measured by RT-qPCR. *YCR101C*, *YCR099C*, and *YCR094C* mRNA levels were normalized to that of *ACT1*. Results are represented relative to untagged wild-type set as 1.0. Error bars represent standard errors of the means for three independent experiments.

significantly higher compared to *yaf9*Δ single mutant (Fig. 3.5D). This suggested that the *swc4 1-400* mutant partially suppressed the *yaf9*Δ single mutant defect at subtelomeric heterochromatin boundaries.

3.3 Discussion

In this chapter, we conceptually took advantage of Yaf9 and Swc4 being unique members of the four-proteins module shared between NuA4 and SWR1-C to derive broader principles of functional associations of biochemically shared entities with their distinct resident complexes. Specifically, we studied the role of the shared module in yeast strains lacking Yaf9 or the C-terminus of Swc4 using a combination of genetic, biochemical, and functional genomic assays. Taken together, the data from the two genomic approaches used here suggested that as an entity, the coordinated shared module contributed predominantly to SWR1-C function as opposed to NuA4, although analysis also revealed that Swc4 behaved more akin to a role in NuA4, while Yaf9 behaved more similarly to SWR1-C. However, *yaf9*Δ and *swc4 1-400* mutants both had a similar defect in H2A.Z chromatin association and H2A.Z acetylation. In contrast, these mutants had slightly different phenotypes when exposed to genotoxic agents and in forming heterochromatin boundaries, with strains lacking *YAF9* generally being more sensitive. Interestingly, epistasis analysis showed that the sensitivity of the *yaf9*Δ mutant to genotoxic agents as well as its defects in maintenance of heterochromatin boundaries were partly rescued when combined with the *swc4 1-400* allele. Overall, this study exposed important aspects of the intricate relationship between chromatin modifying complexes and their shared

modules while also pointing to distinct functional associations of different components of shared modules.

Using E-MAP analysis, we first interrogated the relationship of *YAF9* and *SWC4* with other genes involved in chromatin biology and gene regulation, and how these corresponded to the interactions specific for genes encoding either NuA4 or SWR1-C subunits. Overall, we observed closer clustering of the *swc4 1-400* and *yaf9*Δ mutants with the *swr1*Δ mutant compared to mutants encoding NuA4 subunits. This suggested that the shared subunits engage in a central role in SWR1-C, but play a more auxiliary one in NuA4 function. This was best exemplified by the genetic interaction profiles with mutants encoding subunits of the prefoldin complex, with the negative interactions of *yaf9*Δ, *swc4 1-400* and *swr1*Δ contrasting the lack of interactions displayed by *eaf1*Δ and *epl1 1-380*. Given that the prefoldin complex is involved in actin folding, it is tempting to speculate that these genetic interactions are reflective of a role for actin in SWR1-C function (Vainberg et al., 1998). In principle, this could occur by at least two distinct mechanisms. First, although filamentous actin is not present in chromatin-remodeling complexes, it has been shown that chromatin regulatory complexes might attach to the predominant actin filaments, which in turn might facilitate their anchoring or movement within the nucleus (Fenn et al., 2011; Gottschalk et al., 2008; Rando et al., 2002; Szerlong et al., 2008; Vignali et al., 2000). Second, it is of note that the formation of the actin-Arp4 heterocomplex is required for stability of the yeast INO80 chromatin-remodeling complex, a close relative of SWR1-C (Nishimoto et al., 2012). However, it is not clear at this point whether prefoldin has a role in regulating the non-filamentous form

of actin commonly found in chromatin remodeling complexes, nor has it been investigated whether actin and Arp4 exert a similar structural role in SWR1-C or NuA4. Regardless, the genetic interactions observed here suggested that in contrast to NuA4, SWR1-C regulation might require proper actin folding or actin-mediated complex stability.

Further support for the generally closer association of the shared subunits with SWR1-C compared to NuA4 was provided by genetic interactions profiles with complex components more proximal to chromatin. Specifically, whereas *eaf1*Δ and *epl1 1–380* had negative interactions with genes encoding subunits of several HDAC and HAT complexes, *swr1*Δ, *yaf9*Δ, and *swc4 1-400* mutants did not have significant interactions with these. In conjunction with fact that Yaf9 and the C-terminus of Swc4 were also dispensable for bulk H4 acetylation, the genetic interaction profiles suggested that the shared module did not make a major contribution to the balance of proper histone acetylation levels maintained by the core NuA4 complex.

Reminiscent to the principles derived from the E-MAP results, gene expression analysis revealed as well a closer clustering of the shared module to *swr1*Δ. Again, the gene expression overlaps were not exclusive to genes dependent on SWR-1C, as clustering of profiles and GO term enrichment analysis demonstrated that the shared module was also important for some genes regulated more generally by NuA4 (Fig. 3.3C). In addition to the closer association of the shared module as an entity with SWR1-C, in particular the E-MAP profiles revealed situations in which there was an apparent disjoint between the

specific functional affiliations of Yaf9 and Swc4 and their two resident complexes. For example, a *yaf9* mutant displayed aggravating interactions with genes encoding subunits of RES complex, which is involved in pre-mRNA retention and splicing, contrasting with the alleviating interactions observed with *swc4 1-400*, *eaf1*Δ and *epl1 1-380* mutants.

The biochemical analyses performed here might provide an explanation for the closer clustering of Yaf9 and Swc4 with SWR1-C as opposed to NuA4 in genetic interaction and gene expression analysis. In the context of the model proposing two sequential actions of NuA4 and SWR1-C in depositing and acetylating H2A.Z through complex exchange mediated by the shared subunits, we propose that the shared module might not be required for the initial NuA4 recruitment step, which leads to H4 acetylation. Instead, compromising the function of the shared submodule might result in defects in subsequent SWR1-C recruitment or functional coordination and H2A.Z deposition, with the latter being a prerequisite for NuA4-dependent acetylation of chromatin-associated H2A.Z. Strong support for this arises from my findings of *yaf9*Δ and *swc4 1-400* mutants having defects in bulk H2A.Z acetylation, but not in H4 acetylation (Altaf et al., 2010; Babiarz et al., 2006; Keogh et al., 2006; Le Masson et al., 2003; P. Y. Lu et al., 2009; Wang et al., 2009b; Wu et al., 2009; Zhou et al., 2010). Furthermore, previously published data demonstrates that *swc4* and *yaf9* mutants have a defect in H2A.Z deposition (Zhang et al., 2004; Zhou et al., 2010). Taken together, this suggested that the shared subunits are required for two SWR1-C dependent processes converging on H2A.Z biology: directly through H2A.Z deposition and either directly or indirectly through acetylation of H2A.Z.

Perhaps the most surprising result of these studies was the suppression of phenotypes caused by loss of Yaf9 when the C-terminus of Swc4 was removed simultaneously. While initially motivated by the differences between *yaf9*Δ and *swc4 1-400* uncovered by E-MAP, this somewhat paradox relationship was evident when double mutants were assayed broadly for growth on genotoxic drugs, or specifically for their ability to form heterochromatin boundaries. Given that various reports point to a close connection between the DNA damage response and heterochromatin formation, it is tempting to speculate that the suppression seen here is reflective of a joint intersection of SWR1-C and NuA4 in these processes (Zhang et al., 2004; Zhou et al., 2010). However, my data do not provide direct evidence for such a relationship, and thus the suppression could be indicative of unrelated functions of these two complexes. Regardless, my data points to a mechanistic and temporal uncoupling of Swc4 and Yaf9 in the recruitment of SWR1-C to either site of DNA damage or heterochromatin boundaries. Specifically, we suggest that in a *swc4* mutant, SWR1-C is no longer anchored to chromatin by the shared module. In this scenario, H2A.Z could be available for alternative deposition mechanisms. Consistent with this, *in vitro* studies demonstrated that Nap1 was able to assemble H2A.Z-containing nucleosomes, an activity facilitated by the histone chaperone Chz1 (Luk et al., 2007; Mazurkiewicz et al., 2006; Y. J. Park et al., 2005; Straube et al., 2010).

3.4 Experimental procedures

3.4.1 Yeast strains

All yeast strains used in this study are listed in Table 1 and were created using standard yeast genetic techniques (Ausubel, 1987). Complete gene deletions and integration of the 3xFLAG tag at the 3' end of genes (Gelbart et al., 2001) were achieved using one-step gene integration of PCR-amplified modules (Longtine et al., 1998).

Table 3.1 Yeast strains

Strain	Relevant Genotype
MKY5	W303, *MATα ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 LYS2*
MKY6	W303, *MATA ADE2 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 lys2Δ*
MKY583	BY4741, *MATα his3Δ1 leu2Δ0 LYS2 ura3Δ0 can1Δ::MATαPr-HIS3 lyp1Δ::MATαPr-LEU2*
MKY1432	BY4742, *MATa his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0*
MKY1391	MKY6, *eaf1Δ-3X HA::NATMX6*
MKY1402	MKY6, *epl1 1-380-3XFLAG:: KANMX6*
MKY1446	MKY6, *yaf9Δ-3XFLAG::KANMX6*
MKY1447	MKY6, *swc4 1-400-3XFLAG:: NATMX6*
MKY1448	MKY6, *yaf9Δ-3XFLAG::KANMX6 swc4 1-400-3XFLAG:: NATMX6*
MKY1433	MKY1432, *EAF1-3X HA::NATMX6*
MKY1442	MKY1432, *eaf1Δ-3X HA::NATMX6*
MKY1443	MKY1432, *EPL1-3XFLAG:: NATMX6*
MKY1445	MKY1432, *epl1 1-380-3XFLAG:: NATMX6*
MKY1449	MKY1432, *SWC4-3XFLAG:: NATMX6*
MKY1450	MKY1432, *swc4 1-400-3XFLAG:: NATMX6*
MKY1419	MKY583, *EAF1-3X HA::NATMX6*
MKY1428	MKY583, *eaf1Δ-3X HA::NATMX6*
MKY1429	MKY583, *EPL1-3XFLAG:: NATMX6*
MKY1445	MKY583, *epl1 1-380-3XFLAG:: NATMX6*
MKY1451	MK583, *yaf9Δ::NATMX6*
MKY1452	MKY583, *SWC4-3XFLAG:: NATMX6*
MKY1453	MKY583, *swc4 1-400-3XFLAG:: NATMX6*
MKY1454	MKY583, *swr1Δ::NATMX6*
MKY646	MKY6, *swr1Δ::HIS3*

3.4.2 Drug sensitivity measurements

Overnight cultures grown in YPD at 30°C were diluted to 0.3 $O.D_{600}$. The cells were tenfold serially diluted and spotted onto solid YPD plates plates with 0.015% MMS (Sigma), 100 mM Hydroxyurea (HU) (Sigma), 2% Formamide (Sigma) or 3 mM Caffeine (Sigma). The plates were then incubated at 30°C for 2-3 days before being scanned.

3.4.3 Chromatin association and histone acetylation assays

The chromatin association assay was adapted from a protocol previously described (Liang et al., 1997). Yeast cells were incubated in Pre-Spheroblast Buffer (100 mM Pipes/KOH [pH 9.4], 10 mM DTT, 0.1% sodium azide) for 10 min at room temperature, and spheroblasted with 20 mg/ml Zymolyase-100T (Seikagaku Corporation) in Spheroblast Buffer (50 mM KPO4 [pH 7.5], 0.6 M Sorbitol, 10 mM DTT) at 37 °C for 15 min. Spheroblasts were washed with Wash Buffer (50 mM Hepes/KOH [pH 7.5], 100 mM KCl, 2.5 mM $MgCl_2$, 0.4M sorbitol), resuspended in equal volume of EB (50 mM Hepes/KOH [pH 7.5], 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, and protease inhibitor mixture), and lysed with 1% Triton X-100. Whole cell extract (WCE) was saved, and the remaining lysate was separated into chromatin pellet (Pellet) and supernatant (SUP) fractions by centrifugation through EBSX (EB + 0.25% Triton X-100 and 40% sucrose). WCE, Pellet, and SUP were subjected to SDS/ PAGE and immunoblotted with anti-Htz1 (Abcam), anti- H2A (Upstate) and anti-Pgk (Molecular Probes) antibodies. Immunoblots were scanned with the Odyssey Infrared Imaging

System (Licor) or visualized with SuperSignal enhanced chemiluminescence (Pierce Chemical).

To determine the relative amounts of H4 and H2A.Z K14 acetylation, whole cell extract was subjected to SDS/PAGE, immunoblotted with anti-H2A.Z K14ac (Upstate), H4 tetra-ac (Upstate), and anti-H4 (Abcam) antibodies, and visualized with the Odyssey Infrared Imaging System (Licor).

3.4.4 RT-PCR

Cells were grown in YPD to an OD600 of 0.5. ten $O.D_{600}$. units of cells were harvested for RNA extraction and purification using the Qiagen RNeasy Mini Kit as per the manufacturer protocol. RNA was digested with RNase-free DNase I (Qiagen). cDNA was synthesized using SuperScript III First-Strand Synthesis System for Reverse Transcriptase (RT)–PCR and oligo(dT) (Invitrogen). cDNA was analyzed using a Rotor-Gene 6000 (Corbett Research) and PerfeCTa SYBR Green FastMix (Quanta Biosciences). *YCR101C*, *YCR099C*, and *YCR094C* mRNA were normalized to *ACT1* mRNA. Results are represented relative to untagged wild-type set as 1.0. Samples were analyzed in triplicate for at least three independent RNA preparations. Primers used are listed in Table 2.

Table 3.2 RT -qPCR primers

Name	Method	Primer sequence (5'-3')
YCR101C Fwd	RT-qPCR	GGAGTCATGGAGGGCTTTAAC
YCR101C Rev	RT-qPCR	AGGATTCCCATCATTGCTCA
YCR099C Fwd	RT-qPCR	TCAGATTGCCCTTTCCGATA
YCR099C Rev	RT-qPCR	TCAGATTGCCCTTTCCGATA
YCR094W Fwd	RT-qPCR	TTTCACTCTTTGGTGGCACA
YCR094W Rev	RT-qPCR	TCGCTCTTGGTTGGAAGATT
ACT1 Fwd	RT-qPCR	TGTCCTTGTACTCTTCCGGT
ACT1 Rev	RT-qPCR	CCGGCCAAATCGATTCTCAA

3.4.5 E-MAP

E-MAP assay was performed as described previously (Schuldiner et al., 2006a). Briefly, using a Singer robot, *eaf1*Δ*, epl1 1-380, swc4 1-400, yaf9*Δ*,* and *swr1*Δ alleles were crossed to a library of 1,536 mutants covering a number of processes, including transcription, RNA processing, and chromatin biology. All strains were screened three to four times, and scores were calculated as previously described (Collins et al., 2010; Schuldiner et al., 2006a).

3.4.6 mRNA expression profile

Expression profiling was performed as described previously (van de Peppel et al., 2003). Briefly, *eaf1*Δ*, epl1 1-380, swc4 1-400, yaf9*Δ*,* and *swr1*Δ mutants were processed four times from two independently inoculated cultures. Also, sets of mutants were grown together with additional wild-type cultures in order to correct for other sources of variations. Dual-channel 70-mer oligonucleotide arrays were used with a common reference wild-type RNA. After RNA isolation, all steps were operated using robotic liquid handlers. Scores obtained were calculated as previously described (van Bakel et al., 2004; van de Peppel et al., 2003). Differentially regulated genes were determined by p value < 0.05, Fold change (FC) +/- > 1.7). Enrichment for Gene Ontology Fat terms was performed using the DAVID service (http://david.abcc.ncifcrf.gov/summary.jsp) (Huang da et al., 2009a; Huang da et al., 2009b; Sherman et al., 2007). Statistical significance of gene enrichment at subtelomeric regions was determined using the hypergeometric function (Tavazoie et al., 1999).

Chapter 4

Loss of H3 K79 trimethylation leads to suppression of Rtt107-dependent DNA damage sensitivity through the translesion synthesis pathway[2]

4.1 Introduction

Multiple mechanisms cooperate to maintain genome integrity, thus ensuring proper transmission of genetic information from one generation to the next. DNA damage is detected by sensors that activate the DNA damage checkpoint, which in turn elicits various cellular responses including cell cycle arrest, DNA repair, apoptosis, and/or DNA damage-induced transcriptional programs (Harper et al., 2007; Putnam et al., 2009).

In *Saccharomyces cerevisiae*, the kinase proteins Mec1 and Tel1, the yeast homologues of mammalian ATR (ATM and Rad3-related) and ATM (ataxia-telangiectasia mutated), are crucial for transducing signals in the S phase checkpoint response (Abraham, 2001; Ziv et al., 2006). The downstream signaling cascade leads to cell cycle arrest, replication fork stabilization, and DNA damage repair (Segurado et al., 2009). Following successful DNA repair, the checkpoint must be deactivated to allow resumption of cell cycle and restart of stalled replication forks. While one of the main steps in this process is dephosphorylation of the effector kinase Rad53, checkpoint deactivation is further

---

[2] Lévesque N., Fok A.K., Leung G.P., Schmidt T.I., and Kobor M.S. (2010) Loss of H3 K79 Trimethylation Leads to Suppression of Rtt107-dependent DNA Damage Sensitivity Through TLS Pathway. J Biol Chem; 285(45):35113-22.

coordinated by many different proteins including phosphatases, proteases, and helicases (Conde et al., 2008; Harrison et al., 2006; O'Neill et al., 2007; Szyjka et al., 2008). In the event of irreparable DNA damage, tolerance mechanisms allow bypass of DNA lesions therefore enabling cells to survive (K. Y. Lee et al., 2008). One of these pathways is the translesion synthesis (TLS) pathway which uses special error-prone polymerases to allow replication past DNA lesions, resulting in an increased mutation frequency (Waters et al., 2009).

One of the downstream phosphorylation targets of Mec1 is Rtt107/Esc4, which is required for the reinitiating replication after repair of alkylating DNA damage (Roberts et al., 2006; Rouse, 2004). Accordingly, yeast lacking the non-essential *RTT107* gene or carrying an allele encoding for a non-phosphorylatable Rtt107 protein are hypersensitive to different DNA damaging agents (Rouse, 2004). These include the DNA alkylating agent methyl methane-sulfonate (MMS), the nucleotide reductase inhibitor hydroxyurea (HU), and the topoisomerase I poison camptothecin (CPT) (Chang et al., 2002; Roberts et al., 2006; Rouse, 2004). Moreover, *rtt107*Δ mutants have a chromosome instability phenotype and an increased incidence of Rad52 foci, indicative of homologous recombination occurring due to stalled DNA replication forks (Alvaro et al., 2007; Yuen et al., 2007). Aside from these roles in genome integrity, Rtt107 functions to repress the mobility of Ty1 transposons and to establish silent chromatin (Scholes et al., 2001; Zappulla et al., 2006).

Rtt107 contains several BRCA1 C-terminal (BRCT) homology domains, which often serve as phospho-binding modules to recruit signaling complexes and repair factors to DNA damage-induced lesions (Mohammad et al., 2009; Rouse, 2004). Consistent with a role as a scaffold for protein-protein interactions during the DNA damage response, Rtt107 interacts with a number of DNA repair and recombination proteins (Chin et al., 2006; Roberts et al., 2006; Roberts et al., 2008). Of these, Rtt107's interaction with the structure-specific endonuclease Slx4 is best characterized and indicative of a close functional relationship between the two proteins. Slx4 is required for Mec1-dependent phosphorylation of four SQ/TQ motifs in the C-terminal half of Rtt107 and, like Rtt107, facilitates resumption of DNA replication after DNA damage (Roberts et al., 2006; Rouse, 2004).

In addition to the complex regulation of the DNA damage response by signaling cascades, chromatin structures in the cell also play many roles in regulating access to DNA during the repair process. One example of the emerging interface between chromatin and DNA damage response pathways is the DNA damage-induced recruitment of Rtt107 to chromatin by the H3 K56 acetyltransferase Rtt109 and the cullin Rtt101 (Roberts et al., 2008). There are many other chromatin modifications involved in the DNA damage response, such as the well-studied H2A phosphorylation and H3K79 methylation pathways (van Attikum et al., 2009). H2A S129 is phosphorylated by Mec1 in response to DNA damage, triggering the assembly of many repair proteins and chromatin modifiers acting at subsequent steps (Chambers et al., 2007; Downs et al., 2000; C. Redon et al., 2003; van Attikum et al., 2009). To allow resumption of cell cycle and DNA replication after successful completion of DNA repair, H2A S129 needs to be

dephosphorylated by either Pph3 or Glc7, depending on the exact nature of the initial damage (Bazzi et al., 2010; Keogh et al., 2006). Dot1-mediated H3 K79 methylation, which is regulated by Bre1-mediated H2B K123 ubiquitination, is required for G1 and S phase checkpoints (Dover et al., 2002; Lazzaro et al., 2008; Shilatifard, 2006; Wood et al., 2003; Wysocki et al., 2005). In part, this requirement is mediated through a functional linkage to the Rad9 adaptor protein (Grenon et al., 2007; Wysocki et al., 2005). Several lines of evidence suggest that Dot1 plays an additional role in DNA repair pathways, such as nucleotide excision repair, sister chromatid recombination and repair of ionizing radiation damage (Chaudhuri et al., 2009; Conde et al., 2009; Game et al., 2005). In contrast, Dot1 negatively regulates the error-prone TLS pathway through an unknown mechanism, thereby allowing bypass of DNA replication blocks (Conde et al., 2008). Aside from Dot1's function in DNA damage, it is also involved in gene silencing as well as differential H3 K79 methylation during the cell cycle (Schulze et al., 2009; Singer et al., 1998).

This study established a close connection between Rtt107 and the pathway resulting in a specific chromatin modification, H3 K79 trimethylation. Specifically, loss of H3 K79 trimethylation suppressed the DNA damage sensitivity of *rtt107*Δ and *slx4*Δ mutants. This suppression was not linked to restoration of Rtt107 or Slx4 phosphorylation, but instead was dependent on the presence of a functional TLS pathway. Moreover, deletion of *DOT1* partially suppressed the cell cycle delay and the defect in resuming DNA replication of *rtt107*Δ mutants during the recovery from MMS-induced DNA damage. In

contrast, deletion of *DOT1* rescued neither the chromosome instability phenotype nor the increased incidence of spontaneous Rad52 foci caused by loss of Rtt107.

4.2 Results

4.2.1 Elimination of H3 K79 methylation suppresses the sensitivity of *rtt107*Δ and *slx4*Δ mutants to the DNA-damaging agent MMS

Rtt107 and its interaction partner Slx4 are required for yeast cells to survive exposure to DNA damage conditions, such as those caused by the alkylating agent MMS (Chang et al., 2002; Roberts et al., 2006). Given the importance of the natural chromatin template during the DNA damage response, and the existing link between Rtt107 and the histone acetyltransferase Rtt109 (Bassal et al., 2005; Giannattasio et al., 2005; Roberts et al., 2008), we hypothesized that chromatin modifications might affect the requirement for Rtt107 and Slx4 during DNA damage repair. For this purpose, we created strains that, in addition to deletion of either *RTT107* or *SLX4*, lacked genes encoding several chromatin modifiers with roles in the DNA damage response to test whether their absence enhanced or suppressed the sensitivity of *rtt107*Δ or *slx4*Δ mutants to MMS. While the majority of double mutants grew equally well as *rtt107*Δ or *slx4*Δ mutants, we found that deletion of *DOT1*, a non-essential gene encoding a histone methyltransferase catalyzing mono-, di- and trimethylation of histone H3 K79, almost completely rescued the MMS sensitivity of *rtt107*Δ and *slx4*Δ mutants (Fig. 4.1A).

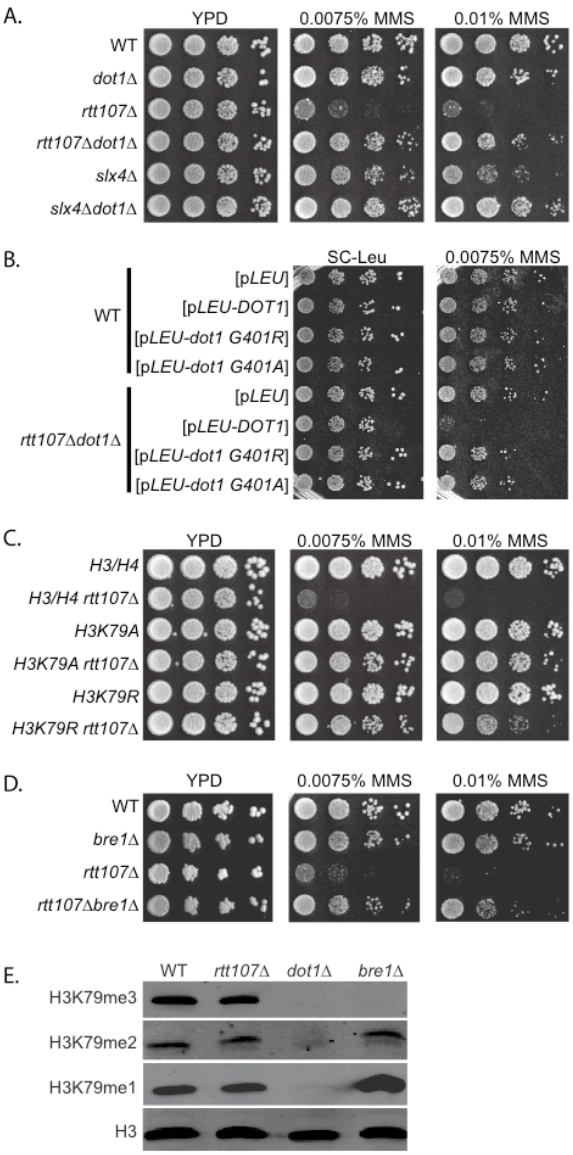


Figure 4.1 Abrogation of H3 K79 trimethylation suppressed the MMS sensitivity of strains lacking Rtt107 or Slx4. Tenfold serial dilutions of the indicated strains were plated onto media containing 0.0075% or 0.01% MMS. (A) Loss of Dot1 suppressed MMS sensitivity of *rtt107*Δ and *slx4*Δ mutants. (B) Loss of Dot1's catalytic activity, (C) H3 K79A, K79R, or (D) loss of Bre1 suppressed MMS sensitivity of *rtt107*Δ mutants. (E) Bre1 affected mainly H3 K79 trimethylation, not di- or monomethylation. Whole cell extracts of indicated strains were analyzed by protein blotting with anti-H3 K79 tri-, di- or monomethyl antibodies. Antibodies against H3 were used as a loading control.

To determine whether this effect was dependent on the catalytic activity of Dot1, alleles encoding catalytically inactive Dot1 proteins were compared to the complete loss of Dot1 and to the presence of wild-type Dot1. The strains carrying *dot1G401R* and *dot1G401A* alleles, encoding for catalytically inactive forms of Dot1, suppressed the MMS sensitivity phenotype similar to the complete deletion (Fig. 4.1B). As expected, re-introducing wild-type *DOT1* in *rtt107*Δ*dot1*Δ double mutants restored MMS sensitivity to levels similar to that of *rtt107*Δ single mutants. These data suggested that eliminating the catalytic activity of Dot1 enabled cells lacking Rtt107 to survive otherwise detrimental conditions during exposure to MMS. The same results were obtained for *slx4*Δ mutants, except that *slx4*Δ mutants were less sensitive to MMS than *rtt107*Δ mutants (Fig. 4.2A).

The only known target of Dot1 methyltransferase activity to date is the K79 residue located in the core of histone H3, but formally it is possible that Dot1, similar to other chromatin modifiers, has other enzymatic targets not yet identified. To examine whether the suppression of the MMS sensitivity of *rtt107*Δ mutants by loss of Dot1 was due to lack of H3 K79 methylation, strains with mutated forms of H3 K79 which cannot be methylated were tested for their ability to survive chronic MMS exposure in the absence of Rtt107. Changing lysine 79 to either alanine or arginine rescued the DNA damage sensitivity of the *rtt107*Δ mutants, analogous to the *DOT1* deletion (Fig. 4.1C). Therefore, we concluded that the reversal of the MMS sensitivity of *rtt107*Δ mutants was due to the loss of Dot1-mediated H3 K79 methylation. Similarly, *H3 K79A* and *H3 K79R* mutants also suppressed the MMS sensitivity of the *slx4*Δ strain (Fig. 4.2B).

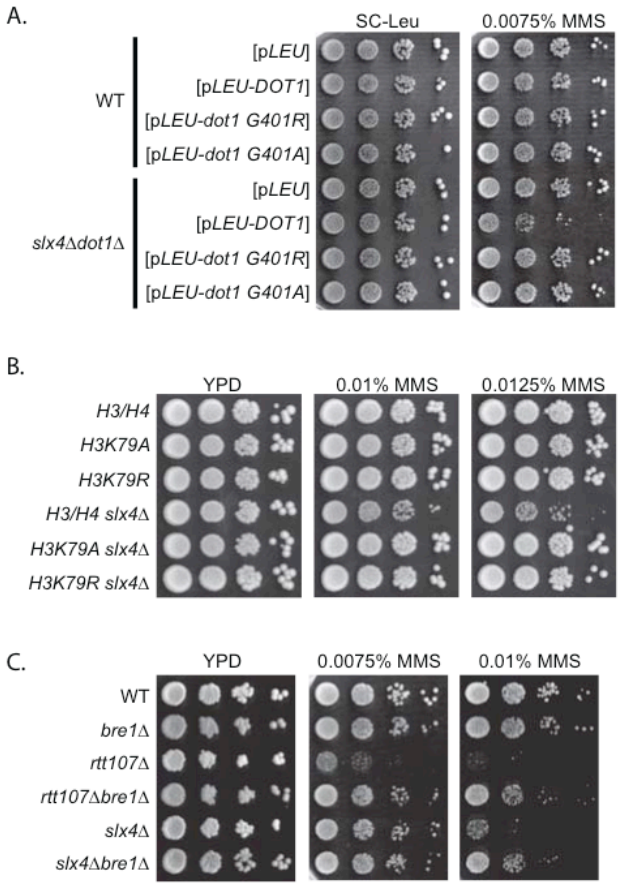


Figure 4.2 Abrogation of H3 K79 trimethylation suppressed the MMS sensitivity of strains lacking Slx4. Tenfold serial dilutions of the indicated strains were plated onto media containing 0.0075%, 0.01%, or 0.0125% MMS. (A) Loss of Dot1's catalytic activity, (B) H3 K79A, K79R, or (C) loss of Bre1 suppressed MMS sensitivity of *slx4*Δ mutants.

Methylation of H3 K79 by Dot1 is regulated through crosstalk with another histone modification, mono-ubiquitination of H2B K123, which is catalyzed by the Bre1/Rad6 enzyme complex (Dover et al., 2002; Foster et al., 2009; Ng, Xu et al., 2002; Shahbazian et al., 2005; Shilatifard, 2006; Wood et al., 2003). Thus we wanted to test whether upstream regulators of H3 K79 methylation would have a similar effect on the MMS sensitivity of *rtt107*Δ and *slx4*Δ mutants. Indeed, deletion of *BRE1* also rescued the MMS sensitivity of the strains lacking Rtt107 or Slx4 (Fig. 4.1D and 4.2C).

To learn more about the biochemical nature underlying the observed effects, we assessed the total levels of mono-, di- or trimethylated H3 K79 in whole cell extracts. Interstingly, whereas Dot1 broadly catalyzes mono-, di- and trimethylation of H3 K79 (Ng et al., 2002; Utley et al., 2005; van Leeuwen et al., 2002), Bre1 primarily affected K79 trimethylation (Fig. 4.1E). These results suggested that specifically a lack of H3 K79 trimethylation caused the suppression of the MMS sensitivity of *rtt107*Δ and *slx4*Δ mutants.

4.2.2 Deletion of *DOT1* suppressed DNA damage sensitivity in the absence of MMS-induced phosphorylation of Rtt107 or Slx4.

In response to DNA damage induced by various agents, Rtt107 and Slx4 are phosphorylated on several S/T residues by the checkpoint kinase Mec1 (Roberts et al., 2006; Rouse, 2004). Phosphorylation of Rtt107 is essential for its function in the DNA damage response and depends on Slx4 (Roberts et al., 2006; Rouse, 2004b). It was in principle possible that, in *slx4*Δ*dot1*Δ double mutants, an alternate pathway directed Rtt107 phosphorylation in the absence of Slx4, thereby enabling cells to survive the otherwise detrimental MMS-induced DNA damage. To test this possibility, Rtt107 phosphorylation was measured in strains lacking Slx4, Dot1, or both simultaneously. As expected, exposure to MMS induced phosphorylation of Rtt107 in wild-type strains but not in strains lacking Slx4 (Fig. 4.3A) (Roberts et al., 2006). Although deletion of *DOT1* suppressed the MMS sensitivity of *slx4*Δ mutants, it did not overcome the requirement of Slx4 for Rtt107 phosphorylation (Fig. 4.3A). Loss of Dot1 had no effect on Rtt107 phosphorylation in response to MMS when Slx4 was present (Fig. 4.3A). Therefore,

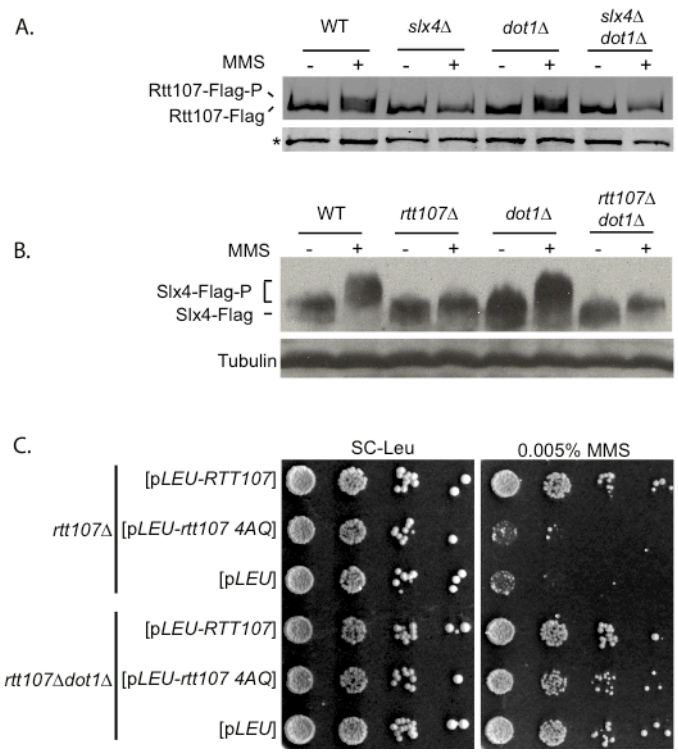


Figure 4.3 Suppression of *rtt107*Δ MMS sensitivity by deletion of *DOT1* was not dependent on the phosphorylation of Slx4 and vice versa. (A) Cells expressing Rtt107-Flag were untreated or treated with 0.03% MMS for 1 h. Analytical-scale immunoprecipitations of Rtt107-Flag were performed and analyzed by protein blotting with anti-Flag antibodies. The reduced mobility of Rtt107-Flag indicated phosphorylation of the protein. Background bands (*) were used as a loading control. (B) Cells expressing Slx4-Flag were treated as described in (A). Whole cell extracts were analyzed by protein blotting with anti-Flag antibodies, with reduced mobility of Slx4-Flag being indicative of phosphorylation. Antibodies against tubulin were used as a loading control. (C) Deletion of *DOT1* suppressed the MMS sensitivity of the mutants expressing the non-phosphorylatable Rtt107-4AQ. Tenfold serial dilutions were plated onto SC-Leu containing 0.005% MMS.

the suppression by *dot1*Δ did not involve a restoration of Rtt107 phosphorylation in the absence of Slx4, arguing against an alternative pathway for Rtt107 phosphorylation. Consistent with the physical interaction and close functional relationship between Rtt107

and Slx4, we found that MMS-induced phosphorylation of Slx4 was dependent on Rtt107, but not on Dot1 (Fig. 4.3B). Analogous to the results obtained for Rtt107, eliminating *DOT1* did not restore Slx4 phosphorylation in the absence of Rtt107 (Fig 4.3B).

To test whether the suppression by deletion of *DOT1* was linked to the phosphorylation of Rtt107 at specific SQ sites, we utilized mutants expressing the non-phosphorylatable form of Rtt107. Using a plasmid expressing *rtt107-4AQ* in cells lacking Dot1, we tested the MMS sensitivity of the double mutants. Consistent with the importance of Rtt107 phosphorylation, deletion of *DOT1* suppressed the MMS sensitivity of the *rtt107 4AQ* mutants (Fig 4.3C). Taken together, these results indicated that MMS sensitivity of mutants lacking Rtt107 phosphorylation was suppressed by deletion of *DOT1*.

4.2.3 The requirement of Rtt107 for resumption of cell cycle after S phase damage was partially suppressed by lack of Dot1.

To further understand the molecular mechanism leading to the suppression of *rtt107*Δ MMS sensitivity, we tested whether loss of Dot1 could compensate for the requirement of Rtt107 during the restart of DNA replication (Roberts et al., 2006; Rouse, 2004b). Cells arrested in G1 were released into S phase in the presence of MMS for 1 h, and restart of DNA replication was directly measured by 5-bromo-2'-deoxyuridine (BrdU) incorporation into nascent genomic DNA (Fig. 4.4A). Since BrdU was added after MMS treatment, it serves as a quantitative indicator of newly replicated DNA during the recovery process. As expected, BrdU levels increased in wild-type cells

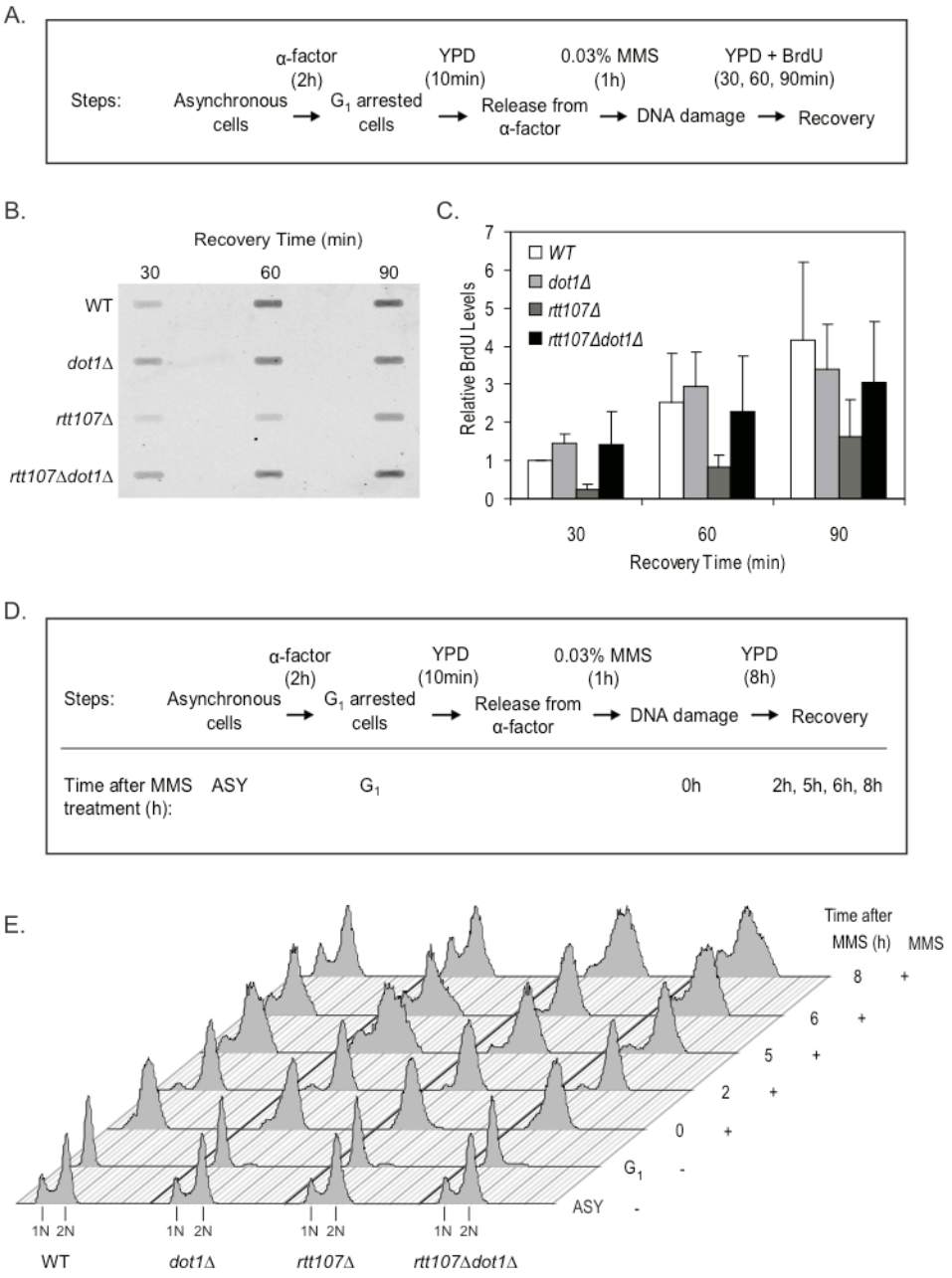


Figure 4.4 Requirement of Rtt107 for resumption of DNA replication and cell cycle after DNA damage was partially suppressed by deletion of *DOT1*. (A) Diagram of experimental strategy used for BrdU incorporation experiment. (B) BrdU incorporation into nascent DNA indicated *rtt107*Δ*dot1*Δ mutants more efficiently resumed DNA replication after DNA damage than *rtt107*Δ mutants. (C) Quantification of newly replicated DNA as measured by BrdU signals. All values are relative to wild-type at 30 min. Error bars represent standard deviations of values from 3 independent experiments. (D) Diagram of experimental strategy used for FACS analysis. (E) FACS analysis showed *rtt107*Δ*dot1*Δ mutants recovered from DNA damage earlier than *rtt107*Δ mutants. ASY, asynchronous cells.

during the course of the experiment, indicating successful resumption of DNA replication (Fig. 4.4B). In contrast, BrdU levels in *rtt107*Δ mutants were consistently lower than the wild-type at each time point. Whereas the levels of BrdU incorporation in *dot1*Δ mutants increased similar to the wild-type cells, *rtt107*Δ*dot1*Δ mutants incorporated BrdU at intermediate levels between wild-type and *rtt107*Δ mutants (Fig. 4.4C). This result suggested that loss of Dot1 could partly suppress the defect of *rtt107*Δ mutants in resuming DNA replication.

Next, we used FACS analysis to test whether loss of Dot1 would have a similar effect on the resumption of cell cycle progression after DNA damage (Fig. 4.4D). At the end of the MMS treatment (0 h), wild-type and *rtt107*Δ mutants were initially arrested in S phase due to activation of the DNA damage checkpoint (Roberts et al., 2006; Rouse, 2004b), whereas *dot1*Δ mutants had proceeded through S phase as judged by the shift of the signal to 2N, consistent with a requirement for Dot1 in the DNA damage checkpoint as described previously (Giannattasio et al., 2005; Wysocki et al., 2005) (Fig. 4.4E). 2 h after removal of MMS, *rtt107*Δ mutants were still in S phase whereas all other strains had progressed to G2/M. Further differences between the mutants were observed as strains continued to recover from DNA damage. For example, at 5 h a substantial fraction of cells in the wild-type and *dot1*Δ mutant had undergone cell division as judged by the appearance of a G1 peak and S phase fraction while *rtt107*Δ mutants had predominantly a 2N peak, suggesting that they were still residing in G2/M. These differences persisted until 8 h after recovery when the G1 peak

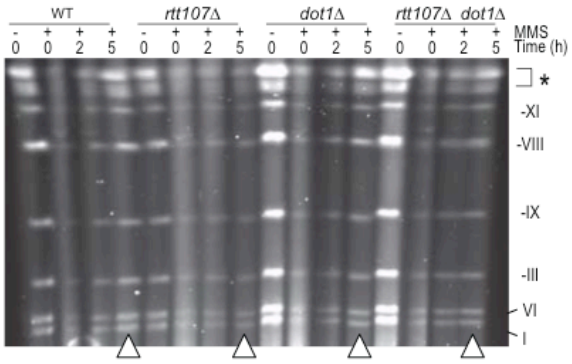


Figure 4.5 Requirement of Rtt107 for resumption of replication after DNA damage was partially suppressed by deletion of *DOT1*. PFGE analysis indicated *rtt107*Δ*dot1*Δ mutants had more efficient DNA replication after DNA damage than *rtt107*Δ mutants. * : unresolved chromosomes.

and S phase fraction first appeared in the *rtt107*Δ mutants. The *rtt107*Δ *dot1*Δ double mutants had an intermediate phenotype, as the G1 peak and S phase fraction became visible at 6 h, which was earlier than *rtt107*Δ mutants but later than wild-type or *dot1*Δ mutants (Fig. 4.4E). Consistent with this, the delayed appearance of intact chromosomes during recovery in the *rtt107*Δ mutants was partially rescued by concurrent loss of Dot1 as visualized by pulsed-field gel electrophoresis (PFGE) (Fig. 4.5).

The defect of *rtt107*Δ mutants in completing the G2/M phase of the cell cycle during recovery from transient DNA damage can also be observed by examining nuclear morphology (Roberts et al., 2006). In wild-type and *dot1*Δ mutants, the percentage of cells in G2/M increased after exposure to MMS, reached a peak at 3 h of recovery and started to decrease as the cells completed mitosis (Fig. 4.6A,B). As expected, the percentage of *rtt107*Δ mutants in G2/M also reached a peak at 3 h, but the increased level lasted up to 5 h after MMS treatment. As judged by the percentage of

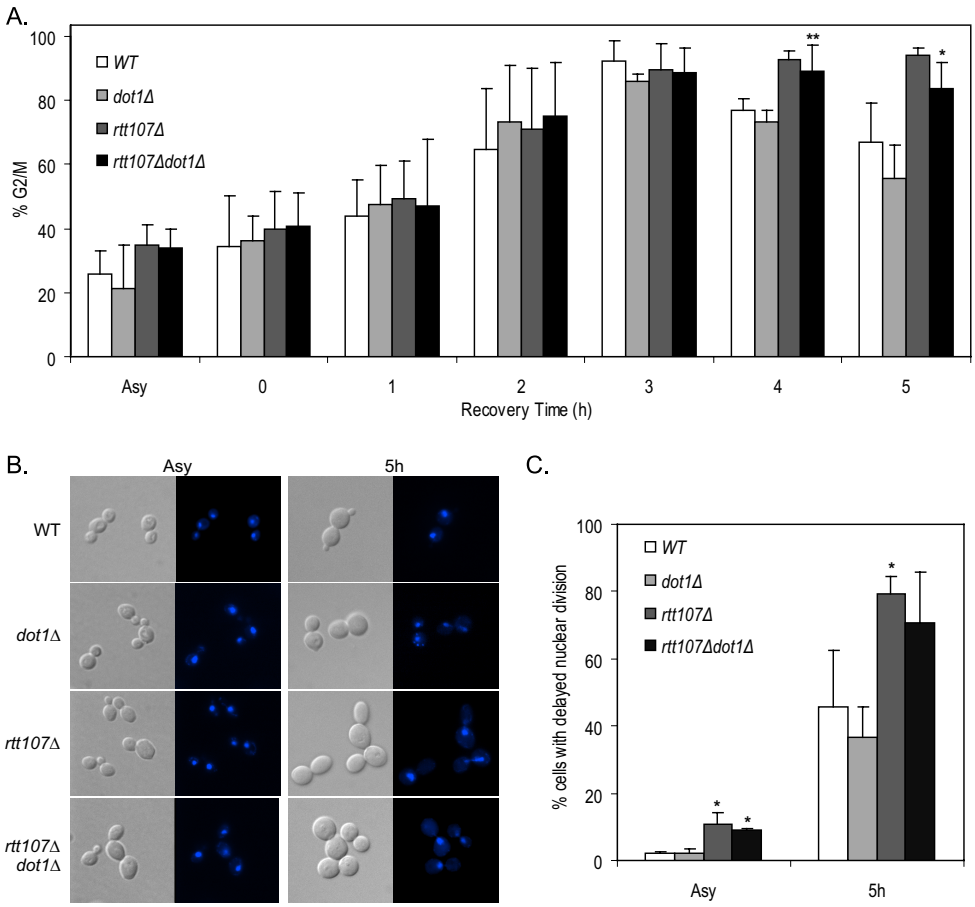


Figure 4.6 Nuclear division delay of *rtt107*Δ mutants was not suppressed by deletion of *DOT1* in the absence and presence of MMS. Cells were treated with 0.03% MMS for 1h, then washed and resuspended in complete media for DNA damage recovery, and stained with DAPI at the indicated timepoints. (A) The increased percentage of cells in G2/M of *rtt107*Δ mutants after DNA damage was not suppressed by deletion of *DOT1*. The percentage of cells with medium to large buds (% G2/M) was calculated by dividing the number of cells with medium to large buds by the total number of cells. (B) Representative differential interference contrast images are shown on the left and the corresponding DAPI images on right. (C) The increased percentage of cells exhibiting nuclear division delay in *rtt107*Δ mutants was not suppressed by loss of Dot1. The percentage of cells with delayed nuclear division was calculated by dividing the number of large-budded cells with unsegregated nuclei by the total number of cells. In both (A) and (C), at least 200 cells were counted in 3 independent experiments. Error bars represent standard deviations of the values. * : $p<0.05$; ** : $p<0.005$ when compared to the wild-type strain in the same timepoint.

G2/M cells, the kinetics of recovery from DNA damage in *rtt107*Δ*dot1*Δ double mutants was slower than wild-type and *dot1*Δ mutants, but faster than *rtt107*Δ mutants, although this did not reach statistical significance (Fig. 4.6A). Another known phenotype of *rtt107*Δ mutants is the delay of nuclear division, as judged by the higher percentage of large budded cells with elongated nuclei spanning the bud neck. Consistent with previous reports, nuclear division was delayed in *rtt107*Δ mutants when compared to wild-type at 5 h after exposure to MMS (Fig. 4.6C). In contrast, *dot1*Δ mutants did not show any delay of nuclear division. A similar phenotype was also observed in asynchronous cultures not exposed to MMS. In both conditions, deletion of *DOT1* did not rescue the defect caused by loss of Rtt107.

4.2.4 The TLS pathway was required for the suppression of the MMS sensitivity of *rtt107*Δ mutants by deletion of *DOT1*.

Next we sought to determine the pathway by which deletion of *DOT1* suppressed the requirement for *RTT107* during MMS exposure. In addition to the suppression of the DNA damage sensitivity of *rtt107*Δ and *slx4*Δ mutants reported here, lack of Dot1 suppresses the MMS sensitivity of strains lacking a variety of repair proteins, and this effect is dependent on the TLS polymerases Polζ and Rev1 (Conde et al., 2008). To address whether the suppression of *rtt107*Δ DNA damage sensitivity by loss of Dot1 was similarly dependent on the TLS pathway, we constructed triple mutants lacking two main components of the TLS pathway: Rev3 (catalytic subunit of Polζ) or Rev1 (dC-transferase) (Kunz et al., 2000). Lack of Dot1 did not suppress the MMS sensitivity of

*rtt107*Δ mutants in the absence of Rev3 (Fig. 4.7A). Similarly, Rev1 was necessary for the reversal of *rtt107*Δ sensitivity by deletion of *DOT1*, suggesting that the *dot1*Δ suppression was dependent on the TLS pathway in general and not specifically on Rev3 (Fig. 4.7B). Very low concentrations of MMS were used in this assay due to the extreme MMS sensitivity of the triple mutants. It is interesting to note that both *dot1*Δ*rev3*Δ and *rtt107*Δ*rev3*Δ double mutants were very sensitive to MMS, and a similar phenotype was also observed for *dot1*Δ*rev1*Δ and *rtt107*Δ*rev1*Δ mutants. This indicated that a functional TLS pathway became more important for DNA damage resistance in the absence of Dot1 or Rtt107.

The TLS pathway is error-prone and its activation would therefore be expected to cause an increased mutation rate. Using the *CAN1* forward mutagenesis assay, we observed a 4 to 5-fold increased mutation rate in the presence of 0.005% MMS in both *dot1*Δ and *dot1*Δ*rtt107*Δ mutants (Fig. 4.7C). Together, these results suggested that deletion of *DOT1* led to activation of the TLS pathway, thereby allowing the survival of *rtt107*Δ mutants.

4.2.5 Rtt107 had functions in maintaining genomic integrity that were independent of Dot1 activity.

Rtt107's role in maintenance of genome stability is not restricted to its specific function of restarting the cell cycle during S phase after DNA damage. During normal cell cycle progression, cells lacking Rtt107 have increased number of Rad52 and Ddc2 foci,

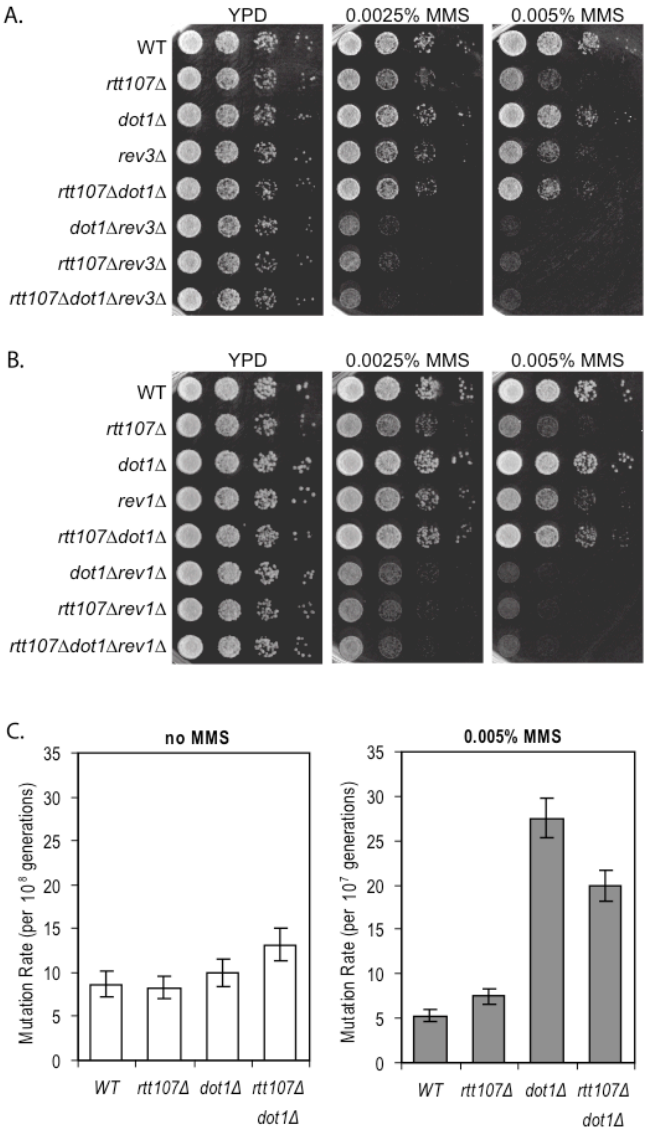


Figure 4.7 Suppression of the *rtt107*Δ MMS sensitivity by deletion of *DOT1* was dependent on the TLS pathway. Deletion of *REV3* or *REV1* in *rtt107*Δ*dot1*Δ mutants resulted in loss of the suppression. (A) *rev3*Δ or (B) *rev1*Δ mutants in combination with the indicated deletions of *RTT107* and/or *DOT1* were plated in tenfold serial dilutions onto YPD containing 0.0025% or 0.005% MMS. (C) Loss of Dot1 resulted in increased mutation rates in presence of MMS. Mutation rates of indicated strains with and without 0.005% MMS were determined using 12 independent colonies. Mutation rates and 95% confidence intervals were calculated using the Ma-Sandri-Sarkar Maximum Likelihood Estimator (MSS-MLE) Method.

indicative of spontaneous DNA damage and /or replication fork stalling (Alvaro et al., 2007; Roberts et al., 2006). Consistent with this, we observed that the percentage of cells with Rad52-GFP foci in S/G2/M phase was approximately 7-fold higher in *rtt107*Δ mutants than in wild-type (Fig. 4.8A, B). Deletion of *DOT1* in the *rtt107*Δ background did not significantly alter the number of cells containing Rad52-GFP foci. However, consistent with published data, we observed a 2-fold increase in the number of cells with Rad52-GFP foci in *dot1*Δ mutants, suggesting that *RTT107* was epistatic to *DOT1* in suppressing spontaneous DNA damage and/or replication fork stalling (Conde et al., 2008).

Further indicative of a broader role of Rtt107 in genome stability is the chromosome instability (CIN) phenotype of *rtt107*Δ mutants. Compared to wild-type cells, *rtt107*Δ homozygous diploid mutants have higher loss of heterozygosity (LOH) at the *MAT*a and *MAT*α loci, which is due to either enhanced mitotic recombination between homologous chromosomes, chromosome loss, rearrangement or gene conversion (Yuen et al., 2007). Using a quantitative version of the original Bimater Screen (BiM) used to define *RTT107* as a CIN gene (Gerring et al., 1990), we tested whether this phenotype was suppressed by loss of Dot1. Consistent with increased LOH, strains lacking Rtt107 had a 4-fold increase in mating rate when compared to wild-type or *dot1*Δ mutants (Fig. 4.8C). Deletion of *DOT1* did not rescue the CIN phenotype of *rtt107*Δ mutants, but rather resulted in a further increase in the mating rate to 6-fold as compared

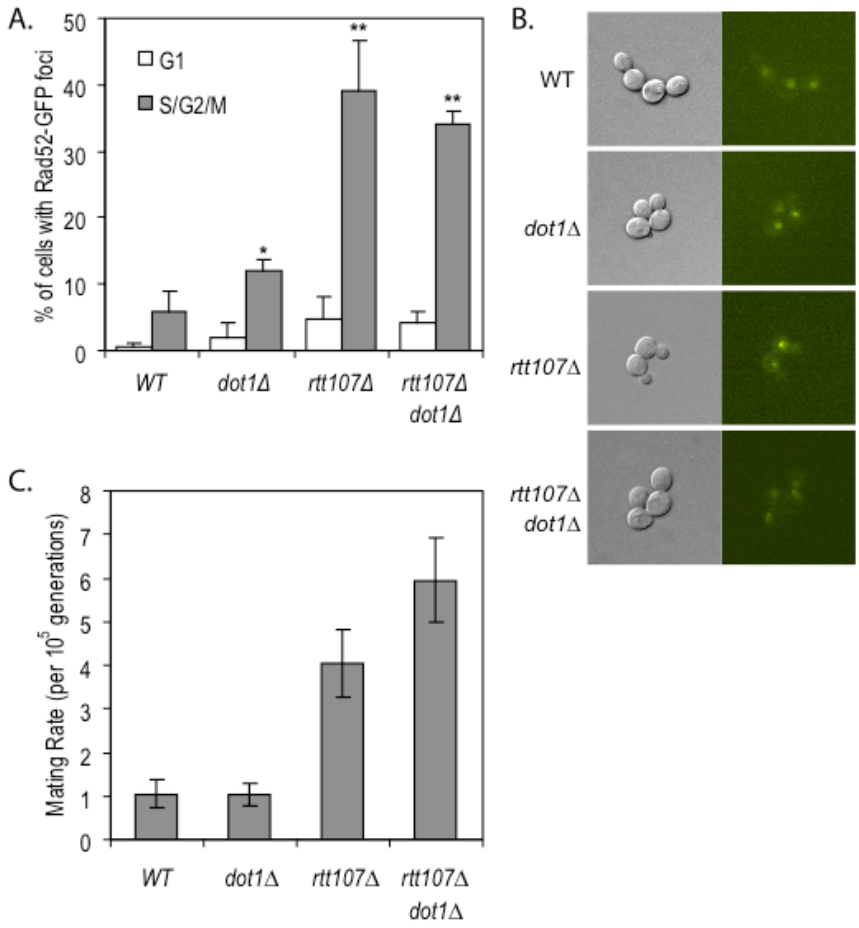


Figure 4.8 Genomic instability of the *rtt107*Δ mutants was not suppressed by deletion of *DOT1*. (A) The increased number of Rad52-GFP foci in *rtt107*Δ mutants was not suppressed in absence of Dot1. The percentage of cells in G1 or S/G2/M phase containing Rad52-GFP foci was calculated by dividing the number of cells in G1 or S/G2/M phase containing Rad52-GFP foci by the total number of cells in G1 or S/G2/M, respectively. At least 200 cells were counted in 3 independent experiments. Error bars represent standard deviations of the values. * : $p<0.05$; ** : $p<0.005$ when compared to the wild-type strain for the same cell cycle phase. (B) Representative differential interference contrast images are shown on the left and the corresponding GFP images on the right. (C) Deletion of *DOT1* did not suppress the increased loss of heterozygosity in *rtt107*Δ mutants. Mating rates of homozygous diploids of indicated strains were determined by using 12 independent colonies. Mating rates and 95% confidence intervals were calculated using the Ma-Sandri-Sarkar Maximum Likelihood Estimator (MSS-MLE) Method.

to wild-type. Hence it appeared that rather than rescuing the requirement for Rtt107 in preventing LOH, Dot1 cooperated with Rtt107 in this process.

4.3 Discussion

In this study, we uncover a close functional relationship between chromatin and the cellular processes regulated by the BRCT domain-containing protein Rtt107 and its interaction partner Slx4. Loss of Dot1, likely mediated by loss of histone H3 K79 trimethylation, suppressed the DNA damage sensitivity of *rtt107*Δ mutants through a mechanism that was dependent on the presence of a functional TLS pathway. The DNA damage-induced phosphorylation of Rtt107 and Slx4, which was mutually dependent, was not restored in the absence of Dot1. Furthermore, deletion of *DOT1* partially reversed the cell cycle progression and replication fork restart defect caused by the lack of Rtt107. In contrast, other genomic instability defects of *rtt107*Δ mutants were worsened or unaffected by loss of *DOT1*. Together, these data point to a complex functional relationship between Rtt107 and Dot1 in both the DNA damage response and preservation of genome integrity.

We propose a model to explain the inhibitory effect of H3 K79 trimethylation on growth during DNA damage conditions in yeast cells lacking Rtt107 or Slx4 (Fig. 4.9). Bre1-mediated H2B K123 ubiquitination is required for Dot1 to catalyze H3 K79 trimethylation, which in turn prevents *rtt107*Δ and *slx4*Δ mutants from surviving DNA damage conditions. This effect likely is mediated through inhibition of the TLS pathway by H3 K79 trimethylation, either directly or indirectly through a nexus to the Dot1-

mediated DNA damage checkpoint. We favour a direct mechanism that could involve binding to the H3 K79 trimethylation mark by a protein that inhibits TLS. Alternatively, H3 K79 trimethylation might directly create a chromatin conformation that in some way is refractory to TLS. An indirect enhancement of TLS might be caused by the compromised DNA damage checkpoint due to loss of Dot1, allowing *rtt107*Δ mutants to survive DNA damage conditions. However, currently there is no evidence linking Dot1's roles in TLS and DNA damage checkpoint. Moreover, UV exposure of DNA damage checkpoint deficient mutants does not result in an increased mutation rate, thereby disfavouring a link between the DNA damage checkpoint and TLS (Pages et al., 2009). Lastly, it is formally possible that the suppression is an indirect effect of an altered transcriptional response caused by lack of Dot1, Bre1 or H3 K79 methylation, which could involve reduced expression of an unknown inhibitor of the TLS. In any case, given our finding that the suppression of *rtt107*Δ phenotypes was linked to loss of trimethylation of H3 K79, it is tempting to speculate that specific genomic regions might be more prone to mediate this effect than others. This is supported by a genome-wide analysis showing that regions containing H3 K79 trimethylation are distinct from those containing H3 K79 dimethylation (Schulze et al., 2009). Further studies are required to elucidate the precise mechanism whereby Dot1-mediated H3 K79 trimethylation inhibits the TLS pathway.

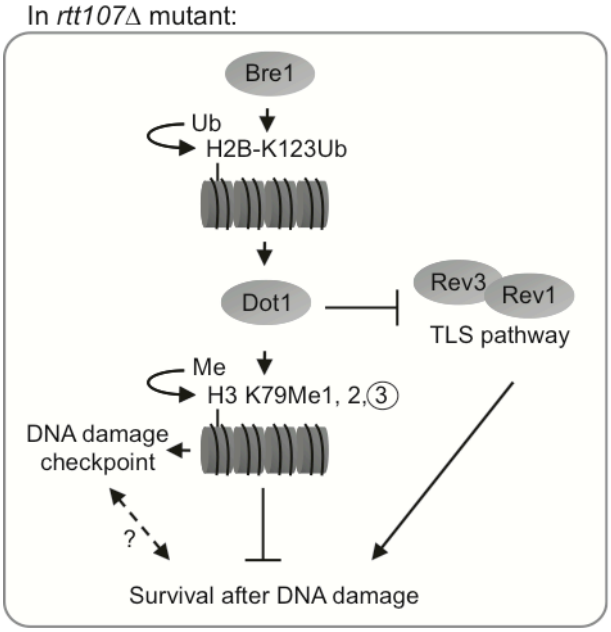


Figure 4.9. Model for repressive effect of chromatin modifications on DNA damage survival in *rtt107*Δ mutants. During DNA damage response, Bre1-mediated H2B K123Ub and by extension, Dot1-mediated H3 K79Me3, are required for checkpoint function. In *rtt107*Δ mutants, the presence of H3K79Me3 is inhibitory to the yeast survival in DNA damage conditions. Loss of Dot1 increases the activity of the TLS pathway which bypasses the requirement of Rtt107 for cell survival.

Our data showed that Mec1-mediated phosphorylation of Rtt107 was dependent on Slx4, consistent with earlier reports (Roberts et al., 2006). We also showed that MMS-induced phosphorylation of Slx4 was dependent on Rtt107, suggesting a mutual requirement of these two proteins for their respective phosphorylation. Interestingly, neither Rtt107 nor Slx4 was phosphorylated in *slx4*Δ or *rtt107*Δ mutants, respectively, when *DOT1* was also deleted. Furthermore, the MMS sensitivity of a strain containing a non-phosphorylatable form of Rtt107 was rescued by deletion of *DOT1*. Together, this biochemical and genetic evidence suggested that DNA damage dependent phosphorylation of Rtt107 is essential for resistance to MMS only when Dot1 is present to methylate H3 K79. Although it is not clear what mechanistic change is triggered by Rtt107 phosphorylation, it is likely to

involve a DNA damage-induced protein-protein interaction. Whatever the mechanism might be, it is clear that Rtt107 phosphorylation in the DNA damage response becomes dispensable when H3 K79 trimethylation is inhibited.

The suppression of *rtt107*Δ by deletion of *DOT1* was restricted to situations of induced DNA damage, suggesting that the functional interaction between Rtt107 and Dot1 was context-dependent. Confirming published data from a high-throughput screen for regulators of Rad52 foci formation, we found that loss of Rtt107 caused a significant increase in the number of Rad52 foci positive cells (Alvaro et al., 2007). In contrast to the suppression of MMS sensitivity of *rtt107*Δ mutants, this phenotype was not rescued by loss of Dot1. This suggested that Rtt107 had a role in preventing spontaneous DNA damage – likely caused by stalled DNA replication forks – which was not negatively regulated by H3 K79 methylation. Furthermore, our work uncovered additional evidence for a complex relationship between Dot1 and Rtt107 in the maintenance of genomic integrity. Rtt107 was required for chromosome stability, as determined by a genetic assay (Yuen et al., 2007). While loss of Dot1 alone did not affect chromosome stability, it enhanced the defect caused by loss of Rtt107. This suggested that in the absence of Rtt107, Dot1 plays a minor role in maintenance of chromosome stability. Taken together, these data point to multiple activities of Rtt107, where only those induced by external DNA damaging agents were suppressed by deletion of *DOT1*. Presumably, both the increased number of Rad52 foci and the chromosome instability in *rtt107*Δ mutants were not suppressed by deletion of *DOT1* because the TLS pathway is unlikely to be activated in these conditions.

The suppression of DNA damage sensitivity by loss of Dot1 reported here is interesting in light of other findings suggesting certain chromatin modifications act as negative regulators of DNA replication, recombination and repair. For example, the H3 K36 histone methyltransferase Set2 and the ATP-dependent chromatin remodeler Chd1 exert an inhibitory effect on DNA replication, as deletions of these genes suppress the HU sensitivity of mutations in several genes involved in DNA replication (Biswas et al., 2008). In addition, deletion of *CHD1* can suppress the lethality normally caused by disruption of the gene encoding either Mec1 or Rad53 DNA damage checkpoint kinases (Biswas et al., 2008). The UV sensitivity and G2/M checkpoint defects of *rad9*Δ and *mec1-21* mutants can be suppressed by loss of genes encoding components of the Rpd3/Sin3 histone deacetylase (Scott et al., 2003). Rpd3 and the aforementioned Set2 also repress meiotic recombination at the *HIS4* meiotic recombination hotspot (Merker et al., 2008). However, not all chromatin modifications involved in DNA metabolism exert a negative effect, as mutations in the genes encoding members of the histone acetyltransferase complex NuA4, the ATP-dependent chromatin remodelers RSC, Swi/Snf, SWR1-C, and INO80 cause sensitivity to MMS, as does loss of the histone variant H2A.Z, or the Mec1-dependent phosphorylation targets in H2A (Karagiannis et al., 2007; Morrison et al., 2009). Together, all these data suggest a complex and differentiated role for the chromatin template in DNA repair, recombination and replication. Our work revealed that Dot1's role in the DNA damage response is multifaceted and extends to regulation of the TLS pathway and maintenance of chromosome stability, although the mechanisms are still unclear. The challenge of future

research will be to uncover the intricate network between chromatin modifiers and DNA damage response effectors.

4.4 Experimental procedure

4.4.1 Yeast strains

All yeast strains used in this study are listed in Table 4.1 and created using standard yeast genetic techniques (Ausubel, 1987). Complete gene deletions and integration of a triple FLAG tag at the 3' end of genes (Gelbart et al., 2001) were achieved using one-step gene integration of PCR-amplified modules (Longtine et al., 1998). Plasmid shuffling experiments were performed using 5-FOA as a counterselecting agent for the *URA3* plasmid (pRS316, *HHT2-HHF2*), and shuffling in plasmids containing histone H3 K79 mutations (pRS314, *hht2-HHF2*) (B. Yang et al., 2008). Catalytically inactive Dot1 mutants were expressed from pRS315 plasmids (van Leeuwen et al., 2002), and a non-phosphorylatable mutant of Rtt107 (four SQ motifs substituted by AQ) was expressed from a plasmid (pRS315, *rtt107-4AQ*) which was a generous gift from Grant Brown and Tania Roberts (University of Toronto). BrdU-Incorporating (BrdU-Inc) wild-type and mutant strains containing constitutively expressed Herpes simplex virus thymidine kinase (HSV-TK) and human equilibrative nucleoside transporter (hENT1) were generated by genetic crosses with a previously published parental strain (Viggiani et al., 2006).

Table 4.1 Yeast strains used in this study

Strain	Relevant Genotype
MKY5	W303, *MATα ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 LYS2*
MKY6	W303, *MATA ADE2 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 lys2Δ*
MKY7	W303, *MATA ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 LYS2*
MKY399	W303, *MATα ADE2 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 lys2Δ*
MKY10	W303, *MATα his4*
MKY11	W303, *MATA his4*
MKY921	MKY5, *dot1Δ::his5MX6*
MKY922	MKY5, *rtt107Δ::KANMX6 lys2Δ*
MKY923	MKY7, *rtt107Δ::KANMX6 dot1Δ::his5MX6*
MKY924	MKY7, *slx4Δ::KANMX6*
MKY925	MKY5, *slx4Δ::KANMX6 dot1Δ::his5MX6*
MKY927	MKY5, *bre1Δ::HYGMX6 lys2Δ*
MKY928	MKY5, *rtt107Δ::KANMX6*
MKY929	MKY5, *rtt107Δ::KANMX6 bre1Δ::HYGMX6 lys2Δ*
MKY930	MKY5, *slx4Δ::KANMX6 lys2Δ*
MKY931	MKY5, *slx4Δ::KANMX6 bre1Δ::HYGMX6 lys2Δ*
MKY932	MKY5, *[pRS315]*
MKY933	MKY5, *[pRS315, DOT1]*
MKY934	MKY5, *[pRS315, dot1G401R]*
MKY935	MKY5, *[pRS315, dot1G401A]*
MKY936	MKY7, *rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315]*
MKY937	MKY7, *rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315, DOT1]*
MKY938	MKY7, *rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315, dot1G401R]*
MKY939	MKY7, *rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315, dot1G401A]*
MKY940	*MATA/α ADE2/ade2-1 can1-100/can1-100 his3-11/his3-11 leu2-3,112/leu2-3,112 LYS2/lys2Δ*
MKY941	MKY940, *rtt107Δ::KANMX6/rtt107Δ::NATMX6*
MKY942	MKY940, *dot1Δ::his5MX6/dot1Δ::his5MX6*
MKY943	MKY940, *rtt107Δ::KANMX6/rtt107Δ::KANMX6 dot1Δ::his5MX6/ dot1Δ::his5MX6*
MKY944	MKY7, *Rad 52-GFP::NATMX6*
MKY945	MKY7, *rtt107Δ::KANMX6 Rad 52-GFP::NATMX6*
MKY946	MKY7, *dot1Δ::his5MX6 Rad 52-GFP::NATMX6*
MKY947	MKY7, *rtt107Δ::KANMX6 dot1Δ::his5MX6 Rad 52-GFP::NATMX6*
MKY949	MKY7, *dot1Δ::his5MX6*
MKY950	MKY7, *rtt107Δ::KANMX6*
MKY951	MKY399, *rtt107Δ::KANMX6*
MKY952	MKY6, *dot1Δ::his5MX6*
MKY953	MKY399, *rev1Δ::HYGMX6*
MKY954	MKY6, *rtt107Δ::KANMX6 dot1Δ::his5MX6*
MKY955	MKY399, *dot1Δ::his5MX6 rev1Δ::HYGMX6*
MKY956	MKY399, *rtt107Δ::KANMX6 rev1Δ::HYGMX6*
MKY957	MKY6, *rtt107Δ::KANMX6 dot1Δ::his5MX6 rev1Δ::HYGMX6*
MKY958	MKY5, *rev3Δ::HYGMX6*

Strain	Relevant Genotype
MKY959	MKY5, *rtt107Δ::KANMX6 dot1Δ::his5MX6*
MKY960	MKY5, *dot1Δ::his5MX6 rev3Δ::HYGMX6*
MKY961	MKY5, *rtt107Δ::KANMX6 rev3Δ::HYGMX6*
MKY962	MKY5, *rtt107Δ::KANMX6 dot1Δ:: his5MX6 rev3Δ::HYGMX6*
MKY963	MKY399, *hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS316, HHT2-HHF2]*
MKY964	MKY6, *rtt107Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS316, HHT2-HHF2]*
MKY965	MKY399, *hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79A-HHF2]*
MKY966	MKY6, *rtt107Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79A-HHF2]*
MKY992	MKY5, *SLX4-3XFLAG::NATMX6*
MKY993	MKY5, *rtt107Δ::KANMX6 SLX4-3XFLAG::NATMX6*
MKY994	MKY5, *dot1Δ::his5MX6 SLX4-3XFLAG::NATMX6*
MKY995	MKY5, *rtt107Δ::KANMX6 dot1Δ::his5MX6 SLX4-3XFLAG::NATMX6*
MKY996	MKY5, *RTT107-3XFLAG::NATMX6 lys2Δ*
MKY997	MKY5, *slx4Δ::KANMX6 RTT107-3XFLAG::NATMX6*
MKY998	MKY7, *dot1Δ::his5MX6 RTT107-3XFLAG::NATMX6 lys2Δ*
MKY999	MKY7, *slx4Δ::KANMX6 dot1Δ::his5MX6 RTT107-3XFLAG::NATMX6 lys2Δ*
MKY1000	MKY6, *hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79R-HHF2]*
MKY1001	MKY399, *rtt107Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79R-HHF2]*
MKY1040	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
MKY1041	MKY1040, *rtt107Δ::his5MX6*
MKY1042	MKY1040, *dot1Δ::HYGMX6*
MKY1043	MKY1040, *rtt107Δ::his5MX6 dot1Δ::HYGMX6*
MKY1055	MKY5, *rtt107Δ::KANMX6 [pRS315, RTT107]*
MKY1056	MKY5, *rtt107Δ::KANMX6 [pRS315, rtt107-4AQ]*
MKY1057	MKY5, *rtt107Δ::KANMX6 [pRS315]*
MKY1058	MKY5, *rtt107Δ::KANMX6 dot1Δ: his5MX6 [pRS315, RTT107]*
MKY1059	MKY5, *rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315, rtt107-4AQ]*
MKY1060	MKY5, *rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315]*
MKY1082	MKY5, *slx4Δ::KANMX6 dot1Δ::his5MX6 [pRS315]*
MKY1083	MKY5, *slx4Δ::KANMX6 dot1Δ::his5MX6 [pRS315, DOT1]*
MKY1084	MKY5, *slx4Δ::KANMX6 dot1Δ::his5MX6 [pRS315, dot1 G401A]*
MKY1085	MKY5, *slx4Δ::KANMX6 dot1Δ::his5MX6 [pRS315, dot1 G401R]*
MKY1086	MKY6, *slx4Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, HHT2-HHF2]*
MKY1087	MKY6, *slx4Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79A-HHF2]*
MKY1088	MKY6, *slx4Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79R-HHF2]*
MKY1094	MKY6, *ars603.5Δ::URA3 ars608Δ::HIS3 ars609Δ::TRP1 LEU2::BrdU-Inc*
MKY1095	MKY6, *ars603.5Δ::URA3 ars608Δ::HIS3 ars609Δ::TRP1 LEU2::BrdU-Inc rtt107Δ::KANMX6*
MKY1096	MKY6, *ars603.5Δ::URA3 ars608Δ::HIS3 ars609Δ::TRP1 LEU2::BrdU-Inc dot1Δ::HYGMX6*
MKY1097	MKY6, *ars603.5Δ::URA3 ars608Δ::HIS3 ars609Δ::TRP1 LEU2::BrdU-Inc rtt107Δ::KANMX6 dot1Δ::HYGMX6*

4.4.2 Sensitivity measurements

Overnight cultures grown in YPD or SC-Leu at 30°C were diluted to 0.3 $O.D_{600}$. The cells were tenfold serially diluted and spotted onto solid YPD plates or SC-Leu plates with MMS (Sigma) at various concentrations. The plates were then incubated at 30°C for 2-3 days.

4.4.3 Protein extracts and protein blot analysis

Overnight cultures were diluted to 0.3 $O.D_{600}$ and grown in YPD to 0.8 $O.D_{600}$, and then treated with 0.03% MMS for 1 h. The FLAG-tagged Slx4 protein was extracted by an alkaline method using 0.2 M NaOH (Kushnirov, 2000). Slx4-FLAG proteins were visualized using anti-FLAG M2 antibodies (Sigma) and SuperSignal enhanced chemiluminescence (Pierce Chemical). The procedure for analytical-scale immunoprecipitation of the FLAG-tagged Rtt107 protein was adapted from a previous report (Kobor et al., 2004a). Briefly, yeast cells were harvested, and lysed in TAP-IP Buffer (50mMTris [pH 7.8], 150 mM NaCl, 1.5 mM MgAc, 0.15% Nonidet P-40, 1 mM DTT, 10 mM NaPPi, 5 mM EGTA, 5 mM EDTA, 0.1mM Na_3VO_4, 5 mM NaF, Complete^{TM} Protease inhibitor mixture) using acid-washed glass beads and mechanically disrupting using a bead beater (BioSpec Products). Rtt107-FLAG fusion proteins were captured using anti FLAG M2 agarose beads (Sigma), and subsequently washed in TAP-IP buffer. Captured material was analyzed by protein blotting with anti-FLAG M2 (Sigma) and visualized using the Odyssey Infrared Imaging System (Licor).

4.4.4 Flow cytometric analysis and BrdU incorporation experiments

Cells were prepared under the same conditions for flow cytometric analysis and BrdU incorporation experiment. For the latter, we used wild-type and mutant strains containing the BrdU-Inc cassette (Viggiani et al., 2006) to allow for BrdU uptake in yeast. Briefly, cells were arrested in G1 by addition of 2 μg/ml of α-factor for 2 h at 30°C in YPD, then washed with sterile 1X PBS and resuspended in YPD containing 0.03% MMS for 1 h. MMS was removed by treating with 2% sodium thiosulfate and washing with sterile 1X PBS. The cells were resuspended in YPD and incubated at 30°C during the recovery phase. For the BrdU incorporation experiment, 400 μg/ml of BrdU was added to the cultures during the recovery phase. Aliquots were removed at the indicated times and processed further for flow cytometric analysis or measurement of BrdU incorporation.

For flow cytometric analysis, cells were collected in 70% ethanol with 0.2M Tris-HCl pH 7.5 and prepared as previously described (Haase et al., 1997). Samples were analyzed using the BD FACS Calibur instrument and the Flow Jo software (Tree Star Inc. OR). For the BrdU incorporation experiments, cells were collected in buffer containing 100 mM EDTA (pH 8.0), 10 mM Tris-HCl (pH 8.0), and 0.1% NaH_3. Total genomic DNA was extracted by bead-beating and use of the DNeasy kit (QIAGEN) and sonicated using Bioruptor (Diagenode). The DNA concentration was adjusted to 20 ng/μl, then heat-denatured and snap-cooled. One microgram of DNA was spotted onto a nitrocellulose membrane (Bio-Rad) pre-soaked with 2X SSC using the Convertible Filtration Manifold System (GIBCO BRL) and subjected to ultraviolet crosslinking in a Stratalinker (Stratagene). Subsequently, the membrane was blocked with 5% milk in TTBS, probed

with an anti-BrdU antibody (GE Healthcare), and visualized using the Odyssey Infrared Imaging System (Licor).

4.4.5 Quantitative bimater assay

The procedure for the bimater assay was modified from a previous method to allow quantification (Yuen et al., 2007). Briefly, 12 independent colonies from each homozygous diploid strain were grown in YPD overnight at 30°C and diluted to 2.0 $O.D_{600}$. Cells were plated on to solid YPD at appropriate dilutions to determine the total number of cells. Equal volumes of *MAT*a mating tester cultures (10.0 $O.D_{600}$) and the homozygous diploid strain cultures (2.0 $O.D_{600}$) were plated onto solid media containing no amino acids and incubated at 30°C for 3-4 days. Mating rates and 95% confidence intervals were calculated with the Ma-Sandri-Sarkar Maximum Likelihood Estimator (MSS-MLE) method using the online Fluctuation AnaLysis CalculatOR (FALCOR) (Hall et al., 2009).

4.4.6 Microscopy

Nuclear morphology was determined by treating cells as for flow cytometric analysis, except α-factor incubation was omitted and SC-complete medium was used to minimize auto-fluorescence. Aliquots were removed at the indicated times and treated with 4% formaldehyde solution (Sigma) for 10 min. Cells were immobilized on a glass slide with a solution of 1 mg/ml polylysine (Sigma) and then stained with 4, 6-diamidino-2-phenylindole (DAPI; Sigma). Cells with medium to large buds were counted as being in

G2/M phase. Ambiguous cases where cells with separate nuclei were insufficiently spread were considered to be in G1 phase.

To visualize Rad52-GFP foci, cells were grown at 30°C in SC-complete medium to logarithmic phase, and then immobilized on a glass slide with a solution of 1.0% agarose in ddH_2O. Multiple images were obtained at 0.3 μm intervals along the z axis and Rad52-GFP foci were counted by inspection of all focal planes. At least 300 cells were counted for each time point. All imaging was done with the Zeiss Axioplan 2 fluorescence microscope using the Metamorph software. Statistical significance was assessed using Student's *t* test.

4.4.7 Measurement of mutation rates

Forward mutation rates were measured by mutations at the *CAN1* locus, which when mutated renders cells sensitive to canavanine (Grenson et al., 1966). Cells from 12 independent colonies for each strain were grown in YPD to logarithmic phase, 0.005% MMS was added to half of each culture, and cells were further incubated at 30°C for 20 hours. Cells plated on SC-Arg were diluted 1 in 200000 while cells plated on SC-Arg containing 50 μg/ml canavanine (Sigma) were diluted by a factor of 2. Plates were incubated at 30°C for 2 days and colonies were counted. The mutation rates and 95% confidence intervals were calculated with the Ma-Sandri-Sarkar Maximum Likelihood Estimator (MSS-MLE) method using the online Fluctuation AnaLysis CalculatOR (FALCOR) (Hall et al., 2009).

4.4.8 Pulsed-field gel electrophoresis

Briefly, cells were arrested in G1 by addition of 2 μg/ml of α-factor for 2 h at 30°C in YPD, then washed with sterile 1X PBS and resuspended in YPD containing 0.03% MMS for 1 h. MMS was removed by treating with 2% sodium thiosulfate and washing with sterile 1X PBS. The cells were resuspended in YPD and incubated at 30°C during the recovery phase. Aliquots were removed at the indicated times and processed further.

Cells were treated using the CHEF Yeast Genomic DNA Plug Kit (Bio-Rad, CA). As described by the manufacturer, cells were washed with cold 50 mM EDTA and resuspended in Cell Suspension Buffer containing 0.75% agarose and Lyticase, and incubated for 2 h at 37oC. The agarose plugs were incubated with Proteinase K overnight at 50oC, then washed and loaded onto a 1.0% agarose gel in 0.5X Tris-borate EDTA. Pulsed-field gel electrophoresis was carried out as described by the manufacturer (GE Healthcare, UK). Briefly, the gel was subjected to electrophoresis at 100 V with a switch time of 60–120 s for 24 h with constant recirculation of the buffer at 8.5°C. Following electrophoresis, the gels were stained with ethidium bromide (0.5 μg/ml) and photographed.

Chapter 5

Discussion

Extensive discoveries in the chromatin field have revealed the importance of post-translational modifications in regulating a wide variety of cellular processes (Ehrenhofer-Murray, 2004). Despite progress in research describing the complex relationship between cellular functions and chromatin modifications, further investigations still need to be done to uncover the complete picture of genome regulation. My work presented here contributes to the understanding of the function of chromatin modifiers such as NuA4, picNuA4, and Dot1, in genome regulation and maintenance in *Saccharomyces cerevisiae*.

In Chapter 2 of this dissertation, I explored the precise nature of the relationship between NuA4 and picNuA4 by using two different approaches thought to liberate picNuA4: a complete deletion of *EAF1* or an allele encoding a C-terminal truncation of Epl1 (Auger et al., 2008; Boudreault et al., 2003; Ginsburg et al., 2009). Interestingly, investigations using genetic, biochemical, and functional genomic assays demonstrated not only commonalities but also distinct phenotypes between the two mutants, suggesting that the two approaches cause different functional consequences. Accordingly, deletion of Eaf1 led to decrease of bulk histone H4 acetylation in contrast to the increase of histone H4 acetylation observed in Epl1 C-terminus mutants. Despite the markedly opposite global histone acetylation levels conferred by these two approaches, they did not lead to any major transcriptional differences as suggested by the similar global expression profiles of *epl1* and *eaf1* mutants. These expression profiles hinted that functionally similar genome-

wide patterns of histone acetylation were present in both mutants. These seemingly contradictory results could be reconciled by a number of explanations. For instance, the increased acetylated histones in the *epl1* mutant might not be incorporated into chromatin or might be localized to intergenic regions. Therefore, if either of these hypotheses are true, the acetylation levels at promoters or open reading frames would remain unchanged, explaining the lack of variation in gene expression in the *epl1* mutant compared to the *eaf1* mutant. Consistent with these hypotheses, mapping of H4 acetylation at promoters of ribosomal protein genes did not reveal any differences between *eaf1* and *epl1* mutants at specific loci. Thus, genome-wide mapping of H4 acetylated residues might provide an answer to these questions. However, an elegant study on single-nucleosome resolution mapping of histone modifications suggested that distinct patterns of histone acetylation did not directly translate to different levels of transcription (Liu et al., 2005). Collectively, the work of this group suggested that transcriptional consequences linked to acetylation are compatible with the presence of untargeted acetylation in the genome (Liu et al., 2005). Therefore, these evidences reconcile the bulk changes in histone acetylation with similar global transcriptional profiles observed in *eaf1* and *epl1*. As a possibility, non-targeted HATs could create a global increase of histone acetylation promoting the binding of activators at genes required upon inducing conditions (Friis et al., 2009). Nevertheless, the mechanisms and transcriptional effects behind non-targeted histone acetylation by HATs remain elusive.

While several studies have exposed the molecular architecture of picNuA4 (Arnold et al., 2011; Berndsen et al., 2007; Chittuluru et al., 2011; Lee et al., 2011), its precise role

remains unclear, as it is difficult to study only picNuA4 since it does not contain any unique subunits different from NuA4. In Chapter 2, global genetic and gene expression analysis revealed potential picNuA4 functions; however, additional investigations are required to get a better picture. For instance, it is still unknown whether picNuA4 is able to also acetylate non-histone targets like NuA4, which was demonstrated to acetylate non-histone substrates playing a role in cellular aging (Lu et al., 2011; Lu, Lin, Zhu et al., 2011). Similarly, a recent study demonstrated that acetylation of the protein kinase ULK1 by TIP60, the human homologue of NuA4, is responsible for regulation of autophagy in metazoans (Lin et al., 2012). Further insight into picNuA4 function could be uncovered through studying HBO1, a mammalian complex that shares similar properties with picNuA4. These include the presence of a catalytic subunit belonging to the same HAT family, having affinity to nucleosomes over free histones, and being involved in untargeted histone acetylation, although picNuA4 has preference for H2A/H4 and HBO1 for H3/H4 (Boudreault et al., 2003; Doyon et al., 2006). Previous work showed that HBO1 interacts with non-histone proteins such as androgen receptors in mammals and the transcription factor/oncogene DJun/DFos in flies (Miotto et al., 2006; Sharma et al., 2000). These interactions suggest that HBO1 is involved in gene-specific recruitment, which could potentially reveal a function in non-histone acetylation. Additionally, whereas the cellular role of picNuA4 is not well understood, HBO1 is involved in gene regulation, cell proliferation, as well as DNA replication (Avvakumov et al., 2007; Avvakumov et al., 2012; Miotto et al., 2008). Consistent with a role in DNA replication, an *epl1* mutant displayed sensitivity when exposed to the nucleotide reductase inhibitor hydroxyurea (HU), which affects DNA synthesis by preventing the dNTP pool

expansion. Therefore, discoveries about HBO1 function could provide promising hypotheses for possible picNuA4 functions that could be further tested.

In Chapter 2, striking differences between *eaf1* and *epl1* on NuA4-dependent phenotypes incited us to study the structure-function relationship of the scaffolding subunit Eaf1, which when deleted, broadly compromises NuA4 function (Auger et al., 2008; Babiarz et al., 2006; Kobor et al., 2004b; Mitchell et al., 2008). In this chapter, we determined that the Eaf1 C-terminal region was required for NuA4-dependent function, such as histone acetylation, NuA4 recruitment to chromatin, and NuA4 complex stability. In addition to the Eaf1 C-terminal region, we also investigated the role of the different Eaf1 domains in NuA4 function. We revealed a requirement of Eaf1 HSA domain in both H4 and H2A.Z acetylation, while deletion of the SANT domain did not lead to any major defect. The HSA domain was previously shown to be responsible for binding of actin and actin-related proteins (Arps) in chromatin modifying complexes, and more precisely, Act1 and Arp4 subunits of the shared module in the NuA4 and SWR1-C (Szerlong et al., 2008). Collectively, impairment of binding of actin or Arps to chromatin modifying complexes might affect their function, such as NuA4-dependent histone acetylation. Interestingly, actin filaments are proposed to anchor or move the complexes they reside in within the nucleus (Gottschalk et al., 2008; Rando et al., 2002; Szerlong et al., 2008). However, this model is hard to reconcile with other data demonstrating that only globular actin might be found in chromatin modifying complexes, as opposed to filamentous actin (Fenn et al., 2011). Other studies also suggest that Arps might play the role of histone chaperones or regulatory complexes involved in recruitment of chromatin remodelers to nucleosomes

through binding of histones (Downs et al., 2004a; Galarneau et al., 2000). Finally, recent studies revealed that formation of an actin-Arp4 heterocomplex is required for complex stability of chromatin remodelling complexes (Nishimoto et al., 2012). Still, mutation in the shared subunits only led to a defect in H2A.Z acetylation, but not H4 acetylation, arguing against a general defect in NuA4 function (Results in Chapter 3, (Wang et al., 2009a)). Evidence from the literature demonstrated that the Eaf1 HSA domain is responsible for binding of the shared module through interaction with Arp4-Act1 (Szerlong et al., 2008). Although it is still not known whether loss of Yaf9 or Swc4 destabilize association of the actin-Arp4 heterocomplex to the HSA domain. Interestingly, our results suggest that not only the shared subunits are lost upon HSA domain deletion, but possibly other NuA4 components such as the picNuA4, which has been shown to bind the N-terminal region of Eaf1.

Previous studies suggested that SANT domains in other chromatin remodelling complexes bind to DNA or interact with histones (Aasland et al., 1996; Baker et al., 2009; Boyer et al., 2004; Park et al., 2010; Ryan et al., 2011; Sterner et al., 2002). Additionally, it is still unclear why NuA4 includes several SANT domain-containing proteins such as Eaf1 and Swc4 (Lu et al., 2009). Do these SANT domains have a redundant function, or do they each have a specific role? What are their targets? Could they be DNA, proteins, or histone post-translational modifications? Different strategies could be used to uncover the interacting partners of these distinct SANT domains, such as a chromatin pull-down assay using a strain with a complete version of the gene of interest and a domain deletion mutant for control (Martin et al., 2006). Another strategy that

allows the identification of specific PTMs binding partners is the yeast tethered catalysis method, a modified two-hybrid system able to identify protein-protein interactions requiring post-translational modifications (Guo et al., 2004). Using a histone peptide sequence in tandem with its modifier enzyme, and the SANT domain as the reader domain, we could identify the specific binding targets of the different SANT domains.

In Chapter 3 of this dissertation, I explored another NuA4 submodule, the shared module between NuA4 and SWR1-C. To decipher the specific contribution of the shared submodule in SWR1-C or NuA4, I used mutants of Yaf9 and Swc4, which are unique components of both complexes. While biochemical assays showed similar defects in H2A.Z deposition and acetylation, global genetic interaction patterns and gene expression changes demonstrated that both Yaf9 and Swc4 had a role more akin to SWR1-C. Additionally, genetic, gene expression, and biochemical assays revealed that Yaf9 and Swc4 similarly contributed to the shared module functions but also have distinct roles. For instance, large-scale genetic interaction profiles exposed noticeable differences between Yaf9 and Swc4, where Swc4 behaved more similarly to NuA4, and Yaf9 to SWR1-C. Consistent with this intricate relationship existing within the shared module, the sensitivity of the *yaf9*Δ mutant to genotoxic agents and its defects in forming heterochromatin boundaries were partly rescued when combined with the *swc4 1-400* allele. Based on the *swc4 1-400 yaf9*Δ double mutant phenotype, it is tempting to speculate that Swc4 may be responsible for the binding of Yaf9 to SWR1-C, where Swc4 recruits the SWR1-C to chromatin through its essential SANT domain while the Yaf9 YEATS domain solidifies the interaction to chromatin by binding to histones H3/H4

(Bittner et al., 2004; Wu et al., 2005). Although this is contrasting with the previous model where Yaf9 recruits Swc4 to SWR1-C via its C-terminus, Swc4 is not totally lost in SWR1-C purification upon *YAF9* deletion (Wu et al., 2005). Moreover, Arp4, another shared subunit, was shown to be able to bind nucleosomes *in vitro* (Downs et al., 2004b; Galarneau et al., 2000), adding to the evidence that suggests the shared module is required for anchoring the NuA4 and SWR1-C complexes to chromatin. However, besides the shared subunits, other components of these two complexes contain domains that are thought to facilitate recruitment to chromatin (Doyon et al., 2004). For instance, the SWR1-C component Bdf1 contains a bromodomain that was shown to interact with acetylated H4 residues, recruiting SWR1-C to chromatin (Raisner et al., 2005; Wu et al., 2009; Zhang, Roberts, and Cairns, 2005a). Nonetheless, the exact molecular mechanism by which the numerous subunits coordinate to regulate their access and activity on chromatin remains unclear. To investigate the described model, a complex purification using a tagged version of Swc4 truncated at its C-terminus could help clarify the structure of the complex.

Specific shared subunits were also shown to play a role in the DNA damage response. For instance, Arp4 was previously shown to be involved in NuA4 recruitment to chromatin upon DNA damage through phosphorylated H2A.X (Downs et al., 2004a). Once NuA4 is associated to sites of DNA damage, spreading of histone H4 acetylation is observed over 2kb from the DSB (Lin et al., 2008). The newly acetylated H4, which possibly leads to a more accessible chromatin structure, together with phosphorylated H2A.X, promote the recruitment of components of the DNA repair machinery and

numerous chromatin remodelling complexes including SWR1-C and INO80 (Bird et al., 2002; Downs et al., 2004b; Sapountzi et al., 2006; Tamburini et al., 2005). Subsequently, this leads to H2A.Z deposition into chromatin adjacent to DSB (Kalocsay et al., 2009), followed by H2A.Z and H2A.X removal from DSBs in an INO80-dependent manner (van Attikum et al., 2007). During the DNA damage response, not only chromatin-remodelling complexes are recruited to DSB but also factors involved in the DNA damage response. For instance, Rtt107, a protein required for reinitiating replication after DNA damage repair, is also recruited to chromatin upon DNA damage, specifically through direct interaction of its BRCT domains with H2A.X (Li et al., 2012; Roberts et al., 2008; Rouse, 2004b). However, the relationship between chromatin remodelling complexes and DNA damage factors is still unclear. For instance, the HAT Rtt109 responsible for acetylation of H3K56 is necessary for Rtt107 recruitment to chromatin, although acetylated H3K56 residues are not required (Li et al., 2012; Roberts et al., 2008). Moreover, *ESA1*, the gene encoding for the catalytic subunit of NuA4, and *RTT107* displayed an alleviating genetic interaction (data not shown), suggesting a commonality in NuA4 and Rtt107's role in the DNA damage response. We could hypothesize that either acetylated H4 is required for direct Rtt107 binding to H2A.X, or for "opening" of chromatin, which normally sterically blocks access to Rtt107.

Several chromatin modifications constitutively integrated across the genome also play a role in the DNA damage response, such as methylation of H3K79 by the methyltransferase Dot1. Methylation by Dot1 does not lead to chromatin rearrangement, but is believed to play roles in Rad9-mediated DNA damage response by activating the

G1/S checkpoint and by the repair of DNA damage during late G2 phase (Grenon et al., 2007). In Chapter 4 of this dissertation, we proposed a model explaining the effect of H3K79 trimethylation during DNA damage in cells lacking Rtt107. Specifically, we showed that loss of H3K79 trimethylation suppressed the DNA damage sensitivity observed in *rtt107*Δ mutant. This suppression was not linked to restoration of Rtt107 phosphorylation, but instead was dependent on the presence of a functional TLS pathway, a tolerance mechanism that allows bypass of DNA lesions enabling cells to survive (Lee et al., 2008). Moreover, deletion of *DOT1* partially suppressed the cell cycle delay and the defect in resuming DNA replication of *rtt107*Δ mutants during the recovery from MMS-induced DNA damage. In this study we proposed that, directly or indirectly, binding of the H3K79 trimethylation by a DNA damage response protein can inhibit TLS. Hypothetically, trimethylation of H3K79 may also generate a change in chromatin conformation that inhibits TLS. Alternatively, use of the TLS pathway may be stimulated by a compromised DNA damage checkpoint due to loss of Dot1. However, there is still lack of evidence to connect the roles of Dot1 in the TLS and DNA damage checkpoint. Interestingly, recent work demonstrated that di- and tri-methylated H3K79 marks are mutually exclusive across the genome (Schulze et al., 2009). Therefore, it is tempting to speculate that some specific genomic regions could be involved in repression of the TLS pathway. Additionally, identifying the factors binding trimethylated H3K79 upon DNA damage through purification followed by MS could help to elucidate the mechanism by which this mark could potentially inhibit TLS.

Methylation of H3K79 plays a role in various biological processes in addition to DNA damage repair, such as gene silencing and cell cycle. Consistent with this, several studies exposed the impact of loss of Dot1's function on human health, as Dot1 was found to have a role in hematopoiesis, and cardiac muscle function, as well as leukemia (Nguyen et al., 2011). Interestingly, Dot1 can bind AF9, one of Yaf9's human homologues (Park et al., 2010). Likewise, AF9 is also involved in spontaneous acute leukemias, as it is a prevalent fusion partner with the mixed lineage leukemia (MLL) protein, resulting in the expression of oncogenic fusion proteins (Daser et al., 2004). However, protein fusion can be prevented by formation of stable complex of Dot1 with AF9 (Biswas et al., 2011). Similarly, Dot1 was shown to counteract MLL translocation with other partners such as components of the elongation complexes (Ayton et al., 2001; Lin et al., 2010; Mueller et al., 2007; Yokoyama et al., 2010). Collectively, this reveals a profound and complex relationship between chromatin modifiers and their impact on cellular functions.

In conclusion, it is essential to remember that chromatin structure not only affects cellular function but also ultimately affects human health and disease (Bhaumik et al., 2007). Research in the field of chromatin has revealed that changes in chromatin neighbourhoods not only play a fundamental role for proper child development, but also for preservation of human health (Petronis, 2010). Ultimately, progress in chromatin research will eventually contribute to the development of therapeutics and treatments for diseases, therefore improving human health.

Bibliography

Aasland, R., Stewart, A. F., and Gibson, T. (1996). The SANT domain: A putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. *Trends in Biochemical Sciences, 21*(3), 87-88.

Abbott, D. W., Ivanova, V. S., Wang, X., Bonner, W. M., and Ausio, J. (2001). Characterization of the stability and folding of H2A.Z chromatin particles: Implications for transcriptional activation. *The Journal of Biological Chemistry, 276*(45), 41945-41949.

Abraham, R. T. (2001). Cell cycle checkpoint signaling through the ATM and ATR kinases. *Genes & Development, 15*(17), 2177-2196.

Adam, M., Robert, F., Larochelle, M., and Gaudreau, L. (2001). H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. *Molecular and Cellular Biology, 21*(18), 6270-6279.

Albert, I., Mavrich, T. N., Tomsho, L. P., Qi, J., Zanton, S. J., Schuster, S. C., et al. (2007). Translational and rotational settings of H2A.Z nucleosomes across the saccharomyces cerevisiae genome. *Nature, 446*(7135), 572-576.

Alberti, S., Gitler, A. D., and Lindquist, S. (2007). A suite of gateway cloning vectors for high-throughput genetic analysis in saccharomyces cerevisiae. *Yeast (Chichester, England), 24*(10), 913-919.

Allard, S., Utley, R. T., Savard, J., Clarke, A., Grant, P., Brandl, C. J., et al. (1999). NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *The EMBO Journal, 18*(18), 5108-5119.

Allfrey, V. G., Faulkner, R., and Mirsky, A. E. (1964). Acetylation and methylation of histones and their possible role in the regulation of rna synthesis. *Proceedings of the National Academy of Sciences of the United States of America, 51*, 786-794.

Allis, C. D., Glover, C. V., Bowen, J. K., and Gorovsky, M. A. (1980). Histone variants specific to the transcriptionally active, amitotically dividing macronucleus of the unicellular eucaryote, tetrahymena thermophila. *Cell, 20*(3), 609-617.

Allis, C. D., Jenuwein, T., Reinberg, D. and Caparros, M. L. (2007). *Epigenetics* (1st edition ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Altaf, M., Auger, A., Monnet-Saksouk, J., Brodeur, J., Piquet, S., Cramet, M., et al. (2010). NuA4-dependent acetylation of nucleosomal histones H4 and H2A directly

stimulates incorporation of H2A.Z by the SWR1 complex. *The Journal of Biological Chemistry, 285*(21), 15966-15977.

Altaf, M., Utley, R. T., Lacoste, N., Tan, S., Briggs, S. D., and Cote, J. (2007). Interplay of chromatin modifiers on a short basic patch of histone H4 tail defines the boundary of telomeric heterochromatin. *Molecular Cell, 28*(6), 1002-1014.

Alvaro, D., Lisby, M., and Rothstein, R. (2007). Genome-wide analysis of Rad52 foci reveals diverse mechanisms impacting recombination. *PLoS Genetics, 3*(12), e228.

Ansari, A., and Hampsey, M. (2005). A role for the CPF 3'-end processing machinery in RNAP II-dependent gene looping. *Genes & Development, 19*(24), 2969-2978.

Arents, G., Burlingame, R. W., Wang, B. C., Love, W. E., and Moudrianakis, E. N. (1991). The nucleosomal core histone octamer at 3.1 A resolution: A tripartite protein assembly and a left-handed superhelix. *Proceedings of the National Academy of Sciences of the United States of America, 88*(22), 10148-10152.

Arnold, K. M., Lee, S., and Denu, J. M. (2011). Processing mechanism and substrate selectivity of the core NuA4 histone acetyltransferase complex. *Biochemistry, 50*(5), 727-737.

Auger, A., Galarneau, L., Altaf, M., Nourani, A., Doyon, Y., Utley, R. T., et al. (2008). Eaf1 is the platform for NuA4 molecular assembly that evolutionarily links chromatin acetylation to ATP-dependent exchange of histone H2A variants. *Molecular and Cellular Biology, 28*(7), 2257-2270.

Ausubel, F. M. (Ed.). (1987). *Current protocols in molecular biology*. New York, N.Y.: J. Wiley.

Avvakumov, N., and Cote, J. (2007). The MYST family of histone acetyltransferases and their intimate links to cancer. *Oncogene, 26*(37), 5395-5407.

Avvakumov, N., Lalonde, M. E., Saksouk, N., Paquet, E., Glass, K. C., Landry, A. J., et al. (2012). Conserved molecular interactions within the HBO1 acetyltransferase complexes regulate cell proliferation. *Molecular and Cellular Biology, 32*(3), 689-703.

Ayton, P. M., and Cleary, M. L. (2001). Molecular mechanisms of leukemogenesis mediated by MLL fusion proteins. *Oncogene, 20*(40), 5695-5707.

Babiarz, J. E., Halley, J. E., and Rine, J. (2006). Telomeric heterochromatin boundaries require NuA4-dependent acetylation of histone variant H2A.Z in saccharomyces cerevisiae. *Genes & Development, 20*(6), 700-710.

Baker, A. M., Fu, Q., Hayward, W., Lindsay, S. M., and Fletcher, T. M. (2009). The Myb/SANT domain of the telomere-binding protein TRF2 alters chromatin structure. *Nucleic Acids Research, 37*(15), 5019-5031.

Bannister, A. J., and Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research, 21*(3), 381-395.

Bannister, A. J., Schneider, R., Myers, F. A., Thorne, A. W., Crane-Robinson, C., and Kouzarides, T. (2005). Spatial distribution of di- and tri-methyl lysine 36 of histone H3 at active genes. *The Journal of Biological Chemistry, 280*(18), 17732-17736.

Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., Wang, Z., et al. (2007). High-resolution profiling of histone methylations in the human genome. *Cell, 129*(4), 823-837.

Bassal, S., and El-Osta, A. (2005). DNA damage detection and repair, and the involvement of epigenetic states. *Human Mutation, 25*(2), 101-109.

Bazzi, M., Mantiero, D., Trovesi, C., Lucchini, G., and Longhese, M. P. (2010). Dephosphorylation of gamma H2A by Glc7/protein phosphatase 1 promotes recovery from inhibition of DNA replication. *Molecular and Cellular Biology, 30*(1), 131-145.

Berndsen, C. E., Selleck, W., McBryant, S. J., Hansen, J. C., Tan, S., and Denu, J. M. (2007). Nucleosome recognition by the piccolo NuA4 histone acetyltransferase complex. *Biochemistry, 46*(8), 2091-2099.

Bettinger, B. T., Gilbert, D. M., and Amberg, D. C. (2004). Actin up in the nucleus. *Nature Reviews.Molecular Cell Biology, 5*(5), 410-415.

Bhaumik, S. R., Smith, E., and Shilatifard, A. (2007). Covalent modifications of histones during development and disease pathogenesis. *Nature Structural & Molecular Biology, 14*(11), 1008-1016.

Bird, A. W., Yu, D. Y., Pray-Grant, M. G., Qiu, Q., Harmon, K. E., Megee, P. C., et al. (2002). Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. *Nature, 419*(6905), 411-415.

Biswas, D., Milne, T. A., Basrur, V., Kim, J., Elenitoba-Johnson, K. S., Allis, C. D., et al. (2011). Function of leukemogenic mixed lineage leukemia 1 (MLL) fusion proteins through distinct partner protein complexes. *Proceedings of the National Academy of Sciences of the United States of America, 108*(38), 15751-15756.

Biswas, D., Takahata, S., Xin, H., Dutta-Biswas, R., Yu, Y., Formosa, T., et al. (2008). A role for Chd1 and Set2 in negatively regulating DNA replication in saccharomyces cerevisiae. *Genetics, 178*(2), 649-659.

Bittner, C. B., Zeisig, D. T., Zeisig, B. B., and Slany, R. K. (2004). Direct physical and functional interaction of the NuA4 complex components Yaf9p and Swc4p. *Eukaryotic Cell, 3*(4), 976-983.

Bohnsack, M. T., Stuven, T., Kuhn, C., Cordes, V. C., and Gorlich, D. (2006). A selective block of nuclear actin export stabilizes the giant nuclei of xenopus oocytes. *Nature Cell Biology, 8*(3), 257-263.

Boudreault, A. A., Cronier, D., Selleck, W., Lacoste, N., Utley, R. T., Allard, S., et al. (2003). Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. *Genes & Development, 17*(11), 1415-1428.

Boyer, L. A., Latek, R. R., and Peterson, C. L. (2004). The SANT domain: A unique histone-tail-binding module? *Nature Reviews.Molecular Cell Biology, 5*(2), 158-163.

Brown, C. E., Howe, L., Sousa, K., Alley, S. C., Carrozza, M. J., Tan, S., et al. (2001). Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science (New York, N.Y.), 292*(5525), 2333-2337.

Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., et al. (1996). Tetrahymena histone acetyltransferase A: A homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell, 84*(6), 843-851.

Cai, Y., Jin, J., Florens, L., Swanson, S. K., Kusch, T., Li, B., et al. (2005). The mammalian YL1 protein is a shared subunit of the TRRAP/TIP60 histone acetyltransferase and SRCAP complexes. *The Journal of Biological Chemistry, 280*(14), 13665-13670.

Cai, Y., Jin, J., Tomomori-Sato, C., Sato, S., Sorokina, I., Parmely, T. J., et al. (2003). Identification of new subunits of the multiprotein mammalian TRRAP/TIP60-containing histone acetyltransferase complex. *The Journal of Biological Chemistry, 278*(44), 42733-42736.

Cano, A., and Pestana, A. (1979). Purification and properties of a histone acetyltransferase from artemia salina, highly efficient with H1 histone. *European Journal of Biochemistry / FEBS, 97*(1), 65-72.

Carrozza, M. J., Utley, R. T., Workman, J. L., and Cote, J. (2003). The diverse functions of histone acetyltransferase complexes. *Trends in Genetics : TIG, 19*(6), 321-329.

Chambers, A. L., and Downs, J. A. (2007). The contribution of the budding yeast histone H2A C-terminal tail to DNA-damage responses. *Biochemical Society Transactions, 35*(Pt 6), 1519-1524.

Chang, M., Bellaoui, M., Boone, C., and Brown, G. W. (2002). A genome-wide screen for methyl methanesulfonate-sensitive mutants reveals genes required for S phase progression in the presence of DNA damage. *Proceedings of the National Academy of Sciences of the United States of America, 99*(26), 16934-16939.

Chaudhuri, S., Wyrick, J. J., and Smerdon, M. J. (2009). Histone H3 Lys79 methylation is required for efficient nucleotide excision repair in a silenced locus of saccharomyces cerevisiae. *Nucleic Acids Research, 37*(5), 1690-1700.

Chin, J. K., Bashkirov, V. I., Heyer, W. D., and Romesberg, F. E. (2006). Esc4/Rtt107 and the control of recombination during replication. *DNA Repair, 5*(5), 618-628.

Chittuluru, J. R., Chaban, Y., Monnet-Saksouk, J., Carrozza, M. J., Sapountzi, V., Selleck, W., et al. (2011). Structure and nucleosome interaction of the yeast NuA4 and piccolo-NuA4 histone acetyltransferase complexes. *Nature Structural & Molecular Biology, 18*(11), 1196-1203.

Clapier, C. R., and Cairns, B. R. (2009). The biology of chromatin remodeling complexes. *Annual Review of Biochemistry, 78*, 273-304.

Clarke, A. S., Lowell, J. E., Jacobson, S. J., and Pillus, L. (1999). Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Molecular and Cellular Biology, 19*(4), 2515-2526.

Clarkson, M. J., Wells, J. R., Gibson, F., Saint, R., and Tremethick, D. J. (1999). Regions of variant histone His2AvD required for drosophila development. *Nature, 399*(6737), 694-697.

Collins, S. R., Miller, K. M., Maas, N. L., Roguev, A., Fillingham, J., Chu, C. S., et al. (2007). Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. *Nature, 446*(7137), 806-810.

Collins, S. R., Roguev, A., and Krogan, N. J. (2010). Quantitative genetic interaction mapping using the E-MAP approach. *Methods in Enzymology, 470*, 205-231.

Conde, F., Ontoso, D., Acosta, I., Gallego-Sanchez, A., Bueno, A., and San-Segundo, P. A. (2010). Regulation of tolerance to DNA alkylating damage by Dot1 and Rad53 in saccharomyces cerevisiae. *DNA Repair, 9*(10), 1038-1049.

Conde, F., Refolio, E., Cordon-Preciado, V., Cortes-Ledesma, F., Aragon, L., Aguilera, A., et al. (2009). The Dot1 histone methyltransferase and the Rad9 checkpoint adaptor contribute to cohesin-dependent double-strand break repair by sister chromatid recombination in saccharomyces cerevisiae. *Genetics, 182*(2), 437-446.

Conde, F., and San-Segundo, P. A. (2008). Role of Dot1 in the response to alkylating DNA damage in saccharomyces cerevisiae: Regulation of DNA damage tolerance by the error-prone polymerases Polzeta/Rev1. *Genetics, 179*(3), 1197-1210.

Cote, J., Quinn, J., Workman, J. L., and Peterson, C. L. (1994). Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science (New York, N.Y.), 265*(5168), 53-60.

Cowell, I. G., Sunter, N. J., Singh, P. B., Austin, C. A., Durkacz, B. W., and Tilby, M. J. (2007). gammaH2AX foci form preferentially in euchromatin after ionising-radiation. *PloS One, 2*(10), e1057.

Daser, A., and Rabbitts, T. H. (2004). Extending the repertoire of the mixed-lineage leukemia gene MLL in leukemogenesis. *Genes & Development, 18*(9), 965-974.

Dover, J., Schneider, J., Tawiah-Boateng, M. A., Wood, A., Dean, K., Johnston, M., et al. (2002). Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. *The Journal of Biological Chemistry, 277*(32), 28368-28371.

Downs, J. A., Allard, S., Jobin-Robitaille, O., Javaheri, A., Auger, A., Bouchard, N., et al. (2004a). Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Molecular Cell, 16*(6), 979-990.

Downs, J. A., Allard, S., Jobin-Robitaille, O., Javaheri, A., Auger, A., Bouchard, N., et al. (2004b). Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Molecular Cell, 16*(6), 979-990.

Downs, J. A., Lowndes, N. F., and Jackson, S. P. (2000). A role for saccharomyces cerevisiae histone H2A in DNA repair. *Nature, 408*(6815), 1001-1004.

Doyon, Y., Cayrou, C., Ullah, M., Landry, A. J., Cote, V., Selleck, W., et al. (2006). ING tumor suppressor proteins are critical regulators of chromatin acetylation required for genome expression and perpetuation. *Molecular Cell, 21*(1), 51-64.

Doyon, Y., and Côté, J. (2004). The highly conserved and multifunctional NuA4 HAT complex. *Current Opinion in Genetics & Development, 14*(2), 147-154.

Dryhurst, D., Thambirajah, A. A., and Ausio, J. (2004). New twists on H2A.Z: A histone variant with a controversial structural and functional past. *Biochemistry and Cell Biology = Biochimie Et Biologie Cellulaire, 82*(4), 490-497.

Eberharter, A., and Becker, P. B. (2004). ATP-dependent nucleosome remodelling: Factors and functions. *Journal of Cell Science, 117*(Pt 17), 3707-3711.

Ehrenhofer-Murray, A. E. (2004). Chromatin dynamics at DNA replication, transcription and repair. *European Journal of Biochemistry / FEBS, 271*(12), 2335-2349.

Eissenberg, J. C., Wong, M., and Chrivia, J. C. (2005). Human SRCAP and drosophila melanogaster DOM are homologs that function in the notch signaling pathway. *Molecular and Cellular Biology, 25*(15), 6559-6569.

Ekwall, K. (2005). Genome-wide analysis of HDAC function. *Trends in Genetics : TIG, 21*(11), 608-615.

Essers, J., Vermeulen, W., and Houtsmuller, A. B. (2006). DNA damage repair: Anytime, anywhere? *Current Opinion in Cell Biology, 18*(3), 240-246.

Faast, R., Thonglairoam, V., Schulz, T. C., Beall, J., Wells, J. R., Taylor, H., et al. (2001). Histone variant H2A.Z is required for early mammalian development. *Current Biology : CB, 11*(15), 1183-1187.

Farrants, A. K. (2008). Chromatin remodelling and actin organisation. *FEBS Letters, 582*(14), 2041-2050.

Farris, S. D., Rubio, E. D., Moon, J. J., Gombert, W. M., Nelson, B. H., and Krumm, A. (2005). Transcription-induced chromatin remodeling at the c-myc gene involves the local exchange of histone H2A.Z. *The Journal of Biological Chemistry, 280*(26), 25298-25303.

Feng, Q., Wang, H., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Struhl, K., et al. (2002). Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Current Biology : CB, 12*(12), 1052-1058.

Fenn, S., Breitsprecher, D., Gerhold, C. B., Witte, G., Faix, J., and Hopfner, K. P. (2011). Structural biochemistry of nuclear actin-related proteins 4 and 8 reveals their interaction with actin. *The EMBO Journal, 30*(11), 2153-2166.

Fink, M., Imholz, D., and Thoma, F. (2007). Contribution of the serine 129 of histone H2A to chromatin structure. *Molecular and Cellular Biology, 27*(10), 3589-3600.

Fishburn, J., Mohibullah, N., and Hahn, S. (2005). Function of a eukaryotic transcription activator during the transcription cycle. *Molecular Cell, 18*(3), 369-378.

Foster, E. R., and Downs, J. A. (2009). Methylation of H3 K4 and K79 is not strictly dependent on H2B K123 ubiquitylation. *The Journal of Cell Biology, 184*(5), 631-638.

Fraser, P. (2006). Transcriptional control thrown for a loop. *Current Opinion in Genetics & Development, 16*(5), 490-495.

Frederiks, F., Tzouros, M., Oudgenoeg, G., van Welsem, T., Fornerod, M., Krijgsveld, J., et al. (2008). Nonprocessive methylation by Dot1 leads to functional redundancy of

histone H3K79 methylation states. *Nature Structural & Molecular Biology, 15*(6), 550-557.

Friis, R. M., and Schultz, M. C. (2009). Untargeted tail acetylation of histones in chromatin: Lessons from yeast. *Biochemistry and Cell Biology = Biochimie Et Biologie Cellulaire, 87*(1), 107-116.

Friis, R. M., Wu, B. P., Reinke, S. N., Hockman, D. J., Sykes, B. D., and Schultz, M. C. (2009). A glycolytic burst drives glucose induction of global histone acetylation by picNuA4 and SAGA. *Nucleic Acids Research, 37*(12), 3969-3980.

Galarneau, L., Nourani, A., Boudreault, A. A., Zhang, Y., Heliot, L., Allard, S., et al. (2000). Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. *Molecular Cell, 5*(6), 927-937.

Game, J. C., Williamson, M. S., and Baccari, C. (2005). X-ray survival characteristics and genetic analysis for nine saccharomyces deletion mutants that show altered radiation sensitivity. *Genetics, 169*(1), 51-63.

Gelbart, M. E., Rechsteiner, T., Richmond, T. J., and Tsukiyama, T. (2001). Interactions of Isw2 chromatin remodeling complex with nucleosomal arrays: Analyses using recombinant yeast histones and immobilized templates. *Molecular and Cellular Biology, 21*(6), 2098-2106.

Georgieva, M., Harata, M., and Miloshev, G. (2008). The nuclear actin-related protein Act3p/Arp4 influences yeast cell shape and bulk chromatin organization. *Journal of Cellular Biochemistry, 104*(1), 59-67.

Gerring, S. L., Spencer, F., and Hieter, P. (1990). The CHL 1 (CTF 1) gene product of saccharomyces cerevisiae is important for chromosome transmission and normal cell cycle progression in G2/M. *The EMBO Journal, 9*(13), 4347-4358.

Gevry, N., Chan, H. M., Laflamme, L., Livingston, D. M., and Gaudreau, L. (2007). p21 transcription is regulated by differential localization of histone H2A.Z. *Genes & Development, 21*(15), 1869-1881.

Giannattasio, M., Lazzaro, F., Plevani, P., and Muzi-Falconi, M. (2005). The DNA damage checkpoint response requires histone H2B ubiquitination by Rad6-Bre1 and H3 methylation by Dot1. *The Journal of Biological Chemistry, 280*(11), 9879-9886.

Ginsburg, D. S., Govind, C. K., and Hinnebusch, A. G. (2009). NuA4 lysine acetyltransferase Esa1 is targeted to coding regions and stimulates transcription elongation with Gcn5. *Molecular and Cellular Biology, 29*(24), 6473-6487.

Gorrini, C., Squatrito, M., Luise, C., Syed, N., Perna, D., Wark, L., et al. (2007). Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. *Nature, 448*(7157), 1063-1067.

Gorzer, I., Schuller, C., Heidenreich, E., Krupanska, L., Kuchler, K., and Wintersberger, U. (2003). The nuclear actin-related protein Act3p/Arp4p of saccharomyces cerevisiae is involved in transcription regulation of stress genes. *Molecular Microbiology, 50*(4), 1155-1171.

Gottschalk, A. J., Conaway, R. C., and Conaway, J. W. (2008). New clues to actin function in chromatin regulation. *Nature Structural & Molecular Biology, 15*(5), 432-433.

Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., et al. (1997a). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: Characterization of an ada complex and the SAGA (Spt/Ada) complex. *Genes & Development, 11*(13), 1640-1650.

Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., et al. (1997b). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: Characterization of an ada complex and the SAGA (Spt/Ada) complex. *Genes & Development, 11*(13), 1640-1650.

Grenon, M., Costelloe, T., Jimeno, S., O'Shaughnessy, A., Fitzgerald, J., Zgheib, O., et al. (2007). Docking onto chromatin via the saccharomyces cerevisiae Rad9 tudor domain. *Yeast (Chichester, England), 24*(2), 105-119.

Grenson, M., Mousset, M., Wiame, J. M., and Bechet, J. (1966). Multiplicity of the amino acid permeases in saccharomyces cerevisiae. I. evidence for a specific arginine-transporting system. *Biochimica Et Biophysica Acta, 127*(2), 325-338.

Grummt, I. (2006). Actin and myosin as transcription factors. *Current Opinion in Genetics & Development, 16*(2), 191-196.

Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. *Nature, 389*(6649), 349-352.

Guillemette, B., Bataille, A. R., Gevry, N., Adam, M., Blanchette, M., Robert, F., et al. (2005a). Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. *PLoS Biology, 3*(12), e384.

Guillemette, B., Bataille, A. R., Gevry, N., Adam, M., Blanchette, M., Robert, F., et al. (2005b). Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. *PLoS Biology, 3*(12), e384.

Guo, D., Hazbun, T. R., Xu, X. J., Ng, S. L., Fields, S., and Kuo, M. H. (2004). A tethered catalysis, two-hybrid system to identify protein-protein interactions requiring post-translational modifications. *Nature Biotechnology, 22*(7), 888-892.

Haase, S. B., and Lew, D. J. (1997). Flow cytometric analysis of DNA content in budding yeast. *Methods in Enzymology, 283*, 322-332.

Hall, B. M., Ma, C. X., Liang, P., and Singh, K. K. (2009). Fluctuation analysis CalculatOR: A web tool for the determination of mutation rate using luria-delbruck fluctuation analysis. *Bioinformatics (Oxford, England), 25*(12), 1564-1565.

Harata, M., Oma, Y., Mizuno, S., Jiang, Y. W., Stillman, D. J., and Wintersberger, U. (1999). The nuclear actin-related protein of saccharomyces cerevisiae, Act3p/Arp4, interacts with core histones. *Molecular Biology of the Cell, 10*(8), 2595-2605.

Hargreaves, D. C., and Crabtree, G. R. (2011). ATP-dependent chromatin remodeling: Genetics, genomics and mechanisms. *Cell Research, 21*(3), 396-420.

Harper, J. W., and Elledge, S. J. (2007). The DNA damage response: Ten years after. *Molecular Cell, 28*(5), 739-745.

Harrison, J. C., and Haber, J. E. (2006). Surviving the breakup: The DNA damage checkpoint. *Annual Review of Genetics, 40*, 209-235.

Hatch, C. L., and Bonner, W. M. (1990). The human histone H2A.Z gene. sequence and regulation. *The Journal of Biological Chemistry, 265*(25), 15211-15218.

Hoke, S. M., Guzzo, J., Andrews, B., and Brandl, C. J. (2008). Systematic genetic array analysis links the saccharomyces cerevisiae SAGA/SLIK and NuA4 component Tra1 to multiple cellular processes. *BMC Genetics, 9*, 46.

Houtsmuller, A. B., Rademakers, S., Nigg, A. L., Hoogstraten, D., Hoeijmakers, J. H., and Vermeulen, W. (1999). Action of DNA repair endonuclease ERCC1/XPF in living cells. *Science (New York, N.Y.), 284*(5416), 958-961.

Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009a). Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Research, 37*(1), 1-13.

Huang da, W., Sherman, B. T., and Lempicki, R. A. (2009b). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols, 4*(1), 44-57.

Iouzalen, N., Moreau, J., and Mechali, M. (1996). H2A.ZI, a new variant histone expressed during xenopus early development exhibits several distinct features from the core histone H2A. *Nucleic Acids Research, 24*(20), 3947-3952.

Ishibashi, T., Dryhurst, D., Rose, K. L., Shabanowitz, J., Hunt, D. F., and Ausio, J. (2009). Acetylation of vertebrate H2A.Z and its effect on the structure of the nucleosome. *Biochemistry, 48*(22), 5007-5017.

Jackson, J. D., and Gorovsky, M. A. (2000). Histone H2A.Z has a conserved function that is distinct from that of the major H2A sequence variants. *Nucleic Acids Research, 28*(19), 3811-3816.

Jeong, J. S., Rho, H. S., and Zhu, H. (2011). A functional protein microarray approach to characterizing posttranslational modifications on lysine residues. *Methods in Molecular Biology (Clifton, N.J.), 723*, 213-223.

Jiang, Y. W., and Stillman, D. J. (1996). Epigenetic effects on yeast transcription caused by mutations in an actin-related protein present in the nucleus. *Genes & Development, 10*(5), 604-619.

Jin, J., Cai, Y., Li, B., Conaway, R. C., Workman, J. L., Conaway, J. W., et al. (2005). In and out: Histone variant exchange in chromatin. *Trends in Biochemical Sciences, 30*(12), 680-687.

Kalocsay, M., Hiller, N. J., and Jentsch, S. (2009). Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. *Molecular Cell, 33*(3), 335-343.

Kamine, J., Elangovan, B., Subramanian, T., Coleman, D., and Chinnadurai, G. (1996). Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 tat transactivator. *Virology, 216*(2), 357-366.

Karagiannis, T. C., and El-Osta, A. (2007). Chromatin modifications and DNA double-strand breaks: The current state of play. *Leukemia : Official Journal of the Leukemia Society of America, Leukemia Research Fund, U.K, 21*(2), 195-200.

Katan-Khaykovich, Y., and Struhl, K. (2005). Heterochromatin formation involves changes in histone modifications over multiple cell generations. *The EMBO Journal, 24*(12), 2138-2149.

Keogh, M. C., Kim, J. A., Downey, M., Fillingham, J., Chowdhury, D., Harrison, J. C., et al. (2006). A phosphatase complex that dephosphorylates gammaH2AX regulates DNA damage checkpoint recovery. *Nature, 439*(7075), 497-501.

Keogh, M. C., Mennella, T. A., Sawa, C., Berthelet, S., Krogan, N. J., Wolek, A., et al. (2006). The saccharomyces cerevisiae histone H2A variant Htz1 is acetylated by NuA4. *Genes & Development, 20*(6), 660-665.

Khorasanizadeh, S. (2004). The nucleosome: From genomic organization to genomic regulation. *Cell, 116*(2), 259-272.

Kim, J. A., Kruhlak, M., Dotiwala, F., Nussenzweig, A., and Haber, J. E. (2007). Heterochromatin is refractory to gamma-H2AX modification in yeast and mammals. *The Journal of Cell Biology, 178*(2), 209-218.

Kimura, A., Matsubara, K., and Horikoshi, M. (2005). A decade of histone acetylation: Marking eukaryotic chromosomes with specific codes. *Journal of Biochemistry, 138*(6), 647-662.

Kleff, S., Andrulis, E. D., Anderson, C. W., and Sternglanz, R. (1995). Identification of a gene encoding a yeast histone H4 acetyltransferase. *The Journal of Biological Chemistry, 270*(42), 24674-24677.

Knutson, B. A., and Hahn, S. (2011). Domains of Tra1 important for activator recruitment and transcription coactivator functions of SAGA and NuA4 complexes. *Molecular and Cellular Biology, 31*(4), 818-831.

Kobor, M. S., Venkatasubrahmanyam, S., Meneghini, M. D., Gin, J. W., Jennings, J. L., Link, A. J., et al. (2004a). A protein complex containing the conserved Swi2/Snf2-

related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biology, 2*(5), E131.

Kobor, M. S., Venkatasubrahmanyam, S., Meneghini, M. D., Gin, J. W., Jennings, J. L., Link, A. J., et al. (2004b). A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biology, 2*(5), E131.

Kossel, A. (1911). Ueber die chemische beschaffenheit des zellkerns. *Munchen Med. Wochenschrift, 58*, 65–69.

Kossel, A. (1928). The protamines and histones. *Longmans, Green and Company, New York.,*

Kouzarides, T. (2007). Chromatin modifications and their function. *Cell, 128*(4), 693-705.

Krogan, N. J., Baetz, K., Keogh, M. C., Datta, N., Sawa, C., Kwok, T. C., et al. (2004). Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4. *Proceedings of the National Academy of Sciences of the United States of America, 101*(37), 13513-13518.

Krogan, N. J., Keogh, M. C., Datta, N., Sawa, C., Ryan, O. W., Ding, H., et al. (2003). A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Molecular Cell, 12*(6), 1565-1576.

Kunz, B. A., Straffon, A. F., and Vonarx, E. J. (2000). DNA damage-induced mutation: Tolerance via translesion synthesis. *Mutation Research, 451*(1-2), 169-185.

Kusch, T., Florens, L., Macdonald, W. H., Swanson, S. K., Glaser, R. L., Yates, J. R.,3rd, et al. (2004). Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. *Science (New York, N.Y.), 306*(5704), 2084-2087.

Kushnirov, V. V. (2000). Rapid and reliable protein extraction from yeast. *Yeast (Chichester, England), 16*(9), 857-860.

Lacoste, N., Utley, R. T., Hunter, J. M., Poirier, G. G., and Cote, J. (2002). Disruptor of telomeric silencing-1 is a chromatin-specific histone H3 methyltransferase. *The Journal of Biological Chemistry, 277*(34), 30421-30424.

Latham, J. A., and Dent, S. Y. (2007). Cross-regulation of histone modifications. *Nature Structural & Molecular Biology, 14*(11), 1017-1024.

Larochelle, M., and Gaudreau, L. (2003). H2A.Z has a function reminiscent of an activator required for preferential binding to intergenic DNA. *The EMBO Journal, 22*(17), 4512-4522.

Lazzaro, F., Sapountzi, V., Granata, M., Pellicioli, A., Vaze, M., Haber, J. E., et al. (2008). Histone methyltransferase Dot1 and Rad9 inhibit single-stranded DNA accumulation at DSBs and uncapped telomeres. *The EMBO Journal, 27*(10), 1502-1512.

Le Masson, I., Yu, D. Y., Jensen, K., Chevalier, A., Courbeyrette, R., Boulard, Y., et al. (2003). Yaf9, a novel NuA4 histone acetyltransferase subunit, is required for the cellular response to spindle stress in yeast. *Molecular and Cellular Biology, 23*(17), 6086-6102.

Lee, J. H., and Paull, T. T. (2007). Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. *Oncogene, 26*(56), 7741-7748.

Lee, J. S., Smith, E., and Shilatifard, A. (2010). The language of histone crosstalk. *Cell, 142*(5), 682-685.

Lee, J. Y., Wei, S., and Lee, T. H. (2011). Effects of histone acetylation by piccolo NuA4 on the structure of a nucleosome and the interactions between two nucleosomes. *The Journal of Biological Chemistry, 286*(13), 11099-11109.

Lee, K. Y., and Myung, K. (2008). PCNA modifications for regulation of post-replication repair pathways. *Molecules and Cells, 26*(1), 5-11.

Levesque, N., Leung, G. P., Fok, A. K., Schmidt, T. I., and Kobor, M. S. (2010). Loss of H3 K79 trimethylation leads to suppression of Rtt107-dependent DNA damage sensitivity through the translesion synthesis pathway. *The Journal of Biological Chemistry, 285*(45), 35113-35122.

Li, B., Pattenden, S. G., Lee, D., Gutierrez, J., Chen, J., Seidel, C., et al. (2005a). Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. *Proceedings of the National Academy of Sciences of the United States of America, 102*(51), 18385-18390.

Li, B., Pattenden, S. G., Lee, D., Gutierrez, J., Chen, J., Seidel, C., et al. (2005b). Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. *Proceedings of the National Academy of Sciences of the United States of America, 102*(51), 18385-18390.

Li, X., Liu, K., Li, F., Wang, J., Huang, H., Wu, J., et al. (2012). Structure of C-terminal tandem BRCT repeats of Rtt107 protein reveals critical role in interaction with phosphorylated histone H2A during DNA damage repair. *The Journal of Biological Chemistry, 287*(12), 9137-9146.

Liang, C., and Stillman, B. (1997). Persistent initiation of DNA replication and chromatin-bound MCM proteins during the cell cycle in cdc6 mutants. *Genes & Development, 11*(24), 3375-3386.

Lin, C., Smith, E. R., Takahashi, H., Lai, K. C., Martin-Brown, S., Florens, L., et al. (2010). AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. *Molecular Cell, 37*(3), 429-437.

Lin, S. Y., Li, T. Y., Liu, Q., Zhang, C., Li, X., Chen, Y., et al. (2012). GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy. *Science (New York, N.Y.), 336*(6080), 477-481.

Lin, Y. Y., Lu, J. Y., Zhang, J., Walter, W., Dang, W., Wan, J., et al. (2009). Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. *Cell, 136*(6), 1073-1084.

Lin, Y. Y., Qi, Y., Lu, J. Y., Pan, X., Yuan, D. S., Zhao, Y., et al. (2008). A comprehensive synthetic genetic interaction network governing yeast histone acetylation and deacetylation. *Genes & Development, 22*(15), 2062-2074.

Lindstrom, K. C., Vary, J. C.,Jr, Parthun, M. R., Delrow, J., and Tsukiyama, T. (2006). Isw1 functions in parallel with the NuA4 and Swr1 complexes in stress-induced gene repression. *Molecular and Cellular Biology, 26*(16), 6117-6129.

Lisby, M., Mortensen, U. H., and Rothstein, R. (2003). Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. *Nature Cell Biology, 5*(6), 572-577.

Liu, C. L., Kaplan, T., Kim, M., Buratowski, S., Schreiber, S. L., Friedman, N., et al. (2005). Single-nucleosome mapping of histone modifications in S. cerevisiae. *PLoS Biology, 3*(10), e328.

Liu, X., Li, B., and GorovskyMA. (1996). Essential and nonessential histone H2A variants in tetrahymena thermophila. *Molecular and Cellular Biology, 16*(8), 4305-4311.

Longtine, M. S., McKenzie, A.,3rd, Demarini, D. J., Shah, N. G., Wach, A., Brachat, A., et al. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in saccharomyces cerevisiae. *Yeast (Chichester, England), 14*(10), 953-961.

Lu, J. Y., Lin, Y. Y., Sheu, J. C., Wu, J. T., Lee, F. J., Chen, Y., et al. (2011). Acetylation of yeast AMPK controls intrinsic aging independently of caloric restriction. *Cell, 146*(6), 969-979.

Lu, J. Y., Lin, Y. Y., Zhu, H., Chuang, L. M., and Boeke, J. D. (2011). Protein acetylation and aging. *Aging, 3*(10), 911-912.

Lu, P. Y., Levesque, N., and Kobor, M. S. (2009). NuA4 and SWR1-C: Two chromatin-modifying complexes with overlapping functions and components. *Biochemistry and Cell Biology = Biochimie Et Biologie Cellulaire, 87*(5), 799-815.

Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature, 389*(6648), 251-260.

Luk, E., Vu, N. D., Patteson, K., Mizuguchi, G., Wu, W. H., Ranjan, A., et al. (2007). Chz1, a nuclear chaperone for histone H2AZ. *Molecular Cell, 25*(3), 357-368.

Lusser, A., and Kadonaga, J. T. (2003). Chromatin remodeling by ATP-dependent molecular machines. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology, 25*(12), 1192-1200.

Martin, D. G., Baetz, K., Shi, X., Walter, K. L., MacDonald, V. E., Wlodarski, M. J., et al. (2006). The Yng1p plant homeodomain finger is a methyl-histone binding module that recognizes lysine 4-methylated histone H3. *Molecular and Cellular Biology, 26*(21), 7871-7879.

Martin, M., Cho, J., Cesare, A. J., Griffith, J. D., and Attardi, G. (2005). Termination factor-mediated DNA loop between termination and initiation sites drives mitochondrial rRNA synthesis. *Cell, 123*(7), 1227-1240.

Martinato, F., Cesaroni, M., Amati, B., and Guccione, E. (2008). Analysis of myc-induced histone modifications on target chromatin. *PLoS ONE, 3*(11), e3650.

Mazurkiewicz, J., Kepert, J. F., and Rippe, K. (2006). On the mechanism of nucleosome assembly by histone chaperone NAP1. *The Journal of Biological Chemistry, 281*(24), 16462-16472.

Meneghini, M. D., Wu, M., and Madhani, H. D. (2003). Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell, 112*(5), 725-736.

Merker, J. D., Dominska, M., Greenwell, P. W., Rinella, E., Bouck, D. C., Shibata, Y., et al. (2008). The histone methylase Set2p and the histone deacetylase Rpd3p repress

meiotic recombination at the HIS4 meiotic recombination hotspot in saccharomyces cerevisiae. *DNA Repair, 7*(8), 1298-1308.

Micialkiewicz, A., and Chelstowska, A. (2008). The essential function of Swc4p - a protein shared by two chromatin-modifying complexes of the yeast saccharomyces cerevisiae - resides within its N-terminal part. *Acta Biochimica Polonica, 55*(3), 603-612.

Miescher, F. (1871). Ueber die chemische zusammensetzung der eiterzellen. *Hoppe-Seyler, Med. Chem. Unters., 4*, 441–460.

Millar, C. B., Xu, F., Zhang, K., and Grunstein, M. (2006). Acetylation of H2AZ lys 14 is associated with genome-wide gene activity in yeast. *Genes & Development, 20*(6), 711-722.

Min, J., Feng, Q., Li, Z., Zhang, Y., and Xu, R. M. (2003). Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell, 112*(5), 711-723.

Miotto, B., Sagnier, T., Berenger, H., Bohmann, D., Pradel, J., and Graba, Y. (2006). Chameau HAT and DRpd3 HDAC function as antagonistic cofactors of JNK/AP-1-dependent transcription during drosophila metamorphosis. *Genes & Development, 20*(1), 101-112.

Miotto, B., and Struhl, K. (2008). HBO1 histone acetylase is a coactivator of the replication licensing factor Cdt1. *Genes & Development, 22*(19), 2633-2638.

Miralles, F., and Visa, N. (2006). Actin in transcription and transcription regulation. *Current Opinion in Cell Biology, 18*(3), 261-266.

Misteli, T. (2007). Beyond the sequence: Cellular organization of genome function. *Cell, 128*(4), 787-800.

Mitchell, L., Lambert, J. P., Gerdes, M., Al-Madhoun, A. S., Skerjanc, I. S., Figeys, D., et al. (2008). Functional dissection of the NuA4 histone acetyltransferase reveals its role as a genetic hub and that Eaf1 is essential for complex integrity. *Molecular and Cellular Biology, 28*(7), 2244-2256.

Mitchell, L., Lau, A., Lambert, J. P., Zhou, H., Fong, Y., Couture, J. F., et al. (2011). Regulation of septin dynamics by the saccharomyces cerevisiae lysine acetyltransferase NuA4. *PloS One, 6*(10), e25336.

Mizuguchi, G., Shen, X., Landry, J., Wu, W. H., Sen, S., and Wu, C. (2004). ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science (New York, N.Y.), 303*(5656), 343-348.

Mohammad, D. H., and Yaffe, M. B. (2009). 14-3-3 proteins, FHA domains and BRCT domains in the DNA damage response. *DNA Repair, 8*(9), 1009-1017.

Morrison, A. J., Highland, J., Krogan, N. J., Arbel-Eden, A., Greenblatt, J. F., Haber, J. E., et al. (2004). INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. *Cell, 119*(6), 767-775.

Morrison, A. J., and Shen, X. (2009). Chromatin remodelling beyond transcription: The INO80 and SWR1 complexes. *Nature Reviews.Molecular Cell Biology,*

Mueller, D., Bach, C., Zeisig, D., Garcia-Cuellar, M. P., Monroe, S., Sreekumar, A., et al. (2007). A role for the MLL fusion partner ENL in transcriptional elongation and chromatin modification. *Blood, 110*(13), 4445-4454.

Natsume-Kitatani, Y., Shiga, M., and Mamitsuka, H. (2011). Genome-wide integration on transcription factors, histone acetylation and gene expression reveals genes co-regulated by histone modification patterns. *PloS One, 6*(7), e22281.

Ng, H. H., Feng, Q., Wang, H., Erdjument-Bromage, H., Tempst, P., Zhang, Y., et al. (2002). Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and sir protein association. *Genes & Development, 16*(12), 1518-1527.

Ng, H. H., Xu, R. M., Zhang, Y., and Struhl, K. (2002). Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *The Journal of Biological Chemistry, 277*(38), 34655-34657.

Nguyen, A. T., and Zhang, Y. (2011). The diverse functions of Dot1 and H3K79 methylation. *Genes & Development, 25*(13), 1345-1358.

Nishimoto, N., Watanabe, M., Watanabe, S., Sugimoto, N., Yugawa, T., Ikura, T., et al. (2012). Heterocomplex formation by Arp4 and beta-actin involved in integrity of the Brg1 chromatin remodeling complex. *Journal of Cell Science,*

Norris, A., and Boeke, J. D. (2010). Silent information regulator 3: The goldilocks of the silencing complex. *Genes & Development, 24*(2), 115-122.

Nourani, A., Utley, R. T., Allard, S., and Cote, J. (2004). Recruitment of the NuA4 complex poises the PHO5 promoter for chromatin remodeling and activation. *The EMBO Journal, 23*(13), 2597-2607.

Ogiwara, H., Ui, A., Kawashima, S., Kugou, K., Onoda, F., Iwahashi, H., et al. (2007). Actin-related protein Arp4 functions in kinetochore assembly. *Nucleic Acids Research, 35*(9), 3109-3117.

Olins, A. L., and Olins, D. E. (1974). Spheroid chromatin units (v bodies). *Science (New York, N.Y.), 183*(4122), 330-332.

O'Neill, B. M., Szyjka, S. J., Lis, E. T., Bailey, A. O., Yates, J. R.,3rd, Aparicio, O. M., et al. (2007). Pph3-Psy2 is a phosphatase complex required for Rad53 dephosphorylation and replication fork restart during recovery from DNA damage. *Proceedings of the National Academy of Sciences of the United States of America, 104*(22), 9290-9295.

O'Sullivan, J. M., Tan-Wong, S. M., Morillon, A., Lee, B., Coles, J., Mellor, J., et al. (2004). Gene loops juxtapose promoters and terminators in yeast. *Nature Genetics, 36*(9), 1014-1018.

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

Pages, V., Santa Maria, S. R., Prakash, L., and Prakash, S. (2009). Role of DNA damage-induced replication checkpoint in promoting lesion bypass by translesion synthesis in yeast. *Genes & Development, 23*(12), 1438-1449.

Papamichos-Chronakis, M., Krebs, J. E., and Peterson, C. L. (2006). Interplay between Ino80 and Swr1 chromatin remodeling enzymes regulates cell cycle checkpoint adaptation in response to DNA damage. *Genes & Development, 20*(17), 2437-2449.

Park, G., Gong, Z., Chen, J., and Kim, J. E. (2010). Characterization of the DOT1L network: Implications of diverse roles for DOT1L. *The Protein Journal, 29*(3), 213-223.

Park, J. H., Sun, X. J., and Roeder, R. G. (2010). The SANT domain of p400 ATPase represses acetyltransferase activity and coactivator function of TIP60 in basal p21 gene expression. *Molecular and Cellular Biology, 30*(11), 2750-2761.

Park, Y. J., Chodaparambil, J. V., Bao, Y., McBryant, S. J., and Luger, K. (2005). Nucleosome assembly protein 1 exchanges histone H2A-H2B dimers and assists nucleosome sliding. *The Journal of Biological Chemistry, 280*(3), 1817-1825.

Park, Y. J., Dyer, P. N., Tremethick, D. J., and Luger, K. (2004). A new fluorescence resonance energy transfer approach demonstrates that the histone variant H2AZ stabilizes the histone octamer within the nucleosome. *The Journal of Biological Chemistry, 279*(23), 24274-24282.

Petronis, A. (2010). Epigenetics as a unifying principle in the aetiology of complex traits and diseases. *Nature, 465*(7299), 721-727.

Pokholok, D. K., Harbison, C. T., Levine, S., Cole, M., Hannett, N. M., Lee, T. I., et al. (2005). Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell, 122*(4), 517-527.

Politi, A., Mone, M. J., Houtsmuller, A. B., Hoogstraten, D., Vermeulen, W., Heinrich, R., et al. (2005). Mathematical modeling of nucleotide excision repair reveals efficiency of sequential assembly strategies. *Molecular Cell, 19*(5), 679-690.

Pruyne, D., and Bretscher, A. (2000). Polarization of cell growth in yeast. *Journal of Cell Science, 113 (Pt 4)*(Pt 4), 571-585.

Putnam, C. D., Jaehnig, E. J., and Kolodner, R. D. (2009). Perspectives on the DNA damage and replication checkpoint responses in saccharomyces cerevisiae. *DNA Repair, 8*(9), 974-982.

Raisner, R. M., Hartley, P. D., Meneghini, M. D., Bao, M. Z., Liu, C. L., Schreiber, S. L., et al. (2005). Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. *Cell, 123*(2), 233-248.

Rando, O. J. (2007). Global patterns of histone modifications. *Current Opinion in Genetics & Development, 17*(2), 94-99.

Rando, O. J., and Chang, H. Y. (2009). Genome-wide views of chromatin structure. *Annual Review of Biochemistry, 78*, 245-271.

Rando, O. J., Zhao, K., Janmey, P., and Crabtree, G. R. (2002). Phosphatidylinositol-dependent actin filament binding by the SWI/SNF-like BAF chromatin remodeling complex. *Proceedings of the National Academy of Sciences of the United States of America, 99*(5), 2824-2829.

Redon, C., Pilch, D. R., Rogakou, E. P., Orr, A. H., Lowndes, N. F., and Bonner, W. M. (2003). Yeast histone 2A serine 129 is essential for the efficient repair of checkpoint-blind DNA damage. *EMBO Reports, 4*(7), 678-684.

Redon, C. E., Nakamura, A. J., Zhang, Y. W., Ji, J. J., Bonner, W. M., Kinders, R. J., et al. (2010). Histone gammaH2AX and poly(ADP-ribose) as clinical pharmacodynamic biomarkers. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 16*(18), 4532-4542.

Reid, J. L., Iyer, V. R., Brown, P. O., and Struhl, K. (2000). Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. *Molecular Cell, 6*(6), 1297-1307.

Ridgway, P., Brown, K. D., Rangasamy, D., Svensson, U., and Tremethick, D. J. (2004). Unique residues on the H2A.Z containing nucleosome surface are important for

xenopus laevis development. *The Journal of Biological Chemistry, 279*(42), 43815-43820.

Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M., and Seraphin, B. (1999). A generic protein purification method for protein complex characterization and proteome exploration. *Nature Biotechnology, 17*(10), 1030-1032.

Roberts, T. M., Kobor, M. S., Bastin-Shanower, S. A., Ii, M., Horte, S. A., Gin, J. W., et al. (2006). Slx4 regulates DNA damage checkpoint-dependent phosphorylation of the BRCT domain protein Rtt107/Esc4. *Molecular Biology of the Cell, 17*(1), 539-548.

Roberts, T. M., Zaidi, I. W., Vaisica, J. A., Peter, M., and Brown, G. W. (2008). Regulation of rtt107 recruitment to stalled DNA replication forks by the cullin rtt101 and the rtt109 acetyltransferase. *Molecular Biology of the Cell, 19*(1), 171-180.

Rogakou, E. P., Boon, C., Redon, C., and Bonner, W. M. (1999). Megabase chromatin domains involved in DNA double-strand breaks in vivo. *The Journal of Cell Biology, 146*(5), 905-916.

Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., and Bonner, W. M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *The Journal of Biological Chemistry, 273*(10), 5858-5868.

Rouse, J. (2004a). Esc4p, a new target of Mec1p (ATR), promotes resumption of DNA synthesis after DNA damage. *The EMBO Journal, 23*(5), 1188-1197.

Rouse, J. (2004b). Esc4p, a new target of Mec1p (ATR), promotes resumption of DNA synthesis after DNA damage. *The EMBO Journal, 23*(5), 1188-1197.

Ruhl, D. D., Jin, J., Cai, Y., Swanson, S., Florens, L., Washburn, M. P., et al. (2006). Purification of a human SRCAP complex that remodels chromatin by incorporating the histone variant H2A.Z into nucleosomes. *Biochemistry, 45*(17), 5671-5677.

Ryan, D. P., Sundaramoorthy, R., Martin, D., Singh, V., and Owen-Hughes, T. (2011). The DNA-binding domain of the Chd1 chromatin-remodelling enzyme contains SANT and SLIDE domains. *The EMBO Journal, 30*(13), 2596-2609.

Santisteban, M. S., Kalashnikova, T., and Smith, M. M. (2000). Histone H2A.Z regulats transcription and is partially redundant with nucleosome remodeling complexes. *Cell, 103*(3), 411-422.

Sapountzi, V., Logan, I. R., and Robson, C. N. (2006). Cellular functions of TIP60. *The International Journal of Biochemistry & Cell Biology, 38*(9), 1496-1509.

Sarma, K., and Reinberg, D. (2005). Histone variants meet their match. *Nature Reviews.Molecular Cell Biology, 6*(2), 139-149.

Scholes, D. T., Banerjee, M., Bowen, B., and Curcio, M. J. (2001). Multiple regulators of Ty1 transposition in saccharomyces cerevisiae have conserved roles in genome maintenance. *Genetics, 159*(4), 1449-1465.

Schuldiner, M., Collins, S. R., Weissman, J. S., and Krogan, N. J. (2006a). Quantitative genetic analysis in saccharomyces cerevisiae using epistatic miniarray profiles (E-MAPs) and its application to chromatin functions. *Methods (San Diego, Calif.), 40*(4), 344-352.

Schuldiner, M., Collins, S. R., Weissman, J. S., and Krogan, N. J. (2006b). Quantitative genetic analysis in saccharomyces cerevisiae using epistatic miniarray profiles (E-MAPs) and its application to chromatin functions. *Methods (San Diego, Calif.), 40*(4), 344-352.

Schulze, J. M., Jackson, J., Nakanishi, S., Gardner, J. M., Hentrich, T., Haug, J., et al. (2009). Linking cell cycle to histone modifications: SBF and H2B monoubiquitination machinery and cell-cycle regulation of H3K79 dimethylation. *Molecular Cell, 35*(5), 626-641.

Schulze, J. M., Wang, A. Y., and Kobor, M. S. (2009). YEATS domain proteins: A diverse family with many links to chromatin modification and transcription. *Biochemistry and Cell Biology = Biochimie Et Biologie Cellulaire, 87*(1), 65-75.

Scott, K. L., and Plon, S. E. (2003). Loss of Sin3/Rpd3 histone deacetylase restores the DNA damage response in checkpoint-deficient strains of saccharomyces cerevisiae. *Molecular and Cellular Biology, 23*(13), 4522-4531.

Segurado, M., and Tercero, J. A. (2009). The S-phase checkpoint: Targeting the replication fork. *Biology of the Cell / Under the Auspices of the European Cell Biology Organization, 101*(11), 617-627.

Selleck, W., Fortin, I., Sermwittayawong, D., Cote, J., and Tan, S. (2005). The saccharomyces cerevisiae piccolo NuA4 histone acetyltransferase complex requires the enhancer of polycomb A domain and chromodomain to acetylate nucleosomes. *Molecular and Cellular Biology, 25*(13), 5535-5542.

Shahbazian, M. D., Zhang, K., and Grunstein, M. (2005). Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1. *Molecular Cell, 19*(2), 271-277.

Sharma, M., Zarnegar, M., Li, X., Lim, B., and Sun, Z. (2000). Androgen receptor interacts with a novel MYST protein, HBO1. *The Journal of Biological Chemistry, 275*(45), 35200-35208.

Sherman, B. T., Huang da, W., Tan, Q., Guo, Y., Bour, S., Liu, D., et al. (2007). DAVID knowledgebase: A gene-centered database integrating heterogeneous gene annotation

resources to facilitate high-throughput gene functional analysis. *BMC Bioinformatics, 8*, 426.

Shilatifard, A. (2006). Chromatin modifications by methylation and ubiquitination: Implications in the regulation of gene expression. *Annual Review of Biochemistry, 75*, 243-269.

Shiloh, Y. (2003). ATM and related protein kinases: Safeguarding genome integrity. *Nature Reviews.Cancer, 3*(3), 155-168.

Shroff, R., Arbel-Eden, A., Pilch, D., Ira, G., Bonner, W. M., Petrini, J. H., et al. (2004a). Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. *Current Biology : CB, 14*(19), 1703-1711.

Shroff, R., Arbel-Eden, A., Pilch, D., Ira, G., Bonner, W. M., Petrini, J. H., et al. (2004b). Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. *Current Biology : CB, 14*(19), 1703-1711.

Singer, M. S., Kahana, A., Wolf, A. J., Meisinger, L. L., Peterson, S. E., Goggin, C., et al. (1998). Identification of high-copy disruptors of telomeric silencing in saccharomyces cerevisiae. *Genetics, 150*(2), 613-632.

Smith, E. R., Eisen, A., Gu, W., Sattah, M., Pannuti, A., Zhou, J., et al. (1998). ESA1 is a histone acetyltransferase that is essential for growth in yeast. *Proceedings of the National Academy of Sciences of the United States of America, 95*(7), 3561-3565.

Sterner, D. E., Wang, X., Bloom, M. H., Simon, G. M., and Berger, S. L. (2002). The SANT domain of Ada2 is required for normal acetylation of histones by the yeast SAGA complex. *The Journal of Biological Chemistry, 277*(10), 8178-8186.

Straube, K., Blackwell, J. S.,Jr, and Pemberton, L. F. (2010). Nap1 and Chz1 have separate Htz1 nuclear import and assembly functions. *Traffic (Copenhagen, Denmark), 11*(2), 185-197.

Sunada, R., Gorzer, I., Oma, Y., Yoshida, T., Suka, N., Wintersberger, U., et al. (2005). The nuclear actin-related protein Act3p/Arp4p is involved in the dynamics of chromatin-modulating complexes. *Yeast (Chichester, England), 22*(10), 753-768.

Suto, R. K., Clarkson, M. J., Tremethick, D. J., and Luger, K. (2000). Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. *Nature Structural Biology, 7*(12), 1121-1124.

Svotelis, A., Gevry, N., and Gaudreau, L. (2009). Regulation of gene expression and cellular proliferation by histone H2A.Z. *Biochemistry and Cell Biology = Biochimie Et Biologie Cellulaire, 87*(1), 179-188.

Szerlong, H., Hinata, K., Viswanathan, R., Erdjument-Bromage, H., Tempst, P., and Cairns, B. R. (2008). The HSA domain binds nuclear actin-related proteins to regulate chromatin-remodeling ATPases. *Nature Structural & Molecular Biology, 15*(5), 469-476.

Szyjka, S. J., Aparicio, J. G., Viggiani, C. J., Knott, S., Xu, W., Tavare, S., et al. (2008). Rad53 regulates replication fork restart after DNA damage in saccharomyces cerevisiae. *Genes & Development, 22*(14), 1906-1920.

Talbert, P. B., and Henikoff, S. (2010). Histone variants--ancient wrap artists of the epigenome. *Nature Reviews.Molecular Cell Biology, 11*(4), 264-275.

Tamburini, B. A., and Tyler, J. K. (2005). Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand DNA repair. *Molecular and Cellular Biology, 25*(12), 4903-4913.

Tavazoie, S., Hughes, J. D., Campbell, M. J., Cho, R. J., and Church, G. M. (1999). Systematic determination of genetic network architecture. *Nature Genetics, 22*(3), 281-285.

Thatcher, T. H., and Gorovsky, M. A. (1994). Phylogenetic analysis of the core histones H2A, H2B, H3, and H4. *Nucleic Acids Research, 22*(2), 174-179.

Tolstorukov, M. Y., Kharchenko, P. V., Goldman, J. A., Kingston, R. E., and Park, P. J. (2009). Comparative analysis of H2A.Z nucleosome organization in the human and yeast genomes. *Genome Research, 19*(6), 967-977.

Trojer, P., and Reinberg, D. (2007). Facultative heterochromatin: Is there a distinctive molecular signature? *Molecular Cell, 28*(1), 1-13.

Unal, E., Arbel-Eden, A., Sattler, U., Shroff, R., Lichten, M., Haber, J. E., et al. (2004). DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Molecular Cell, 16*(6), 991-1002.

Utley, R. T., and Cote, J. (2003). The MYST family of histone acetyltransferases. *Current Topics in Microbiology and Immunology, 274*, 203-236.

Utley, R. T., Lacoste, N., Jobin-Robitaille, O., Allard, S., and Cote, J. (2005). Regulation of NuA4 histone acetyltransferase activity in transcription and DNA repair by phosphorylation of histone H4. *Molecular and Cellular Biology, 25*(18), 8179-8190.

Vainberg, I. E., Lewis, S. A., Rommelaere, H., Ampe, C., Vandekerckhove, J., Klein, H. L., et al. (1998). Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin. *Cell, 93*(5), 863-873.

van Attikum, H., Fritsch, O., and Gasser, S. M. (2007). Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. *The EMBO Journal, 26*(18), 4113-4125.

van Attikum, H., and Gasser, S. M. (2009). Crosstalk between histone modifications during the DNA damage response. *Trends in Cell Biology,*

van Bakel, H., and Holstege, F. C. (2004). In control: Systematic assessment of microarray performance. *EMBO Reports, 5*(10), 964-969.

van Daal, A., and Elgin, S. C. (1992). A histone variant, H2AvD, is essential in drosophila melanogaster. *Molecular Biology of the Cell, 3*(6), 593-602.

van de Peppel, J., Kemmeren, P., van Bakel, H., Radonjic, M., van Leenen, D., and Holstege, F. C. (2003). Monitoring global messenger RNA changes in externally controlled microarray experiments. *EMBO Reports, 4*(4), 387-393.

van Driel, R., Fransz, P. F., and Verschure, P. J. (2003). The eukaryotic genome: A system regulated at different hierarchical levels. *Journal of Cell Science, 116*(Pt 20), 4067-4075.

van Leeuwen, F., Gafken, P. R., and Gottschling, D. E. (2002). Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell, 109*(6), 745-756.

Viggiani, C. J., and Aparicio, O. M. (2006). New vectors for simplified construction of BrdU-incorporating strains of saccharomyces cerevisiae. *Yeast (Chichester, England), 23*(14-15), 1045-1051.

Vignali, M., Hassan, A. H., Neely, K. E., and Workman, J. L. (2000). ATP-dependent chromatin-remodeling complexes. *Molecular and Cellular Biology, 20*(6), 1899-1910.

Wang, A. Y., Aristizabal, M. J., Ryan, C., Krogan, N. J., and Kobor, M. S. (2011). Key functional regions in the histone variant H2A.Z C-terminal docking domain. *Molecular and Cellular Biology, 31*(18), 3871-3884.

Wang, A. Y., Schulze, J. M., Skordalakes, E., Gin, J. W., Berger, J. M., Rine, J., et al. (2009a). Asf1-like structure of the conserved Yaf9 YEATS domain and role in H2A.Z deposition and acetylation. *Submitted,*

Wang, A. Y., Schulze, J. M., Skordalakes, E., Gin, J. W., Berger, J. M., Rine, J., et al. (2009b). Asf1-like structure of the conserved Yaf9 YEATS domain and role in H2A.Z deposition and acetylation. *Proceedings of the National Academy of Sciences of the United States of America, 106*(51), 21573-21578.

Wang, Z., Zang, C., Rosenfeld, J. A., Schones, D. E., Barski, A., Cuddapah, S., et al. (2008). Combinatorial patterns of histone acetylations and methylations in the human genome. *Nature Genetics, 40*(7), 897-903.

Waters, L. S., Minesinger, B. K., Wiltrout, M. E., D'Souza, S., Woodruff, R. V., and Walker, G. C. (2009). Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiology and Molecular Biology Reviews : MMBR, 73*(1), 134-154.

Wei, Y., Mizzen, C. A., Cook, R. G., Gorovsky, M. A., and Allis, C. D. (1998). Phosphorylation of histone H3 at serine 10 is correlated with chromosome condensation during mitosis and meiosis in tetrahymena. *Proceedings of the National Academy of Sciences of the United States of America, 95*(13), 7480-7484.

Wong, M. M., Cox, L. K., and Chrivia, J. C. (2007). The chromatin remodeling protein, SRCAP, is critical for deposition of the histone variant H2A.Z at promoters. *The Journal of Biological Chemistry, 282*(36), 26132-26139.

Wood, A., Krogan, N. J., Dover, J., Schneider, J., Heidt, J., Boateng, M. A., et al. (2003). Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Molecular Cell, 11*(1), 267-274.

Wu, W. H., Alami, S., Luk, E., Wu, C. H., Sen, S., Mizuguchi, G., et al. (2005). Swc2 is a widely conserved H2AZ-binding module essential for ATP-dependent histone exchange. *Nature Structural & Molecular Biology, 12*(12), 1064-1071.

Wu, W. H., Wu, C. H., Ladurner, A., Mizuguchi, G., Wei, D., Xiao, H., et al. (2009). N terminus of Swr1 binds to histone H2AZ and provides a platform for subunit assembly in the chromatin remodeling complex. *The Journal of Biological Chemistry, 284*(10), 6200-6207.

Wysocki, R., Javaheri, A., Allard, S., Sha, F., Cote, J., and Kron, S. J. (2005). Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9. *Molecular and Cellular Biology, 25*(19), 8430-8443.

Yang, B., Britton, J., and Kirchmaier, A. L. (2008). Insights into the impact of histone acetylation and methylation on sir protein recruitment, spreading, and silencing in saccharomyces cerevisiae. *Journal of Molecular Biology, 381*(4), 826-844.

Yang, X. J. (2004). The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Research, 32*(3), 959-976.

Yang, X. J., and Seto, E. (2007). HATs and HDACs: From structure, function and regulation to novel strategies for therapy and prevention. *Oncogene, 26*(37), 5310-5318.

Yokoyama, A., Lin, M., Naresh, A., Kitabayashi, I., and Cleary, M. L. (2010). A higher-order complex containing AF4 and ENL family proteins with P-TEFb facilitates oncogenic and physiologic MLL-dependent transcription. *Cancer Cell, 17*(2), 198-212.

Yu, J., Li, Y., Ishizuka, T., Guenther, M. G., and Lazar, M. A. (2003). A SANT motif in the SMRT corepressor interprets the histone code and promotes histone deacetylation. *The EMBO Journal, 22*(13), 3403-3410.

Yuen, K. W., Warren, C. D., Chen, O., Kwok, T., Hieter, P., and Spencer, F. A. (2007). Systematic genome instability screens in yeast and their potential relevance to cancer. *Proceedings of the National Academy of Sciences of the United States of America, 104*(10), 3925-3930.

Zanton, S. J., and Pugh, B. F. (2004). Changes in genomewide occupancy of core transcriptional regulators during heat stress. *Proceedings of the National Academy of Sciences of the United States of America, 101*(48), 16843-16848.

Zappulla, D. C., Maharaj, A. S., Connelly, J. J., Jockusch, R. A., and Sternglanz, R. (2006). Rtt107/Esc4 binds silent chromatin and DNA repair proteins using different BRCT motifs. *BMC Molecular Biology, 7*, 40.

Zhang, H., Richardson, D. O., Roberts, D. N., Utley, R., Erdjument-Bromage, H., Tempst, P., et al. (2004). The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation, and Htz1 replacement near telomeres. *Molecular and Cellular Biology, 24*(21), 9424-9436.

Zhang, H., Roberts, D. N., and Cairns, B. R. (2005a). Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell, 123*(2), 219-231.

Zhang, H., Roberts, D. N., and Cairns, B. R. (2005b). Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell, 123*(2), 219-231.

Zhou, B. O., Wang, S. S., Xu, L. X., Meng, F. L., Xuan, Y. J., Duan, Y. M., et al. (2010). SWR1 complex poises heterochromatin boundaries for antisilencing activity propagation. *Molecular and Cellular Biology, 30*(10), 2391-2400.

Ziv, Y., Bielopolski, D., Galanty, Y., Lukas, C., Taya, Y., Schultz, D. C., et al. (2006). Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nature Cell Biology, 8*(8), 870-876.

Zlatanova, J., and Thakar, A. (2008). H2A.Z: View from the top. *Structure (London, England : 1993), 16*(2), 166-179.