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GENOME-WIDE ROLE OF HISTONE ACETYLTRANSFERASES AND CHROMATIN REGULATORS IN REGULATING YEAST CHROMATIN AND GENE TRANSCRIPTION

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ABSTRACT

The regulation of gene expression is a dynamic process involving interplay between a multitude of activators and repressors, ultimately serving to regulate the accessibility of DNA coding sequences to RNA Polymerase. Initiation of gene transcription is a complex process, but can be summarized in a few key steps: activator binding; chromatin remodeling; assembly of general transcription factors; Pol II recruitment; transcription initiation. Insights into chromatin remodeling over the past decade have shifted the focus of research to this regulatory mechanism, and the resulting discoveries have led to a new understanding of how cells initiate gene transcription. The functional contributions of many histone acetyltransferases (HATs) to gene expression and their relationships with each other remain largely undefined. Current models of gene activation suggest that acetylation of promoter nucleosomes serve to recruit factors containing protein domains with a specific affinity for binding acetylated histone tails. Furthermore, evidence that bromodomain factors associate with a variety of chromatin remodeling and transcription factor complexes implies that bromodomain factors play an important role in linking histone acetylation with transcription factor recruitment. The work presented in this thesis explores the relationship between histone acetylation, bromodomain factors, and the transcription complex, TFIID, and extends the study of these transcription components to a genome-wide scale. Evidence from genome-wide studies in this thesis show that activation of TFIID-regulated genes requires hyper-acetylation of histone H4 tails by the HAT, Esa1, and that this relationship is exclusive to TFIID genes. Association of the bromodomain subunit of TFIID, Bdf1, is dependent on

H4 acetylation, and is an important step for the binding of TFIID to nucleosome-bound promoters. Genome-wide evidence is also presented that contradicts previous models that the TAF1 subunit of yeast TFIID acetylates histones in vivo. Furthermore, I find that only the Esa1 and Gcn5 HATs contribute substantially to global gene expression. In summary, the findings resulting from these genome-wide studies support a model whereby acetylation of promoter nucleosomes occurs early in the activation process and establishment of these histone acetylation patterns are crucial for the recruitment of TFIID through interaction of the bromodomain subunit, Bdf1, with acetylated histone tails.

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LIST OF ABBREVIATIONS

Ac-	Acetyl-
ADA	Ada2-Ada3-Gcn5 complex
Bdf1	Bromodomain Factor 1
Bdf2	Bromodomain Factor 2
chIP	Chromatin Immunoprecipitation
chIP/chip	Chromatin Immunoprecipitation on chip
dTAF1	*Drosophila melanogaster* TAF1
GNAT	Gcn5-related N-acetyltransferase
GTF	General transcription factor
HAT	Histone Acetyl-Transferase
HDAC	Histone De-Acetylase
hTAF1	Human TAF1
MYST	MOZ, Ybf2/Sas3, Sas2, and Tip60
NFR	Nucleosome free region
ORF	Open Reading Frame
PIC	Pre-Initiation Complex
Pol II	Polymerase II
SAGA	Spt20-Ada2-Gcn5-Ada3 complex
SAS	Something About Silencing
SIR	Silence Information Regulators
SLIK/SALSA	SAGA variant complex
snRNP	Small nuclear ribo-nuclease proteins
TAF1	TBP-Associated Factor 1
TBP	TATA-Binding Protein
TFIID	Transcription Factor II Complex D
yTAF1	Yeast TAF1
T-H	Tail to Head
H-H	Head to Head
T-T	Tail to Tail
WT	Wild type

ACKNOWLEDGEMENTS

There are many people who have contributed to the work presented in this thesis.

Expression microarrays

I was fortunate to join the Pugh lab at a time when the microarray technology was at an optimal stage for productivity. Former members of the Pugh lab, specifically Lata Chitikila, had been instrumental in working out all of the kinks and statistical problems involved with getting technology like this started. While I never had the chance to work with Lata, her legacy for starting the microarrays at Penn State lives on, and for all of her hard work in establishing this successful endeavor, I am truly thankful.

Kate Huisinga, a former graduate student in the lab, took the technology Lata had developed and helped take it to the next level. In addition, Kate passed on all of her microarray knowledge to me, and without her assistance, the expression array experiments presented in this thesis would not have been possible. She also helped guide me through the confusing process of interpreting and analyzing the data generated from these experiments, and her guidance and expertise was highly valued.

chIP-on-chip technology

Lata and Kate were instrumental in establishing the expression microarrays, but the chIP-on-chip technology was made possible by the combined efforts of Joe Reese and Sara Zanton. Joe Reese shared his protocol for generating purified chIP material, and Sara

was responsible for figuring out how to utilize that chIP material on a genome-wide scale. In addition, I would like to thank the efforts of the late Bob Simpson for developing the material for the intergenic slides that were important for the microarray portion of the procedure.

Chapter 1

Thanks to Bryan Venters for helping create the nucleosome structure image in Figure 1.2.

Chapter 2

I would like to thank Bob Simpson for providing the ResGen deletion strains used to make all of the strains used in that study. The Esa1 plasmids were kindly donated by Jacques Côtè at the Laval University Cancer Research Center in Canada. The Gcn5 deletion strains were created by Jaclyn Shingara, and Sara Zanton executed the experiment in Figure 2.3. The *rpb1-1* strain was a gift from Rick Young. Thanks also to Mitra Sharma for technical assistance with the acetylation chIP experiment, Joe Reese for donating several histone acetylation antibodies, and many people for helpful discussions regarding this project.

Chapter 3

I would like to thank Joe Reese for providing ResGen deletion strains used to generate some of the strains used in the study, Sara Zanton for help with the chIP-chip data analysis and Excel scripts, and Jordan Irvin for creating the pJI11 and pJI12 TAF1

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I am extremely grateful to have had the opportunity to be a part of the Pugh lab during my time here at Penn State. When I joined the lab, I greatly admired the older grad students: Kate Huisinga, Jordan Irvin, and Haiping Kou. They not only took me under their wing and taught me everything they knew, but they provided helpful insight, wisdom, coffee breaks, and comic relief when things got tough. John Robinson, Sara Zanton, and Travis Mavrich have also provided valuable guidance and suggestions during my time in the lab. The newest additions to the lab, Bryan Venters and Tom Koerber, have helped to keep me on my toes and provide new insight. I am grateful to each and every lab member than I have had the pleasure of working with; each and every one of them has contributed to my experience at Penn State.

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Most importantly, I would like to thank my family and Mark for their unfailing support in my endeavors and in my abilities. Words cannot express my gratitude for all you have done for me, and my success would not have been possible without your love and support.

Chapter 1

Literature Review

1.1 The importance of regulating gene expression

A constantly changing environment surrounds every organism, from microbes to humans. Each organism must adapt to these ever-present variations in order to survive. While many of these adaptations manifest themselves as physical changes, such as an increase in fat storage or sporulation, the root of these changes is gene expression. In the afore-mentioned example of microbial sporulation, a cell has sensed that nutrients are low and environmental conditions are harsh. This stimulus leads to a response where genes that are no longer required for cell growth are shut down, and genes that are required for sporulation events are activated. It is easy to imagine that the cell does not want these changes to occur when they are not needed, especially since alterations in homeostatic processes require a good deal of costly energy. Thus, it is vital for a cell to maintain tight control over the regulation of all the genes in the genome.

Gene regulation occurs at a variety of different levels, from control over transcription factor access to DNA to the control of mRNA degradation. From a general "bird's-eye" view, there are five main regulatory steps that lead to transcription initiation, diagrammed in Figure **1.1**: (1) chromatin remodeling; (2) activator binding; (3) general transcription factor recruitment/binding; (4) Polymerase II [Pol II] recruitment; and (5) transcription initiation. Each of these outlined steps will be discussed in further detail below, with more focus placed on those steps that will be investigated in this thesis.

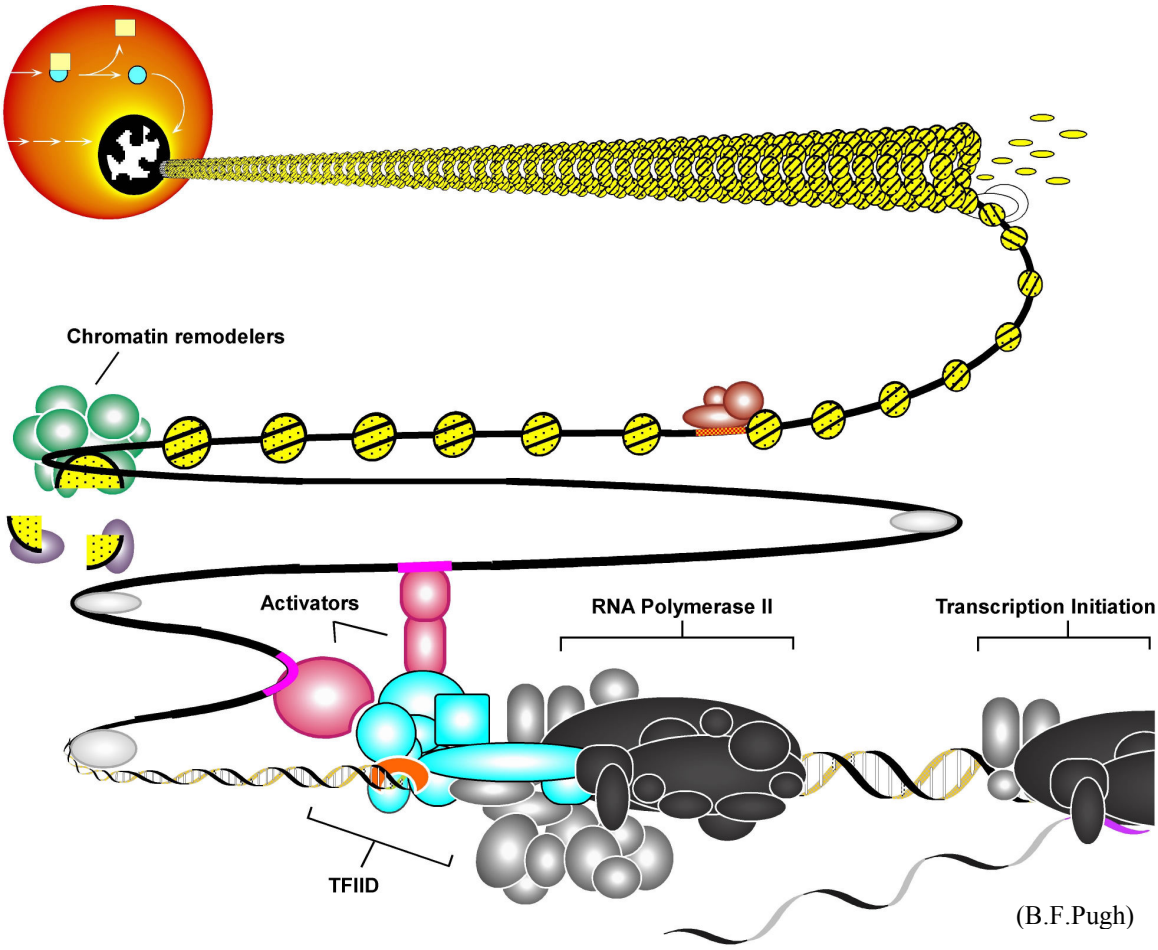

Figure 1.1**: Overview of gene regulation**

Gene regulation involves the recruitment and assembly of multiple protein complexes at the promoter. To allow for PIC assembly, chromatin remodeling complexes (light green) must first unpackage the nucleosome (yellow) bound DNA (black). Increased accessibility of DNA then allows sequence-specific activators (pink) to bind to upstream activating sequences and recruit additional co-activator complexes (light blue), such as TFIID and SAGA. TFIID and SAGA both work to deliver TBP (orange) to promoter DNA, allowing for further transcription factor assembly and, ultimately, the recruitment of the RNA Polymerase II enzyme (black).

To better understand each of these regulatory events, I have chosen to use the yeast species *Saccharomyces cerevisiae* as a model organism. This organism is a great tool for studying gene regulation because it represents a simplified version of the regulatory mechanisms that are well conserved in higher eukaryotes. In addition, genetic

manipulations are relatively easy to perform in yeast, making it easier to study the roles and effects of different proteins and complexes involved in gene regulation. This literature review will therefore focus more specifically on the proteins and complexes found in *Saccharomyces cerevisiae*.

1.2 Activators

Activators play an important role in the early stages of gene activation; however, they will be only briefly reviewed here, as they are not a central part of the work presented in this thesis. Cells contain a multitude of different, sequence-specific activators, many of which regulate only a small subset of genes in the genome. Despite differences in gene activation targets, their overall function is the same: to provide a "kick-start" to the gene activation process. Many activators are not required for transcription initiation, but instead aid to increase transcription rates above basal levels by recruiting various regulatory complexes, sometimes referred to as co-activators. One example of this general mechanism is the Gcn4 activator, which has been shown to interact with co-activator subunits of multiple complexes, including TFIID, SWI/SNF, and Ada2-Ada3-Gcn5 (Drysdale et al., 1998; Yudkovsky et al., 1999). Evidenced by the previous example, multiple types of co-activator complexes can be targeted by activators, including chromatin remodeling complexes. While it is thought that chromatin remodeling complexes are primarily recruited at the early stages of initiation, they have also been shown to play important roles throughout the initiation process and even into elongation (Steger and Workman, 1996; Wittschieben et al., 1999). For activators to

increase the rate of transcription initiation, it seems logical that they would recruit proteins to help remodel chromatin, thus allowing for increased access of general transcription factors and polymerase to their target DNA sequences.

1.3 Chromatin and chromatin remodeling

1.3.1 Chromatin structure

Cells must overcome a number of physical barriers throughout the activation process; one of the first obstacles encountered is limited accessibility of DNA to transcription factors. Nuclear DNA is typically found in the form of chromatin, which consists of repeating structural elements called nucleosomes, which are connected by linker DNA. A complete nucleosome consists of the nucleosome core particle, linker DNA, and the histone H1 protein (Ramakrishnan, 1997). While the histone H1 protein is not required for formation of the nucleosome core particle, it is involved in the formation of higher order chromatin structures (Thoma and Koller, 1977; Ramakrishnan, 1997; Thomas, 1999). The nucleosome core particle, shown in Figure **1.2**, is made up of 147bp of DNA and two copies each of four distinct histone proteins: H2A, H2B, H3, H4 (Davey et al., 2002). H2A and H3 dimerize with H2B and H4, respectively, and two copies of each dimer interact with one another to ultimately form the octamers around which DNA is wound. Protruding from these nucleosomes are the N-terminal tails of the histone proteins. Histone tails are 25 – 40 residues in length and are relatively unstructured with respect to the nucleosome core particle. We have since learned that these histone tails,

while seemingly unimportant to the overall structure of the nucleosome core particle, play a significant role in gene regulation.

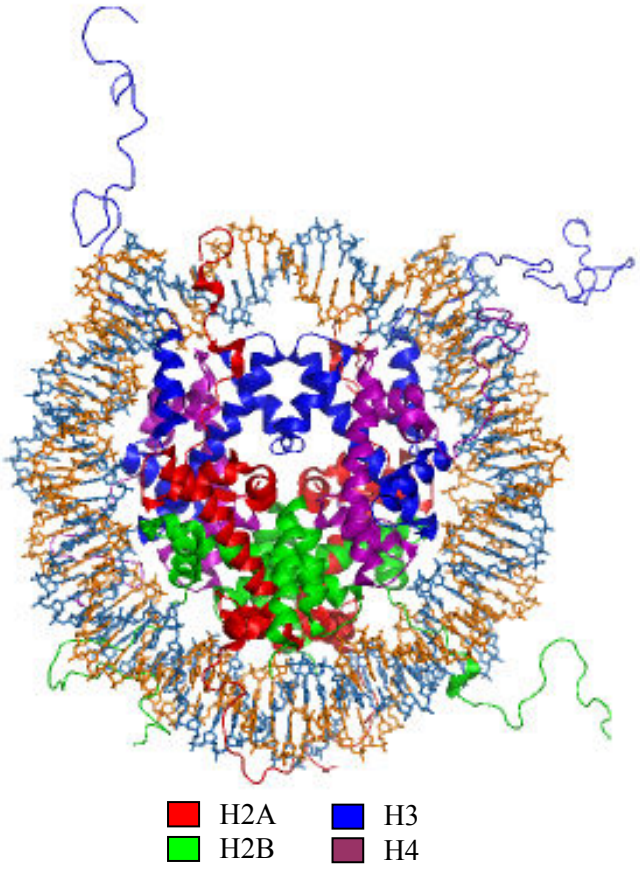

Figure 1.2**: Structure of the nucleosome core particle.**

The nucleosome core particle consists of two pairs of histone dimers; two H2A:H2B dimers, and two H3:H4 dimers. The four dimers interact with one another to form a histone octamer, around which 147bp of DNA is wound to form a nucleosome. Extending from the histone octamer through the DNA gyres are the eight N-terminal histone tails. The crystal structure coordinates (PDB code 1kx5) used in making this image were originally published in Davey et al., 2002, and were generated using PyMOL software (DeLano, 2002).

1.3.2 Histone tail modification

Histone tail modification is one of many mechanisms that cells use to alter chromatin structure with the intended outcome of increasing DNA accessibility to transcription factors (Strahl and Allis, 2000). Histone tails can undergo a variety of different posttranslational modifications, including acetylation, phosphorylation, methylation, ubiquitination, and sumolyation; while multiple types of histone tail modifications can contribute to gene activation, the modification most often associated with gene activation is acetylation (Allfrey et al., 1964; Chicoine et al., 1987; Strahl and Allis, 2000). Acetylation of lysine residues on the histone tail results in two main outcomes, the first of which is a change in the overall charge of the histone tail (Hong et al., 1993; Bauer et al., 1994). Acetylation aids in neutralizing the positively charged histone tails, resulting in a less favorable interaction with the negatively charged DNA (Hong et al., 1993). Some histone tails can also acquire acetylation modifications at multiple lysine residues on the same tail (Figure **1.3**), resulting in a more "open" form of chromatin (Davie et al., 1981; Tse et al., 1998).

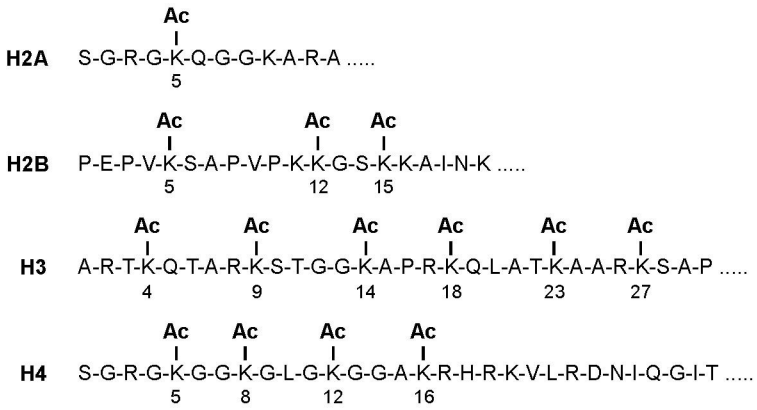

Figure 1.3**: Known acetylation targets of histone tails**

Histone tails are primarily targeted for acetylation at lysine residues. The lysine residues indicated above have been shown to be acetylated *in vitro* and *in vivo* by various HATs. This is not to suggest that other residues within the histone tail or the core histone particle are not targeted for histone acetylation, but rather these are the modifications best associated with gene regulation.

1.3.2.1 Histone Acetyl-Transferases [HATs]

Histone tails are acetylated by a specific class of proteins, appropriately called Histone Acetyl-Transferases (HATs). To date, yeast have been found to contain eight different HAT proteins, briefly summarized in Table **1.1** (Sterner and Berger, 2000). It is interesting to note that while each HAT protein performs the same basic catalytic function, organisms have evolved to contain a variety of HATs, each a member of a distinct protein complex and with its own unique set of target residues (Sterner and Berger, 2000). Much speculation still exists as to why multiple HATs exist to target the same residue, and since little is known about the *in vivo* contribution of a number of these factors, this question remains unanswered.

Table **1.1**: Yeast HATs and their general characteristics

HAT	Protein Complex	Acetylation Target(s) *	
Elp3	Elongator	H3	K14
		H4	K8
Esa1	NuA4, Piccolo	H2A	K4, K7
		H2B	K16
		H4	K5, K8, K12, K16
Gcn5	SAGA/SLIK/SALSA, ADA	H2A	K4
		H2B	K11
		H3	K9, K14, K18, K23, K27
		H4	K8, K16
Hat1	HAT B	H2A	K7
		H4	K5, K12
Hpa2	unknown	H3	K4, K14
		H4	K5, K12
Sas2	SAS	H4	K16
Sas3	NuA3	H3	K14, K23
TAF1	TFIID	H3	K14

* Acetylation targets include both *in vitro* and *in vivo* targets
Table adapted from information in Peterson and Laniel (2004) (Peterson and Laniel, 2004)

There are two main models that describe how HAT complexes might work together to regulate gene expression. One theory, dubbed the "histone code" hypothesis, proposes that each HAT complex makes a unique contribution to histone tail acetylation, and depending on the combination of histone modifications generated, different outcomes in gene regulation can be achieved (Jenuwein and Allis, 2001). A comparison between the Hat1 and Esa1 HATs are a good example of this model. Hat1 is a cytoplasmic HAT that targets free histone H4 for acetylation at lysines 5 and 12; acetylation of H4 K5,12 by Hat1 is important for the deposition of free histone H4 in nuclear chromatin (Ruiz-Garcia et al., 1998; Ai and Parthun, 2004). Since deposition of histones onto DNA results in the downregulation of gene expression, acetylation of H4K5, K12 by Hat1 can

be viewed as an inhibitory modification (Parthun et al., 1996; Adams and Kamakaka, 1999). Esa1, a nuclear HAT, also acetylates H4 K5 and K12, but in combination with K8 (Clarke et al., 1999); in contrast to Hat1, the outcome of H4 acetylation by Esa1 leads to gene activation (Allard et al., 1999; Clarke et al., 1999). According to the histone code, the different effects on gene regulation occur because the cell reads an Ac-H4 K5, K8, K12 pattern differently than an Ac-H4 K5, K12 pattern. This would allow for a potentially complex method of gene regulation, given the endless number of different combinations, or "codes", that could be generated from 14 acetylation sites, not to mention alternate forms of histone modification (methylation, phosphorylation, ubiquitination, summoylation).

In contrast, a different model suggests that many of the histone modifications are redundant with one another and the "histone code" is more of a simple signaling pathway rather than a complex code (Dion et al., 2005). Most HATs are dispensable for cellular function, indicating that there might be a significant amount of overlap between the functions of multiple HAT proteins. Work from Dion et al. has also shown that three of the four histone H4 lysines targeted for acetylation make functionally redundant contributions to gene regulation (Dion et al., 2005). Mutation of H4K5, K8, or K12 to arginine, mimicking an unacetylated state, resulted in the same overall changes in gene expression. Furthermore, double and triple mutations of these residues in any combination only increased the magnitude of the effects seen in the single mutations. If these residues had been part of a "histone code", the predicted outcome would be unique patterns of gene expression depending on the combination of mutations made.

Code or no code, there is still much to be learned with regards to histone acetylation and HATs. Much of what is known about HATs comes from *in vitro* characterization studies, such as HAT assays. While these types of methods help provide a starting point for future studies, they turn out to be misleading in many cases. For example, in vivo characterization of Gcn5 discovered additional acetylation targets compared to the known *in vitro* targets (Zhang et al., 1998). This result can be explained by the hypothesis that association of Gcn5 with a multi-subunit complex, such as SAGA, directs Gcn5's HAT activity to specific residues (Grant et al., 1997; Grant et al., 1999; Robert et al., 2004). Thus, while information from *in vitro* HAT assays should not be discounted, conclusions drawn from *in vitro* assays of individual HAT proteins should not be automatically assumed true *in vivo*.

HATs have been classified into two main groups: the GNAT family and the MYST family. The HATs studied in this thesis will be briefly reviewed within their respective superfamily. Because the TAF1 HAT is unlike other HATs and in a classification by itself, it will be reviewed in section **1.4.1** with the general transcription complex, TFIID.

1.3.2.2 The GNAT family: Gcn5, Elp3, Hat1, Hpa2

The GNAT (Gcn5-related N-acetyltransferase) family contains HATs that have a high degree of similarity in their functional domains and structural motifs (Vetting et al., 2005). Each of the proteins in this family contains four distinct motifs, labeled motif A through D. Motif A has been identified to contain a region responsible for recognizing

and binding the acetyl-coA substrate necessary for acetylation (Dutnall et al., 1998). While the functions of motifs C-D are not known, their high degree of conservation suggests that the structure of these HAT proteins is important for their function.

Gcn5 was the first nuclear HAT discovered in yeast, and to date is the best-characterized HAT (Brownell et al., 1996). Originally identified in *Tetrahymena thermophila*, Gcn5 has been identified in a multitude of eukaryotic species, suggesting that Gcn5 provides an important function that has been conserved through higher eukaryotes (Georgakopoulos and Thireos, 1992; Brownell et al., 1996; Candau et al., 1996; Smith et al., 1998). Gcn5 is a major H3 HAT, as shown by greatly reduced acetylation levels at multiple H3 residues in a *gcn5Δ* strain (Zhang et al., 1998; Suka et al., 2001). A variety of HAT complexes include Gcn5 as a subunit, including SAGA, ADA, SLIK/SALSA, and HAT A2 (Eberharter et al., 1999; Sendra et al., 2000; Balasubramanian et al., 2002; Pray-Grant et al., 2002). *In vitro* HAT experiments with purified Gcn5-containing complexes have shown that the acetylation targets of Gcn5 is contingent upon which complex Gcn5 is associated with, suggesting that other proteins are important for conferring specificity for Gcn5's HAT activity (Grant et al., 1999; Balasubramanian et al., 2002).

Given that Gcn5 is important for maintaining bulk histone H3 acetylation levels, it is surprising that loss of Gcn5 results in the down-regulation of only a small portion of the genome (Lee et al., 2000; Huisinga and Pugh, 2004). It was originally speculated that the main function of Gcn5 existed within its role in the SAGA complex; however, SAGA function does not appear to be compromised in a *gcn5Δ* strain, indicating that the contribution of Gcn5 to gene expression is not though its involvement in SAGA-mediated

TBP delivery (Bhaumik and Green, 2001; Larschan and Winston, 2001). Gcn5 might also make many small contributions to gene expression through it's involvement with other protein complexes beyond SAGA, including ADA and SLIK. More recently, it has been shown that Gcn5 also contributes to gene activation by maintaining non-specific H3 acetylation levels (Imoberdorf et al., 2006). Thus, it could be speculated that the effects of *gcn5Δ* on gene expression are simply due to the general compaction of chromatin resulting from a significant loss of global H3 acetylation.

The lack of dependence on Gcn5 for gene expression has also been explained by the functional overlap between Gcn5 and other HATs, specifically Elp3 and Sas3 (Wittschieben et al., 2000; Howe et al., 2001). Since most HATs are not required for cell viability, it has been suggested that multiple proteins overlap in function, allowing for some degree of compensation if one is deleted (Ruiz-Garcia et al., 1998; Wittschieben et al., 2000). In accordance with this functional redundancy model, individual deletions of Gcn5 and Sas3 are viable; however, deletion of both proteins results in synthetic lethality (Howe et al., 2001). Evidence has also shown that a *gcn5Δ elp3Δ* strain has larger decreases in global H3 K9,14Ac levels as compared to the single deletions (Wittschieben et al., 2000). These and other studies support the idea that Gcn5 is responsible for higher H3 acetylation levels, but also shares some minor functions with other H3 HATs (Howe et al., 2001). The functional redundancy shown between Gcn5 and Sas3 does not extend to all HATs (Howe et al., 2001). For example, Elp3 and Sas3 show no indication of functional overlap, suggesting that each factor has a unique functional relationship with Gcn5 (Howe et al., 2001). The functional relationships between Gcn5 and other HATs, in addition to the association of Gcn5 with multiple protein complexes, suggests that this

protein plays a complex role in gene regulation; thus, many questions still remain regarding the role of Gcn5 in gene activation.

The afore-mentioned Elp3 HAT is a subunit of the Elongator complex and is thus believed to be important for facilitating elongation of Pol II by acetylating nucleosomes located in the ORF (Winkler et al., 2002). This proposed role would indicate that Elp3 plays a significant role in the elongation process, and Elp3 is not required for cell viability (Wittschieben et al., 1999). The indicated functional relationship between Elp3 and Gcn5 suggests that Gcn5 or another HAT might be compensating for the loss of Elp3; but in general, it appears that H3 acetylation by Elp3 is not essential for gene regulation (Wittschieben et al., 2000).

Another member of the GNAT superfamily is the Hat1 protein. This HAT is unique from the others in this family in that it is the only known cytoplasmic HAT (Ruiz-Garcia et al., 1998). Hat1 has been shown to reside primarily in the cytoplasm and is involved in acetylating free histones prior to their deposition onto nuclear DNA (Ruiz-Garcia et al., 1998; Ai and Parthun, 2004). Hat1 has been characterized *in vitro* as a H4 HAT that targets lysine residues K5 and K12 (Ai and Parthun, 2004). An additional role for Hat1 in DNA double strand break repair has also been discovered, but much still remains to be uncovered about this HAT (Qin and Parthun, 2006).

The last HAT in the GNAT family, Hpa2, is the least understood HAT. This protein has been purified and shown to acetylate lysine residues in H3 and H4 *in vitro*, but beyond this, little has been published about this protein or its function *in vivo* (Angus-Hill et al., 1999). No phenotypes are present in an hpa2Δ strain, and it has not yet been determined what other proteins Hpa2 interacts with *in vivo*.

1.3.2.3 The MYST family: Esa1, Sas2, Sas3

The MYST family is named for the human and yeast proteins that originally characterized this group, MOZ, Ybf2/Sas3, Sas2, and Tip60, and contains the yeast HATs Esa1, Sas2, and Sas3 (Utley and Cote, 2003). Proteins in the MYST family share sequence homology and even contain one of the domains found in the GNAT family of HATs; what distinguishes these proteins is that they do not contain the other three domains found in GNAT HATs (Utley and Cote, 2003).

Esa1 is the only HAT other than TAF1 that is essential for cell viability, indicating an important role for this protein in gene regulation (Clarke et al., 1999). Inactivation of Esa1 using a temperature sensitive allele results in arrest of cells in the G_2/M phase and a significant loss of global H4 acetylation levels (Clarke et al., 1999). Esa1 has been purified with two different complexes: NuA4 and Piccolo (Allard et al., 1999; Boudreault et al., 2003). The Piccolo complex is actually a subcomplex of NuA4 and consists of a core group of proteins responsible for NuA4's catalytic function: Esa1, Epl1, and Yng2 (Boudreault et al., 2003). This subcomplex is highly active and has a strong preference for acetylating nucleosomes over free histones (Boudreault et al., 2003; Selleck et al., 2005). Similar to the difference between Gcn5 in ADA and SAGA, it is thought that Piccolo represents a more global, untargeted form of the Esa1 HAT, and the addition of other proteins in NuA4, including the activator-targeted Tra1 subunit, results in a more regulated HAT complex (Boudreault et al., 2003).

The Sas2 and Sas3 proteins are named for their identification in a screen for defects in Sir protein silencing, giving them the SAS (Something About Silencing)

acronym (Reifsnyder et al., 1996). Sas2 has been characterized as the HAT primarily responsible for maintaining H4K16Ac, a modification that plays an important role in maintaining telomere boundaries (Kimura et al., 2002; Suka et al., 2002). Telomeres are silenced by the spreading of a group of repressor proteins called the SIR proteins (Guarente, 1999). The mechanism by which the SIR proteins function involves deacetylation of H4 K16 by Sir2, and assembly of Sir2/Sir3/Sir4 at the deacetylated tails (Guarente, 1999). Thus, maintenance of acetylated H4 K16 by Sas2 is important to prevent the spreading of the Sir proteins into sub-telomeric regions (Suka et al., 2002). The function of Sas3 is also presumed to be involved in silencing, but specifics about its role have not yet been defined (Reifsnyder et al., 1996). Sas3 has been identified as a subunit of the NuA3 HAT complex, and interacts genetically with Gcn5 (Eberharter et al., 1998; Howe et al., 2001). However, little else has been published regarding the contribution of Sas3 *in vivo*.

1.3.3 ATP-dependent remodeling

Histone tail modification is one of two main mechanisms by which DNA accessibility is altered. Another class of chromatin modification complexes increase DNA accessibility to transcription factors by physically moving histone octamers; these complexes are generally referred to as ATP-dependent remodelers, as many require ATP for their catalytic functions (Imbalzano, 1998). While there is no unified mechanism for ATP-dependent remodelers, all of the complexes in this class are involved in making larger changes in chromatin structure, allowing for an even further increase in DNA

accessibility (Johnson et al., 2005). Many ATP-dependent remodeling complexes also contain bromodomain subunits (Horn and Peterson, 2001). Bromodomains, discussed in further detail in section **1.4.4.2**, are domains that preferentially bind acetylated histone tails (Zeng and Zhou, 2002). The fact that multiple chromatin remodeling complexes, including SWR-C, SWI/SNF, and RSC, contain at least one bromodomain subunit has led to the hypothesis that chromatin remodeling complexes are specifically recruited to acetylated promoters with the intent of helping to further increase transcription factor access to promoter DNA (Cairns et al., 1999; Muchardt and Yaniv, 1999; Krogan et al., 2003; Kobor et al., 2004).

Multiple mechanisms for nucleosome remodeling have been proposed. Some ATP-dependent remodeling complexes have been shown to reposition nucleosomes using a sliding mechanism, where the histone octamer is not removed, but moved along DNA to a new location (Lomvardas and Thanos, 2001); other complexes are thought to completely eject histone octamers from DNA (Lorch et al., 2006). The recent characterization of the SWR-C remodeling complex has led to the discovery of yet another mechanism for ATP-dependent remodelers – histone swapping (Mizuguchi et al., 2004).

1.3.3.1 The histone variant H2A.Z

The idea of exchanging canonical histones for histone variants is not a new concept, but the discovery of an ATP-dependent remodeling enzyme as the catalyst for this function is. Histone variants are present in multiple species, with more types of

variants present in higher eukaryotes compared to yeast (Pusarla and Bhargava, 2005). Only two variants have been discovered in yeast: Cse4 and H2A.Z (Stoler et al., 1995; Jackson and Gorovsky, 2000). Cse4 is similar to the human variant, CENP-A, which is localized to centromeres and is required for mitotic events, such as kinetochore formation and chromosome segregation (Palmer et al., 1987; Stoler et al., 1995; Howman et al., 2000). Association of histone H4 with CENP-A results in a structurally rigid nucleosome, making this variant well suited for its involvement in higher levels of chromatin compaction (Black et al., 2004). Since Cse4 is able to substitute for CENP-A both functionally and structurally, it is assumed that the structural properties described above for CENP-A hold true for Cse4 as well (Wieland et al., 2004).

In contrast to the condensation-favored Cse4 variant, incorporation of the H2A.Z variant is believed to result in the formation of a less stable nucleosome, potentially making it an easier target for chromatin remodeling complexes (Fan et al., 2002; Ramaswamy et al., 2005). Evidence that H2A.Z-H2B nucleosomes dissociate much easier than canonical nucleosomes adds support to this model (Zhang et al., 2005). Approximately 5-10% of genome-wide nucleosomes are thought to contain H2A.Z, indicating an important role for this variant in chromatin structure and gene regulation (Leach et al., 2000). Interestingly, while deletion of the canonical histones is lethal, deletion of the gene that encodes for H2A.Z, *HTZ1*, is not lethal, but does result in slow growth and formamide sensitivity (Jackson and Gorovsky, 2000).

Recent studies on the role of H2A.Z have led to the intriguing discovery that this histone variant is preferentially localized at promoter regions (Raisner et al., 2005; Zhang et al., 2005). Genome-wide chIP-chip studies have found that the presence of H2A.Z

inversely correlates with gene expression levels, in that H2A.Z is typically found at quiescent promoters (Li et al., 2005); furthermore, H2A.Z is removed with the other histones upon PIC assembly. These observations have led to the idea that H2A.Z might somehow act as a hallmark of promoters poised for transcription activation (Zhang et al., 2005).

1.3.3.2 SWR-C

The SWR-C complex was simultaneously discovered by two different research groups, both of which arrived at very similar conclusions regarding the nature of the complex (Kobor et al., 2004; Mizuguchi et al., 2004). Both studies sought to identify the protein complex responsible for depositing H2A.Z into nucleosomes. H2A.Z co-purified with a complex containing 12-15 proteins, including select subunits of the NuA4 HAT complex, the Bdf1 bromodomain protein, and an ATP-dependent catalytic subunit, Swr1 (Kobor et al., 2004; Mizuguchi et al., 2004).

The discovery of these previously characterized subunits made for some simple conjectures about SWR-C. Identification of the NuA4 subunits (Act1, Arp4, Eaf1/Swc4, Yaf9) suggested a functional link between the two complexes. The isolation of Bdf1 with the SWR-C complex also made for interesting speculations that SWR-C might utilize Bdf1's bromodomains for binding to acetylated promoter nucleosomes. Chromatin Immunoprecipitation (chIP) evidence from Zhang et al. (2005) went on to show that deletion of Bdf1 results in decreased H2A.Z deposition genome-wide, lending

further support to a possible link between NuA4, SWR-C, and Bdf1. However, these models have yet to be rigorously tested.

1.4 General transcription factor recruitment

The large number of regulatory mechanisms utilized within a cell serves to regulate transcription factor access to regulatory DNA sequences. Once the chromatin state at the promoter is optimized for factor recruitment, assembly of the pre-initiation complex (PIC) can begin. PIC assembly can be broken down into three major steps: TFIID recruitment; TBP delivery; Polymerase II recruitment. While it is likely that each of these "steps" occur together rather than in a sequential order, they will be discussed in this manner for simplicity.

1.4.1 TBP delivery complexes: TFIID and SAGA

The TATA-binding protein (TBP) is a central part of PIC assembly (Pugh, 2000). As its name suggests, TBP binds to the highly conserved TATA box sequence, which is usually found 100-150bp upstream of the transcription start site (Basehoar et al., 2004). As TATA-boxes are only found at ~20% of the yeast genome, binding of TBP to promoters is not restricted to this sequence (Basehoar et al., 2004). TBP is a highly regulated transcription factor, and its binding to promoter DNA, regardless of the presence of a TATA-box, is influenced by two major co-activator complexes: TFIID and SAGA (Pugh, 2000).

Regulation of the genome is divided between these two complexes, but not in an absolute sense (Huisinga and Pugh, 2004). Approximately 10% of the genome is preferentially regulated by the SAGA complex; genes included in this class are involved in stress response pathways, such as heat shock and nutrient deprivation, and are therefore repressed under normal homeostatic growth conditions. Because these genes are only expressed under specific circumstances, they are negatively regulated by a large number of factors, including Mot1, NC2, and histone deacetylases (HDACs). The other 90% of the genome is made up largely of "housekeeping" genes that are regulated by the TFIID complex. These genes are expressed at constitutively low levels and are positively regulated by histone tails through their involvement with chromatin remodeling and modification complexes.

In addition to their common function as TBP-delivery complexes, TFIID and SAGA also have similarities in their subunit composition (Wu et al., 2004). Both complexes share a number of subunits, called TAFs (TBP-associated factors); TFIID contains 14 known TAFs, five of which are also associated with SAGA (Sanders et al., 2002; Wu et al., 2004). Interestingly, a number of TAFs contain a histone-fold domain that has been speculated to be involved in complex structure (Gangloff et al., 2001; Selleck et al., 2001). Some subunits of SAGA and TFIID also provide functions outside of TBP delivery and structural integrity (Pugh and Tjian, 1990; Pugh and Tjian, 1991; Eisenmann et al., 1992; Lee and Young, 1998; Dudley et al., 1999). Both complexes contain subunits that possess HAT activity and bromodomains. Gcn5 provides both functions in SAGA; TFIID's has been proposed to exhibit HAT activity via the TAF1 subunit, and a double bromodomain is located in TAF1 in higher eukaryotes and Bdf1 in

lower eukaryotes (Mizzen et al., 1996; Sterner et al., 1999; Matangkasombut et al., 2000). Similarities in subunit composition and enzymatic functions suggest that these complexes utilize similar mechanisms to deliver TBP to promoters. In short, it has been proposed that both complexes utilize their HAT subunits to acetylate promoter nucleosomes to which the bromodomains can bind; anchoring of the complex to acetylated nucleosomes via the bromodomains then allows the complex to recruit other general transcription factors, ultimately leading to the recruitment of Pol II.

1.4.4.1 TAF1

TAF1 is one of 14 TAFs found in TFIID, and at 145kDa, is the largest subunit in the complex (Sanders et al., 2002). Because of its large size, TAF1 is believed to play an important role in maintaining the structural integrity of the complex through interaction with multiple TAFs, (Yatherajam et al., 2003; Leurent et al., 2004; Singh et al., 2004). The functional domain within TAF1 that allows for TAF-TAF interactions has been mapped, as well as a number of other functional domains in TAF1, including a TBP interaction domain, a HAT domain, and a DNA binding domain (Kokubo et al., 1998; Mencia and Struhl, 2001; Singh et al., 2004). Given the many roles of TAF1 within TFIID, it is not surprising that TAF1 is essential for cell viability; loss of TAF1 by inactivation of a temperature sensitive allele has been shown to result in cell arrest at the G_2/M transition phase of the cell cycle (Walker et al., 1997). TAF1 has also been shown to exhibit additional functions *in vitro* that would presumably aid in gene activation *in vivo*; these functions include HAT activity, kinase activity, and ubiquitin ligase activity

(Mizzen et al., 1996; Tansey and Herr, 1997; Pham and Sauer, 2000). Higher eukaryotic TAF1 also contains a double bromodomain at the C-terminus (Jacobson et al., 2000). While the HAT, kinase, and ubiquitin ligase activities of TAF1 are well characterized in higher eukaryotes, there is little evidence for their existence in lower eukaryotes. The finding that Bdf1 corresponds to the C-terminal portion of hTAF1 suggests that despite their high degree of conservation, there is a good deal of separation between the functions of mammalian and yeast TAF1 (Matangkasombut et al., 2000).

One dubious function of yTAF1 is its HAT activity. Mizzen et al. simultaneously identified and characterized TAF1's HAT activity in human TAF1 (hTAF1), *Drosophila melanogaster* TAF1 (dTAF1), and yeast TAF1 (yTAF1) (Mizzen et al., 1996). Since this discovery, there has been no published evidence to expand upon TAF1's role as a HAT in yeast. Most of the evidence characterizing TAF1's HAT activity has been executed in mammalian systems. A temperature sensitive allele isolated in the ts13 hamster cell line, caused by the G716D mutation, was found to be defective in HAT activity and affected H3K14 acetylation levels at the cyclin D1 promoter, coinciding with the arrest of cells in the G_2/M phase of the cell cycle (Hayashida et al., 1994; Dunphy et al., 2000). It is currently unknown if these findings carry over to other species, including yeast.

Based on the work from Mizzen et al., the protein domain responsible for yTAF1's HAT activity was mapped to amino acids 612 to 1140 in hTAF1, 1 to 1140 in dTAF1, and 354 to 817 in yTAF1 (Mizzen et al., 1996; Wassarman et al., 2000). Comparison of these protein domains reveals surprising dissimilarities between yTAF1 and higher eukaryotic TAF1. While there is ~70% identity between the hTAF1 and dTAF1 HAT domains, scTAF1 only shares ~30% identity with either homolog. This

would suggest that either the HAT domain of yTAF1 is mechanistically different from its higher eukaryotic homologs, or the HAT domain simply does not exist in yeast. The lack of resemblance between TAF1's HAT domain and the HAT domains of GNAT and MYST HAT proteins also provides a challenge to understanding TAF1's acetylation mechanism (Marmorstein, 2001). In addition, the mechanism by which TAF1 transfers Ac-CoA to its substrate is unknown.

Additional doubt regarding the relevance of yTAF1's HAT activity *in vivo* has resulted from evidence that the kinase and ubiquitin ligase functions of hTAF1 are absent in yTAF1 (Wassarman and Sauer, 2001). If yTAF1 lacks kinase activity, ubiquitin ligase activity, and bromodomains, does HAT activity follow suit? The only *in vivo* evidence to suggest that yTAF1 might play a role in acetylating histone tails comes from gene expression studies with TAF1 and Gcn5 (Lee et al., 2000). Changes in gene expression profiles were observed in a temperature sensitive TAF1 allele (taf1-ts), a Gcn5 deletion strain (*gcn5Δ*), and the double mutant (*taf1*^{ts2} *gcn5Δ*). While ~30% of the yeast genome decreased expression levels by 2-fold or more in the taf1-ts strain and ~12% in the *gcn5Δ* strain, another ~25% of the genome was only affected when both TAF1 and Gcn5 were compromised. This led to the hypothesis that TAF1 and Gcn5 were functionally redundant, possibly due to their common function as histone acetyltransferases in TBP-delivery complexes. This idea is easy to accept considering the functional relationships that Gcn5 exhibits with the Elp3 and Sas3 HATs (Howe et al., 2001).

While the TAF1/Gcn5 expression study suggests that TAF1 and Gcn5 might have a functional relationship based on their roles as HATs, this does not lend direct proof that (1) the foundation of the relationship between these two factors is HAT activity, and (2)

that yTAF1 is contributing to histone acetylation *in vivo*. The question of whether or not yTAF1 acetylates histones *in vivo* is an important one, as it holds the key to better understanding the mechanism by which TFIID regulates gene expression.

1.4.4.2 The bromodomain factor, Bdf1

The bromodomain motif was first discovered in the *Drosophila melanogaster* protein, brahma, and has since been identified in a number of proteins involved in chromatin modification and transcription regulation (Haynes et al., 1992; Tamkun et al., 1992). Structural and functional studies of bromodomain factors have demonstrated the ability of these domains to bind to acetylated-lysine residues on histone tails (Dhalluin et al., 1999; Hudson et al., 2000; Jacobson et al., 2000; Owen et al., 2000). This discovery has led to the general hypothesis that bromodomain factors aid in the recruitment and/or binding of protein complexes to nucleosomes, providing a nice link between histone tail acetylation and gene activation (Brownell and Allis, 1996; Zeng and Zhou, 2002). However, bromodomains have been largely studied *in vitro*, resulting in a lack of evidence for the importance of these domains *in vivo*.

The yeast protein, Bdf1, provides a good candidate for studying the role of bromodomains *in vivo*. Bdf1 was originally identified in a genetic screen for genes that affect the synthesis of snRNPs, suggesting from the start that Bdf1 was involved in gene regulation (Lygerou et al., 1994). But the involvement of Bdf1 with TFIID was unknown until a yeast-two hybrid screen discovered an interaction between Bdf1 and TAF7 (Matangkasombut et al., 2000). Sequence alignments also indicated that Bdf1 was highly

homologous to the C-terminus of hTAF1, thus representing a missing portion of yeast TAF1 (Matangkasombut et al., 2000). Association of Bdf1 with TFIID through interaction with TAF7 has led to the hypothesis that Bdf1 plays an important role in tethering TFIID to nucleosome-bound promoters.

Interestingly, while most of the TAF subunits of TFIID are required for cell survival, loss of Bdf1 is not lethal (Giaever et al., 2002). This result is initially surprising, since a role for Bdf1 as the "anchor" for TFIID to promoters might suggest that Bdf1 plays a central role in TFIID-mediated gene activation. One potential explanation could be that Bdf1 is involved in TFIID-mediated gene activation at only a subset of genes, presumably genes that are not required for cell viability. However, the discovery of Bdf2, a protein with a high degree of homology to Bdf1, has led to an alternative hypothesis that Bdf1 is dispensable due to the presence of Bdf2 (Matangkasombut et al., 2000). Like Bdf1, Bdf2 has also been shown to interact with TAF7 *in vitro* and bind acetylated histone tails (Matangkasombut et al., 2000; Ladurner et al., 2003; Matangkasombut and Buratowski, 2003); additionally, a *bdf1Δ bdf2Δ* strain is not viable, strengthening the idea of a functionally redundant relationship between these two factors (Matangkasombut et al., 2000).

Bdf1 also functions in pathways outside of its role in TFIID gene regulation. Genome-wide expression analysis of a *bdf1Δ* strain revealed a non-random distribution of Bdf1-dependent genes in the subtelomere (Ladurner et al., 2003). Additional evidence showed that loss of Bdf1 resulted in the spreading of SIR proteins (described in section **1.3.2.3**) into the subtelomeric region. Knowing that Bdf1 preferentially binds hyper-Ac H4 tails, a modification that inhibits the formation of repressive SIR protein complex, it

was proposed that Bdf1 functions in a TFIID-independent manner to regulate heterochromatin boundaries at the subtelomere (Ladurner et al., 2003).

Another TFIID-independent function of Bdf1 is found in its involvement with the SWR-C histone remodeling complex. As reviewed in section **1.3.3.2**, there is evidence that suggests a functional link between the H4 HAT complex, NuA4, Bdf1, and SWR-C. Purification of the SWR-C complex results in co-purification of Bdf1 (Kobor et al., 2004; Krogan et al., 2004), and chIP-on-chip studies have shown that H2A.Z deposition is negatively affected in *bdf1Δ* strains, strengthening the model that Bdf1 is involved in SWR-C-mediated H2A.Z deposition (Zhang et al., 2005). The involvement of NuA4 in this process is a bit more elusive; two hypotheses have emerged to explain the link between NuA4, Bdf1, and SWR-C. The first model stems from evidence that Bdf1 preferentially binds to hyper-acetylated H4 tails *in vitro* (Ladurner et al., 2003; Matangkasombut and Buratowski, 2003); NuA4-mediated H4 acetylation might regulate Bdf1 binding, which in turn aids in the recruitment of SWR-C and H2A.Z deposition. Alternatively, recent evidence from Keogh et al. indicates that NuA4 acetylates H2A.Z (Keogh et al., 2006); while this provides an explanation for the relationship between NuA4 and H2A.Z, it does not offer a model for the involvement of Bdf1 with these two complexes. Thus, there is still much to be investigated regarding the relationship between Bdf1, TFIID, NuA4, SWR-C, and H2A.Z.

1.5 Polymerase II recruitment and initiation

Once a TBP-associated complex, such as TFIID, is recruited to promoter DNA, additional general transcription factors associate with TFIID, eventually resulting in the recruitment of the Polymerase II holoenzyme to form the Pre-Initiation Complex (PIC). The classic model regarding PIC formation begins with recruitment of TBP through TFIID, followed by binding of TFIIA and TFIIB, which aid in stabilizing the TBP-DNA complex (Martinez, 2002). TFIIF and Pol II are subsequently recruited to the TFIID-TBP-TFIIA-TFIIB complex, and are followed by the association of TFIIE and TFIIH (Martinez, 2002). TFIIH is a DNA helicase important for "melting" the DNA at the transcription start site, allowing for transcription initiation by Pol II after it dissociates from the PIC (Zurita and Merino, 2003). While this is a generally accepted model for PIC formation and transcription initiation, many of the defined steps in the process have been determined through *in vitro* assays with reconstituted components. Thus, the order of recruitment for each component in the initiation process could vary *in vivo*; it has also been proposed that portions of the PIC can assemble in solution and bind to the promoter as a holoenzyme (Roeder, 1996; Zurita and Merino, 2003).

Once Pol II has cleared the promoter and initiated transcription of the downstream gene, it is believed that the remaining components of the PIC disassemble from the promoter, and the promoter "re-sets" itself, the degree to which has yet to be determined (Roeder, 1996). The cleared promoter is then ready to re-assemble the transcription machinery again if transcription of the downstream gene is still required. If transcription

is no longer required, changes are made to the promoter, such as de-acetylation or repressor binding, which prevent reassembly of the PIC.

1.6 A genome-wide approach to studying gene regulation

This literature review has hopefully shown that the process of gene regulation is very complex, and it is unlikely that any one mechanism can be applied to every gene in the genome. Up until ~10 years ago, scientists were limited in their approach to studying gene regulation in that only a few genes could be studied at one time. As a result, regulatory mechanisms were modeled after a handful of well-studied genes, such as *PHO5*, *HIS3*, and *GAL1*. While the investigation of specific "model" genes has led to the development of some basic principles in the regulatory field, we have since learned that some of these mechanisms are gene specific and do not necessarily apply to the other ~6000 genes in the yeast genome.

With the advancement of genome-wide microarray technology, we have been able to study gene regulation on a much larger scale and elucidate multiple mechanisms at one time. In the work presented in this thesis, I have utilized genome-wide expression and chromatin-immunoprecipitation (chIP) technology to study the complex involvement of histone acetylation and factor recruitment throughout the genome. While the expression studies help understand the positive and negative regulation of mRNA expression, the chIP microarray experiments analyze changes in transcription factor recruitment and/or binding on a genome-wide level.

Discussion in this literature review of the extent to which yeast TAF1 contributes to histone acetylation *in vivo* has shown that much doubt remains in the transcription field regarding this particular function of TAF1. The focus of the work presented in Chapter 2 of this thesis is further investigation of the role of yeast TAF1 as a HAT *in vivo*. Expression microarrays were a primary catalyst for investigating the question of TAF1's HAT activity, and through these microarray experiments, we learned not only that TAF does not contributed to histone acetylation levels in yeast, but furthermore that the H4 HAT, Esa1, has a unique relationship with TFIID-regulated genes. Given the previously hypothesized relationship between H4 acetylation, TFIID, and Bdf1, I further investigated the relationship between Esa1-mediated H4 acetylation and recruitment of the protein complexes, TFIID and SWR-C, by Bdf1 in Chapter 3 using chIP-on-chip assays. The results from these overlapping experiments showed that while the model regarding bromodomain-mediated recruitment of protein complexes is true at some genes, this model is not necessarily applicable to every gene throughout the genome. In general, the findings and models presented here reinforce the idea that not every gene is regulated by the same mechanism; however, they also serve to provide a better understanding of the larger dynamics involved in gene regulation.

1.7 References

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Chapter 2

Genome-Wide Analysis of Yeast Histone Acetyl-Transferases

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2.1 Summary

Histone acetylation regulates gene expression. Yet, the functional contributions of the numerous histone acetyltransferases (HATs) to gene expression and their relationships with each other remain largely undefined. The central role of the putative HAT-containing TAF1 subunit of TFIID in gene expression raises the fundamental question as to what extent, if any, TAF1 contributes to acetylation *in vivo*, and to what extent it is redundant with other HATs. Our findings, herein, do not support the basic tenet that TAF1 is a major HAT in yeast. Nor do we find that TAF1 is functionally redundant with other HATs, including Gcn5, Elp3, Hat1, Hpa2, Sas3, or Esa1, which is in contrast to previous conclusions regarding Gcn5. Our findings do reveal that, of these HATs, only Gcn5 and Esa1 contribute substantially to gene expression genome-wide. Interestingly, histone acetylation at promoter regions throughout the genome does not require TAF1 or RNA polymerase II, indicating that most acetylation is likely to precede

transcription and not depend upon it. TAF1 function has been linked to Bdf1, which binds TFIID and acetylated histone H4 tails, but no linkage between TAF1 and the H4 HAT Esa1 has been established. Here we not only present evidence for such linkage through Bdf1, but also demonstrate that genes utilizing this assembly pathway can be rate-limited by Esa1 and TAF1.

2.2 Introduction

Eukaryotic genes are packaged into chromatin that is largely composed of histone proteins. Access to these genes requires mobilization of the histones, which is thought to involve specific lysine acetylation of their amino terminal tails, as well as other types of modifications (Carrozza et al., 2003; Peterson and Laniel, 2004). Cells contain a multitude of histone acetyltransferases (HATs), some of which play important roles in transcriptional activation (Sterner and Berger, 2000). In addition to mobilizing nucleosomes, histone acetylation provides binding sites for bromodomain proteins, many of which are part of the transcription machinery (Loyola and Almouzni, 2004).

The transcription machinery assembles at promoters via two alternative pathways directed by two related multi-subunit complexes, TFIID and SAGA (Kuras et al., 2000; Lee et al., 2000; Li et al., 2000; Cheng et al., 2002; Basehoar et al., 2004; Huisinga and Pugh, 2004). Interestingly, both complexes contain subunits (TAF1 and Gcn5, respectively) that harbor bromodomains and HAT activity (Brownell et al., 1996; Mizzen et al., 1996), thereby linking histone acetylation and recruitment of the transcription

machinery. In yeast, TAF1 lacks bromodomains, which instead appear to be located on the TFIID-interacting protein Bdf1 (Matangkasombut et al., 2000).

Much of what is currently known about the HAT activity of TAF1 stems from studies on higher eukaryotic TAF1. TAF1 was first identified as a HAT through *in vitro* acetyltransferase assays, and shown to possess similar substrate specificity as Gcn5 (Mizzen et al., 1996). A naturally occurring mutation (ts13) in mammalian TAF1 renders cells temperature-sensitive for both cell cycle progression (Sekiguchi et al., 1991; Hisatake et al., 1993; Ruppert et al., 1993) and expression of the cyclin D1 and cyclin A genes (Wang and Tjian, 1994; Wang et al., 1997). This mutation maps to the TAF1 HAT domain and impairs its HAT activity *in vitro* (Dunphy et al., 2000). Additional mutations that eliminate TAF1 HAT function have been defined, and expression of these mutant TAF1 proteins *in vivo* leads to defects in H3 acetylation at the cyclin D1 promoter (Hilton et al., 2005). Taken together, these observations provide evidence for TAF1 being a physiologically relevant HAT, at least in higher eukaryotes.

Both TAF1 and Gcn5 acetylate histone H3 at lysine 14 (H3K14) (Mizzen et al., 1996; Zhang et al., 1998; Kuo et al., 2000; Suka et al., 2001), and both TAF1 and Gcn5 have been reported to play functionally redundant roles in yeast (Lee et al., 2000). At face value, this relationship fits well with the notion that TFIID and SAGA play functionally redundant roles by acetylating the same targets and nucleating assembly of a transcription complex by two alternative pathways. However, many aspects of this relationship have not been rigorously tested. For example, it is not known to what extent histone acetylation *in vivo* is dependent upon TAF1 versus Gcn5. In addition, yeast TAF1 has not been demonstrated to have robust HAT activity *in vitro*, or to have HAT

activity as a full-length protein or when part of TFIID. The relationship between TAF1 and Gcn5 is important to clarify because their potentially parallel function, if it exists, could be central to the mechanism of transcription complex assembly. Moreover, the putative HAT redundancy between yeast TAF1 and Gcn5 raises the question as to whether other HATs are functionally redundant with TAF1. HATs such as Elp3 and Sas3 have been suggested to be functionally redundant with Gcn5 (Wittschieben et al., 2000; Howe et al., 2001), and thus have the potential to be redundant with TAF1.

TAF1 function might also be intertwined with the HAT Esa1, a subunit of the NuA4 complex, inasmuch as Esa1 acetylates histone H4 tails (Allard et al., 1999) and the TAF1-containing TFIID complex might bind to acetylated H4 tails via bromodomains contained within TAF1 in higher eukaryotes or Bdf1 in yeast (Jacobson et al., 2000; Matangkasombut et al., 2000). Moreover, TFIID-regulated genes tend to have high levels of H4 acetylation (Huisinga and Pugh, 2004). Transcriptional dependencies on Esa1 have not been previously conducted on a genome-wide scale, and so it remains uncertain as to whether gene regulation by TFIID and Esa1 are correlated.

Here we examine a number of basic tenets of the hypothesis that TAF1 and Gcn5 are functionally redundant *in vivo*, by examining whether each are necessary and sufficient to acetylate H3 *in vivo*. We further test whether TAF1 is functionally redundant with other HATs *in vivo*, genome-wide. In addition to finding no evidence of TAF1 HAT activity or functional redundancy in yeast, we also find that genome-wide promoter-specific acetylation does not require TAF1 or RNA polymerase II (pol II). We do however detect a strong correlation between genes regulated by TAF1 and Esa1, which suggests that the two function in the same pathway. Interestingly, Bdf1 occupancy

correlates more with H4 acetylation than does TAF1, which is consistent with Bdf1 recruitment being more central to H4 acetylation than TFIID recruitment.

2.3 Results

2.3.1 TAF1 and Gcn5 are not functionally equivalent

The premise upon which we began our inquiry is that TAF1 is a HAT that targets similar histone residues as Gcn5, and thus is at least in part functionally redundant with Gcn5. Since TAF1 and Gcn5 reportedly acetylate histone H3 tails, we focused our attention on lysine acetylation of H3. If TAF1 and Gcn5 are both major HATs that acetylate the same H3 residues, then elimination of one or the other should have less of an impact on H3 acetylation when compared to loss of both. TAF1 was eliminated using a temperature-sensitive *taf1*^{ts2} allele, which upon shifting to 37˚C for 45 min. results in degradation of nearly all of TAF1 (Walker et al., 1996), and at least a partial shutdown of ~90% of all expressed genes (Holstege et al., 1998; Huisinga and Pugh, 2004). Inasmuch as TAF1 is physically eliminated at the nonpermissive temperature, it is reasonable to assume that any HAT activity within TAF1 is eliminated as well. Gcn5 was eliminated using a *gcn5Δ* strain. This strain was also shifted to 37˚C for 45 min. to remain consistent with the conditions used to inactivate TAF1. As shown by the immunoblot in Figure **2.1** , loss of TAF1 had no significant effect on bulk H3 acetylation at K9, K14, K18, K23, and K27. In contrast, loss of Gcn5 resulted in a ~85% drop in bulk K9, K14, and K27 acetylation, and a modest effect at K18, findings that confirm previous results on Gcn5 specificity towards bulk histones at K9, K14, and K18 *in vivo*

(Zhang et al., 1998). Elimination of both TAF1 and Gcn5 had no further effect than elimination of Gcn5 alone.

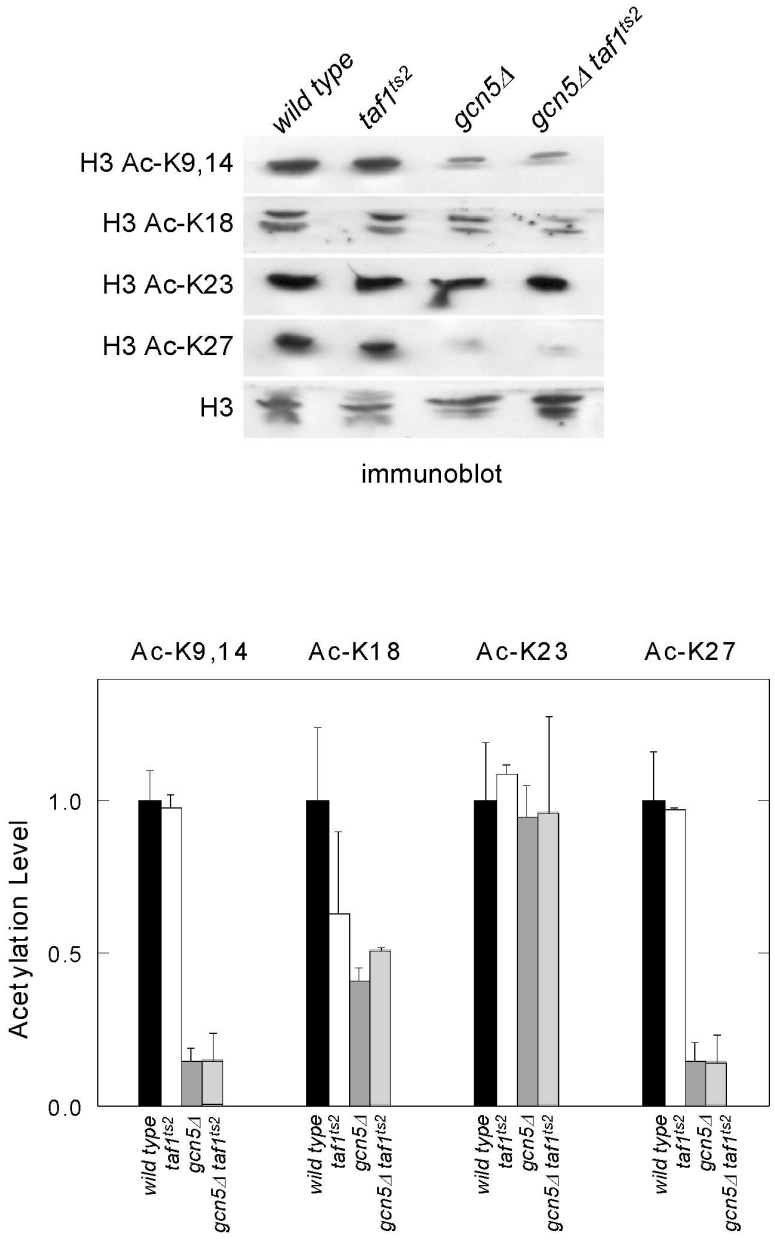

Figure 2.1**: Requirement of Gcn5 and TAF1 on bulk H3 histone acetylation levels**

All strains were grown in CSM –His medium at 25ºC and then shifted to 37ºC for 45min to inactivate the taf1ts2 allele, when present. Crude whole-cell lysates ere subjected to SDS-polyacrylamide gel electrophoresis and immunoblots analysis using antibodies recognizing the indicated histone H3 modifications or total H3 (bottom immunoblots). Quantitation of three independent replicates is shown.

A number of conclusions can be drawn from these results. 1) Gcn5 is the major HAT operating at bulk H3 K9, K14, and K27 under these growth conditions, which reconfirms similar conclusion drawn elsewhere on H3 K9, K14 (Zhang et al., 1998). This does not exclude smaller contributions from other HATs such as Sas3 (Howe et al., 2001). 2) Yeast TAF1 is not a major physiological HAT of bulk H3 histones. 3) Gcn5 either does not acetylate the bulk of H3 K23 *in vivo*, or does so in a redundant manner with another HAT that is not TAF1. 4) Ongoing transcription throughout most of the genome, which is lost in the *taf1*^{ts2} strain, is not required to maintain bulk H3 acetylation. Collectively, the data indicate that TAF1 is not similar to Gcn5 with respect to bulk histone H3 acetylation.

The immunoblot in Figure **2.1** examined bulk histone H3 acetylation regardless of its location in the genome. In principle, if acetylation of H3 lysines is spread throughout the genome, including at promoters, within ORFs, and downstream of genes, then it is plausible that putative H3 acetylation by TAF1 might be missed if its HAT activity is concentrated over promoter regions, where it normally binds. This would seem unlikely since H3 acetylation appears to be concentrated near promoters (Pokholok et al., 2005; Yuan et al., 2005). Nevertheless, to address this possibility, we used genome-wide location analysis (chIP-chip) to determine if promoter-specific acetylation was affected by loss of TAF1, Gcn5, or both.

In this analysis, we focused on acetylation at H3 K9,14, a major target of Gcn5. Mutant (*gcn5Δ*, *taf1*^{ts2}, and *gcn5Δtaf1*^{ts2}) and wild type cells were subjected to formaldehyde crosslinking. The chromatin was sheared and immunoprecipitated with H3 Ac-K9,14 antibodies. Immunoprecipitated DNA was assayed with microarray probes

covering approximately 6000 intergenic regions (including regions that lack promoters). Ratios of mutant/wild-type occupancy of H3 Ac-K9,14 were determined and the dataset was centered to the median value of the upper 5th percentile of the data (i.e., mutant/wild-type ratios that were the highest). In order to compare data sets, it was necessary to set those genomic regions whose H3 acetylation was truly unaffected by the mutants to be equivalent (i.e. centering). Since it is unknowable from this assay whether any region of the genome meets this criterion, we arbitrarily define those intergenic regions that are in the upper 5th percentile (representing 5% of the data set) as having acetylation levels, if any, that are unaffected by loss of TAF1 and/or Gcn5. This normalization attempts to steer clear of the 90% of the genome that is at least partially dependent upon TAF1. Implicit in this normalization is that increases in acetylation in the mutants do not occur. This approach makes no assumptions about acetylation in promoter and non-promoter intergenic regions, and thus allows them to be evaluated independently. The conclusions drawn here can be attained from other normalization methods as well (e.g. centering to nonpromoter region, not shown).

Log_2 ratios for intergenic regions that contain promoters (n = 3823) were binned and plotted as smoothed frequency distributions in Figure **2.2** (panel A). A control co-hybridization of two independent wild-type samples resulted in a tight distribution, reflecting intrinsic experimental variance, the peak of which we define as zero (no change). Loss of Gcn5 resulted in a drop in H3K9,14 acetylation over nearly all promoter regions (manifested by a leftward shift of the profile relative to wild type). The broad distribution of the data relative to the wild type control indicates that the drop in acetylation was not uniform at all promoter regions. This profile indicates that Gcn5

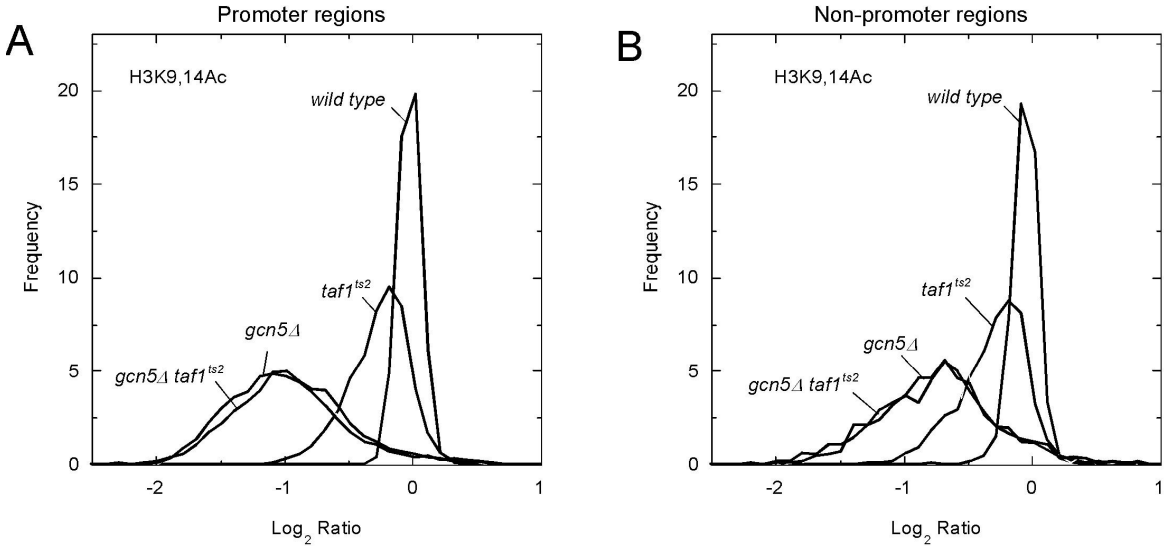

Figure 2.2**: Assessment of gene-specific changes in H3 K9,14-Ac levels in *gcn5Δ* and *taf1^{ts2}* mutant strains through chIP on chip**

Frequency distribution of H3 Ac-K9,14 occupancy in intergenic regions containing (A) or lacking (B) a promoter, the latter being intergenic regions located between two convergently transcribed genes. chIP assays were performed on cross-linked, sheared chromatin from *WT*, *taf1^{ts2}*, *gcn5Δ*, and *taf1^{ts2} gcn5Δ* strains that were grown at 25ºC and then shifted to 37ºC for 45min to inactivate the taf1ts2 allele. Immunoprecipitated DNA from test strains was labeled and cohybridized to intergenic microarrays along with an independent wild-type sample. H3 Ac-K9,14 occupancy levels relative to the wild type were converted to a log2 scale and binned in 0.05 intervals, and the resulting frequency histogram was converted to an interpolated frequency distribution using Kaleidagraph software.

contributes to H3 K9,14 acetylation at most promoters, but at some promoters the contribution is more and at others the contribution is less.

Loss of TAF1 resulted in a minor leftward shift of the population, which was centered over -0.2 ($\log_2$ scale), reflecting a negligible (~1.1 fold) decrease in acetylation. The *taf1^{ts2}gcn5Δ* double mutant was indistinguishable from the *gcn5Δ* single mutant. In both TAF1 mutants (*taf1^{ts2}* and *taf1^{ts2}gcn5Δ*) it is clear that loss of TAF1 had no major impact on H3 K9,14 acetylation in promoter regions. Any minor effects are likely to be indirect since similar changes in H3 K9,14 acetylation were observed in non-promoter

intergenic regions as well (i.e., regions downstream of two convergently transcribed genes, Figure **2.2** panel B), where TAF1 is not expected to bind. Loss of Gcn5 showed an overall larger decrease in H3 K9,14 acetylation for the promoter regions as compared to non-promoter regions (Figure **2.2**, panel A vs. B). This observation is consistent with promoter regions having higher H3 K9, K14 acetylation levels (and thus more acetylation to lose) than non-promoter regions. Nevertheless, Gcn5 contributes to low levels of histone acetylation in nonpromoter regions as well, which is consistent with reports of targeted and nontargeted acetylation by Gcn5 (Vogelauer et al., 2000).

The following conclusions can be drawn from the chIP-chip data: 1) TAF1 is not a major physiological H3 K9,14 HAT at the vast majority of yeast promoters. 2) Gcn5 is a physiological HAT at most yeast promoters, which confirms genome-wide Gcn5 occupancy data and genome-wide transcriptional dependencies on Gcn5 (Lee et al., 2000; Huisinga and Pugh, 2004; Robert et al., 2004), but represents the first genome-wide assessment of Gcn5-dependent acetylation. 3) Loss of TAF-dependent transcription, which occurs upon inactivation of TAF1, leads to little or no changes in H3 K9,14 acetylation in promoter regions, indicating that TFIID occupancy at promoters is not needed to maintain promoter-specific H3 K9,14 acetylation. We conclude from these studies that if yeast TAF1 is a physiological HAT, it lacks the same specificity as Gcn5.

Histone acetylation occurs independently of transcription. Inasmuch as the SAGA complex can partially compensate for the loss of TFIID in transcription (Lee et al., 2000; Huisinga and Pugh, 2004), the experiments described above could not ascertain whether transcription was necessary to maintain H3 K9,14 acetylation. To address this, we performed a similar H3 K9,14 chIP-chip experiment in a strain harboring the

temperature-sensitive pol II allele, *rpb1-1*. In this experiment, H3 K9,14 acetylation levels that exist after a 45 minute heat inactivation of the *rpb1-1* allele were compared to equivalently treated wild type cells. As shown by the scatter plot in Figure **2.3**, promoter occupancy of H3 acetylated at K9,14 in the *rpb1-1* strain strongly correlated with occupancy levels occurring in wild type cells, indicating that transcription is not necessary to maintain H3 K9,14 acetylation in promoter regions. This finding confirms on a genome-wide scale what has been observed at selected loci (Kuo et al., 2000).

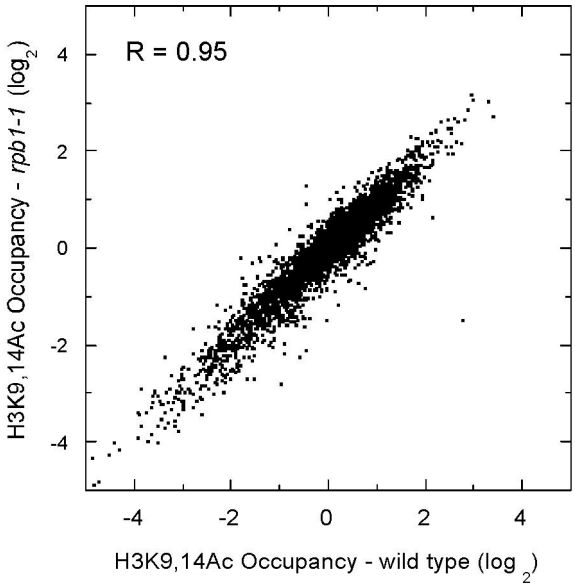

Figure 2.3**: Changes global H3 acetylation levels are not due to inactivation of Pol II transcription**

Cells growth and chIP assays were performed as described for Figure 2.2 using a wild-type of *rpb1-1* strain. He Ac-K9,14 occupancy data were normalized to a nonspecific immunoprecipitated chIP DNA set, converted to a $\log_2$ scale, and centered to the median value for nonpromoter intergenic regions. Shown is a scatter plot comparison between H3 Ac-K9,14 in an rpb1-1 strain versus a wild-type strain at the nonpermissive temperature (37ºC).

Since histone acetylation occurs at a variety of histone sites, we further examined whether sustained transcription is essential for maintaining bulk acetylation at a variety of H3 and H4 residues. As shown in the immunoblot in Figure **2.4**, loss of transcription upon inactivation of the *rpb1-1* allele resulted in no observable changes in bulk H3 acetylation at the residues tested, suggesting that ongoing transcription is not necessary for global maintenance of histone H3 acetylation. This does not exclude the possibility of widespread transcription-coupled acetylation in open reading frames that occurs transiently during elongation (Kristjuhan and Svejstrup, 2004), and is thus a minor contributor to bulk acetylation.

In contrast, the levels of H4 acetylation at K8 decreased in the *rpb1-1* strain, even at the permissive temperature. Little or no effects were observed at H4 K5, K12, and K16. Acetylation at H4 K5, K8, and K12 has been largely linked together as providing additive changes in the overall charge on H4 tails, which incrementally affect transcription (Dion et al., 2005). Although the basis for the sensitivity of K8 acetylation to the *rpb1-1* mutation is unclear, it does suggest a functional link between pol II and K8 acetylation that might not exist with the other sites. It is intriguing that the pol II-associated HAT Elp3 specifically acetylates H4K8 (Winkler et al., 2002). Conceivably, H4K8 acetylation might occur via one or more HATs such as Elp3 that are associated with an elongating pol II, whereas acetylation at other sites might be less pol II-dependent.

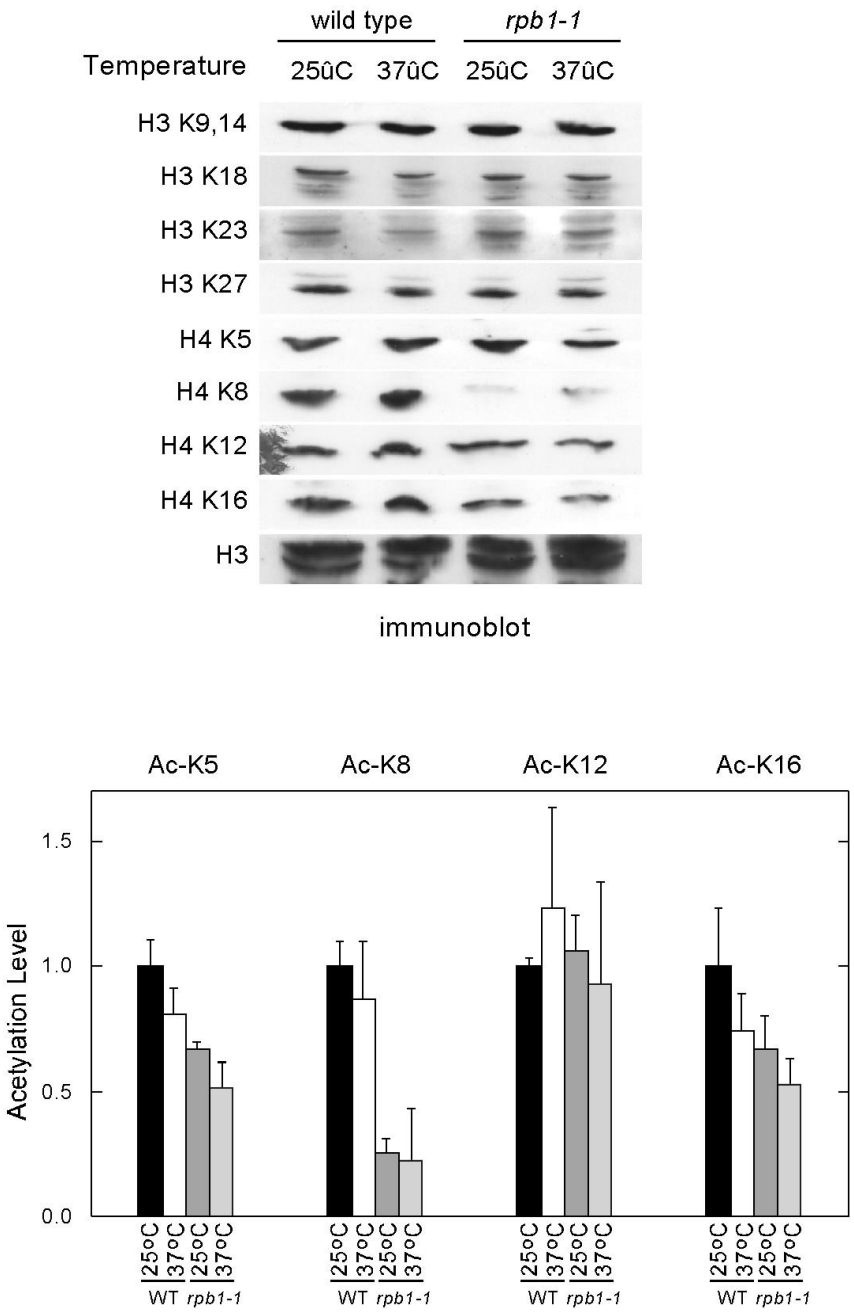

Figure 2.4**: Bulk H3 and H4 acetylation levels are not dependent on Pol II transcription**

Immunoblots were performed on *WT* and *rpb1-1* strains at the permissive temperature (25ºC) and nonpermissive temperature (37ºC) using antibodies against the indicated histone modifications or total H3. Quantitation of three independent H4 modification replicates is shown below the immunoblots.

H4K5 and H4K12 hyperacetylation at promoters is linked to transcription (Yuan et al., 2005), yet we find that pol II is not required to maintain acetylation at K5 and K12, as we observed for H3 acetylation (Figure **2.4**). Thus transcription is not necessary to maintain acetylation.

2.3.2 Functional redundancy reassessed by genome-wide expression profiling

The previous conclusion that TAF1 and Gcn5 are functionally redundant was based in part on the observation that changes in gene expression for about 25% of the yeast genome was apparent only when both Gcn5 and TAF1 were eliminated (Lee et al., 2000). Thus far, we find no evidence to support the basic tenet of this hypothesis. We sought to reconcile this difference by collecting similar genome-wide expression profiles using strains and conditions in our study. Rather than utilizing an arbitrary cut-off (e.g. 2-fold) for delineating real changes in gene expression, we chose to involve all data in a gene-by-gene comparison. Functional redundancy should be manifested by a larger fold-change in gene expression in the *gcn5Δtaf1*^{ts2} double mutant than can be explained by changes arising from independent effects of single mutants.

In Figure **2.5**, $\log_2$ changes in gene expression are plotted in terms of a frequency distribution akin to the chIP analysis described in Figure **2.2** and elsewhere (Huisinga and Pugh, 2004). The horizontal "error" bars represent the standard error of the two replicate data sets; thus they indicate the peak positions of the distributions for each individual data set. The small size of each error bar indicates a high degree of reproducibility between the independent data sets. Both *gcn5Δ* and *taf1*^{ts2} single mutants generate a leftward shift

of the profile relative to wild type, and the double mutant generates a further leftward shift. If Gcn5 and TAF1 each make independent contributions to transcription, then loss of both should be equivalent to the multiplicative result (additive on a log scale) of losing each individually. Thus, if *gcn5Δ* causes a 2-fold drop in expression, and *taf1*^{ts2} causes a 4-fold drop, then the double mutant should cause an 8-fold drop, which was observed. If the two are functionally redundant, then the double mutant should result in an effect that is substantially greater than the multiplicative effects of the individual mutants, and the individual mutants should have small effects. The distribution for the *gcn5Δtaf1*^{ts2} double mutant, calculated from the single mutants, is shown in Figure **2.5**. The calculated and observed distributions were not significantly different either in terms of a bulk population distribution (panel A) or on a gene-by-gene comparison (panel B), indicating that TAF1 and Gcn5 make independent contributions to gene expression, and thus are not functionally redundant. Nonetheless TAF1 and Spt3, as components of TFIID and SAGA respectively, display some functional redundancy (Huisinga and Pugh, 2004). Thus, TFIID and SAGA appear to be partially redundant with respect to TBP function but not histone acetylation, at least in yeast.

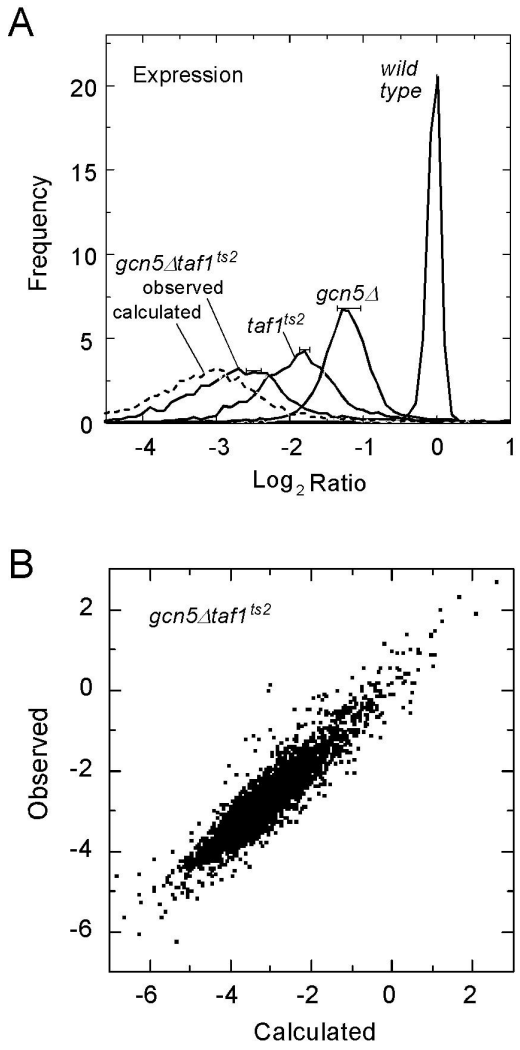

Figure 2.5**: TAF1 and Gcn5 make independent contributions to gene expression**

(A) Genome-wide changes in gene expression in *WT*, *taf1*^{ts2}, *gcn5Δ*, and *taf1*^{ts2} *gcn5Δ* strains relative to an independent wild-type strain. Expression changes were determined after cultures were shifted to 37ºC for 45min to inactivate the *taf1*^{ts2} allele. Frequency distributions are plotted as described in the legend of Figure 2.2. This experiment is a repeat of an experiment described previously (Huisinga and Pugh, 2004) but was performed in the context of the experiments described in the legend of Figure 2.6. The dashed line represents the calculated distribution for the double mutant obtained by adding the log2 ratios of the single mutants. (B) Gene-by-gene scatter plot relating calculated and observed values for the *taf1*^{ts2} *gcn5Δ* strain.

The previous conclusion (Lee et al., 2000), arrived at via different methods of analysis, arises because the distribution of values (fold changes in gene expression) is largely Gaussian rather than linear. Thus, at a fixed and arbitrary cut-off (e.g. 2-fold) typically used to establish significance, small lateral shifts in the distribution shown in Figure **2.5A** can lead to large and nonlinear changes in the number of genes meeting the cut-off criteria. Thus, comparing numbers of genes that meet an arbitrary cut-off should not be used to assess functional redundancy. Arbitrary cut-offs, however, can be valid for other types of statistical analyses.

2.3.3 TAF1 displays no functional redundancy with other HATs

Since frequency distribution profiles are established with as many as 6000 data points replicated multiple times, they can provide a robust reflection of overall changes in gene expression and a robust means for assessing functional redundancy. Detecting no functional redundancy between TAF1 and Gcn5, we asked whether any functional redundancy exists between TAF1 and other cellular HATs. Genome-wide expression profiles were conducted on a variety of yeast HAT deletion strains in the context of either wild type TAF1 or *taf1*^{ts2}, after a 45 min. shift to 37˚C. In one case (Esa1) where the HAT is essential for cell growth, we employed the temperature-sensitive *esa1-414* allele. No prior study has reported genome-wide dependencies on these HATs.

Figure **2.6** displays the distribution profiles for changes in expression for each of the single and double mutants. The same datasets were used for wild type (WT) and *taf1*^{ts2} in all plots. Also shown is the predicted distribution for the double mutants,

calculated from the single mutants. In each case, the observed distribution for the double mutants did not show a significant leftward shift (larger decrease in gene expression) relative to the predicted distribution, reflecting a lack of functional redundancy between TAF1 and each of the tested HATs (Sas3, Elp3, Hpa2, Hat1, and Esa1).

Interestingly, the *hat1Δ taf1*^{ts2} distribution was significantly shifted to the right of its predicted location. Thus, loss of Hat1 partially compensated for the loss of TAF1, suggesting that Hat1 contributes to the genome-wide dependency on TAF1. Hat1 acetylates cytoplasmic histones prior to their deposition onto chromosomal DNA (Ai and Parthun, 2004; Ye et al., 2005). However, its contribution to transcription is largely unknown. A plausible, but highly speculative, interpretation of the data is that loss of TAF1 results in a loss of the major TFIID-directed transcription complex assembly pathway with the consequence of chromatin re-assembling at promoters. Such chromatin might antagonize alternative assembly pathways, such as that directed by SAGA. Indeed, histones are more repressive towards the SAGA pathway than to the TFIID pathway (Huisinga and Pugh, 2004). If the accumulation of chromatin depends upon Hat1 for deposition, then loss of Hat1 might keep promoter regions more accessible to SAGA-directed transcription complex assembly, and thus less dependent upon TAF1.

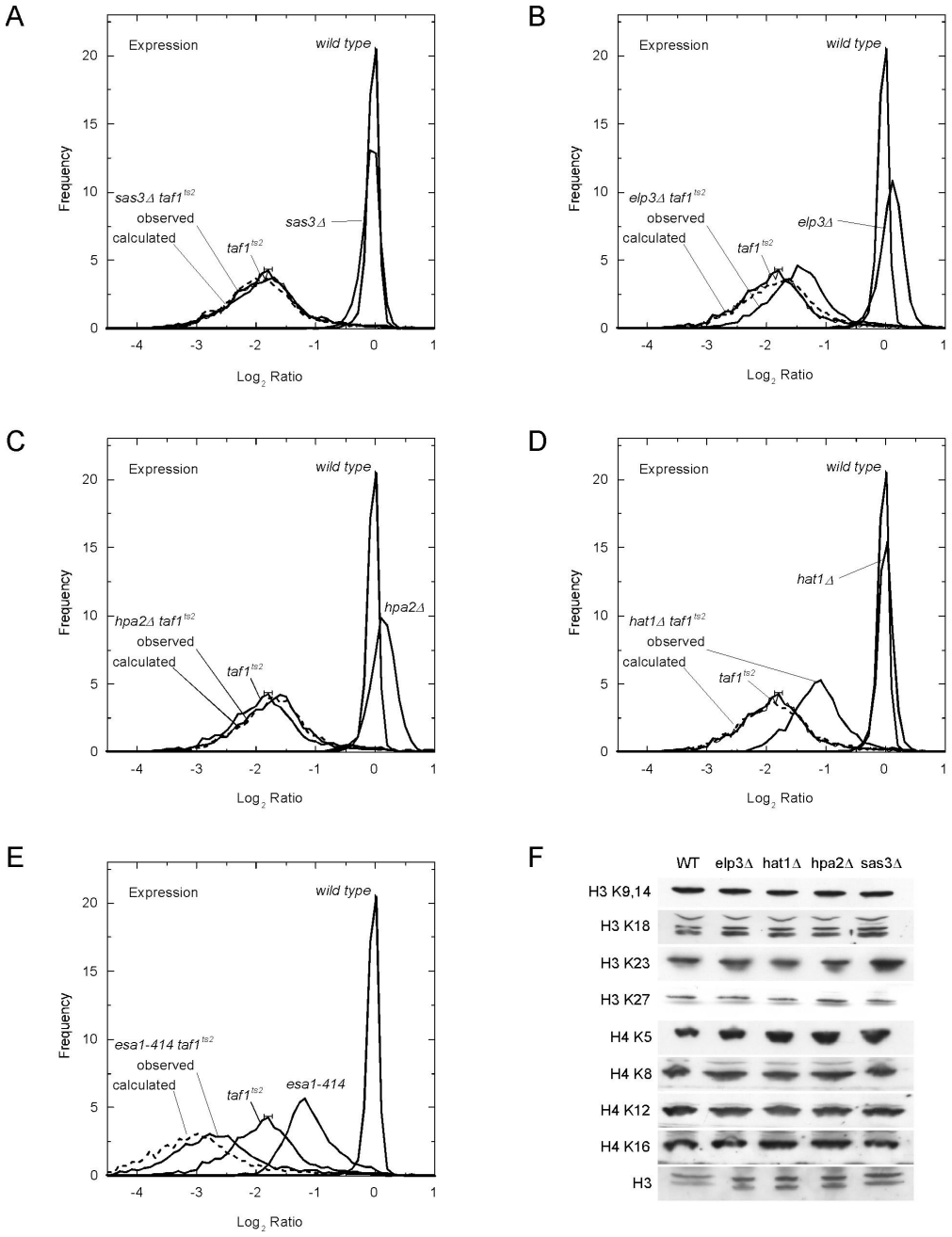

Figure 2.6**: Genome-wide analysis of the contribution of various HATs to gene expression levels**

(A to E) Changes in genome-wide mRNA expression levels were determined in mutant HAT strains in a wild-type or *taf1*^{ts2} background, as described in the legend of Figure 2.5. Predicted values (dashed line) were calculated for the double mutants by adding the $\log_2$ ratios of the single mutants. (F) Changes in bulk H3 or H4 acetylation levels at specific lysine residues in each of the mutant HAT strains were assayed by immunoblotting, as described in the legend of Figure 2.1.

With the exception of Gcn5 (Figure **2.6**A) and Esa1 (Figure **2.6**E), no other HAT by itself made a broad or substantially unique contribution to gene expression, as evidenced by the largely identical distribution of the expression profiles in the HAT deletion mutants with the wild type profile (Figure **2.6**A-D). These HATs might be functionally redundant with one or more other HATs, have physiological roles that are unrelated to transcription, or target only a few genes. In fact, <0.3% of the yeast genome is uniquely dependent (2-fold or more) upon any one of these HATs (Elp3, Hat1, Hpa2, or Sas3) for expression under the growth conditions studied. Those that are moderately affected show no particularly distinguishing property, except having a tendency to be stress-induced (Table **2.1**, Table **2.2**). Interestingly, genes that displayed modest positive regulation by Sas3 tended to be Ntd80-regulated sporulation-induced genes, which are lowly expressed under our growth conditions. Sas3 has not been implicated in the sporulation response, and the growth conditions in this study did not involve sporulation. Therefore, it is conceivable that genes might display greater dependency on Sas3 under conditions of spore formation.

Table **2.1**: Overlap between Bottom 10% of published expression data sets and Bottom 10% of mutant HAT expression data sets

Bottom 10% of Expression Data Set	Bottom 10% of HAT Data Set														
	WT			epl3Δ			hat1Δ			hpa2Δ			sas3Δ		
Characteristic	**% overlap**	**Value**	**P-value**	**% overlap**	**Value**	**P-value**	**% overlap**	**Value**	**P-value**	**% overlap**	**Value**	**P-value**	**% overlap**	**Value**	**P-value**
Sporulation															
0 hr Sporulation	1%	53	2.1E-01	1%	55	8.4E-01	1%	50	8.9E-01	1%	57	2.7E-01	1%	59	4.0E-01
0.5 hr Sporulation	1%	80	4.6E-02	1%	71	2.5E-02	2%	71	2.9E-03	1%	65	3.6E-02	2%	85	2.1E-05
11.5 hr Sporulation	1%	83	6.9E-03	1%	71	1.1E-02	1%	53	4.9E-01	1%	50	8.3E-01	2%	88	5.5E-07
2 hr Sporulation	1%	54	1.7E-01	1%	55	9.5E-01	1%	57	3.8E-01	1%	60	2.1E-01	1%	68	7.3E-02
5 hr Sporulation	1%	71	2.4E-01	1%	58	4.6E-01	1%	53	4.9E-01	1%	49	9.4E-01	1%	70	1.2E-02
7 hr Sporulation	1%	75	1.3E-01	1%	73	8.1E-03	1%	55	4.1E-01	1%	48	8.4E-01	2%	77	9.7E-04
9 hr Sporulation	1%	79	3.1E-02	1%	75	2.2E-03	1%	55	3.4E-01	1%	45	6.0E-01	2%	81	6.3E-05
ntd80 del [Sporulation]	1%	53	2.3E-01	1%	60	3.4E-01	1%	57	2.3E-01	1%	55	4.4E-01	1%	68	4.2E-02
ntd80 del [Sporulation]	1%	65	8.3E-01	1%	59	4.8E-01	1%	55	4.2E-01	1%	50	8.7E-01	1%	67	4.4E-02
Stress Response															
Amino acid starvation [Rapamycin 15-30']	1%	55	5.6E-01	1%	59	1.7E-01	1%	43	6.2E-01	1%	51	4.6E-01	1%	43	2.9E-01
Amino acid starvation [Rapamycin 90-120']	0%	17	2.2E-04	1%	27	1.9E-01	1%	17	2.1E-03	1%	21	6.3E-02	1%	21	1.3E-02
Nutrient deprivation [Rapamycin 0']	1%	42	4.8E-01	1%	39	8.9E-01	1%	35	7.6E-01	1%	50	7.9E-02	1%	40	9.9E-01
Nutrient deprivation [Rapamycin 15-30']	1%	26	2.6E-03	1%	35	6.3E-01	1%	30	2.8E-01	0%	17	4.4E-04	1%	35	4.4E-01
UPR (Unfolded Protein Response) [Tunicamycin]	1%	80	3.9E-02	1%	66	9.8E-02	1%	50	9.3E-01	1%	43	2.7E-01	1%	64	1.8E-01
PIC factors															
bdf1 del	1%	55	1.9E-02	1%	55	2.8E-01	1%	60	8.0E-01	1%	31	3.2E-04	2%	82	6.1E-03
bdf1 YF186/YF353	1%	1	4.1E-01	1%	2	7.3E-01	1%	1	7.1E-01	1%	2	9.4E-01	4%	5	2.4E-02
taf10-ts1	0%	7	3.2E-01	1%	8	7.2E-01	1%	9	9.6E-01	1%	11	5.3E-01	1%	18	3.5E-05
taf12-23ts	1%	38	4.0E-01	1%	32	5.0E-01	1%	42	4.3E-02	1%	25	1.9E-01	1%	43	7.9E-03
taf1-ts2	0%	16	7.5E-02	1%	18	5.7E-01	1%	29	3.1E-01	1%	21	3.6E-01	0%	15	2.7E-01
taf1-ts2 gcn5 del	1%	22	4.8E-02	1%	28	9.4E-01	1%	36	2.3E-01	1%	27	2.8E-01	1%	21	3.7E-01
taf1-ts2 spt3 del	1%	67	1.4E-01	1%	56	2.4E-01	1%	46	9.1E-01	1%	30	2.0E-02	1%	55	3.7E-01
taf2-1	1%	21	6.5E-01	1%	23	5.0E-01	1%	23	9.5E-01	1%	22	5.8E-01	1%	19	9.0E-01
taf5 ts9-12	1%	24	8.6E-01	1%	17	4.0E-01	1%	40	9.4E-05	1%	22	5.9E-01	1%	36	2.3E-04
taf6-19	0%	15	2.9E-02	1%	22	9.7E-01	1%	24	8.5E-01	1%	19	8.1E-02	1%	18	3.9E-01
taf9-ts2	0%	3	3.0E-03	0%	6	4.9E-02	0%	5	1.4E-02	0%	5	5.4E-03	0%	4	3.1E-02
kin28-ts3 [TFIIH]	1%	19	7.7E-01	0%	12	1.9E-01	1%	17	6.0E-01	0%	7	1.7E-03	0%	11	1.5E-01
tfa1-21 [TFIIE]	1%	31	1.7E-01	1%	40	2.9E-01	1%	46	9.7E-02	1%	29	1.7E-01	1%	37	6.5E-01
rpb1-1 [Pol II - calc]	0%	16	1.4E-05	0%	19	4.3E-03	1%	26	1.1E-01	1%	24	3.8E-02	0%	20	7.4E-03
Chromatin/Histones															
GAL-HH4	0%	18	2.4E-03	1%	32	9.6E-01	0%	17	4.4E-03	0%	15	8.1E-04	0%	12	2.1E-04
H3?(1-28)	1%	38	1.6E-01	1%	53	8.1E-02	1%	48	2.5E-01	1%	29	5.5E-02	1%	40	9.6E-01
H4?2-26	1%	37	2.4E-03	1%	37	4.6E-02	1%	36	1.2E-01	0%	16	5.8E-06	1%	40	1.9E-01
htz1?	1%	71	2.9E-01	1%	58	5.1E-01	2%	78	2.6E-05	0%	19	1.0E-05	1%	68	4.4E-02
htz1? hmr?	1%	74	1.5E-01	1%	64	1.4E-01	2%	74	2.8E-04	0%	20	2.0E-05	1%	64	1.4E-01
htz1?sir2?	1%	74	1.5E-01	1%	58	5.1E-01	2%	74	2.8E-04	1%	44	3.9E-01	2%	78	7.0E-04
HATs															
gcn5 del	1%	45	1.5E-01	1%	52	5.6E-01	1%	45	9.8E-01	1%	35	1.2E-01	1%	45	7.1E-01
gcn5(KQL)	1%	47	4.2E-01	1%	50	5.9E-01	1%	36	2.5E-01	0%	21	8.9E-04	1%	48	6.4E-01
Methyltransferases															
set1 del	1%	46	4.6E-02	1%	40	7.9E-02	1%	41	3.2E-01	1%	47	7.3E-01	1%	51	8.6E-01
HDACs															
hda1 del 1(Rosetta)	1%	75	1.4E-03	2%	73	4.5E-06	1%	58	2.5E-02	1%	59	2.2E-03	2%	72	1.7E-04
hos3 del	1%	52	8.5E-02	1%	51	2.4E-02	1%	38	8.6E-01	1%	58	7.5E-04	1%	42	2.6E-01
rpd3 del 2	1%	76	5.2E-02	1%	74	2.3E-03	1%	70	9.6E-04	1%	38	1.2E-01	2%	85	3.1E-06
ATP-dependent remodelers															
rsc30 del ave	1%	85	3.0E-03	1%	68	1.8E-02	1%	66	8.8E-03	2%	73	3.2E-04	1%	53	9.3E-01
rsc3-2 ave	1%	54	3.2E-01	1%	39	8.9E-02	1%	45	6.7E-01	1%	57	2.0E-01	1%	55	7.1E-01
snf2 del MM	2%	92	7.9E-05	1%	73	3.5E-03	1%	61	5.8E-02	1%	69	4.5E-03	1%	57	4.6E-01
swi1 del MM	2%	91	1.6E-04	1%	74	2.5E-03	1%	65	1.3E-02	1%	62	7.1E-02	1%	59	3.3E-01
swi2(K798A)	1%	37	7.2E-01	1%	34	5.5E-01	1%	48	6.1E-03	1%	35	8.2E-01	1%	51	1.8E-04

Table **2.1** (Cont.)

Bottom 10% of Expression Data Set				Bottom 10% of HAT Data Set													
	WT			epl3Δ			hat1Δ			hpa2Δ			sas3Δ				
Characteristic	% overlap	Value	P-value	% overlap	Value	P-value	% overlap	Value	P-value	% overlap	Value	P-value	% overlap	Value	P-value		
Chromatin Repressors																	
sir2 ?	1%	50	1.1E-01	1%	35	1.2E-02	1%	43	4.1E-01	1%	44	3.9E-01	1%	71	1.5E-02		
sir2 del	1%	38	2.5E-01	1%	35	1.8E-01	1%	40	9.6E-02	1%	36	4.3E-01	1%	50	1.6E-05		
sir3 del	1%	47	1.5E-02	1%	52	6.2E-06	1%	45	1.4E-02	1%	45	2.6E-02	1%	44	3.1E-03		
sir4 del	1%	39	8.6E-02	1%	32	4.2E-01	1%	37	2.8E-01	1%	44	1.8E-02	1%	38	3.1E-02		
tup1 del	1%	51	1.3E-01	1%	35	1.1E-02	1%	49	9.7E-01	1%	63	5.3E-02	1%	61	2.8E-01		
Mediator																	
med2 del [Mediator]	1%	72	7.5E-02	1%	60	1.5E-01	1%	50	5.0E-01	1%	67	2.0E-03	1%	37	8.1E-02		
med2 del [Mediator]	1%	65	4.9E-01	1%	49	7.8E-01	1%	50	5.6E-01	1%	43	7.3E-01	1%	40	1.6E-01		
med6-ts [Mediator}	1%	34	8.6E-01	1%	36	2.3E-01	1%	48	5.6E-03	1%	38	3.5E-01	1%	34	2.8E-01		
srb10-3 [Mediator}	1%	29	5.2E-01	1%	37	2.4E-01	1%	38	3.7E-01	0%	18	1.1E-02	1%	27	8.2E-01		
srb4-138 ts [Mediator]	0%	13	7.6E-02	1%	20	9.3E-01	1%	23	6.6E-01	0%	11	1.2E-02	0%	14	2.4E-01		
srb5-del1 [Mediator}	1%	25	7.8E-03	1%	21	8.1E-02	1%	22	5.2E-02	1%	30	3.4E-03	1%	25	1.9E-03		
Miscellaneous																	
bur6-1	1%	30	1.9E-02	0%	16	7.7E-05	1%	39	5.9E-01	1%	32	4.9E-02	1%	46	2.4E-01		
Crt1 [Rpressor]	1%	13	1.7E-01	1%	14	7.8E-01	1%	13	9.5E-01	1%	11	2.6E-01	1%	14	6.0E-01		
fcp1-1 [GTF - Fcp phosphatase]	1%	35	5.7E-01	1%	20	1.1E-01	1%	47	1.5E-03	1%	34	8.8E-01	1%	28	9.5E-01		
hmr?	1%	54	2.7E-01	1%	43	1.6E-01	1%	43	4.1E-01	1%	42	2.6E-01	1%	44	2.0E-01		
mot1-1	1%	22	6.4E-01	1%	17	6.0E-01	1%	18	6.8E-01	1%	35	8.4E-03	1%	34	8.9E-04		
Polyribosome membrane fraction / cytosolic fraction	1%	56	5.2E-01	1%	60	2.3E-01	1%	48	9.9E-01	1%	61	1.0E-01	1%	48	5.8E-01		
rap1-17	1%	34	2.0E-01	1%	26	6.8E-01	1%	41	5.5E-03	1%	47	3.5E-04	1%	40	7.5E-04		
rvb2/tih2-160 [INO80 complex]	1%	39	2.2E-01	1%	39	9.8E-01	1%	58	8.1E-03	1%	46	3.5E-02	1%	50	1.1E-01		
sap30 del	1%	54	1.4E-02	1%	44	1.4E-01	1%	44	1.7E-01	1%	43	2.6E-01	1%	54	1.3E-04		
sin3 del 1(Affy)	1%	41	2.0E-01	1%	38	1.4E-01	1%	34	6.2E-01	1%	34	7.8E-01	1%	30	7.2E-01		
Ume6 [Repressor]	1%	47	3.0E-01	1%	43	2.9E-01	1%	35	8.3E-01	1%	46	1.4E-01	1%	45	7.2E-02		
Yap1 [Activator]	1%	89	4.3E-03	2%	77	4.9E-03	1%	56	5.3E-01	1%	52	9.7E-01	1%	55	9.5E-01		

Table **2.2**: Overlap between Bottom 10% of published expression data sets and Top 10% of mutant HAT expression data sets

Top 10% of Expression Data Set	Bottom 10% of HAT Data Set														
	WT			epl3Δ			hat1Δ			hpa2Δ			sas3Δ		
Characteristic	% overlap	Value	P-value	% overlap	Value	P-value	% overlap	Value	P-value	% overlap	Value	P-value	% overlap	Value	P-value
Sporulation															
0 hr Sporulation	1%	43	2.1E-02	1%	49	6.9E-01	1%	33	3.4E-02	1%	24	5.5E-04	0%	25	2.2E-04
0.5 hr Sporulation	1%	84	1.4E-03	1%	53	7.4E-01	1%	53	3.3E-01	1%	34	6.3E-02	1%	53	6.8E-01
11.5 hr Sporulation	1%	75	7.5E-02	1%	59	3.3E-01	1%	56	2.3E-01	1%	63	2.9E-02	1%	54	7.2E-01
2 hr Sporulation	1%	70	2.1E-01	1%	51	9.6E-01	1%	49	7.8E-01	1%	41	3.6E-01	1%	29	2.1E-03
5 hr Sporulation	1%	67	4.2E-01	1%	54	7.5E-01	1%	46	8.4E-01	1%	54	3.5E-01	1%	40	1.2E-01
7 hr Sporulation	1%	68	3.7E-01	1%	54	7.8E-01	1%	46	8.1E-01	1%	62	4.0E-02	1%	39	8.3E-02
9 hr Sporulation	1%	68	3.6E-01	1%	60	2.6E-01	1%	52	5.2E-01	1%	67	5.2E-03	1%	44	3.1E-01
Sporulation - early [ntd80]	1%	46	6.4E-02	1%	51	9.6E-01	1%	63	2.0E-02	1%	43	5.3E-01	1%	46	5.0E-01
Sporulation - late [ntd80]	1%	71	2.0E-01	1%	58	3.9E-01	1%	51	6.2E-01	1%	41	3.2E-01	1%	30	2.8E-03
Sporulation - mid [ntd80]	1%	59	8.2E-01	1%	39	7.5E-02	1%	37	1.3E-01	1%	24	5.8E-04	0%	24	1.4E-04
StressResponse															
Amino acid starvation [Rapamycin 15-30']	1%	62	6.8E-01	1%	50	8.7E-01	1%	48	7.5E-01	2%	81	1.3E-07	1%	69	6.6E-03
Amino acid starvation [Rapamycin 90-120']	1%	48	1.9E-01	1%	30	4.8E-01	1%	45	7.6E-02	1%	51	2.9E-04	1%	54	1.6E-03
Nutrient deprivation [Rapamycin 0']	1%	64	7.2E-03	1%	47	1.1E-01	1%	48	4.6E-02	1%	42	5.5E-01	1%	56	7.3E-03
Nutrient deprivation [Rapamycin 15-30']	1%	70	3.5E-04	1%	42	4.7E-01	1%	37	8.9E-01	2%	83	4.7E-13	1%	58	2.9E-03
UPR (Unfolded Protein Response) 2Kbeta overexp	1%	54	4.9E-01	1%	44	3.7E-01	1%	68	1.2E-03	1%	72	3.4E-04	1%	44	3.6E-01
UPR (Unfolded Protein Response) Tunicamycin	1%	61	9.9E-01	1%	52	9.7E-01	1%	66	6.7E-03	1%	61	8.0E-02	1%	57	4.8E-01
PICfactors															
bdf1 YF186/YF353	2%	4	1.9E-01	0%	0	2.2E-01	1%	1	7.5E-01	0%	0	1.8E-01	0%	0	1.8E-01
bdf2 del	1%	58	9.0E-01	1%	46	5.8E-01	1%	59	4.5E-02	1%	59	4.8E-02	1%	50	7.5E-01
taf12-23ts	1%	55	9.8E-05	1%	50	3.3E-05	1%	39	1.2E-01	2%	61	2.9E-07	1%	33	4.0E-01
taf1-ts2 spt3 del	1%	61	4.5E-01	1%	33	3.5E-02	1%	37	2.4E-01	1%	63	7.7E-03	1%	55	3.3E-01
taf2-1	1%	44	1.1E-05	1%	32	6.0E-03	1%	27	3.4E-01	1%	35	3.3E-02	1%	33	2.0E-03
taf9-ts2	1%	27	4.9E-04	1%	25	8.4E-04	1%	21	6.5E-02	2%	38	3.4E-08	1%	18	3.8E-02
tfa1-21 [TFIIE]	1%	57	4.1E-03	1%	44	6.9E-02	1%	36	9.6E-01	2%	73	2.4E-09	1%	50	5.9E-03
Chromatin/Histones															
htz1?	1%	88	9.7E-04	1%	76	1.3E-03	1%	50	8.1E-01	2%	98	4.4E-12	1%	61	2.6E-01
htz1? hmr?	1%	91	2.3E-04	1%	68	3.5E-02	1%	51	7.0E-01	2%	110	5.7E-18	1%	67	5.0E-02
HDACs															
hda1 del 1(Affy)	1%	38	8.2E-01	1%	28	4.4E-01	1%	29	4.0E-01	1%	28	2.9E-01	1%	25	3.1E-01
hos3 del	1%	28	5.2E-02	1%	30	2.7E-01	1%	22	1.7E-02	1%	25	5.1E-02	1%	27	1.9E-01
rpd3 del 2	2%	92	3.6E-05	1%	56	5.2E-01	1%	61	3.7E-02	2%	93	7.4E-11	1%	51	1.0E+00
sap30 del [Rpd3 complex]	1%	39	9.1E-01	1%	33	7.6E-01	1%	30	3.5E-01	1%	49	2.7E-02	1%	25	2.1E-01
ATP-dependentremodelers															
rsc3-2 ave	1%	80	1.6E-02	1%	62	1.1E-01	1%	64	1.6E-02	2%	75	7.0E-05	1%	44	2.8E-01
snf2 del MM	1%	79	1.5E-02	1%	61	1.6E-01	1%	51	5.7E-01	1%	72	6.1E-04	2%	80	3.9E-05
swi1 del MM	1%	77	2.8E-02	1%	52	8.9E-01	1%	51	5.3E-01	1%	68	4.1E-03	2%	78	1.2E-04
swi2(K798A)	1%	52	2.8E-03	1%	45	7.9E-03	1%	46	1.4E-02	2%	60	3.4E-06	1%	35	3.7E-01
ChromatinRepressors															
sir2 ?	2%	93	8.2E-05	2%	80	1.6E-04	1%	61	6.6E-02	1%	70	3.4E-03	1%	69	2.6E-02
sir2 del	1%	32	9.0E-01	1%	26	7.4E-01	1%	36	3.2E-01	1%	37	3.1E-01	1%	33	2.7E-01
sir3 del	1%	37	4.5E-01	1%	26	7.3E-01	1%	34	5.8E-01	1%	43	5.1E-02	1%	33	3.4E-01
sir4 del	1%	31	2.1E-01	1%	29	7.7E-01	0%	19	5.4E-03	1%	37	8.0E-01	1%	27	2.9E-01
sir4 del	1%	42	2.0E-02	1%	33	7.6E-01	1%	49	9.1E-04	1%	34	5.4E-01	1%	44	7.1E-04
Mediator															
med2 del [Mediator]	1%	86	1.2E-04	1%	66	1.3E-02	1%	45	8.8E-01	1%	42	7.5E-01	1%	57	1.7E-01
med6-ts {Mediator]	1%	36	5.6E-01	1%	36	2.0E-01	1%	29	6.0E-01	1%	32	9.5E-01	1%	25	5.7E-01
srb4-138 ts [Mediator]	1%	26	2.7E-01	1%	25	2.0E-01	1%	17	4.0E-01	1%	41	1.3E-04	1%	23	3.5E-01
srb5-del1 [Mediator]	0%	9	1.4E-01	0%	8	9.5E-02	1%	11	3.6E-01	1%	14	3.9E-01	0%	4	9.6E-03

Table **2.2** (Cont.)

Top 10% of Expression Data Set	Bottom 10% of HAT Data Set														
	WT			epl3Δ			hat1Δ			hpa2Δ			sas3Δ		
Characteristic	% overlap	Value	P-value	% overlap	Value	P-value	% overlap	Value	P-value	% overlap	Value	P-value	% overlap	Value	P-value
SAGA															
spt3 del	1%	40	2.0E-02	1%	28	2.4E-03	1%	32	4.1E-02	1%	59	5.1E-02	1%	42	2.8E-01
spt3(E240K)	1%	38	8.1E-02	1%	26	3.7E-03	1%	22	1.9E-03	1%	64	1.5E-03	1%	37	2.1E-01
Miscellaneous															
Crt1 repressor	1%	14	2.8E-01	1%	16	3.7E-01	1%	7	1.1E-01	1%	12	4.1E-01	1%	11	7.7E-01
fcp1-1 [GTF - Fcp phosphatase]	1%	50	9.8E-04	1%	40	2.5E-02	1%	31	7.7E-01	1%	55	1.0E-04	1%	37	6.7E-02
hmr?	1%	77	5.8E-02	1%	68	3.5E-02	1%	65	1.6E-02	1%	66	1.9E-02	1%	74	3.4E-03
mot1-1	1%	36	2.3E-04	1%	27	7.0E-02	1%	28	5.8E-02	2%	44	4.1E-06	1%	16	4.6E-01
Polyribosome membrane fraction / cytosolic fraction	1%	61	8.8E-01	1%	52	8.4E-01	1%	47	9.9E-01	1%	51	7.3E-01	1%	53	7.8E-01
Rap1	1%	35	1.2E-01	1%	35	2.0E-02	1%	23	5.0E-01	1%	27	8.7E-01	1%	19	3.6E-01
rvb2/tih2-160 [INO80 complex]	1%	72	6.5E-05	1%	46	1.6E-01	1%	54	1.8E-02	1%	43	5.7E-02	1%	43	4.3E-01
sin3 del 1(Rosetta)	1%	69	1.7E-02	1%	55	5.7E-02	1%	50	2.8E-01	1%	63	1.4E-04	1%	44	7.5E-01
Ume6 repressor	1%	44	5.3E-01	1%	40	5.3E-01	1%	30	3.2E-01	1%	39	6.9E-01	1%	32	7.1E-01
Yap1 activator	1%	59	9.4E-01	1%	54	6.3E-01	1%	35	9.0E-02	1%	55	2.2E-01	1%	59	2.1E-01

Since loss of Elp3, Hat1, Hpa2, or Sas3 had little impact on the expression of most genes, we next examined whether this was reflected in bulk histone acetylation states. Bulk acetylation levels were examined at H3 K9, 14, 18, 23 and 27 and at H4 K5, 8, 12, and 16 (Figure **2.6**F). In no case did loss of these HATs affect bulk acetylation at specific lysine residues. The lack of effect could have any number of sources including: 1) having highly selective gene targets, 2) having lysine specificities other than those tested, 3) making transient contributions, and 4) being highly redundant with other HATs.

2.3.4 Genome-wide linkage between Esa1, Bdf1, and TAF1

In yeast, TFIID interacts with the bromodomain protein Bdf1 and the two function at many of the same genes (Matangkasombut et al., 2000; Huisinga and Pugh, 2004). In higher eukaryotes, *BDF1* is fused to *TAF1*, which physically links Bdf1 function to TFIID (Matangkasombut et al., 2000). Bdf1 interacts with acetylated histone H4 tails (Jacobson et al., 2000; Ladurner et al., 2003; Matangkasombut and Buratowski, 2003), and the presence of H4 acetylation is correlated with TFIID function (Huisinga and Pugh, 2004). Esa1 is part of the NuA4 (promoter-specific acetylation) and Piccolo (global acetylation) complexes that are thought to be responsible for the majority of histone H4 tail acetylation (Allard et al., 1999; Boudreault et al., 2003). Together these findings suggest an intimate relationship between TFIID, Bdf1, Esa1, and H4 acetylation. Given the putative HAT function in TAF1, we explored the relationship between TAF1 and Esa1 by examining whether TAF1 and Esa1 both acetylated histone H4 *in vivo*. As shown by the immunoblot in Figure **2.7**, loss of TAF1 had no effect on bulk H4

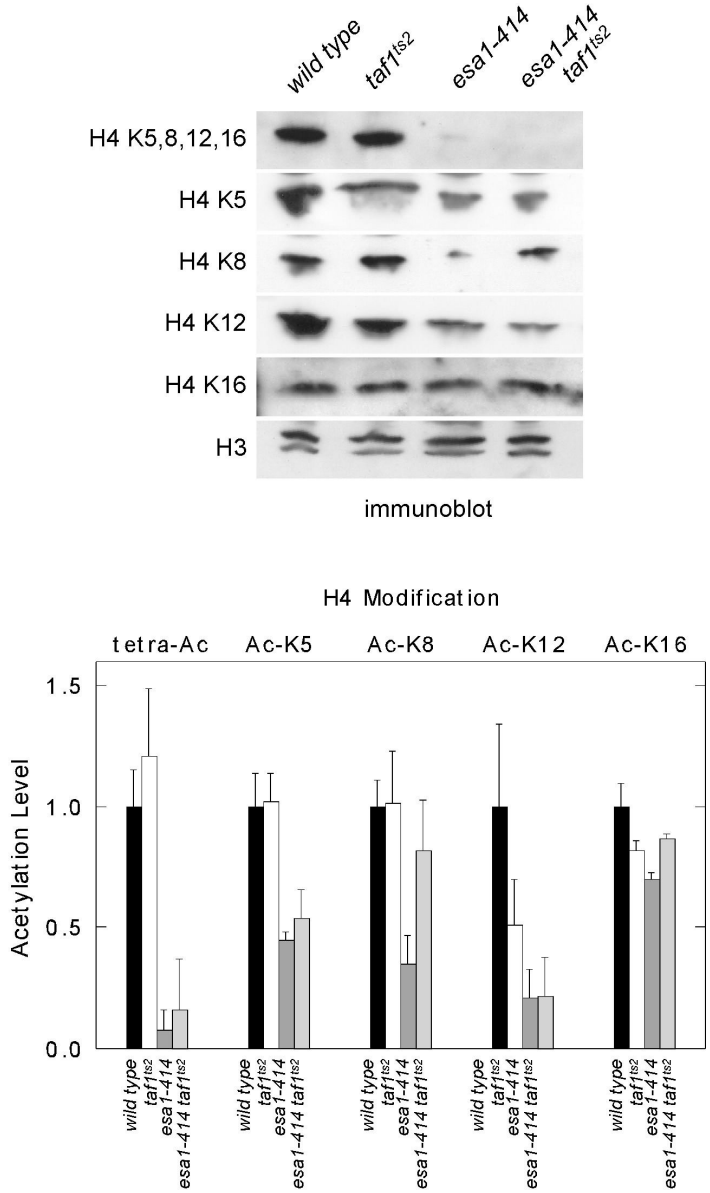

Figure 2.7**: Esa1, but not TAF1, is important for bulk histone H4 acetylation levels**

Wild-type, *taf1*^{ts2}, *esa1-414*, and *taf1*^{ts2} *esa1-414* strains ere grown and shifted to the nonpermissive temperature as described in the legend of Figure 2.1. Blots were probed with the indicated antibody. Quantitation of three independent replicates is shown in the bar graph.

acetylation at K5, K8, K12, and K16, indicating that TAF1 is not a major H4 HAT. In contrast, loss of Esa1 resulted in decreased acetylation at K5, K8, and K12, but not K16, as previously shown (Clarke et al., 1999; Loewith et al., 2000). The lack of effect at K16

is consistent with Sas2, rather than Esa1, acetylation of K16 (Sutton et al., 2003). Changes in the *taf1^{ts2}esa1-414* double mutant were no different than the effects seen from the *esa1-414* mutant alone, ruling against the idea that TAF1 and Esa1 are redundant HATs at H4. Taken together, these findings demonstrate that Esa1 is the main HAT for bulk H4 acetylation at K5, K8, and K12, and further suggest that if TFIID is to be recruited to promoters via Bdf1 interactions with acetylated histone H4 tails, it is likely to be Esa1 rather than TAF1 that provides the H4 tail acetylation.

The close working relationship between TFIID and Esa1 was examined in more detail by cluster analysis of genome-wide expression profiles (Eisen et al., 1998). Genes whose expression changed by more than 2-fold in the *taf1^{ts2}*, *esa1-414*, or the double mutant were clustered by K-means into eight groups (4754 total genes). Average values for the *taf1^{ts2}* and *esa1-414* data sets were plotted against each other in Figure **2.8** . As expected, most values were negative (in the lower left quadrant) reflecting losses in gene expression when either TAF1 or Esa1 were inactivated. A finer trend was observed within the entire data set. Gene clusters that depended more on TAF1 tended to depend less on Esa1, as evidence by the inverse relationship on the left side of the graph. The rise on the right side of the graph reflects a trend toward Esa1-independence as genes become more TAF1-independent. These genes tend to be dominated by the alternative SAGA assembly pathway (Table **2.3**).

Table **2.3**: Esa1 preferentially regulates TFIID-dominated genes

Cluster #	% of genome	% SAGA-dominated*	P-value
1	7	7	2.E-01
2	13	3	4.E-11
3	11	5	4.E-04
4	21	2	9.E-22
5	13	6	3.E-03
6	9	13	9.E-04
7	2	34	5.E-25
8	0.3	95	4.E-37

*Genome-wide = 9% SAGA-dominated

Previous studies have suggested a model for TFIID recruitment where NuA4 hyper-acetylates H4, allowing TFIID to dock at promoters via Bdf1 (illustrated in Figure **2.8**) (Huisinga and Pugh, 2004; Martinez-Campa et al., 2004). We wondered whether the trend observed on the left side of the graph in Figure **2.8**A could be reconciled in terms of this model. If the acetylation step catalyzed by Esa1 is at least partially rate-limiting for the expression of certain genes (Figure **2.8**B), then transcription will be particularly dependent upon Esa1 and less dependent upon TFIID. At other genes, acetylation may not be limiting. Transcription might instead be limited in part by the recruitment of TFIID. These genes would be more dependent upon TAF1 than Esa1. Nevertheless, all genes regulated by Esa1 and TAF1 would be highly dependent upon both.

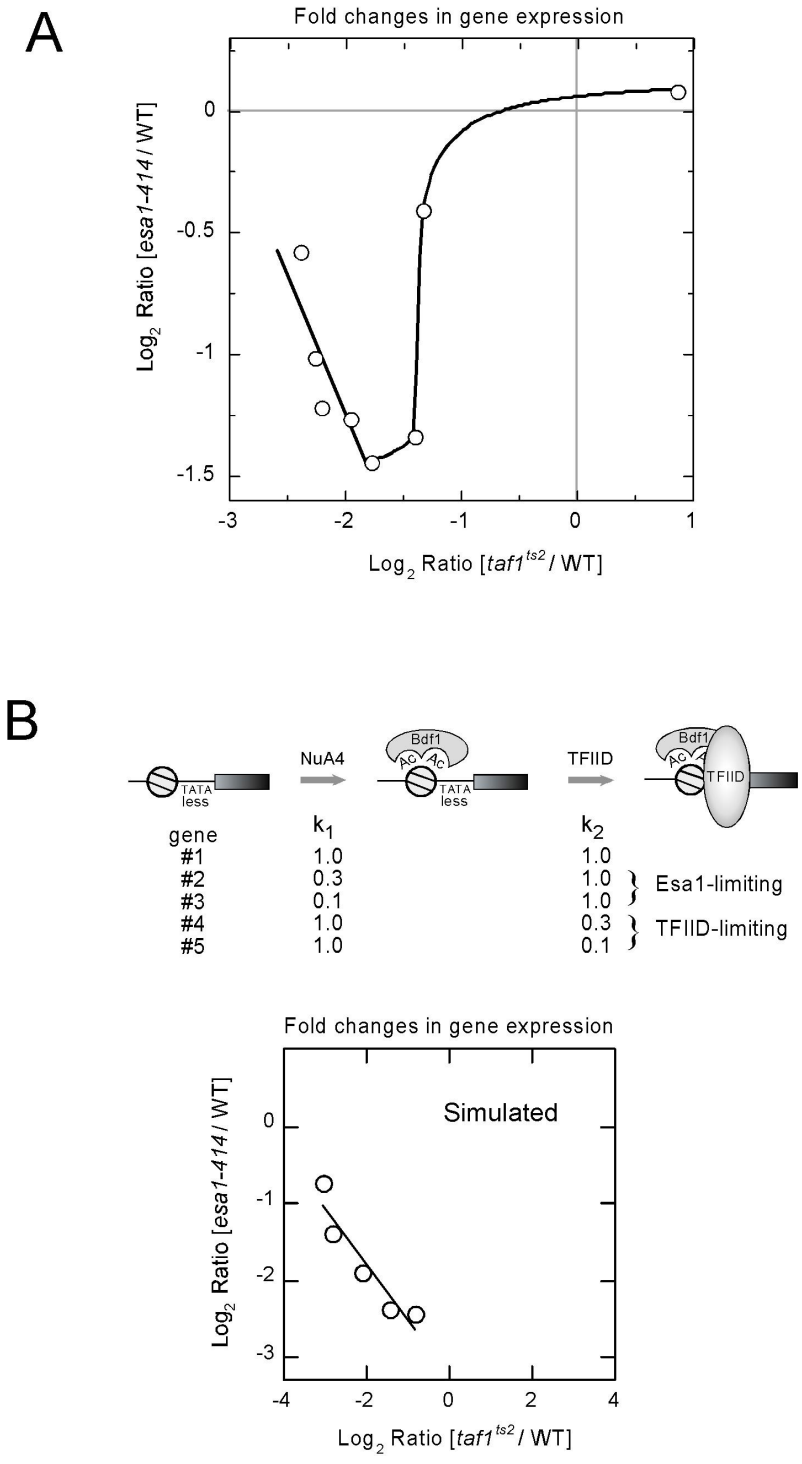

Figure 2.8**: Esa1 and TAF1 are functionally linked**

(A) Data from Figure 2.6E were filtered for a two-fold cutoff and clustered into eight groups by K means. Average values for each group in the *taf1*^{ts2} and *esa1-414* data sets

(Figure 2.8 legend, cont.) are plotted against each other. (B) A general mechanism describing the potential interrelationship between Esa1 (NuA4) and TAF1 (TFIID) in gene activation. According to the model, recruitment of NuA4 to TATA-less promoters results in H4 tail acetylation, allowing Bdf1 to dock and escort in TFIID. Two potentially rate-limiting steps are shown. Below these steps are arbitrary rate constants in which either NuA4 or TFIID activity, as indicated, is limiting for transcription at five hypothetical genes. The graph represented simulated $\log_2$ changes in gene expression (RNA output) in a hypothetical *esa1-414* mutant versus a hypothetical *taf1*^{ts2} mutant for each of the five genes. See Materials and Methods for a detailed description of the simulation using KinTekSim software.

To examine the plausibility of a model where genes might be more rate-limited by Esa1 and others more rate-limited by TAF1, we performed a computational simulation of the reaction scheme illustrated in Figure **2.8**B using KinTekSim software. In this simplified scheme, a chromatin-containing promoter becomes acetylated by Esa1 (NuA4), which is governed by a "rate constant" k_1. Next, TAF1 (TFIID) binds, resulting in transcription (i.e., RNA output) that is governed by "rate constant" k_2. Arbitrary values for both rate constants were established for five different hypothetical genes, representing two situations where Esa1 is limiting ($k_1 < k_2$), two where TAF1 is limiting ($k_2 < k_1$), and one where both are equally limiting ($k_1 = k_2$). RNA output from the simulator was recorded. Next, RNA output was measured in a hypothetical *esa1-414* mutant, which was computationally modeled by decreasing k_1 by ten fold, reflecting an elimination of 90% of Esa1 activity. The same was done with k_2 to simulate the *taf1*^{ts2} mutant, reflecting 90% loss of TAF1 activity. Figure **2.8**B plots the $\log_2$ fold change (mutant / WT) in simulated RNA output for each of the five hypothetical genes. The similarity of the response pattern in Figure **2.8**B to the left side of the graph in Fig. 6A, indicates that the simulated model represents a valid, albeit speculative, interpretation of

the data. This interpretation is consistent with how Esa1 (NuA4) and TAF1 (TFIID) are thought to work, and conceptualizes how different genes may be rate-limited by distinct steps in a common assembly pathway.

Previously we have shown by chIP-chip that Bdf1 largely tracks with TAF1 throughout the genome, which is consistent with their functional connection via TFIID (Zanton and Pugh, 2004). However, we noted that Bdf1 comes and goes in many places in a way that is unlinked to TAF1. This raises the question as to whether the presence of H4 acetylation is more closely linked to Bdf1 binding or to TAF1 binding. To address this, genome-wide promoter occupancy data for TAP-tagged Bdf1 and TAF1 were compared to genome-wide H4 acetylation occupancy data (Bernstein et al., 2002; Zanton and Pugh, 2004), using a sliding window analysis. As shown in Figure **2.9**, panels A and B, H4 acetylation was more strongly linked to Bdf1 occupancy than to TAF1 occupancy. But TAF1 nevertheless displayed a strong linkage with Bdf1 (Figure **2.9**C). A chIP-chip experiment in a strain lacking a TAP tag showed no correlation (Figure **2.9**D). The strong linkage of Bdf1 to H4 acetylation and TAF1, but weaker linkage between H4 acetylation and TAF1, support the model in Figure **2.8**B, where acetylated H4 tails primarily bind Bdf1 which generally (but not always) binds TFIID.

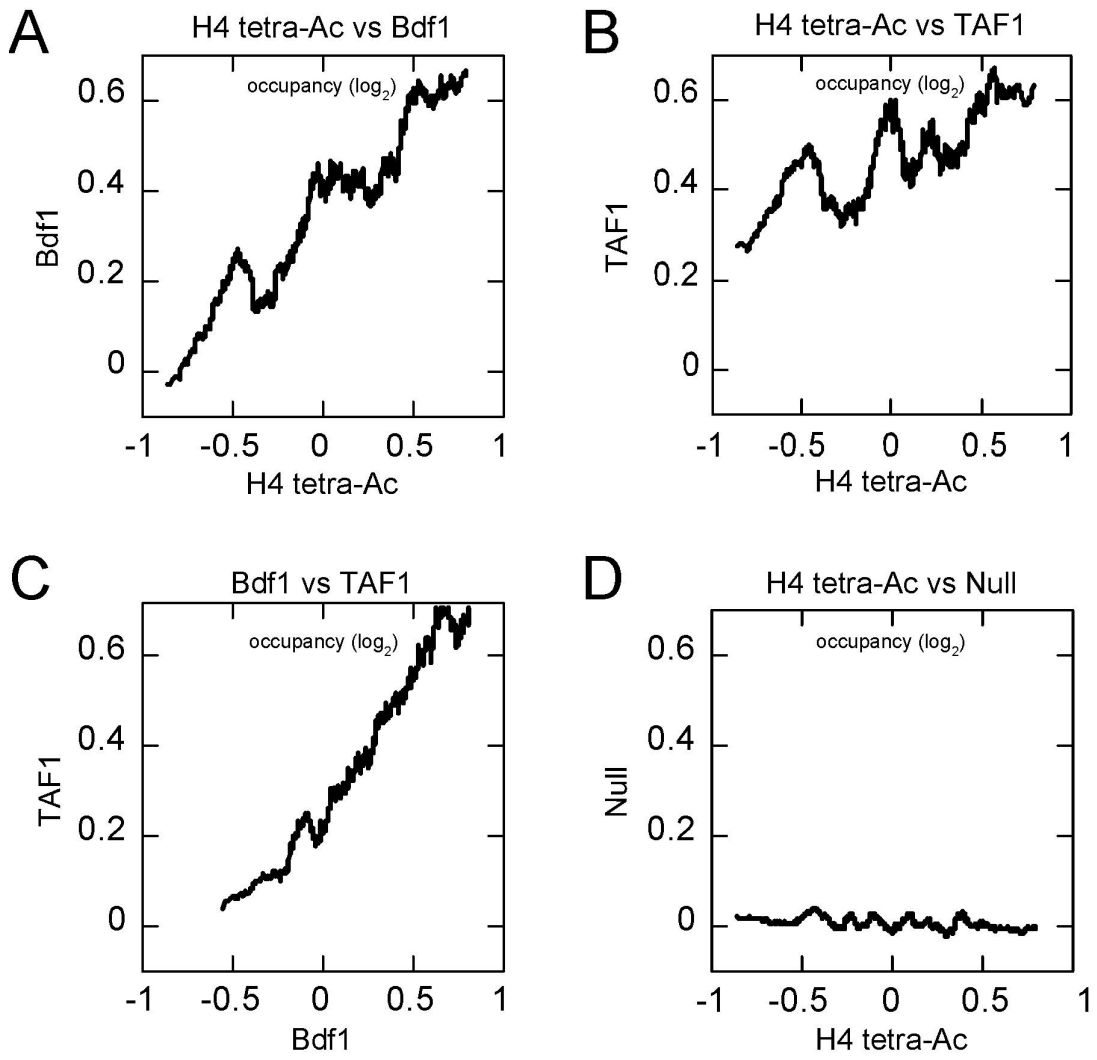

Figure 2.9**: H4 acetylation is linked to Bdf1 occupancy, which is linked to TAF1 occupancy**

Bdf1-TAP, TAF1-TAP, and "null" (no tag) $\log_2$ occupancy levels at 25ºC were derived from data reported previously (Zanton and Pugh, 2004). The data were centered to the median value for non-promoter regions. The H4 tetra-acetylated $\log_2$ occupancy levels at 30ºC were derived from data reported previously (Bernstein et al., 2002) and centered to the genome-wide median. The x-axis data were sorted by value, and 100-gene sliding-window averages (step size = 1) were plotted against the corresponding y-axis sliding-window average.

2.4 Discussion

The fact that the putative HAT region of yeast TAF1 is poorly conserved with higher eukaryotic TAF1 further questions whether yeast TAF1 is a physiological HAT. The evidence for TAF1 being a physiologically important HAT in higher eukaryotes is strong (Hilton et al., 2005). Higher eukaryotic TAF1 has a number of properties including protein kinase activity, bromodomains, and ubiquitin ligase activity that have not been shown to be present in yeast TAF1 (Wassarman and Sauer, 2001). To this list we now add histone acetyltransferase. It remains to be answered why higher eukaryotes have accumulated these activities or why yeast has lost them. These features may have evolved to accommodate the increased complexity of gene regulation in higher eukaryotes.

2.4.1 Transcription is dependent upon acetylation, but acetylation is not dependent upon transcription

Histone acetylation has been linked to transcription (Kurdistani et al., 2004; Robert et al., 2004; Pokholok et al., 2005; Yuan et al., 2005), yet we find that a shutdown of pol II transcription via inactivation of either TAF1/TFIID or Rpb1/pol II has little effect on H3 and H4 acetylation. This suggests that most H3 and H4 acetylation is not absolutely coupled to transcription. However, acetylation does correlate with transcription (Pokholok et al., 2005; Yuan et al., 2005), and loss of acetylation impairs

transcription, which taken together suggest that acetylation precedes and facilitates transcription in a way that does not depend upon transcription. More transient acetylation, such as any occurring in ORFs and linked to a transcribing RNA polymerase, cannot be excluded (Winkler et al., 2002). This type of transcription-coupled acetylation likely represents a small fraction of the steady-state level of acetylated histones.

2.4.2 Gcn5 and Esa1 are major gene regulatory HATs, and Hat1, Elp3, Hpa2, and Sas3 are minor gene regulatory HATs

Our findings demonstrate that loss of Hat1, Elp3, Hpa2, or Sas3 does not affect global acetylation levels on bulk nucleosomes or transcription *in vivo*, suggesting that they do not regulate most genes, or are highly redundant with other HATs. Selective redundancy exists between Gcn5 and Elp3 (Wittschieben et al., 2000), and between Gcn5 and Sas3 (Howe et al., 2001), but not between Elp3 and Sas3 (Kristjuhan et al., 2002). Thus, Gcn5 has a broad overlapping function with other HATs that are functionally distinct from each other. Our results support the notion that Gcn5 and Esa1 are the major yeast HATs at H3 and H4, respectively. As the major HATs, any redundancy with other HATs would be limited to a relatively small fraction of the genome, and thus would not be highly redundant. Our study provides the first genome-wide functional comparison of six yeast HATs plus one putative HAT, allowing their relative contributions to gene regulation to be directly compared.

2.4.3 Mechanisms linking Esa1, H4 acetylation, Bdf1 binding, and TFIID recruitment

The work presented here strengthens the relationship between Esa1, H4 acetylation, Bdf1, and TAF1. These linkages can be rationalized if the major function of Esa1 is to acetylate H4 tails at K5, K8, and K12 so that TFIID can bind via Bdf1. While each step in this model is supported on a broad genome-scale, we do not exclude the likelihood that additional mechanisms contribute to TFIID recruitment. Our finding that H4 acetylation at promoters is more highly linked to Bdf1 than to TAF1 suggests that promoter regions that are H4 acetylated and occupied by Bdf1 are not absolutely committed to TFIID recruitment.

2.5 Materials and Methods

Yeast strain construction: A list of the strains used in this study is provided in Table **2.4**. The parent strain for all of the created strains was *Y13.2*, which is a haploid strain containing a chromosomal deletion of *TAF1* and *WT TAF1* on a URA-marked plasmid. To create the deletions of the HAT proteins, genomic DNA was from Open Biosystems yeast deletion strains. Each Open Biosystems deletion strain had the entire ORF replaced by a Kanamycin resistance marker. PCR products extending ~250bp up and downstream of the desired ORF deletion were generated from the desired deletion

Table **2.4**: Yeast strains used in this study

Strain	Chromosomal Deletions	Plasmid 1	Plasmid 2
yJS4	*taf1Δ, gcn5Δ*	pSW104-WT TAF1	-----
yJS5	*taf1Δ, gcn5Δ*	pSW104-taf1 ts2	-----
yMD5	*taf1Δ, elp3Δ*	pSW104-WT TAF1	-----
yMD6	*taf1Δ, elp3Δ*	pSW104-taf1 ts2	-----
yMD7	*taf1Δ, hat1Δ*	pSW104-WT TAF1	-----
yMD8	*taf1Δ, hat1Δ*	pSW104-taf1 ts2	-----
yMD9	*taf1Δ, hpa2Δ*	pSW104-WT TAF1	-----
yMD10	*taf1Δ, hpa2Δ*	pSW104-taf1 ts2	-----
yMD11	*taf1Δ, sas3Δ*	pSW104-WT TAF1	-----
yMD12	*taf1Δ, sas3Δ*	pSW104-taf1 ts2	-----
yMD13	*taf1Δ*	pSW104-WT TAF1	-----
yMD14	*taf1Δ*	pSW104-taf1 ts2	-----
yMD25	*taf1Δ, esa1Δ*	pSW104-WT TAF1	pSAPE1 [WT ESA1]
yMD26	*taf1Δ, esa1Δ*	pSW104-taf1 ts2	pSAPE1 [WT ESA1]
yMD27	*taf1Δ, esa1Δ*	pSW104-WT TAF1	pSAPE2 [esa1-414]
yMD28	*taf1Δ, esa1Δ*	pSW104-taf1 ts2	pSAPE2 [esa1-414]
RY262*	*rpb1-1*	-----	-----

* Reference (Nonet et al., 1987)

strain and transformed into the target yeast strain; yeast colonies that had incorporated the deletion were isolated by selecting for Kanamycin resistance. For the Esa1 strains, *ESA1* was deleted on the chromosome and a plasmid with either the *ESA1* or *esa1-414* allele was expressed from a plasmid to support viability. Each mutant HAT strain was then transformed with a plasmid expressing either a *WT* or *taf1*^{ts2} copy of TAF1, and colonies that had lost the original *WT TAF1-URA* plasmid were selected for using 5-FOA.

Immunoblotting. Cells (1.5 ml for H3, 3.5 ml for H4) were grown at 25°C to OD_{600} = 0.8-0.9 and heat shocked for 45 min. at 37°C. Harvested cells were resuspended in cell lysis buffer (0.2 M Tris-acetate pH 7.5, 0.39 M $(NH_4)SO_4$, 1 mM EDTA, 20% Glycerol, 2 mM DTT, 1 complete protease inhibitor (CPI) tablet (Roche), 0.5 mM phenylmethylsulfonyl fluoride), and lysed with silica/zirconium beads in a mini-bead beater for 6 sessions, 20 seconds each, cooling on ice between sessions. Chromatin was pelleted by centrifugation, washed with cold ddH_2O, and resuspended in SDS sample buffer. Samples were electrophoresed on 16.5% SDS polyacrylamide gels and transferred to PVDF membranes. Membranes were probed with histone modification antibodies obtained from Upstate, and developed by enhanced chemiluminescence.

Microarray Analysis. Strains and plasmids are presented in Table **2.4**. Strains were grown in CSM-His media at 25ºC to OD_{600} = ~0.8. All cultures were shifted to 37ºC with an equal volume of heated media. After 45 min. at 37ºC, cells were quickly harvested at room temperature. Sample preparation and hybridizations were performed as described (Chitikila et al., 2002), and data normalization and analysis performed as described (Huisinga and Pugh, 2004). Raw data is accessible at GEO

(http://www.ncbi.nlm.nih.gov/geo/), accession numbers GSM75452-63, 75470-71, 75473-78, 75480-83, 75491-504.

ChIP-chip. Cultures were grown in CSM media at 25ºC to an OD_{600} = 0.8 then rapidly shifted to 37˚C for 45 min. Cells were crosslinked with 1% formaldehyde for 15 min. and simultaneously cooled to 25˚C. The crosslinking was quenched with 125 mM glycine for 5 min, and cells were subsequently harvested. Cell lysis and sonication of DNA was performed as previously described (Zanton and Pugh, 2004). Sonicated DNA was immunoprecipitated with H3 Ac-K9,14 antibodies to produce enriched DNA. The enriched DNA for each test strain was then co-hybridized with enriched DNA from an independent wild type strain onto microarrays containing PCR-amplified probes corresponding to ~6000 intergenic regions of the yeast genome. Raw data is accessible at GEO, accession numbers GSM75526-33.

Background signal was subtracted from the intensity for each spot, and values that were less than one standard deviation above background were removed. Intensity values were converted to ratios of mutant over wild type, and $\log_2$ transformed.

Computer simulations. KinTekSim freeware was downloaded from http://www.kintek-corp.com/members/. The input mechanism was A==B; B==C; C==A. Output was C. A, B, and C represent each species illustrated in Fig. 6B, from left to right respectively. "==" represents a reaction sequence (e.g., A==B means A converts to B). In this simplified simulation, C is synonymous with RNA output. In order to allow the system to reach steady state, reflecting the physiologic state of the cell, C was allowed to convert back to A (equivalent to disassembly of the transcription machinery and RNA turnover). The forward rate constants k_1 (governing A==B) and k_2 (governing B==C) for

"wild type" are indicated in Fig. 6B. k_1 was reduced by ten fold when simulating *esa1-414*, and k_2 was reduced by ten fold when simulating *taf1*^{ts2}. For all simulations, k_3 was set to 1, reverse rate constants were set to 0.01, starting concentrations of A, B, and C were set to 3, 0, and 0, respectively, Time was set to 100, and f1 (scaling factor) was set to 1. These values were largely arbitrary. The outcome of the simulation was largely independent of these parameters. Values for "C" were obtained at the completion of the time course, in which "C" had reached steady state (unchanged over time). In Fig. 6B, abscissa and ordinate values are $x = \log_2(C^{taf1\text{-}ts2} / C^{WT})$; $y = \log_2(C^{esa1\text{-}414} / C^{WT})$.

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Chapter 3

Investigating the Roles of Histone H4 acetylation and the Bromodomain Factors, Bdf1 and Bdf2, in TFIID Gene Regulation

3.1 Summary

Transcription initiation is a complex process involving the precise orchestration of many proteins to ultimately recruit Polymerase II. This process can also be simplified into a few key steps: activator binding; chromatin remodeling; GTF recruitment; Pol II recruitment; transcription initiation. The current working model of gene regulation by TFIID embodies each of these steps, starting with the hyper-acetylation of H4 tails by NuA4 and then recruitment of TFIID. In-between these two events, the bromodomain subunit, Bdf1, is thought to play an important role in helping anchor TFIID to nucleosome-bound promoters, however this has not been rigorously tested to date. As a result, many questions regarding the mechanism of TFIID-mediated gene activation remain unanswered. For example, if Bdf1 is an important player in the recruitment of TFIID to acetylated promoters, why isn't it essential for cell viability? Does the hypothesis that Bdf1 and Bdf2 are functionally redundant account for the cell's survival? Additional evidence has also suggested a possible link between NuA4-mediated acetylation, Bdf1 recruitment, and H2A.Z deposition by the SWR-C complex. How much do these complexes depend on one another for function? I have attempted to address these questions and more on a genome-wide scale using chIP-on-chip. By looking at changes in the recruitment of various factors in TAF1, Esa1, and Bdf1 mutant

strain backgrounds, I find validation of the general model for TFIID-mediated transcription initiation, as well as some further insight into how all these complexes work together to establish active promoters.

3.2 Introduction

Transcription initiation requires the recruitment and assembly of general transcription factors at promoter DNA to form the pre-initiation complex (PIC). Parts of promoter DNA maybe wrapped around histone octamers to form nucleosomes, the presence of which occludes transcription factor binding (Grunstein, 1990; Owen-Hughes and Workman, 1994). As a result, the compaction of DNA into chromatin was initially viewed as a mechanism of gene repression. We have since learned that chromatin is not strictly a negative regulator of gene transcription, evidenced by the observation that mutating the histone H4 tail results in the up- and down-regulation of genes throughout the genome (Sabet et al., 2003; Sabet et al., 2004; Dion et al., 2005). Thus, it is believed that histones are utilized in mechanisms of gene repression and activation.

The involvement of histones in gene activation is best exemplified by the mechanism of histone tail modification. Histone tails can receive a variety of post-translational modifications, including acetylation, methylation, phosphorylation, and ubiquitilation (Grant, 2001; Marmorstein, 2001). Hyper-acetylation of histone tails, particularly histones H3 and H4, is the modification best associated with gene activation (Hebbes et al., 1988; Turner, 1993). In addition to weakening the charged interaction between the histones and DNA (Hong et al., 1993; Steger and Workman, 1996), these

modifications provide binding sites for bromodomain factors (Dhalluin et al., 1999; Hudson et al., 2000; Jacobson et al., 2000; Owen et al., 2000). Bromodomains have been identified in a variety of chromatin and transcription regulators, suggesting that they play an important role in tethering large protein complexes to nucleosome-bound DNA (Brownell and Allis, 1996; Travers, 1999; Manning et al., 2001; Yang, 2004). Furthermore, the existence of bromodomains indicates that histone tail acetylation acts as a mark to directly facilitate the recruitment of complexes involved in transcription initiation (Brownell and Allis, 1996; Sterner et al., 1999; Travers, 1999; Brown et al., 2001; Manning et al., 2001).

The general transcription factor complexes, TFIID and SAGA, contain the bromodomain subunits, Bdf1 and Gcn5, respectively (Hudson et al., 2000; Owen et al., 2000; Sanders et al., 2002). In higher eukaryotes, Bdf1 is fused to the C-terminal end of TAF1, suggesting that Bdf1's double bromodomain play an important role in TFIID function (Matangkasombut et al., 2000). Given that the majority of TFIID subunits are essential for cell viability (Walker et al., 1996; Giaever et al., 2002), it is surprising to find that *bdf1Δ* cells are viable, exhibiting only mild growth defects such as temperature sensitivity and the inability to sporulate (Lygerou et al., 1994; Chua and Roeder, 1995). On the surface, this observation suggests that bromodomains are dispensable for TFIID function; however, the discovery of Bdf2, a protein highly homologous to Bdf1, has led to an alternative model that proposes functional redundancy between Bdf1 and Bdf2 (Matangkasombut et al., 2000). Additional support for this model of functional substitution is found in the following observations from Matangkasombut et al. (2000): (1) double deletion of Bdf1 and Bdf2 does not support cell viability; (2) overexpression

of Bdf2 suppresses the *bdf1Δ* temperature sensitive phenotype; (3) Bdf2 interacts with TAF7 and can associate with TFIID if Bdf2 is overexpressed. Taken altogether, these observations suggest that bromodomains play an important role in TFIID function, presumably through their role as acetyl-lysine binding motifs; however, this model has not been rigorously tested *in vivo*.

Bdf1 has also been functionally linked to other complexes *in vivo*, including the histone-H4 HAT complex, NuA4, and the H2A.Z-deposition complex, SWR-C (Krogan et al., 2003; Matangkasombut and Buratowski, 2003; Bianchi et al., 2004; Kobor et al., 2004; Raisner et al., 2005; Zhang et al., 2005). The root of the evidence linking Bdf1 to NuA4 is histone H4 acetylation. *In vitro* studies have indicated that Bdf1 is capable of binding acetylated histone H3 and H4 tails with a preference for hyper-acetylated histone H4 tails (Pamblanco et al., 2001; Ladurner et al., 2003; Matangkasombut and Buratowski, 2003), a mark that is primarily achieved by Esa1, the catalytic subunit of the NuA4 and Piccolo HAT complexes (Allard et al., 1999; Clarke et al., 1999; Selleck et al., 2005). In addition, genetic interactions are evident between Bdf1 and Esa1 in that deletion of Bdf1 is lethal when combined with a temperature sensitive allele of Esa1 or with non-acetylatable histone H4 variants (Matangkasombut and Buratowski, 2003). Work presented earlier in this thesis also showed a link between Esa1 and TFIID-regulated genes on a genome-wide scale; given the evidence linking TFIID, Bdf1, and Esa1, is seems natural to propose that Bdf1 acts as the link between NuA4 and TFIID functions (Figure **3.1**) (Durant and Pugh, 2006).

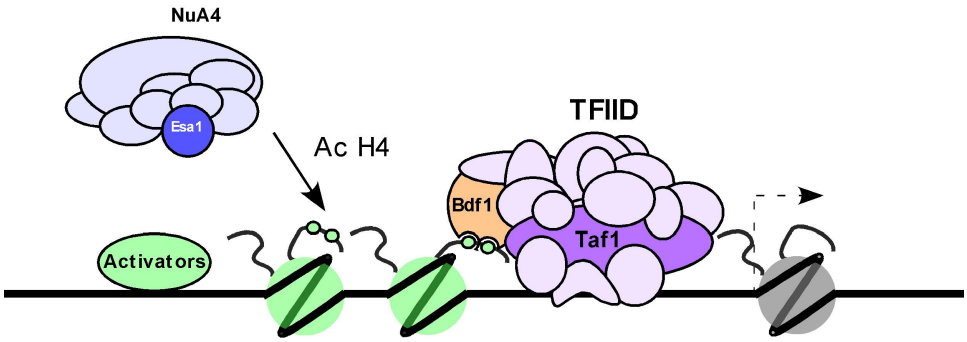

Figure 3.1**: Proposed model for NuA4, Bdf1, and TFIID gene regulation**
NuA4 is recruited to promoters to hyper-acetylate histone H4 tails in nucleosomes. The bromodomain of Bdf1 binds to the acetylated H4 tails and interacts with the TFIID general transcription complex, helping to anchor TFIID at nucleosome-bound promoters.

Bdf1 has only recently emerged as a potential player in the SWR-C-mediated deposition of H2A.Z. SWR-C has been well characterized as the ATP-dependent remodeling complex responsible for exchanging histone H2A for the histone variant, H2A.Z (Krogan et al., 2003; Kobor et al., 2004; Mizuguchi et al., 2004). Little is known about the role of this particular histone variant in gene regulation and/or chromatin structure, but increasing amounts of evidence have led to the development of a model suggesting that H2A.Z-containing nucleosomes are easier to remodel, thus increasing the efficiency of transcription initiation (Zhang et al., 2005). Support for this model stems from recent genome-wide studies showing specific localization of H2A.Z to promoter nucleosomes, particularly those upstream of quiescent genes (Guillemette et al., 2005; Raisner et al., 2005; Zhang et al., 2005). In addition, it has been shown that H2A.Z is more readily released from chromatin compared to other canonical histones (Zhang et al., 2005).

Assuming that the presence of H2A.Z at promoters is important for proper initiation of gene transcription, the next question to address becomes the mechanism by

which SWR-C is targeted to promoters for H2A.Z deposition. One clue to the potential mechanism for SWR-C recruitment might lie in the finding that Bdf1 co-purifies with the SWR-C complex (Krogan et al., 2003). SWR-C has also been shown to contain a number of subunits that are part of the NuA4 HAT complex (Kobor et al., 2004; Krogan et al., 2004; Zhang et al., 2004). It is still uncertain whether the subunit overlap between SWR-C and NuA4 translates to a functional overlap, or if these subunits serve to target SWR-C and NuA4 localization. Our current knowledge of Bdf1 and NuA4 function would suggest that the mechanism behind SWR-C recruitment and promoter-specific H2A.Z deposition might center around H4 acetylation (Figure **3.2**). Initial evidence shows that loss of Bdf1 does affect H2A.Z deposition on a genome-wide scale, albeit modestly (Zhang et al., 2005). However, this has not yet been attributed to defects in SWR-C recruitment and/or H4 acetylation. H2A.Z deposition is also compromised in *elp3Δ*, *gcn5Δ*, and *sas3Δ* strains, suggesting that decondensed chromatin might be important for SWR-C function (Raisner et al., 2005; Zhang et al., 2005).

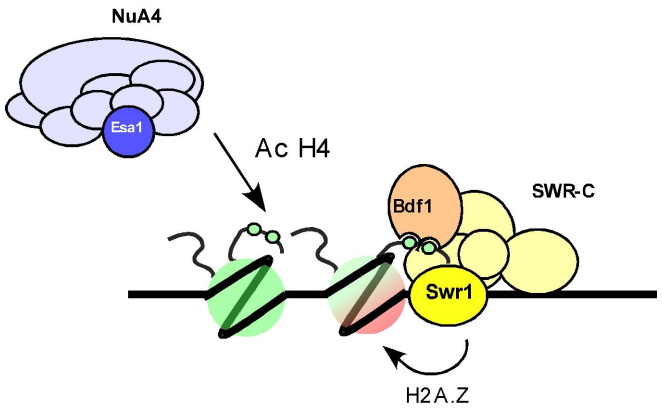

Figure 3.2**: Model for NuA4 and Bdf1 in mediating SWR-C function**

NuA4 hyper-acetylates histone H4 tails in promoter nucleosomes, allowing for the binding of Bdf1. Bdf1 help localize SWR-C at promoters, allowing for deposition of H2A.Z by the catalytic subunit, Swr1.

Given the substantial amount of evidence that links NuA4-mediated H4 acetylation, Bdf1 recruitment, TFIID recruitment, and SWR-C-mediated H2A.Z deposition, it seems valid to propose that H4 acetylation is important for facilitating multiple regulatory events via recruitment of Bdf1 to acetylated promoters. However, no studies to date have directly linked all of these regulatory events together. I present evidence here that not only links NuA4, Bdf1, TFIID, and SWR-C/H2A.Z together, but also provides the first evidence that Bdf2 binds to select promoters in the absence of Bdf1 to aid in stabilization of TFIID. These results implicate a central role for bromodomain factors in multiple steps during transcription initiation.

3.3 Results

3.3.1 Experimental approach and design

The current working models of transcription initiation are rooted in a long history of biochemical *in vitro* studies and gene-specific *in vivo* studies. While these classic studies have been invaluable in the quest for elucidating regulatory mechanisms, we know that cellular genomes are complex and do not rely on only one or two mechanisms to regulate the entire genome. Thus, the findings from studying a handful of "model" genes, such as GAL1, HIS3, and PHO5, do not necessarily apply to the other ~6200 genes in the genome. Technological advancements over the past decade, such as the development of genome-wide microarrays, have exponentially increased the possibilities for studying gene regulation mechanisms on a global scale.

One good example of an experimental finding that might not have been possible without the use of genome-wide microarrays is the discovery of a divided, yet overlapping, mechanism of genome regulation by the TFIID and SAGA complexes (Huisinga and Pugh, 2004). It was found that ~90% of the genome is preferentially regulated by the TFIID general transcription factor complex, while the remaining ~10% of the genome is regulated by the SAGA complex (Huisinga and Pugh, 2004). Summarized in the literature review in Chapter 1, it has been determined that SAGA-regulated genes are negatively regulated by factors such as Mot1, NC2, and HDACs, where as TFIID-regulated genes are largely un-regulated by these repressor proteins

(Huisinga and Pugh, 2004). Instead, the general mechanism by which TFIID regulated genes are activated is thought to be as follows: (1) acetylation of promoter nucleosomes by NuA4; (2) recruitment of Bdf1 to acetylated histone tails; (3) recruitment of TFIID/TBP to bromodomain-bound nucleosomes; (4) recruitment of Pol II holoenzyme. However, it remains to be determined what percentage of TFIID regulated genes utilize this mechanism, and whether or not each of the steps in this pathway are essential for proper PIC assembly.

To gain a better understanding of the role of various proteins and/or complexes in PIC assembly, I utilized chIP-on-chip technology to assess transcription factor recruitment in mutant strain backgrounds. Bdf1, H2A.Z, and proteins representing the TFIID and SWR-C complexes were TAP-tagged in WT and mutant strain backgrounds, generating the strains listed in Table **3.1**. By tagging these factors in various mutant strain backgrounds, such as *bdf1Δ*, *taf1*^{ts2}, and *esa1-414*, we can observe the changes in

Table **3.1**: TAP-tagged strains used in this study

Strain	Chromosomal Deletions	Plasmid 1	Plasmid 2	Tagged Protein
yMD52	taf1Δ, esa1Δ	pJI12 [WT TAF1]	pSAPE1 [WT ESA1]	BDF1-TAP
yMD55	taf1Δ, esa1Δ	pJI12 [WT TAF1]	pSAPE1 [WT ESA1]	SWR1-TAP
yMD58	taf1Δ, esa1Δ	pJI12 [WT TAF1]	pSAPE1 [WT ESA1]	HTZ1-TAP
yMD59	taf1Δ, esa1Δ	pJI11 [taf1-ts2]	pSAPE1 [WT ESA1]	BDF1-TAP
yMD67	taf1Δ, esa1Δ	pJI12 [WT TAF1]	pSAPE2 [esa1-414]	BDF1-TAP
yMD69	taf1Δ, esa1Δ	yCP1 [WT TAF1]	pSAPE2 [esa1-414]	TAF1-TAP
yMD70	taf1Δ, esa1Δ	pJI12 [WT TAF1]	pSAPE2 [esa1-414]	SWR1-TAP
yMD73	taf1Δ, esa1Δ	pJI12 [WT TAF1]	pSAPE2 [esa1-414]	HTZ1-TAP
yMD75	bdf1Δ	-----	-----	TAF1-TAP
yMD76	bdf1Δ	-----	-----	SWR1-TAP
yMD79	bdf1Δ	-----	-----	HTZ1-TAP
yMD87	bdf1Δ	-----	-----	BDF2-TAP
BDF2-TAP	-----	-----	-----	BDF2-TAP
SWR1-TAP	-----	-----	-----	SWR1-TAP
TAF1-TAP	-----	-----	-----	TAF1-TAP

factor occupancy on a genome-wide scale when the function of other factors/complexes are compromised. In each experiment, sonicated chromatin from a mutant "test" strain was immunopurified alongside a WT "reference" strain, each containing the same TAP-tagged factor. Enriched chIP DNA from the test and reference strain were co-hybridized to a microarray slide containing ~8000 intergenic regions of the yeast genome. For each intergenic region, the ratio of the test/reference signal was determined and $\log_2$ transformed. Each data set was then adjusted by the median signal of 1834 converging, or tail-to-tail (T-T), intergenic regions (Figure **3.3**). These intergenic regions represent the known non-promoter regions; since the proteins addressed in this study are presumed to function predominantly at promoter regions, we expect little or no change in the non-promoter regions. Thus, adjusting all of the ratios by the median T-T ratio attempts to correct for any differences in signal intensity between the two channels.

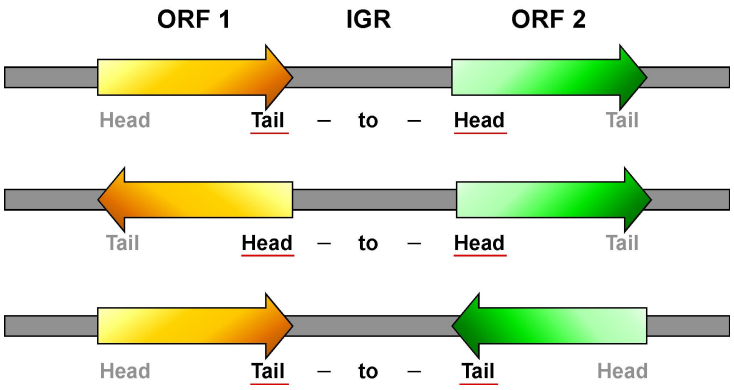

Figure 3.3**: Classification of different intergenic regions.**

Each intergenic region can be classified as a T-H, H-H, or T-T orientation. The "Head" (H) of a gene refers to the 5' end of the ORF, and the "Tail" (T) region corresponds to the 3' end of the ORF. Thus, intergenic regions flanked by the 3' end (Tail) of one ORF and the 5' end (Head) of a second ORF are classified as Tail-to-Head genes, and so on. For the experiments included in this study, only intergenic regions assigned to one promoter (T-H) were used for analysis; these T-H genes were normalized by the median $\log_2$ ratio of the non-promoter, or T-T, genes as they were not expected to show changes in occupancy in the test experiments.

Various controls were included in the experiment to assess the degree of variability in the procedure. Each chIP experiment was repeated with independent samples, and the dye labeling of the samples reversed, referred to as a dye swap. Comparison of the two independent dye swap experiments by a scatter plot helps determine the reproducibility of the data sets and the amount of noise in the data sets. Dye swap sets that correlated well with one another were averaged together before further analysis.

The averaged data sets for ten unique experiments were then analyzed using K-means clustering. This method of analysis allows for the extraction of patterns present among different data sets. Figure **3.4** shows the cluster plot obtained from grouping T-H genes into four distinct clusters. Each experiment is identified at the top of Figure **3.4** with the tagged protein and strain mutation for easy identification. In addition, each of the experiments, labeled 1 – 10, were clustered hierarchically. Hierarchical clustering sorts the included experiments by their degree of similarity to one another. The length of the hierarchical tree branches reflects the relationship between two data sets; experiments joined by shorter branches are more closely related, and vice versa. In addition, a more quantitative representation of the occupancy changes in each cluster are presented in Figure **3.5**.

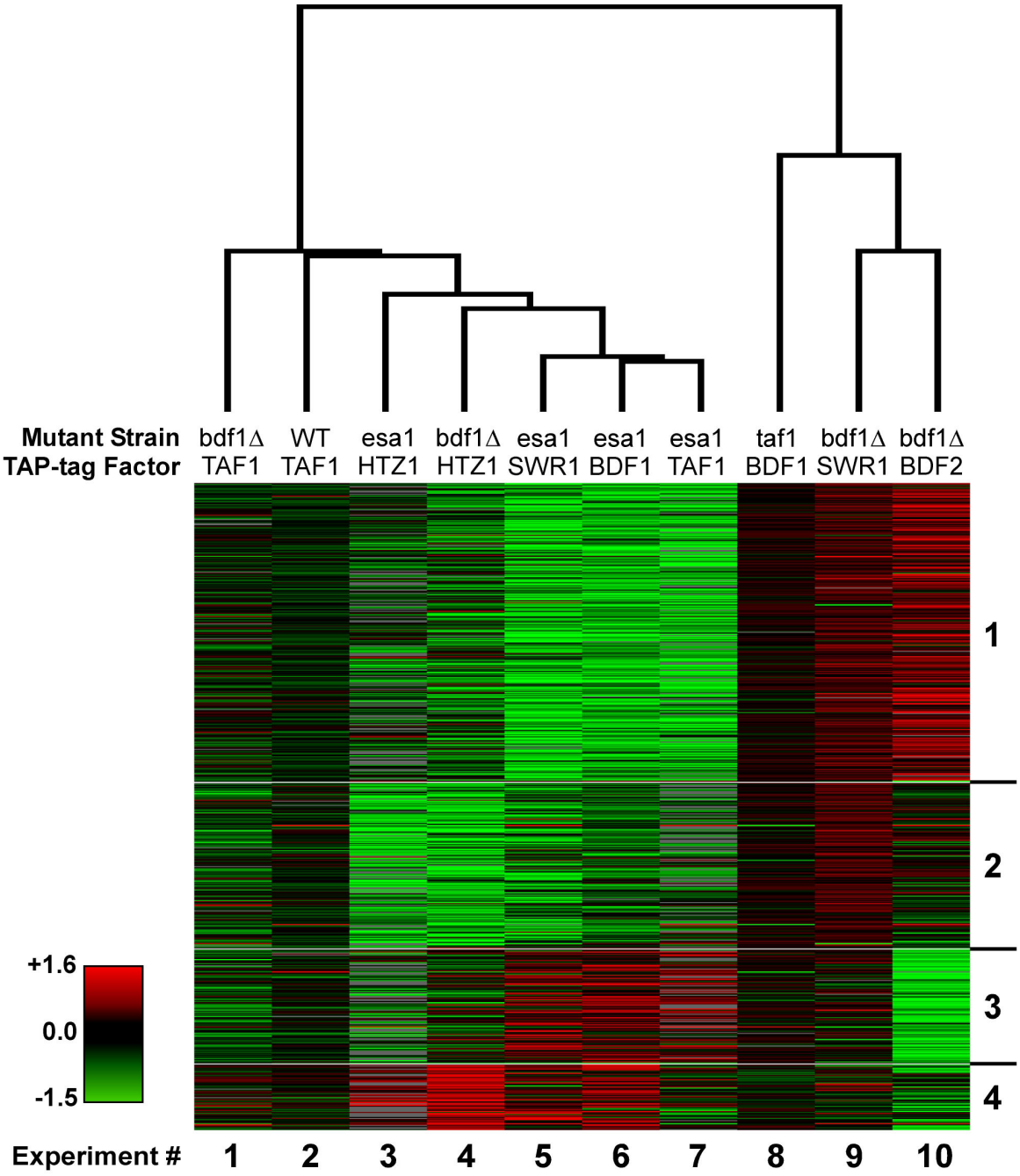

Figure 3.4**: Different groups of genes are regulated by various mechanisms**

(Figure 3.4 cont.) Ten independent chIP experiments were performed, each in duplicate with the incorporation of a dye swap. Intergenic regions assigned to only one promoter (T-H regions) were normalized to the median value of the non-promoter (T-T) regions. Ratios for each gene reflect the occupancy levels for the specific TAP-tag protein in the mutant strain compared to the wild type strain. Log_2 ratios for the dye swap experiments were averaged together and filtered for a 1.4 fold cutoff. Filtered genes for each experiment were then clustered into four groups, and the experimental arrays clustered hierarchically. The average log2 ratio for the genes in each cluster, separated by experiment, are also plotted as a bar graph in Figure 3.5. (esa1 = *esa1-414*; taf1 = *taf1^{ts2}*)

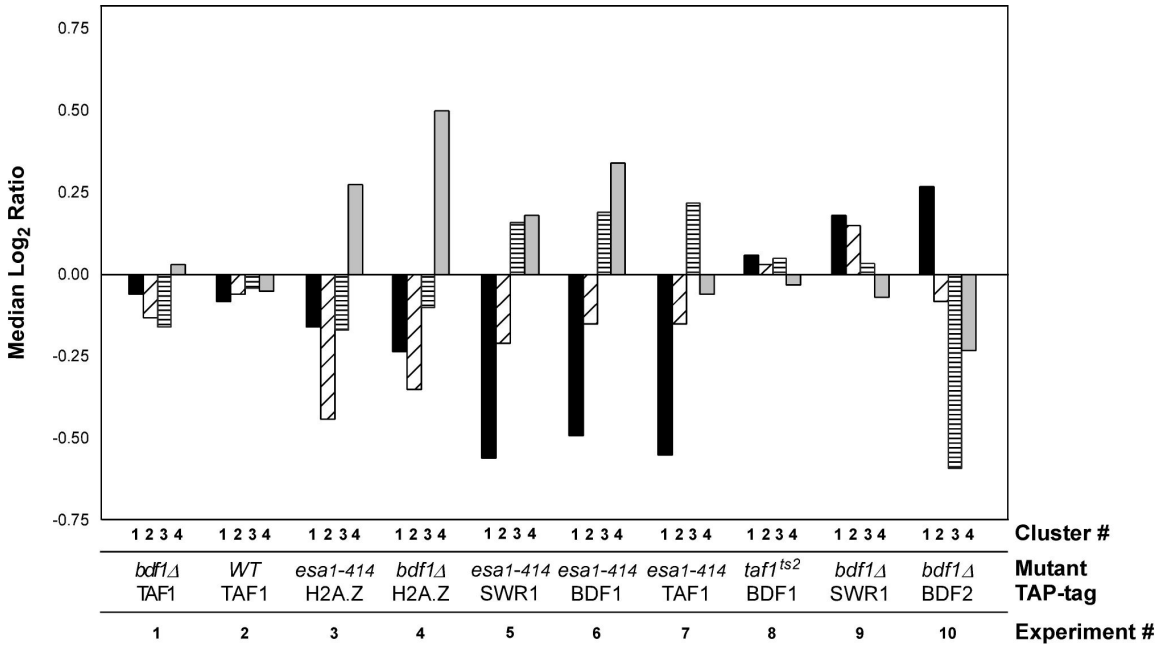

Figure 3.5**: Quantitative representation of log_2 changes in clusters**

For each experiment, the median value of all the genes in a given cluster was determined. Like the cluster plot, patterns are similar between experiments 3 and 4, and between 5, 6, and 7.

3.3.2 Evaluation of starting factor occupancy levels in different clusters

The ratios in the cluster plot for Figure **3.4** are useful in determining the change in factor occupancy in the mutant strain compared to wild type. However, they do not provide information regarding the starting levels of occupancy in the wild type strain. Thus, while the average log ratio for a cluster of genes might indicate that factor occupancy is decreasing, it does not inform us whether the factor is starting out with high or low levels of binding. The occupancy levels for each TAP tag factor in a WT background were determined by normalizing the raw signal to a Null, or untagged, chIP-chip experiment. Execution of a chIP-chip experiment with an untagged strain does result in signal after the hybridization; this signal is mainly attributed to background. Thus, normalization of the raw data to a Null experiment attempts to correct for any background "noise" and also factors in any internal structure in the data set. After normalization, the percent rank for the T-H genes was determined, and the frequency distribution for the genes in each cluster was plotted (Figure **3.6**).

At this point in the data analysis, only a few general conclusions will be made regarding the starting occupancy levels for each factor. The relevance of these findings will be discussed in more detail later on in the context of the observations made from other methods of analysis. Looking at the distribution of TAF1 between the four clusters, there is a striking trend apparent where TAF1 is preferentially located at cluster 1 genes, and is noticeably absent from the genes in cluster 3. Cluster 4 shows no real

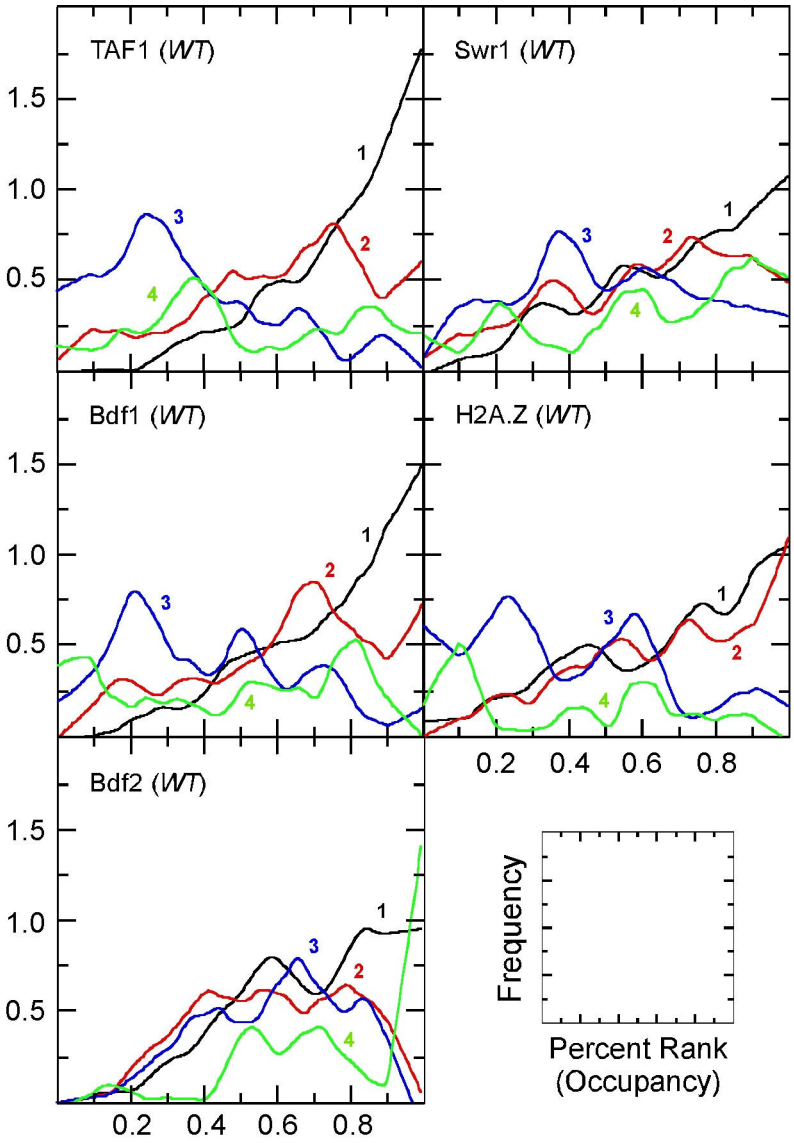

Figure 3.6**: Analysis of relative factor occupancy levels in wild type strain for each cluster**

Raw signal intensity data for each of the TAP-tag factors in a wild type strain were normalized to a Null (untagged) data set, taking into account noise and internal structure in the data set. The percent rank for the occupancy values was determined, and the percent rank values for the genes within each cluster were plotted as a frequency distribution, shown above. The small graph in the bottom right corner indicates the labels for the x and y axis for each graph shown in this figure. For each graph, the percent rank of the occupancy is plotted on the x-axis, and the frequency is plotted on the y-axis.

bias for or against TAF1 binding, and TAF1 shows some preference for cluster 2 genes, but not to the same degree as the genes in cluster 1. Interestingly, the pattern of distribution for Bdf1 bears a strong resemblance to the pattern observed for TAF1. This indicates that not only is Bdf1 is preferentially located at cluster 1 and cluster 2 genes and relatively absent from cluster 3 genes, but also that Bdf1 typically found at the same locations as TAF1. In contrast to the localization patterns observed for TAF1 and Bdf1, Bdf2 does not shown much distinction between the four clusters. There are a small number of genes in cluster 4 that strongly bind Bdf2, evidenced by the sharp peak at the right-hand side of the distribution curve. In addition, Bdf2 is also found in slightly higher levels at some of the genes in cluster 1. But unlike TAF1 and Bdf1, there are no genes that seems to be particularly void of Bdf2.

The frequency distributions for Swr1 indicate that Swr1 is highly occupied at the genes in clusters 1 and 2. There is also a small peak in cluster 4 that indicates high Swr1 occupancy at some genes. Similar to Swr1, there are high levels of H2A.Z occupancy at cluster 1 and cluster 2 genes. H2A.Z is somewhat absent from cluster 3 genes, with even less H2A.Z at cluster 4 genes, indicated by the sharp peak on the left side of the graph. These observations suggest that occupancy of Swr1 does not necessarily correlate with occupancy of H2A.Z, demonstrated by comparing H2A.Z and Swr1 occupancy for cluster 4.

In general, this occupancy analysis indicates that cluster 1 genes are bound by all of the observed factors, and cluster 3 genes have low levels of TAF1, Bdf1, and H2A.Z. Cluster 2 is occupied by H2A.Z and Swr1 with some TAF1 and Bdf1 present, and cluster

4 is not specifically enriched for any factor with the exception of Swr1 and Bdf2 at a select subset of genes.

3.3.3 Histone H4 acetylation is important for the recruitment of multiple protein complexes

Histone acetylation is thought to contribute to gene activation through two mechanisms: alteration of the charged interaction between histone tails and DNA, and binding of bromodomain proteins to acetylated histone tails. Genome-wide expression studies presented in Chapter 2 show a specific relationship between the H4 HAT, Esa1, and TFIID, leading to the hypothesis that TFIID is recruited via Bdf1, which binds H4 tails acetylated by Esa1. Given that Esa1 plays a role in the activation of TFIID-regulated genes, I first wanted to address whether loss of Esa1 would affect the recruitment of TFIID to promoters. Since TAF1 has been shown to be important for the structural integrity of TFIID, any changes observed in TAF1 binding can be assumed to reflect changes in TFIID binding, as well (Singh et al., 2004). Because Esa1 is an essential protein, a temperature sensitive allele, *esa1-414*, was used; this allele has been previously characterized and shown to rapidly turn over upon heat shock at 37ºC (Clarke et al., 1999).

Figure **3.7** shows that loss of Esa1 results in a slight decrease in TAF1 binding at promoters genome-wide compared to a homotypic control (WT curve). The distribution of the genes in the histogram shows that not every promoter is negatively affected. Some genes in the distribution are localized at or near zero, indicating no change on a $\log_2$

scale. Other promoters show a decrease in TAF1 binding when H4 acetylation is lost, ranging from 1.4 fold to 2.4 fold.

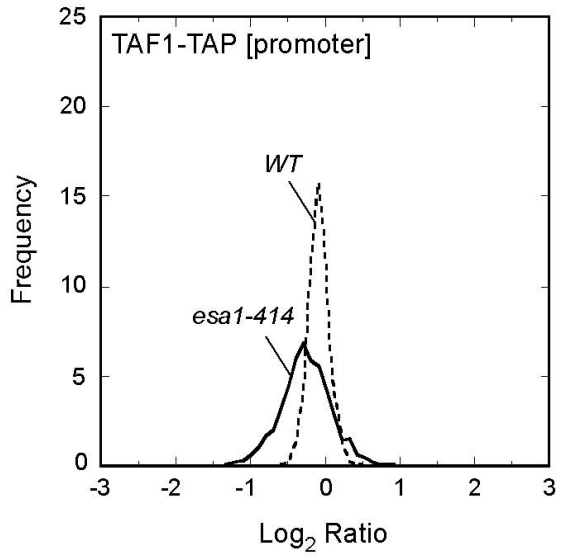

Figure 3.7**: H4 Acetylation by Esa1 contributes to TAF1 recruitment at select promoters**

The log ratios for all promoter-containing intergenic regions in the homotypic (experiment 2, TAF1 *WT* vs. TAF1 *WT*) and the TAF1-TAP *esa1-414* (experiment 7) experiments were binned and plotted as a frequency distribution. The tight distribution of the homotypic experiment (WT) represents no change overall between dye swap experiments, indicating a high level of reproducibility. The frequency distribution of the TAF1-TAP *esa1-414* experiment (esa1) is more dispersed and slightly shifted to the left, indicating a negative effect on TAF1 binding at certain genes compared to the wild type.

Clustering of the T-H genes (Experiment 7, Figure **3.4**) in this experiment reveals a pattern similar to the dispersed distribution observed in Figure **3.7**. The genes included in cluster 1 (experiment 7, Figure **3.4**) represent the left hand part of the histogram, indicating a stronger dependency of TAF1 on H4 acetylation by Esa1. Cluster 2 also shows a decrease in TAF1 binding, but not with the same magnitude as the genes in cluster 1, evident in Figure **3.5**. In contrast to clusters 1 and 2, TAF1 binding was un-compromised for the genes in clusters 3 and 4. Looking back at the occupancy analysis

in Figure **3.6**, TAF1 was primarily located at clusters 1 and 2, with little TAF1 occupancy at clusters 3 and 4. The changes in TAF1 binding in the cluster plot therefore indicate that loss of Esa1 affects genes with the highest levels of TAF1 occupancy. Furthermore, we can speculate that the reason there are not many changes in TAF1 binding at cluster 3 and 4 genes is because these genes are not highly occupied with TAF1 to start with.

Evidence that Esa1-mediated H4 acetylation is important for TAF1 recruitment leads us to ask the next obvious question: Is TAF1's dependency on H4-Ac linked to the bromodomain subunit of TFIID, Bdf1? There is a strong case for preferential binding of Bdf1 to hyper-acetylated H4 tails, potentially leading to the recruitment and/or stabilization of other general transcription factor complexes, such as TFIID (presented in Chapter 2) (Ladurner et al., 2003; Matangkasombut and Buratowski, 2003). However, there is no evidence to date showing that Bdf1 is dependent on acetylation for binding to promoters *in vivo*. A decrease in Bdf1 binding occurred upon loss of Esa1, similar in magnitude to the TAF1-TAP *esa1-414* experiment (Figure **3.8**A). Looking at the cluster plot in Figure **3.4**, it is striking that pattern of changes in Bdf1 binding are very similar to those observed for TAF1 (compare columns 6, 7), further evidenced by the tight correlation and large R-value in Figure **3.8**B. This indicates not only that Bdf1 is dependent on H4 acetylation by Esa1 for promoter binding, but also that a subset of genes relies on H4 acetylation for both Bdf1 and TAF1 binding. Looking back at the model presented in Figure **3.1**, these results confirm our prediction that Bdf1 is recruited to promoters with hyper-Ac H4 tails, and that Bdf1 might be the link between Esa1 and TAF1. The next logical question is whether deletion of Bdf1 results in the same effects

on TAF1 binding as seen in the *esa1-414* strain. The experiment to test this relationship will be temporarily deferred until section **3.3.4**.

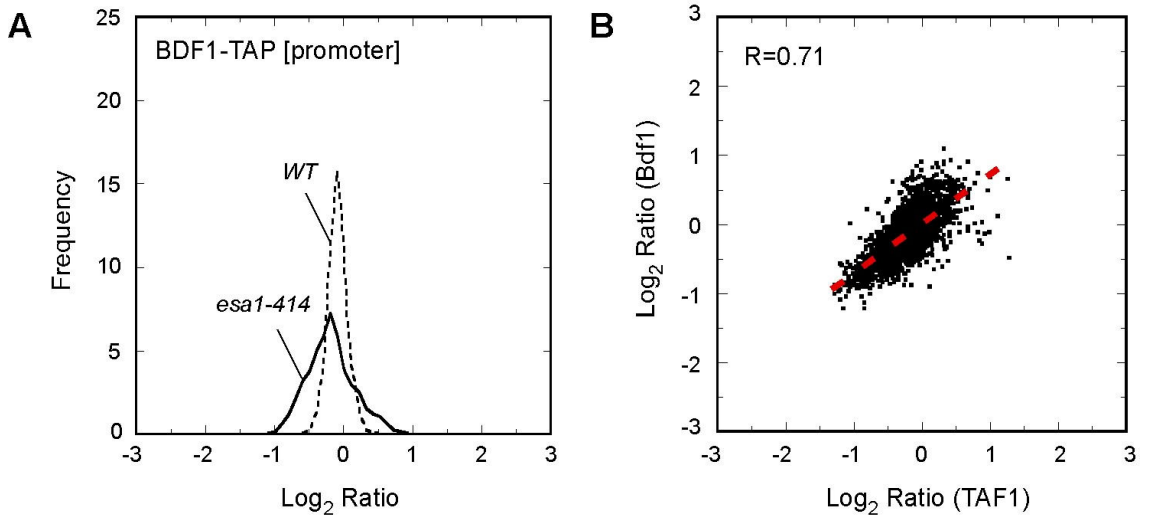

Figure 3.8**: Dependency of TAF1 on Esa1-mediated H4 acetylation is linked to Bdf1**

(A) Log ratios for the homotypic and Bdf1-TAP *esa1-414* data sets were binned and plotted as a frequency distribution as described in Figure **3.7**.

(B) Changes in factor occupancy for all promoter-containing regions in the TAF1-TAP *esa1-414* and Bdf1-TAP *esa1-414* experiments were plotted against one another on a $\log_2$ scale. The R value represented the correlation coefficient between the two experiments; the closer the R value is to 1.0, the closer the relationship between the two data sets.

H4-acetylation has also been implicated in regulating SWR-C function. SWR-C been shown to co-purify with Bdf1, and deletion of Bdf1 results in a loss of H2A.Z deposition. Since H2A.Z occupancy is thought to be a mark of active promoters, it stands to reason that H2A.Z deposition could be dependent on the acetylation status of promoter nucleosomes (Raisner et al., 2005). To test this model, I started out by looking at changes in the binding of Swr1-TAP, the catalytic subunit of SWR-C, in a mutant Esa1 strain (Figure **3.9**). The frequency distribution for the Swr1-TAP *esa1-414* experiment shows a general decrease in Swr1 binding. Upon further examination of this data set in

the cluster plot (Figure **3.4**, column 5), we find that Swr1-TAP recruitment is compromised at the genes grouped in clusters 1 and 2, with little or no change in clusters 3 and 4. Changes in Swr1-TAP binding are also similar to the changes observed for the Bdf1-TAP and TAF1-TAP experiments (compare columns 5, 6, 7). This observation further strengthens the idea that the genes in clusters 1 and 2 are particularly dependent on H4 acetylation for the recruitment of other protein complexes, including TFIID and SWR-C.

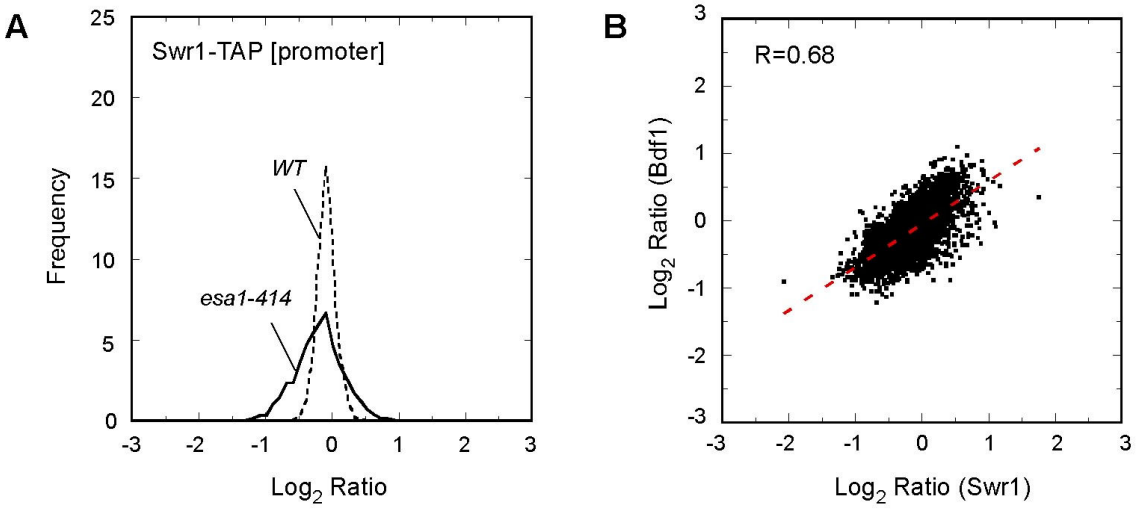

Figure 3.9**: Loss of H4 acetylation results in similar affects on Swr1 and Bdf1 recruitment across the genome**

(A) Log ratios for the homotypic and Swr1-TAP *esa1-414* data sets were binned and plotted as a frequency distribution as described in Figure 3.7.
(B) Changes in factor occupancy for all promoter-containing regions in the Swr1-TAP *esa1-414* and Bdf1-TAP *esa1-414* experiments were plotted as described in Figure **3.8**.

Knowing that cluster 1 genes are dependent on H4 acetylation for the recruitment of Swr1, we next addressed the question of whether or not this observation extended to H2A.Z deposition, perhaps at the same genes that were defective in Swr1 binding. According to the model presented in Figure **3.2**, we predicted that loss of Esa1 would

result in a decrease in H2A.Z deposition. While the histogram for the H2A.Z *esa1-414* experiment shows a slight decrease in H2A.Z binding as expected, the pattern observed in the cluster plot is different from the TAF1, Bdf1, and Swr1 experiments, further evidenced by the distance separating these experiments in the hierarchical tree (Figure **3.10**). The most striking differences between experiment 3 and experiments 5, 6, and 7 are the differences in magnitude between clusters 1 and 2. Comparing the average log ratios for these experiments in Figure **3.5**, it appears that loss of Esa1 primarily affects Bdf1, TAF1, and Swr1 recruitment in cluster 1 genes, where as H2A.Z deposition is most severely affected in cluster 2 genes. Further discussion regarding these observations will occur later on in this chapter.

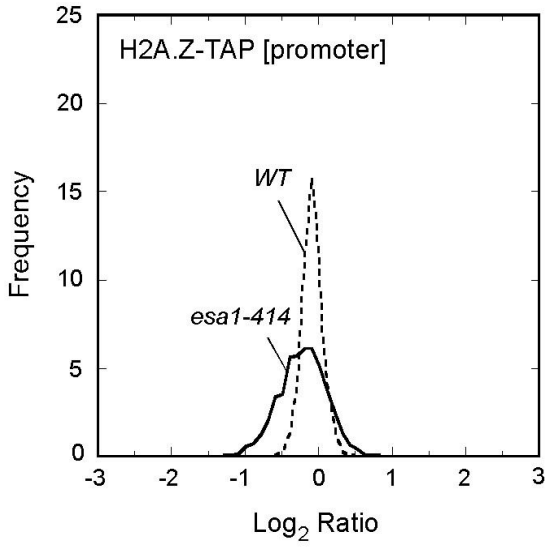

Figure 3.10**: Loss of Esa1 negatively affects H2A.Z deposition at a subset of genes**
Log ratios for the homotypic and H2A.Z-TAP *esa1-414* data sets were binned and plotted as a frequency distribution as described in Figure **3.7**.

From these chIP-chip studies, we can conclude the following: (1) H4 acetylation by Esa1 contributes TAF1, Bdf1, and Swr1 recruitment; (2) H4 acetylation by Esa1 is

important for H2A.Z deposition; (3) some genes depend more on H4 acetylation for factor recruitment than others, with the genes in cluster 1 showing the greatest dependency; (4) loss of Swr1 recruitment by inactivation of Esa1 does not necessarily lead to loss of H2A.Z deposition.

3.3.4 Bdf1 is not required for TFIID recruitment

Up to this point, we have seen that H4 acetylation by Esa1 is important for recruitment of TAF1, Bdf1, and Swr1 to promoters. Our current model for transcription initiation proposes that the link between nucleosome acetylation and the recruitment of general transcription complexes, like TFIID, is bromodomain factors. In the case of TFIID, the bromodomain subunit, Bdf1, is thought to help stabilize TFIID binding at nucleosome-containing promoters. If this is true, our model in Figure **3.1** predicts that the effects on TAF1 recruitment in a *bdf1Δ* strain should mirror those observed in the *esa1-414* strain. Surprisingly, the data in column 1 of Figure **3.4** shows that loss of Bdf1 had no effect on TAF1 binding overall. This result can be explained two different ways. One interpretation is that the dependency of TAF1 on H4 acetylation is completely unrelated to the bromodomain. In other words, the loss of TAF1 binding in an *esa1-414* strain could be the result of chromatin compaction due to a general decrease in acetylation, etc. An alternative explanation might be that TAF1 does not uniquely depend on Bdf1 for recruitment to acetylated promoters.

3.3.5 Bdf2 is recruited to TFIID-dominated promoters in the absence of Bdf1

The finding that TAF1 recruitment was virtually unaffected in a *bdf1Δ* strain was somewhat surprising given the large amount of evidence linking Bdf1 and TAF1 together. Previous studies have hinted at the possible functional redundancy between Bdf1 and Bdf2, leading us to wonder if Bdf2 might be compensating for loss of Bdf1 (Matangkasombut et al., 2000; Matangkasombut and Buratowski, 2003). These proteins share a large degree of structural homology, and over expression of Bdf2 suppresses the temperature sensitivity in *bdf1Δ* (Matangkasombut et al., 2000). While these pieces of evidence strongly suggest that Bdf2 can functionally compensate for Bdf1, this model has not been specifically tested.

To see if maintenance of TAF1 binding coincided with increased recruitment of Bdf2, changes in Bdf2-TAP binding were measured in a *bdf1Δ* strain compared to *WT*. Figure **3.4**, column 10, shows that Bdf2 occupancy changes at a significant number of promoters upon the loss of Bdf1. Interestingly, we observe both increases (cluster 1) and decreases (cluster 3, 4) in Bdf2 binding genome-wide. This general result indicates that in the absence of Bdf1, Bdf2 is leaving some promoters and being re-distributed to others. Analysis of Bdf2 occupancy in a *WT* strain (Figure **3.6**) indicates that Bdf2 is already present at cluster 1 genes. Given that both Bdf1 and Bdf2 are present at cluster 1 genes, the increase in Bdf2 binding could be simply due to the loss of competition by Bdf1. However, the loss of Bdf2 binding at cluster 3 genes does suggest that Bdf2 might also be redistributed to "higher priority" genes, such as those in cluster 1. The preferential recruitment of Bdf2 to cluster 1 genes, which are dependent on H4-Ac for

factor recruitment, further supports our model that histone acetylation plays an important role in PIC assembly through the recruitment of bromodomain proteins, such as Bdf1 or Bdf2.

3.3.6 Bdf1 is not required for SWR-C recruitment, but is required for H2A.Z deposition

The results presented so far have shown that H4 acetylation is important not only for TFIID recruitment, but also for SWR-C recruitment and H2A.Z deposition. Since Bdf1 has been shown to associate with SWR-C and is important for proper H2A.Z deposition, it seemed natural to assume that Bdf1 might help recruit SWR-C to nucleosome-bound promoters, shown in the model in Figure **3.2**. To test this part of the model, I started out by looking at the changes in H2A.Z-TAP deposition in a *bdf1Δ* strain. Consistent with previous findings (Zhang et al., 2005), loss of Bdf1 resulted in a loss of H2A.Z deposition (Figure **3.11**, Figure **3.4**, column 4). Furthermore, comparison of the H2A.Z-TAP chIP experiments in the *bdf1Δ* and *esa1-414* strains shows a significant amount of correlation between the two experiments, suggesting that the defects in H2A.Z deposition resulting from inactivation of Esa1 are linked to the loss of Bdf1 recruitment.

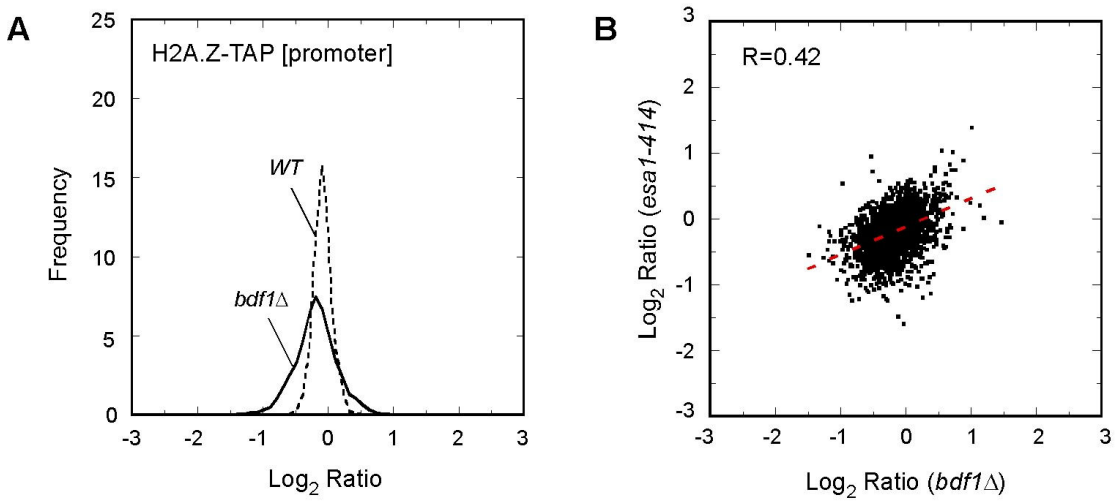

Figure 3.11**: Bdf1 is involved in H2A.Z deposition, possibly linked to H4 acetylation**

(A) Log ratios for the homotypic and H2A.Z-TAP *bdf1*Δ data sets were binned and plotted as a frequency distribution as described in Figure 3.7.
(B) Changes in factor occupancy for all promoter-containing regions in the H2A.Z-TAP *bdf1Δ* and H2A.Z-TAP *esa1-414* experiments were plotted as described in Figure **3.8**.

So far, we have seen that the loss of H4 acetylation or Bdf1 results in a loss of H2A.Z deposition at select promoters. To test if this defect in H2A.Z deposition was due to an inability to recruit SWR-C to promoters, the changes in Swr1-TAP occupancy were assessed in the *bdf1Δ* strain. Surprisingly, loss of Bdf1 had little to no effect on Swr1 recruitment (Figure **3.4** and Figure **3.5**, column 9). This result indicates that Bdf1 is expendable for SWR-C recruitment, but required for H2A.Z deposition. Evidence presented in section **3.3.5** indicated that Bdf2 binding increases at select promoters in the absence of Bdf1. Applying this result to the observations seen in the *bdf1Δ* Swr1-TAP and *bdf1Δ* H2A.Z-TAP experiments, we could make a prediction that Bdf2 is still allowing for recruitment of SWR-C to promoters, but it not sufficient for H2A.Z deposition. However, this hypothesis would predict that genes where both Bdf1 and

Bdf2 are absent, like those in cluster 2, should be defective in SWR-C recruitment. We instead see that loss of Bdf1 (Figure **3.4**, experiment 9) and lack of increased Bdf2 binding (Figure **3.4**, experiment 10) does not affect Swr1 binding. Thus, these results suggest that SWR-C recruitment and H2A.Z deposition do not follow one universal mechanism.

These *bdf1Δ* chIP-chip experiments suggest the following general conclusions: (1) Bdf1 is not uniquely required for TFIID or SWR-C recruitment to promoters; (2) Bdf1 is important for H2A.Z deposition; (3) loss of Bdf1 and Esa1 results in similar patterns of H2A.Z localization, suggesting that the role of Esa1 in H2A.Z deposition might be linked to Bdf1.

3.3.7 TFIID is not required for Bdf1 recruitment to promoters

Studies characterizing the TFIID complex and other PIC components have not reached a consensus as to the sequential order of factor/complex assembly at promoters, assuming that only one mechanism of PIC formation exists. Equally undecided is the degree to which Bdf1 associates with the other subunits of TFIID. Purification of the intact TFIID complex does not always co-purify with Bdf1, suggesting that Bdf1 might not always associate with TFIID (Auty et al., 2004). This leaves open the question of how much of a role Bdf1 plays in TFIID function. From the above chIP-chip studies, we have seen that loss of Bdf1 does not significantly affect the recruitment of the TAF1 subunit of TFIID. We can also ask the opposite question: does Bdf1 require TFIID for promoter association? Studies have suggested that Bdf1 has other functions independent

from its role as a subunit of TFIID, so it is unlikely that loss of TFIID would affect Bdf1 recruitment at all promoters (Ladurner et al., 2003). Interestingly, the results from the BDF1-TAP *taf1*^{ts2} experiment (Figure **3.4** and Figure **3.5**, column 8) show that Bdf1 binding is unaffected when TFIID function is compromised. This finding suggests that Bdf1 is capable of binding its target promoters without assistance from TFIID, and furthermore, suggests the possibility that Bdf1 might bind to promoters prior to TFIID recruitment.

3.3.8 The role of Esa1 in regulating gene expression is linked to Esa1-dependent recruitment of transcription factors

The observation that led me to explore the connection between Esa1 and TFIID was the observation of functional relationship between Esa1 and TAF1 in regulating gene expression. Presented in Chapter 2, we observed that genes with a higher dependency on Esa1 for gene expression had slightly less dependency on TAF1 for gene expression, and vice versa. The model proposed from this and other observations was that some genes were limited primarily by Esa1-mediated histone acetylation, where as others were more limited by the recruitment of TAF1. Applying this model to the clusters in Figure **3.4**, we can predict that genes with a greater dependency on Esa1/H4 acetylation for factor recruitment, such as those in cluster 1, might also be the genes that show the greatest dependency on Esa1 for gene expression levels. To compare the *esa1-414* chIP and expression data sets, the T-H genes from each data set were sorted according to their change in gene expression. Using a 200-gene sliding window, the median log ratio of the chIP data was determined and plotted against the average log ratio of expression levels

(Figure **3.12**). A striking trend is visible in comparing the TAF1 and Bdf1 chIP data to the *esa1-414* expression data. The positive slope for the TAF1 and Bdf1 chIP data indicates that genes which decrease in expression upon loss of Esa1 also lose TAF1 and Bdf1, providing a nice explanation for the results observed in Figure **2.8**. In contrast, the Bdf2-TAP *bdf1Δ* data set shows an inverse correlation to the *esa1-414* expression data. In line with observations from the cluster plot in Figure **3.4**, Bdf2 is recruited to the genes that rely the most on H4 acetylation for factor recruitment and gene expression.

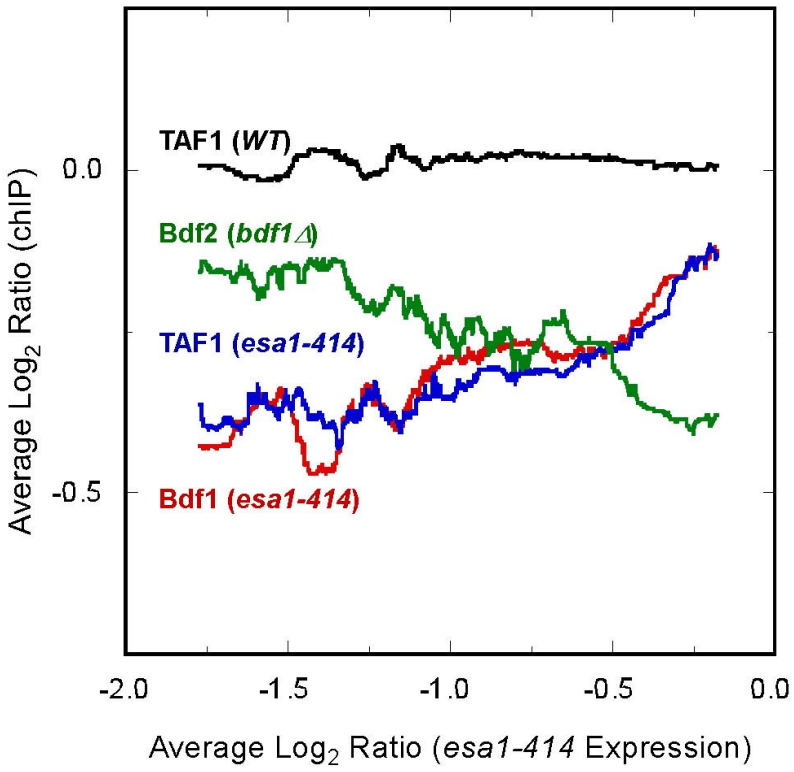

Figure 3.12**: Genes dependent on Esa1 for gene expression require Esa1 for Bdf1 and TAF1 recruitment**

Expression microarray data for *esa1-414* was aligned with the chIP-chip data for TAF1-TAP (WT), TAF1-TAP (*esa1-414*), Bdf1-TAP (*esa1-414*), and Bdf2-TAP (*bdf1Δ*). Intergenic regions assigned to only one ORF (T-H) were aligned with the corresponding expression data for that gene, and the data sorted according to the $\log_2$ change in gene expression. Moving-averages for a 200-gene window were measured for each chIP data set and plotted against the average expression ratio.

3.3.9 Further investigation of chIP-chip clusters by comparison with public microarray data

Clustering the chIP-chip data sets allows us to draw general conclusions based on the overall patterns observed within the various experiments. While this information is very valuable in answering the questions initially posed for this project, our conclusions are rather limited. To try and gather additional information on the genes included in each cluster, we can utilize published microarray data and gene information to see if there are certain characteristics enriched in the gene clusters. Characteristics that are over-represented in a cluster might lend additional information to the roles of TFIID, Bdf1, Esa1, SWR-C, and H2A.Z in gene regulation.

Two classifications that are helpful in discerning regulatory mechanisms are the SAGA vs. TFIID group and TATA-containing vs. TATA-less group. These categories were well-defined by our lab through genome wide studies. The SAGA/TFIID classification stemmed from a genome-wide expression study in TFIID and SAGA mutant strains; from this study, it was determined that ~90% of the genome is preferentially regulated by TFIID, and the other ~10% is SAGA-regulated (Huisinga and Pugh, 2004). These values are not meant to represent an "all-or-none" regulation principle, but rather give a guideline as to whether a group of genes is preferentially regulated by one complex over the other. The second group classifies genes based on the presence of a TATA box sequence in their upstream promoter. A consensus TATA-box sequence (TATA(A/T)A(A/T)(A/G)) was determined by comparing promoter sequences between various yeast species (Basehoar et al., 2004); approximately 20% of the genome was found to contain this TATA-box consensus sequence. Further characterization of

each of these gene properties has led to the definition of some general mechanisms of gene regulation, briefly reviewed in Chapter 1. Thus, by beginning with the classification of a cluster as enriched for TFIID/SAGA regulated genes and TATA-containing/TATA-less promoters, we can gain a quick idea of possible regulatory mechanisms for each cluster.

The percentage of SAGA-dominated and TATA-containing genes in each cluster was determined and reported in Table **3.2**. The majority of the clusters contain predominantly TFIID-regulated genes, with cluster 1 having less SAGA-dominated genes than expected and cluster 3 having more than expected. From this observation, it can be expected that cluster 3 genes would be regulated by many of the same mechanisms that have been previously described for SAGA-regulated genes (Huisinga and Pugh, 2004).

Table **3.2**: Classification of clusters as SAGA/TFIID dominated and TATA-containing/TATA-less

Cluster #	Value	% overlap	P-value
SAGA-dominated		**(10%)+**	
1	9*	3%	5.E-04
2	18	11%	4.E-01
3	22	20%	3.E-04
4	9	14%	2.E-01
TATA-containing		**(20%)+**	
1	50*	18%	1.E-01
2	31*	20%	6.E-01
3	38	35%	3.E-03
4	25	40%	3.E-03

* = under-represented

+ = expected overlap

Since SAGA-dominated genes are also typically characterized as containing a TATA box in their promoter region, it seems fitting that the representation of TATA-containing genes in each cluster matches up nicely with the representation of SAGA-dominated genes. For example, cluster 3 was enriched for genes regulated by the SAGA complex and is also enriched for genes with TATA boxes; the opposite is true for cluster 1 with respect to enrichment for SAGA-dominated genes. Thus, we can conclude in general that genes which depend the most on Esa1 and H4 acetylation for transcription factor recruitment typically lack TATA boxes and are preferentially regulated by the TFIID complex.

We have complied a database consisting of 613 chIP data sets, 336 expression data sets, and 234 gene groups/properties. For comparison purposes, the upper and lower extremes of each data set are extracted. I chose to use a 10% cutoff for each data set, thus isolating the top and bottom 10% from each data set. For genome wide chIP experiments where the occupancy of a given factor was measured, only the top 10% was used; the bottom 10%, or genes with the lowest factor occupancy, are not used because the lowest levels of factor occupancy can not truly be discerned from noise intrinsic to the experiment. The top and bottom 10% extremes of each data set are then compared to the series of genes included in each cluster. For each comparison, the number of genes present in both the database data set and the cluster are counted and the percentage overlap determined. This observed overlap value is compared to the number of genes predicted to exist in the cluster by chance. Using the CHITEST function in excel, a p-value is calculated for the observed and expected values, signifying the possibility that the number of genes overlapped between the two data sets could occur by chance. This

process was done with all of the database data sets and each of the four clusters in Figure **3.4**. The complete results of the analysis are included in Supplementary Table 1, with the highlights of the results presented in Tables **3.3** through Table **3.6**.

Table **3.3** shows the data sets with the strongest overlap to the genes included in cluster 1 of Figure **3.4**. General observations of cluster 1 from Figure **3.4** indicated that the genes in this cluster were positively regulated by histone H4 acetylation and were particularly dependent on bromodomain factors for transcription factor recruitment. Fitting with these conclusions, genes that were previously characterized as having high levels of histone acetylation, Bdf1, TFIID, Swr1, and H2A.Z occupancy were enriched in cluster 1. This observation also provides a nice validation of the results from our experiments. However, this information does not provide any new insight into the genes regulated by these mechanisms. I also utilized other databases through the SGD (Saccharomyces Genome Database), such as GO terms, to try and figure out what these genes might have in common functionally; however, this relationship also did not provide any useful information.

Table **3.3**: Cluster 1 overlap analysis

Cluster 1				(1.0%)
Category	Protein/Characteristic	Pvalue	Value	% OVERLAP
High Occupancy Levels				
Nucleosome	H2B	5.E-06	50	1.9%
	H3	6.E-06	50	1.9%
	H3 Ac-K9, 14	3.E-05	46	1.9%
	H3 Ac-K9,14	2.E-08	55	2.0%
	H4 Ac-K5, 8, 12, 16	1.E-10	57	2.3%
H2A.Z	Htz1	2.E-08	45	2.2%
	Htz1 (25°C)	5.E-06	36	1.7%
	Htz1 (37°C, 30min)	3.E-04	32	1.5%
	Htz1 (37°C, 30min)	3.E-04	32	1.5%
	Htz1 (bdf2Δ)	5.E-04	30	1.5%
	Htz1 (Diauxic Shift T=0)	2.E-06	44	2.0%
	Htz1 (Diauxic Shift T=8hr)	4.E-07	35	1.9%
	Htz1 (gcn5Δ)	5.E-04	25	1.3%
	Htz1 (H3K14G)	2.E-15	48	2.6%
	Htz1 (H3K14Q)	1.E-11	37	2.3%
	Htz1 (H3K14R)	6.E-13	45	2.2%
	Htz1 (H3K4A)	4.E-05	30	1.6%
	Htz1 (H3K4R)	7.E-09	39	2.1%
	Htz1 (H4K16Q)	5.E-07	33	1.8%
	Htz1 (H4K16R)	4.E-11	41	2.3%
	Htz1 (H4K5,12R)	1.E-05	34	1.9%
	Htz1 (H4K8,16R)	2.E-12	51	2.6%
	Htz1 (HS Recovery)	5.E-05	30	1.6%
	Htz1 (sas3Δ)	3.E-06	36	1.8%
	Htz1 (set1Δ)	1.E-07	33	1.7%
Nucleosome remodelers	RSC	5.E-05	45	1.9%
	Swr1 (SWR-C)	7.E-11	28	1.5%
General Transcription Factors	SAGA (Spt3)	4.E-09	50	2.3%
	Bdf1	1.E-06	39	2.2%
	TFIID (TAF1)	3.E-04	40	1.8%
DNA Motif	Known: ABF1	2.E-04	50	1.8%
Low Occupancy Levels				
Nucleosome	H3 K36-Me2	9.E-04	5*	0.3%

* = under-represented

Genes included in cluster 2 were observed to be less dependent on histone acetylation for the recruitment of general transcription factors, but lost H2A.Z more easily than the genes included in the other clusters. Fitting with this observation, Table **3.4** shows a strong overlap between cluster 2 genes and genes that have been characterized as having high levels of H2A.Z occupancy. Interestingly, cluster 2 genes also had a significant overlap with genes that are known to gain TAF1 upon heat shock. Since TAF1 was not severely affected by the loss of Esa1 or Bdf1 at these genes, this observation suggests that these genes are still regulated by TFIID, but can recruit TFIID independently of histone acetylation and/or bromodomain factors. Thus, cluster 2 genes

might represent genes with an acetylation independent mechanism for gene activation. It is also interesting to note that cluster 2 genes are enriched for genes that recruit a variety of sequence-specific activators, listed in Table **3.4**. Thus, it is possible that these activators utilize a mechanism of recruitment that is less dependent on histone H4 acetylation.

Table **3.4**: Cluster 2 overlap analysis

Cluster 2				**(0.6%)**
Category	**Protein/Characteristic**	**Pvalue**	**Value**	**% OVERLAP**
High Occupancy Levels				
Sequence-specific activators	AFT2	8.E-06	18	1.7%
	IME1	7.E-04	13	1.5%
	GAL4	2.E-04	16	1.5%
	AFT2	6.E-06	17	1.5%
	HSF (39°C)	2.E-04	3	3.7%
H2A.Z	Htz1 (WT)	3.E-08	24	1.3%
	Htz1 (bdf2Δ)	7.E-04	19	0.9%
	Htz1 (H4 K16Q)	3.E-04	18	1.0%
SAGA	Spt3 (Gain upon HS)	1.E-07	31	1.4%
TFIID	TAF1 (Gain upon HS)	2.E-09	34	1.5%
	TBP (Gain upon HS)	3.E-09	33	1.5%
Top 10% Expression				
	Gcn5 HAT activity (gcn5(KQL))	9.E-04	26	1.1%
	Environmental Stress (9.5 hrs Diauxic Shift)	3.E-05	27	1.3%
	Mediator (med12Δ)	2.E-04	30	1.1%
	NC2 (bur6-1)	8.E-04	24	1.1%
Bottom 10% Expression				
	Acid Stress (msn2/4 acid 20')	5.E-05	25	1.3%
	hat2	6.E-04	28	1.1%
Group				
	Common Environmental Stress Response Induced	4.E-04	20	0.7%

Table **3.5** shows the data sets with the strongest overlap to the genes in cluster 3. Cluster 3 genes were largely undefined with the exception that they appeared to be occupied by Bdf2 under normal conditions; upon loss of Bdf1, Bdf2 levels were decreased at these genes, perhaps being redistributed to other genes, such as those in cluster 1. Like the genes in cluster 2, cluster 3 genes also appear to be highly regulated by a variety of sequence specific activators. In addition, this cluster is positively regulated by the mediator, Spt3, and TBP. There is also a slight enrichment for SAGA-dominated genes in this cluster, suggesting that these genes probably utilize regulatory

mechanisms alternative to TFIID. This is further evidenced by the overlap between cluster 3 genes and TAF1-independent genes (top 10% of multiple *taf1*^{ts2} data sets).

Table **3.5**: Cluster 3 overlap analysis

Cluster 3				**(0.4%)**
Category	**Protein/Characteristic**	**Pvalue**	**Value**	**% OVERLAP**
High Occupancy Levels				
Sequence-specific activators	CIN5	9.E-08	15	1.4%
	INO4	7.E-05	11	1.0%
	PHD1	2.E-04	11	1.0%
	SWI4	1.E-04	12	1.1%
	ASH1	7.E-09	29	1.1%
	YAP6	3.E-04	22	0.8%
	MSN2 (Acidic medium)	5.E-05	12	1.1%
	SOK2 (Filamentation inducing)	3.E-04	11	1.1%
	MBP1 (High hypoxic)	6.E-04	10	0.9%
	ROX1 (High hypoxic)	6.E-05	11	1.0%
General transcription factors	Mot1 (Gain upon HS)	3.E-05	18	1.0%
	Bdf1 (Gain upon HS)	2.E-15	33	1.4%
Nucleosome	H4 Mononucleosomes (Mnase Dig.)	9.E-04	16	0.9%
Top 10% Expression				
	Mediator (cyccΔ)	2.E-04	23	0.8%
	Mediator (med13Δ)	5.E-05	24	0.9%
	SSN6-TUP1 (tup1Δ)	9.E-05	23	0.9%
	SWI/SNF (swi1Δ MM)	4.E-05	24	0.9%
	taf1 (TANDΔ)	6.E-04	21	0.8%
	taf1-ts2 gcn5(KQL)	8.E-04	20	0.8%
	taf1-ts2 gcn5Δ	4.E-04	21	0.8%
Bottom 10% Expression				
	Environmental Stress (9 hrs Diauxic Shift)	4.E-04	11	1.1%
	Mediator (med18Δ)	7.E-04	22	0.8%
	Mediator (med2Δ cdk8Δ)	7.E-04	22	0.8%
	Mediator (med2Δ)	2.E-07	28	1.0%
	Mediator (med31Δ)	6.E-05	24	0.9%
	Mediator (med9Δ)	7.E-04	22	0.8%
	SAGA (spt3(E240K))	3.E-04	21	0.9%
	SWI/SNF (swi2(K798A))	7.E-04	19	0.9%
	GAL-TBP(V161E)	5.E-04	21	0.8%
	GAL-TBP(V161E) + (V71E)	8.E-04	21	0.8%
	GAL-TBP(V161E/V71E) ave	8.E-04	21	0.8%
	GAL-TBP(V161R[L])	8.E-04	21	0.8%
Group				
	Cell Cycle; G1 phase	7.E-04	14	0.5%
	Mating-specific gene	8.E-04	8*	0.3%
	Rap1 ChIP; non Ribosomal protein	2.E-06	12	0.4%
DNA Motif	Novel: Phd1	5.E-04	9*	0.3%
	Novel: SOK2	8.E-05	9*	0.3%

* = underrepresented

Table **3.6**: Cluster 4 overlap analysis

Cluster 4				(0.2%)
Category	Protein/Characteristic	Pvalue	Value	% OVERLAP
High Occupancy Levels				
Nucleosome	H2A (HS Recovery)	2.E-04	7	0.3%
	H2B	9.E-04	14	0.5%
	H3	1.E-04	12	0.6%
	H3	8.E-04	12	0.6%
	H3 Ac-K9, 14	2.E-07	18	0.7%
	H3 Ac-K9, 14	4.E-04	15	0.6%
	H4	1.E-04	13	0.6%
	H4 (37ºC)	2.E-10	19	0.9%
	H4 Ac-K16	3.E-04	8	0.8%
	H4 Ac-K5, 8, 12, 16	5.E-12	22	0.9%
SAGA/ADA	Gcn5	1.E-04	12	0.5%
Sequence-specific regulator	GAL4	5.E-04	13	0.6%
	GAT3	6.E-05	16	0.6%
	Ifh1	3.E-05	14	0.7%
	MAL13	1.E-04	15	0.6%
	RAP1	2.E-06	14	0.5%
	RGM1	7.E-06	17	0.7%
SIR proteins	Sir2 ORF	3.E-08	12	0.5%
	Sir3-Myc (WT)	9.E-08	19	0.7%
	Sir3-Myc (yaf9Δ)	1.E-10	19	0.9%
Top 10% Expression				
	Mediator (med2Δ CyccΔ)	9.E-05	16	0.6%
Nucleosome	H3Δ(1-28)	2.E-05	16	0.6%
	H4 dep. (1 hr)	6.E-04	14	0.6%
	H4 dep. (1.5 hr)	2.E-05	16	0.6%
	H4 dep. (2 hr)	6.E-04	14	0.6%
	H4 dep. (4 hr)	3.E-05	16	0.6%
	H4 dep. (6 hr)	2.E-04	15	0.6%
	H4 K8, 12, 16R	1.E-04	15	0.6%
	H4Δ2-26 #1	3.E-04	15	0.6%
	H4Δ2-26 #2	9.E-06	17	0.6%
	hht2 (K4,9,14,18,23,27G)	2.E-09	21	0.8%
Histone Methyltransferase	set1Δ	3.E-08	20	0.7%
SWI/SNF	snf2Δ MM	9.E-05	16	0.6%
	snf2Δ YPD	6.E-05	16	0.6%
	swi1Δ MM	4.E-04	15	0.6%
TBP	KV	3.E-05	9	0.8%
	KVF	4.E-05	10	0.8%
HDAC	rpd3 H3Δ1-28	2.E-07	19	0.7%
Bottom 10% Expression				
	19 hrs Diauxic Shift	3.E-05	**17**	0.6%
	Mediator (cdk8Δ)	1.E-04	16	0.6%
	Mediator (med12Δ)	5.E-04	15	0.6%
	Mediator (med13Δ)	5.E-04	15	0.6%
	Mediator (srb4-138 ts)	1.E-04	11	0.7%
SAGA	SAGA (spt3(E240K))	2.E-06	17	0.7%
	SAGA (spt3Δ)	8.E-09	20	0.8%
TFIID	TFIID (taf1-ts2)	8.E-04	14	0.6%
	TFIID (taf1-ts2 spt3Δ)	3.E-05	16	0.6%
	TFIID (taf1-ts2 spt3 (E240K))	6.E-04	14	0.6%
	TFIID (taf9-ts2)	6.E-04	8	0.7%
Feature				
	intron internal to ORF	8.E-04	8	0.3%
Group				
	DNA element = SBF site	1.E-04	5	0.2%
	HAST domain (AcH3-K18 hda1Δ)	4.E-08	8	0.3%
	Sporulation temporal class; late	1.E-10	1*	0.0%

* = underrepresented

Cluster 4 genes showed significant overlap with a larger group of characteristics compared to the other clusters. The genes included in cluster 4 were largely uncharacterized from the cluster plot in Figure **3.4**. A general observation from the pattern in cluster 4 is that these genes are largely unaffected in each of the experiments tested. The only experiment that did show a significant change in occupancy levels was the H2A.Z *bdf1Δ* experiment, where H2A.Z occupancy increased upon loss of Bdf1. Looking at the results from the overlap analysis, it appears that genes included in cluster 4 have high histone occupancy levels, but also that histones H3 and H4 are playing a negative role in gene regulation. These genes are also negatively regulated by the Rpd3 histone deacetylase and the SWI/SNF remodeling complex.

Bdf1 is also thought to be involved in mediating heterochromatin boundaries in subtelomeres (Ladurner et al., 2003). Without Bdf1, a complex of SIR proteins spreads into the subtelomeres, resulting in decreased levels of gene expression. One possibility is that in the process of shutting down gene expression in the subtelomeres coincides with deposition of H2A.Z nucleosomes. The enrichment of HAST domain genes in cluster 4 fits with this model that H2A.Z is being deposited in subtelomeric regions upon loss of Bdf1.

3.4 Discussion

This project began with a goal of addressing specific questions regarding transcription activation at TFIID-regulated promoters. The questions posed were: (1) Is H4 acetylation by Esa1 important for TFIID recruitment? (2) Does Bdf1 act as a link between TFIID and Esa1? (3) Is the role of Bdf1 in H2A.Z deposition related to SWR-C recruitment? (4) Is SWR-C recruitment and H2A.Z deposition dependent on histone H4 acetylation by Esa1? (5) Is the lack of cellular dependence on Bdf1 due to functional compensation by Bdf2? Circumstantial evidence in the published literature suggests what the answers to each of these questions should be; however, we can never be sure that the predicted answers will match the experimental answers. Thus, I sought to address these unaddressed questions from a genome-wide approach using chIP-on-chip technology. While the resulting answers help validate some previously proposed models regarding TFIID-gene regulation, they also open up a new set of questions that can be addressed in the future.

3.4.1 Esa1-mediated H4 acetylation is an important precursor for Bdf1 and TFIID recruitment to promoters

Acetylation has long-since been associated with transcriptionally active genes; however, the mechanisms that directly link histone acetylation and transcription activation have not been investigated completely. A good example of this is the Esa1 protein. Previous studies of this histone H4 HAT have revealed a role for Esa1 in the

regulation of ribosomal protein genes, repair of DNA double-strand breaks, and cell cycle progression (Allard et al., 1999; Clarke et al., 1999; Reid et al., 2000). A relationship between Esa1 and TFIID was uncovered by accident through genome-wide expression studies, presented in Chapter 2. However, this important finding became the catalyst for additional ideas that allowed for further exploration of the mechanisms of TFIID recruitment.

Given that Esa1 is known to be a primary histone H4 acetyltransferase *in vivo*, it has been logically assumed that the link between H4 acetylation and TFIID-mediated gene activation was due to an intermediary factor: the bromodomain protein, Bdf1. I have presented evidence here that H4 acetylation is crucial for recruitment of Bdf1 and TAF1 to significant number of promoters genome-wide. However, this finding is not meant to be generalized to all TFIID-regulated genes, which comprise ~90% of the genome (Huisinga and Pugh, 2004). Gene expression studies presented in Chapter 2 showed that not all genes are equally dependent on Esa1 for gene expression (Figure **2.8**). This result fits well with the results from the chIP-chip studies presented here, showing that not all genes are dependent on Esa1 for TAF1 recruitment. Furthermore, the strong correlation between the chIP-chip and expression experiments (Figure **3.12**) adds further evidence that dependency on Esa1 for gene expression is linked to TAF1 and Bdf1 recruitment. These observations allow for the validation of a model previously proposed in Chapter 2 whereby Esa1 contributes to gene expression by establishing an acetylation pattern at promoter nucleosomes, leading to the recruitment of Bdf1 and TFIID (Figure **3.13**).

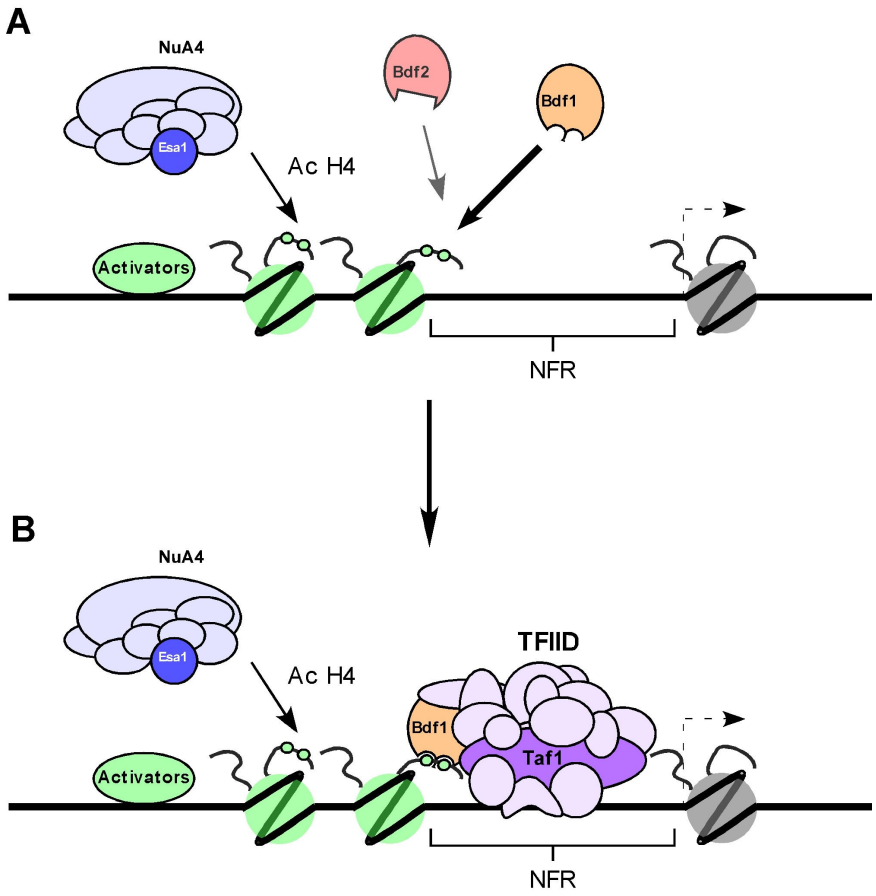

Figure 3.13**: Model for TFIID Gene Regulation by Esa1, Bdf1**

(A) NuA4 is recruited to promoters to hyper-acetylate histone H4 tails in nucleosomes. Bdf1 and Bdf2 are both capable of binding to nucleosome tails, but binding by Bdf1 is favored over Bdf2. (B) Binding of either bromodomain factor then allows for association of Bdf1 with TFIID, and Bdf1 might work to help position TFIID in the nucleosome free region. Loss of Bdf1 allows for less competition between the bromodomain factors, allowing for increased binding of Bdf2 to promoters and recruitment of TFIID.

The next step in developing this model would be further exploration of the characteristics of cluster 1 genes that result in their dependency on Esa1 and H4 acetylation for PIC assembly. Some recent findings of chromatin organization at promoters might give us a clue for which leads to follow in future explorations. Breakthrough studies from two groups showed that promoters are not entirely covered by nucleosomes, but actually contain a sizeable nucleosome-free region of DNA (NFR)

(Pokholok et al., 2005; Yuan et al., 2005). Interestingly, this NFR region is found ~200bps upstream of the transcription start site, the location where TFIID is expected to bind. While this region is large enough to allow for binding of some factors, it is not large enough for the assembly of an entire PIC. This observation fits with the hypothesis that bromodomain factors, such as Bdf1, might be important for the recruitment and/or stabilization of large transcription complexes at promoter DNA. It is also important to note that the location of the NFR relative to the transcription start site is not identical at every gene tested. With these observations in mind, we can postulate that the location of the NFR and the position of the surrounding nucleosomes might dictate the level of Esa1 dependency for TFIID recruitment and gene expression. Furthermore, we can use this model to predict which nucleosomes might be targeted for histone H4 acetylation and Bdf1 binding. All of these proposed ideas will warrant further testing, but the results could really expand what we currently know about TFIID-mediated gene activation.

3.4.2 Bromodomain factors are an important link between histone acetylation and TFIID recruitment

Strong evidence has previously suggested that Bdf1 functions to help TFIID bind to nucleosome-containing promoters. In higher eukaryotes, the Bdf1 gene is even fused to the C-terminus of the TAF1 gene to permanently incorporate bromodomains into TFIID, hinting at an important function for the Bdf1 protein in yeast (Matangkasombut et al., 2000). However, evidence presented here shows that loss of Bdf1 has relatively little effect on the recruitment of TAF1 to promoters. While this finding is somewhat surprising, especially since heat shocking the cells should have aggravated the *bdf1Δ*

phenotype, the theory that Bdf2 substitutes functionally for Bdf1 can help rationalize this result. As expected, Bdf2 localization was found to be re-localized in a *bdf1Δ* strain, being preferentially recruited to the Esa1-dependent cluster 1 genes. While this result does not directly prove that the presence of Bdf2 allows for the maintenance of TAF1 recruitment, it does provide the first bit of *in vivo* evidence to support the Bdf1/Bdf2 hypothesis.

If Bdf1 is involved in the binding of TFIID to acetylated promoters, a new question arises regarding the order of recruitment. Bdf1 is fused to TAF1 in higher eukaryotes, suggesting that Bdf1 might function within the TFIID complex (Matangkasombut et al., 2000). However, loss of TFIID through inactivation of TAF1 shows no effect on the recruitment of Bdf1. This suggests that Bdf1 can bind to promoters independently of the TFIID complex, supporting a model whereby Bdf1 is recruited to hyper-Ac promoters prior to TFIID recruitment. The data presented here thus supports a model for cluster 1 genes (Figure **3.13**) where both Bdf1 and Bdf2 are preferentially present at H4 acetylated promoters, with Bdf1 occupancy being higher than Bdf2, followed by recruitment of TFIID. In the absence of Bdf1, more Bdf2 is able to assume the role of Bdf1 and can achieve higher occupancy levels due to a lack of competition from Bdf1.

Lastly, the results from the Bdf2-TAP *bdf1Δ* experiment also serve to identify a group of genes where Bdf2 might be found when both Bdf1 and Bdf2 are present in the cell. To date, little is known about the function of Bdf2 in a wild type strain, especially since a *bdf2Δ* strain shows few changes in gene expression and exhibits very minor phenotypes (Matangkasombut et al., 2000). Unfortunately, the chIP-chip and overlap

studies presented here do not shed much more light on the role of Bdf2 in a wild type strain aside from identifying a group of genes that might utilize Bdf2 for gene regulation.

3.4.3 Esa1 and Bdf1 are involved in SWR-C-mediated H2A.Z deposition via multiple mechanisms

At the onset of this study, little was known about the relationship between NuA4, H2A.Z, SWR-C, and Bdf1. Purification of the SWR-C complex co-purified Bdf1, although the association of Bdf1 with SWR-C appeared to be weak (Krogan et al., 2003). Bdf1 was also shown to be important for H2A.Z deposition on a genome-wide level, although details of how Bdf1 contributes to SWR-C function had not yet been elucidated (Zhang et al., 2005). The evidence linking NuA4 with SWR-C and H2A.Z was even more indirect. Each of the NuA4 and SWR-C complexes contain four identical subunits, suggesting some degree of functional overlap (Kobor et al., 2004; Krogan et al., 2004; Zhang et al., 2004). These observations led me to develop a simplistic model to explain the functional relationship between NuA4, Bdf1, SWR-C, and H2A.Z (Figure **3.2**). Since Bdf1 had been strongly implicated as an Ac-histone H4 binding protein, it seemed reasonable to suggest that SWR-C utilized Bdf1 to bind to H4-acetylated nucleosomes at promoters, resulting in deposition of H2A.Z.

Experiments testing the effects of SWR-C and H2A.Z occupancy upon loss of Esa1 support some aspects of the proposed model. Cluster 1 genes are the most dependent on Esa1 for factor recruitment, and similar to the results observed for Bdf1 and TAF1, Esa1 is also important for the recruitment of SWR-C at these genes. Based on the proposed model, the link between Esa1 and SWR-C at these genes might be Bdf1.

However, no evidence was found to support this hypothesis, as SWR-C recruitment was unaffected upon deletion of Bdf1. It is possible that lack of SWR-C dependency on Bdf1 could be the result of Bdf2 recruitment. However, this model does not explain all of the observations for cluster 1 genes. Comparing experiments 3 and 5, we see that loss of H4 acetylation via inactivation of Esa1 negatively affects SWR-C recruitment, but does not have much effect on H2A.Z deposition. This shows that the role of H4 acetylation in H2A.Z deposition is not due to recruitment of SWR-C, suggesting a SWR-C independent mechanism for H2A.Z deposition at cluster 1 genes.

Evidence from a number of other studies have previously hinted at the idea of multiple mechanisms for H2A.Z deposition. Kobor et al. (2004) showed that while *swr1Δ* and *htz1Δ* strains resulted in similar gene expression profiles, there were also a large number of genes that were distinctly affected in each strain. Thus, some genes were dependent on Swr1 and H2A.Z-independent, and vice versa. More recently, Keogh et al. (2006) and Millar et al. (2006) presented evidence that H2A.Z can be acetylated by Esa1 at multiple lysine residues and that these modifications are important for re-deposition of H2A.Z after transcription initiation. It was also found that acetylation of H2A.Z by Esa1 only occurred once H2A.Z was incorporated into chromatin by SWR-C (Babiarz et al., 2006; Keogh et al., 2006). Lastly, evidence has shown that H2A.Z deposition is linked to other HAT complexes, including Elp3 and Gcn5 (Raisner et al., 2005; Babiarz et al., 2006; Millar et al., 2006). All of these findings, in addition to the distinct patterns observed for cluster 1 and cluster 2 genes presented here, strongly suggest that multiple mechanisms exist for SWR-C recruitment and H2A.Z deposition.

Re-evaluation of the cluster patterns in Figure **3.4** with multiple H2A.Z deposition models in mind helps make the results easier to interpret. H2A.Z is clearly dependent on Esa1 for deposition at cluster 2 genes, yet these genes are not severely affected in SWR-C recruitment in the *esa1-414* strain. Thus, cluster 2 genes provide the best evidence to support the model of acetylation-dependent H2A.Z deposition presented by Millar et al. (2006). In contrast, cluster 1 genes show more of a role for Esa1 in SWR-C recruitment, possibly through the Bdf1/Bdf2 proteins; however, H2A.Z can be continually deposited in the absence of SWR-C, suggesting another Esa1-independent mechanism for H2A.Z deposition. Thus, while the starting model proposed in Figure **3.2** is still a possible explanation for H2A.Z deposition, it is clear from the results presented here that the mechanism of H2A.Z deposition is more complex than initially believed.

The more intriguing question now becomes why is Bdf1, and not SWR-C, required for H2A.Z deposition? Since little is known about how SWR-C functions to deposit H2A.Z, it is difficult to hypothesize what the answer might be. One attractive possibility is that Bdf1 might function within SWR-C to directly bind H2A.Z. Histone tail binding preferences of Bdf1 have for H2A.Z have never been addressed, so there is no evidence to refute this idea. This model would also help to explain why Bdf1 is not required for SWR-C recruitment, but is required for H2A.Z deposition.

The general conclusions from the results presented here suggest that Esa1 and Bdf1 are both involved in H2A.Z deposition in a potentially SWR-C independent manner. Furthermore, the data shows that SWR-C is dependent on Esa1 for recruitment to select promoters, perhaps requiring the presence of either Bdf1 or Bdf2 for binding to the nucleosome.

3.5 Materials and Methods

Yeast strains: A list of the strains used in this study is provided in Table **3.1**. To create the chromosomal ORF deletions, genomic DNA was from Open Biosystems yeast deletion strains. Each Open Biosystems deletion strain had the entire ORF replaced by a Kanamycin resistance marker. PCR products extending ~250bp up and downstream of the desired ORF deletion were generated from the desired deletion strain and transformed into the target yeast strain; yeast colonies that had incorporated the deletion were isolated by selecting for Kanamycin resistance. Proteins were then tagged in the created yeast deletion strains in a manner similar to the genetic deletions. The C-terminal TAP tag and adjacent HIS3 marker in the Open Biosystems TAP-tag strains were amplified by PCR and incorporated into the WT or mutant yeast strains by homologous recombination. Yeast colonies expressing TAP-tagged proteins were initially selected on HIS – media and expression of the TAP tagged proteins were verified by western blot.

chIP on chip: Cultures were grown, crosslinked, and harvested as described in Chapter 2 (Durant and Pugh, 2006). The only exception was that cells expressing H2A.Z-TAP were crosslinked for 15 minutes instead of 2 hours. Data was filtered as previously described (Durant and Pugh, 2006).

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Chapter 4

Discussion and Conclusions

4.1 Summary of study

Histone acetylation has been associated with gene activation for half a century, but the mechanisms linking these two events together was not understood until the discovery of histone acetyltransferases about twenty years ago (Allfrey et al., 1964; Chicoine et al., 1987). Since the discovery of HATs, much work has been done to characterize these enzymes and understand the nature of their role in potentiating gene transcription. While our understanding of the general role of HATs has expanded, we have also learned that much still remains to be investigated regarding their effect on downstream initiation events.

The work presented in this thesis provides a better understanding of the involvement of multiple yeast HATs in gene regulation, particularly with respect to TFIID regulated genes. Bio-informatic assays, such as expression microarrays and chIP-chip microarrays, proved to be a powerful tool in studying the genome-wide contribution of multiple HAT proteins in gene regulation. One key finding from the studies presented in Chapter 2 was the conclusion that yeast TAF1 is not required for histone acetylation *in vivo*. Scientists in the transcription field have questioned the existence of yTAF1's HAT activity for years, and the experiments presented here finally provide an answer to this important question. The histone acetyltransferase studies in Chapter 2 also showed that many other characterized yeast HATs do not play a unique role in global histone

acetylation or gene expression. Again, this finding provides valuable information for future studies regarding histone acetylation. The histone code hypothesis has previously proposed that the multitude of cellular HATs is important for instituting the complex code needed to regulate gene activation (Strahl and Allis, 2000; Jenuwein and Allis, 2001). This would suggest that each HAT might make a unique contribution to the "code"; yet, we see from the work in chapter 2 that loss of most HAT proteins has virtually no effect on gene expression or bulk histone acetylation levels, indicating that perhaps more redundancy exists between these factors. Thus, it seems that more investigation is required for many of these HATs to better establish their true function *in vivo*.

In contrast, Gcn5 and NuA4 were confirmed as the major H3 and H4 HATs *in vivo* and contribute significantly to maintaining mRNA expression levels. Evidence linking Esa1-mediated gene regulation with the TFIID complex led to further investigation of the mechanism linking these two complexes together. From these studies, presented in Chapter 3, I found that H4 acetylation by Esa1 is an important precursor to the recruitment of multiple protein complexes, including TFIID, Bdf1, and the H2A.Z remodeling complex, SWR-C. Further investigation of these H4-Ac dependent genes revealed a mechanism for TFIID-mediated gene activation whereby recruitment of TFIID to acetylated promoters required the presence of either the Bdf1 or Bdf2 bromodomain factors. Bdf1 and Bdf2 have previously been suggested to possess the ability to functionally compensate for one another, yet this model has never been directly tested *in vivo*. The chIP-chip studies presented in Chapter 3 provide the first evidence for the additional recruitment of Bdf2 to acetylation-dependent genes when

Bdf1 is deleted. Lastly, I also present evidence of a role for Esa1 in SWR-C recruitment and H2A.Z deposition, albeit through multiple mechanisms which will require further study.

4.2 Yeast TFIID does not acetylate histone tails *in vivo*

Much speculation has existed surrounding the function of yeast TAF1 as a histone acetyltransferase. The lack of homology between higher eukaryotic TAF1 and other HAT proteins, in addition to poor sequence similarity between yeast TAF1 and other TAF1 homologs has led to speculation regarding the true nature of TAF1s involvement as a true HAT *in vivo*. Attempting to investigate the possible role of TAF1s HAT activity *in vivo*, I was unable to find evidence to support the existence of this function in yeast. This finding is important for the understanding of TFIID-mediated gene regulation, as it suggests that TFIID is not capable of chromatin remodeling or histone modification during gene activation. It is still tempting to suggest that TAF1 might possess acetyltransferase activity, but that its substrate might be a non-histone protein. Factor acetylation has been shown to play an important role in the activation of certain transcription factors, such as p53 in higher eukaryotes (Sterner and Berger, 2000). Thus, investigation is still open for TAF1 as a factor acetyltransferase.

4.3 Bromodomain factors: The direct link between histone acetylation and transcription factor recruitment

Bdf1 has been previously shown to track with occupancy changes of TAF1, but the evidence presented here links the dependence of TAF1 and Bdf1 binding at promoters to Esa1-mediated H4 acetylation (Zanton and Pugh, 2004). While the chIP-chip data presented in Chapter 3 does not directly attribute the loss of Bdf1 binding to its inability to bind hypo-acetylated histone H4 tails, prior evidence has shown preferential binding of Bdf1 to hyper-Ac histone H4 tails *in vitro* (Ladurner et al., 2003; Matangkasombut and Buratowski, 2003). Thus, it seems likely that loss of Bdf1 binding in the *esa1-414* strain is directly due to the loss of a binding site for the bromodomain.

Much speculation has previously existed regarding the relationship between Bdf1 and Bdf2. From the chIP-chip studies presented here, I find the first indication that Bdf2 recruitment is re-directed upon deletion of Bdf1. The idea of a functionally redundant relationship between Bdf1 and Bdf2 has been proposed before, but no evidence has been provided to directly support this model until now (Matangkasombut et al., 2000). However, while the chIP-chip data provides better support for the Bdf1/Bdf2 functional redundancy model, it does not help us better understand the native role of Bdf2. Little is known about the role of Bdf2 apart from Bdf1, and because deletion of Bdf2 does not provoke much of a phenotype, it has been difficult to study this protein. We do know that Bdf1 and Bdf2 have different acetylation substrate binding affinities, so the delocalized occupancy of Bdf2 indicated by the chIP-chip experiments might be simply due to a lack of binding preference (Matangkasombut and Buratowski, 2003). The intriguing question that still remains is why does the cell contain two bromodomain

proteins with such similar functions? Thus, much still remains to be explored regarding the roles of Bdf1 and Bdf2 in gene regulation.

Recent studies mapping nucleosome positioning at promoters genome-wide have reported the existence of a nucleosome-free region just upstream of the transcription start site (Yuan et al., 2005). With the average size of a NFR being ~150bp, it would be difficult to assemble the entire PIC at the promoter. Thus, it is an attractive idea that bromodomain factors might bind to a nucleosome that borders the NFR to help assemble additional transcription factor complexes at the NFR. Newer technology, such as tiling arrays, have the capability of distinguishing between different promoter regions; these more sensitive assays will be valuable for future studies regarding the nature of PIC assembly at these structurally defined promoters.

4.4 Future directions

The work presented in this thesis has attempted to provide a better understanding of the complex mechanisms involved in initiating gene transcription at nucleosome bound promoters. While the studies conducted within the realm of these projects present a clearer picture of how histone acetylation is involved in TFIID-mediated gene activation, it is obvious that these findings and models do not apply to every gene in the genome. In particular, the chIP-chip studies presented in Chapter 3 show the complex nature of gene regulation, as deletion of one factor does not have the same effect on every gene across the genome. For this thesis, I chose to focus on complexes and factors that were strongly suggested to be involved in regulating the TFIID complex, but there are

still a multitude of factors and pathways yet to be explored. The intricate nature of the results obtained from chIP-chip experiments emphasizes the need to study the involvement of transcription factors in regulating the entire genome, rather than just a handful of genes. If by chance only genes in one cluster had been studied, a model would have been developed that, while true for those genes, does not necessarily apply to the rest of the genome. Thus, these genome-wide assays should be utilized for every known pathway. Applying the methods used here to other regulatory complexes, such as SAGA, Elongator, chromatin remodelers, etc., would provide valuable insight into the inter-relationships between protein complexes and significantly contribute to the understanding of gene regulation on a global level.

Looking more specifically at the TFIID pathway studied here, many questions still remain regarding the roles of various transcription factors, such as the bromodomain factors, Bdf1 and Bdf2. While the experiments in Chapter 3 contribute additional information regarding the role of these two proteins in TFIID-mediated gene activation, it is also evident that the functions of Bdf1 and Bdf2 extend beyond the realm of TFIID. The lack of information regarding Bdf2 provides an open area for future exploration. What is the role of this protein aside from its relationship with Bdf1? If Bdf2 really is just a "backup" for Bdf1, in that it does not have a function distinct from Bdf1, then why does the cell take the trouble to have two functionally identical bromodomain factors around? It would also be interesting to know what other factors or complexes Bdf2 co-purifies with in the presence and/or absence of Bdf1. We already know that over-expression of Bdf2 results in its association with TFIID, but what about other complexes,

such as SWR-C? These pieces of information would aid in the better understanding of how these bromodomain factors work to regulate gene transcription.

The work pertaining to the SWR-C complex and H2A.Z in Chapter 3 also left room for many questions that would warrant further investigation. Previous studies, in addition to the data presented here, provide a strong case for multiple mechanisms of H2A.Z deposition. As this particular area of research is still under intense investigation, as much still remains to be learned regarding the mechanism of H2A.Z deposition and the true purpose of H2A.Z at promoter nucleosomes.

From a broader perspective, it is important to ponder how the findings reported in this thesis relate to the histone code hypothesis. The histone code proposed that the role of histone modifications in gene regulation was a complex network of code combinations to be "read" by the cell. However, data presented by other labs suggest that perhaps the involvement of histone acetylation is not so complicated. While the results and models presented in this work do not refute this idea, they do seem to favor a less complicated model of gene regulation by histone modification. The question of the histone code is still largely open-ended, and future investigations in the field of chromatin remodeling will provide a better understanding of the complex process of gene regulation.

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