Identification of non-histone acetylation targets in *Saccharomyces cerevisiae*

by

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ABSTRACT

Lysine acetylation is a conserved post-translational modification (PTM) which was traditionally believed to be limited to histones and the regulation of gene expression. However, recent proteomic studies have identified lysine acetylation on proteins implicated in virtually all cellular processes indicating that this PTM plays a global regulatory role. Indeed, in humans, aberrance of lysine acetyltransferase (KAT) activity is associated with various pathogenesis. To date, over 2500 human proteins are known to be acetylated *in vivo*, but very few acetylations have been linked to specific KATs. Hence, to understand the biological relevance of KATs and acetylation in human pathology, it is important to learn about the mechanism regulating KAT activity and the identity of their *in vivo* targets. This is a complex task and will require the use of model organisms and system biology approaches. The work presented here explores the significance of self-acetylation in regulating KAT function by focusing on the highly NuA4 lysine acetyltransferase in the model organism *Saccharomyces cerevisiae* or budding yeast. Using genetics and biochemical assays I have identified NuA4 subunit Epl1 as a novel *in vivo* NuA4 substrate. I have also shown that Epl1 acetylation regulates NuA4 function at elevated temperatures. In an attempt to identify new biological processes regulated by yeast KATs and putative novel substrates, I have also performed a genome-wide synthetic dosage lethality screen with six non-essential yeast KATs; Hat1, Rtt109, Hpa2, Sas3, Sas2, and Elp3. My screen identified largely distinct sets of genetic interactions for each KAT suggesting that each KAT has specific cellular functions. Together, this study demonstrates the importance of auto-acetylation in regulating KAT function and the diversity of cellular processes impacted by KAT activity *in vivo*.

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and

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LIST OF ABBREVIATIONS

ACT1	Actin
ARP4	Actin Related Protein
ATM	Ataxia Telangiectasia Mutated
ChIP	Chromatin Immunoprecipitation
DNA	Deoxyribonucleic Acid
EAF1	Esa1-associated factor 1
EAF3	Esa1-associated factor 3
EAF5	Esa1-associated factor 5
EAF6	Esa1-associated factor 6
EAF7	Esa1-associated factor 7
ECO1	Establishment of cohesion 1
EPL1	Enhancer of Polycomb Like
ESA1	Essential SAS Family Acetyltransferase
ELP3	Elongator Protein 3
GNAT	Gcn5-related N-acetyltransferase
HAT	Histone Acetyltransferase
HU	Hydroxyurea
IgG	Immunoglobulin
KAT	Lysine Acetyltransferase
KDAC	Lysine Deacetyltransferase
Lys	Lysine
mChIP	Modified Chromatin Immunoprecipitation
MMS	Methyl Methanesulfonate
MOF	Male absent on the First
MS	Mass Spectrometry
MYST	Moz, Ybf2/Sas3, Sas2, Tip60
NLS	Nuclear Localization Signal
NuA3	Nucleosomal Acetyltransferase of Histone H3
NuA4	Nucleosome Acetyltransferase of Histone H4
OD_{600}	Optical Density at 600nm
PCAF	p300/CBP-associated Factor

PicNuA4	Piccolo nucleosome acetyltransferase histone H4
PrA	Protein A
PTM	Post-translational Modification
rDNA	Ribosomal DNA
Rtt109	Regulator of Ty1 Transposition protein109
SAS2	Something About Silencing 2
SAS3	Something about Silencing 3
SDL	Synthetic Dosage Lethal
SDS	Synthetic Dosage Sick
SDR	Synthetic Dosage Rescue
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
Sir	Silent Information Regulator
SL	Synthetic Lethal
SS	Synthetic Sick
SWC4	SWR Complex 4
TAF1	TATA box binding protein (TBP)-associated Factor
TAP	Tandem Affinity Purification
TIP60	Tat Interactive Protein, 60kDa
TBD	Tandem Bromodomain
TRA1	Similar to TRRAP
TRRAP	Transformation/Transcription Domain-Associated Protein
WCE	Whole Cell Extract
YAF9	Yeast Homolog of the Human Leukemogenic Protein AF9
YNG2	Yeast Homolog of Mammalian Ing1
YPD	Yeast Peptone Dextrose

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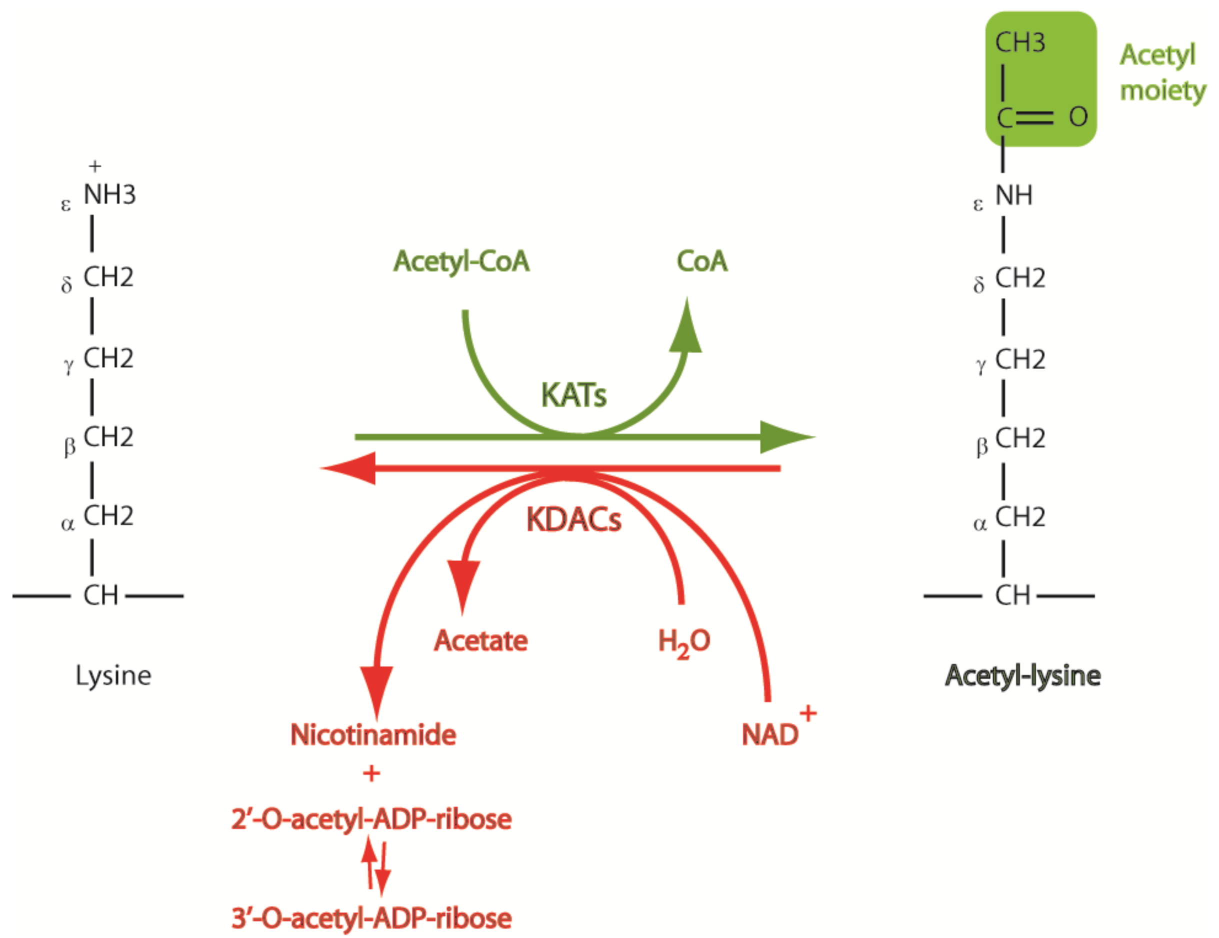
Chapter 1: Introduction

1.1 Lysine acetyltransferases (KATs) – Major biological players

Lysine acetyltransferases (KATs) are a class of highly conserved enzymes that catalyze the transfer of acetyl groups from Acetyl Coenzyme A onto the ε-amino group of a lysine residue to form Ɛ-N-acetyl lysine (Polevoda and Sherman, 2002). Lysine deacetylases (KDACs) on the other hand balance the level of acetylation in the cell by removing acetyl groups from acetylated substrates to regenerate unmodified, positively charged lysine side chains (Figure 1.1). Initially, KATs were thought to be only involved in regulation of transcription through acetylation of histone proteins. Hence, KAT enzyme complexes were traditionally referred to as histone acetyltransferases or HATs. Recently, advances in genetic and proteomic approaches have led to the discovery of thousands of non-histone acetylated proteins, both in prokaryotic and eukaryotic systems, suggesting that protein acetylation is a wide-spread and conserved mechanism by which protein function is regulated. Acetylation of non-histone targets are now implicated in regulating a myriad of biological processes such as transcription regulation, cell cycle control, trafficking, stress response, metabolism and many more. Aberrant KAT function has been linked in a wide range of pathologies, from cancer to neurodegenerative disorders (Table 1.1), and the role of KATs in human diseases cannot solely be attributed to transcriptional regulation. Therefore, to understand the role of KATs in human diseases it is necessary to (i) understand how KAT protein complexes are regulated and (ii) identify their biological targets.

Figure 1.1: Schematic illustration of protein lysine acetylation. Lysine acetylation is a reversible process *in vivo.* The forward reaction (shown in green) is catalyzed by Lysine acetyltransferases (KATs) which transfer an acetyl group from acetyl coenzyme A (acetyl-CoA) to the side chain of lysine residue forming a covalent bond with the Ɛ-amino nitrogen of the lysine residue. The reverse reaction (shown in red) is conducted by lysine deacetylases (KDACs) which remove the acetyl group from acetyl-lysine using either water or NAD^+ to regenerate unmodified lysine side chain.



CH3
C O
Acetyl
moiety
+
ε NH3
δ CH2
γ CH2
β CH2
α CH2
CH
Lysine
Acetyl-CoA
CoA
KATs
KDACs
Acetate
H_2O
Nicotinamide
+
2'-O-acetyl-ADP-ribose
3'-O-acetyl-ADP-ribose
NAD^+
ε NH
δ CH2
γ CH2
β CH2
α CH2
CH
Acetyl-lysine

In this study, I have employed yeast as a model organism to study the role of lysine acetylation in regulating the function of NuA4, an essential yeast KAT, and to identify roles and putative *in vivo* targets for yeast KATs using a genome-wide system biology approach. Below I will provide a brief overview of yeast KATs, a biological significance of protein lysine acetylation, and the role of acetylation in regulating KAT function.

Table 1.1: KATs conservation from yeast to human and their involvement in human pathology

Yeast KATs	Complex	Histone targets	Non-histone targets	Human homolog	Diseases associated
MYST family					
Esa1	NuA4; PicNuA4	H4 (K5,8,12,16); H2A (K4,7); Htz1 (K8,10,14)	Yng2, Pck1, Esa1	TIP60; HBO1	- Prostate cancer (Halkidou et al., 2003) - Lung cancer (Avvakumov and Cote, 2007) - Colon cancer (Avvakumov and Cote, 2007) - Skin cancer (Hobbs et al., 2006) - Alzheimer's disease (Stante et al., 2009) - HIV (Col et al., 2005)
Sas2	SAS	H4 (K16)	None	MOF	-Breast carcinoma and medulloblastoma (Pfister et al., 2008)
Sas3	NuA3	H3 (K14,23)	None	MOZ	- Myeloid leukemia (Liang et al., 1998) - Myelodysplastic syndrome (Imamura et al., 2003)
GNAT family					
Gcn5	SAGA, SLIK, ADA, STAGA	H3 (K9,14,18,23,36) Htz1 (14) H2A	Rsc4	GCN5	- Neural tube closure defects (Bu et al., 2007) - Spinocerebellar ataxia type 7 (Helmlinger et al., 2004) - Retinal degradation (Palhan et al., 2005)
Eco1	---	None	Smc3	ESCO1	- Melanoma cancer (Stockert et al., 1998) - Prostate cancer (Luedeke et al., 2009) - Roberts syndrome (van der Lelij et al., 2009)
Elp3	Elongator	H3	None	ELP3	- Familial dysautomia (Gardiner et al., 2007) - Amyotrophic lateral sclerosis (Simpson et al., 2009)
Hat1	Hat1/2	H4 (K5,12)	None	HAT1	- Colon cancer (Seiden-Long et al., 2006) - Liver cancer (Pogribny et al., 2007)
Hpa2	---	H3 (K4, 14) H4 (5, 12)	None	---	
p300/CBP/Rtt109 family					
Rtt109	trimer with Asf1, Vps75	H3 (K56)	Rtt109, Asf1	p300; CBP	- Colorectal cancer (Iyer et al., 2004) - Breast cancer (Iyer et al., 2004) - Pancreatic cancer (Iyer et al., 2004) - Rubinstein-Taybi syndrome (Petrij et al., 1995)
Spt10	---	---	---	---	
Taf1 (TFIID)	RNA polymerase II	H3>H4 (in vitro)	---	TAF1 (TFIID)	-Parkinson's diseases (Nolte et al., 2003)

1.1.1 Using yeast to study KATs

Saccharomyces cerevisiae or baker's yeast has been extensively used as a model organism in different research laboratories for many years and has contributed greatly to the understanding of eukaryotic biology. Where does the awesome power of yeast come from? Through evolution, there has been a high level of conservation of fundamental cellular mechanics such as DNA replication, recombination, cell division, and metabolism between yeast and higher eukaryotes, including human. The yeast genome is comprised of approximately 6000 genes, compared with the estimated 40,000 genes in human. Importantly, twenty percent of genes involved in human diseases have direct homologues in yeast (Walberg, 2000). Furthermore, genetic redundancy in the yeast genome is low, which facilitates the analysis of gene function in this organism (Costanzo et al., 2011). Yeast have a short generation time and due to their ease in genetic and biochemical manipulation they have become a workhorse model for studying protein function. Given the facts mentioned above along with long history of yeast research has led to development of several post-genomic tools and high throughput screening such as deletion mutants arrays (Winzeler et al., 1999), Synthetic Genetic Array (SGA) (Tong 2001), galactose inducible over-expression arrays (Sopko et al., 2006), and endogenously epitope-tagged arrays used for protein interaction studies and localization (Gavin et al., 2006; Ho et al., 2002; Krogan et al., 2006). Taken together the experimental approaches available makes yeast an excellent platform in modern biological research.

1.1.2 KATs in yeast:

In yeast there are at least nine KAT catalytic proteins with confirmed *in vivo* targets: Esa1 (Smith et al., 1998), Sas2 (Ehrenhofer-Murray et al., 1997), Sas3 (Takechi and

Nakayama, 1999), Hat1 (Kleff et al., 1995), Elp3 (Wittschieben et al., 1999), Hpa2 (Angus-Hill et al., 1999), Rtt109 (Scholes et al., 2001), Gcn5 (Grant et al., 1997), Eco1(Toth et al., 1999), and two putative KATs, Spt10 (Eriksson et al., 2005) and Taf1 (Durant and Pugh, 2006). Based on the sequence similarity of the acetyltransferase domains and the mechanism utilized for catalytic activity, KATs fall within three categories: MYST (Moz, Ybf2/Sas3, Sas2, Tip60) proteins, Gcn5-related N-acetyltransferases (GNATs), and p300/CBP family. The majority of the KATs are found in multi-subunit protein complexes (Table 1.1). Though the functional role of most subunits within KAT complexes is undefined, it is hypothesized that they may contribute to the regulation of KAT catalytic activity, to the scaffolding of the KAT complex, or to target KAT activity to specific loci.

1.1.2.1 Esa1:

Esa1 is a member of MYST family of acetyltransferases and is required for cell viability (Smith et al., 1998). Esa1 comprises the catalytic subunit of two KAT complexes: NuA4 (Allard et al., 1999) and a sub-complex called PiccoloNuA4 (PicNuA4) (Boudreault et al., 2003; Doyon and Cote, 2004). On its own, Esa1 primarily acetylates free histone proteins H4, H2A and H3 *in vitro* (Clarke et al., 1999; Smith et al., 1998). However, to acetylate nucleosomal histones, Esa1 has to be included within either NuA4 or PicNuA4 complex (Boudreault et al., 2003; Chittuluru et al., 2011). In the context of NuA4 or PicNuA4, Esa1 predominantly acetylates lysine residues on the N-terminal tails of histone H4 (Allard et al., 1999; Smith et al., 1998), and to a lesser extent histone H2A variant Htz1(Babiarz et al., 2006; Keogh et al., 2006). Being an essential gene, the study of Esa1's KAT activity has been dependent almost exclusively on creation of temperature sensitive (ts) mutant alleles. Biochemical analysis of the encoded mutant proteins illustrated an

inability to acetylate histone H4 both *in vivo* and *in vitro*, indicating the loss of catalytic activity and suggesting the essential function of Esa1 to be its acetyltransferase activity (Bird et al., 2002; Clarke et al., 1999; Decker et al., 2008). Similarly, deletions of several other NuA4 subunits (Eaf1, Yng2, Esa1, Arp4, Swc4, Tra1, and Epl1) also result in a global reduction of H4 acetylation (Keogh et al., 2006; Kobor et al., 2004) supporting the hypothesis that NuA4 subunits modulate the KAT activity of Esa1 *in vivo.* More recent studies indicate Esa1 also possesses non-histone acetylation targets *in vivo* including the gluconeogenesis regulator PcK1(Lin et al., 2009), NuA4 and PicNuA4 subunit Yng2 (Lin et al., 2008), and itself (Yuan et al., 2011).

1.1.2.2 Sas2:

Sas2 (Something About Silencing protein 2), another member of MYST acetyltransferase family, is the catalytic subunit of the SAS complex (Osada et al., 2001). Biochemical analysis has revealed that Sas2 has intrinsic acetyltransferase activity towards K16 of free histone H4 (Sutton et al., 2003) and minimal acetyltransferase activity toward nucleosomes (Shia et al., 2005). Sas2 activity has been implicated in regulating silencing, albeit differently, at *HML*, *HMR*, telomeres, and rDNA loci (Sherman and Pillus, 1997). For instance, at *HML* and telomeres, *SAS2* activity promotes silencing (Reifsnyder et al., 1996); while, its activity impedes silencing at rDNA locus and restores the silencing defects at the mutant *HMR* with a mutated silencer(Meijsing and Ehrenhofer-Murray, 2001). In addition, mutation in acetyl-CoA binding motif of Sas2 has been shown to eliminate the Sas2-dependent silencing at *HML* and telomeres, suggesting Sas2 mediates its cellular role through its acetyltransferase activity (Osada et al., 2001).

1.1.2.3 Sas3:

Sas3 (Something About Silencing protein 3), also belonging to the MYST family of acetyltransferases was originally isolated as a protein related to Sas2 (Takechi and Nakayama, 1999). Sas3 is the catalytic subunit of yeast NuA3 KAT complex which preferentially acetylates K14 of free histone H3 but not nucleosomal histones (John et al., 2000). Similar to Sas2, Sas3 has been involved in silencing at the *HMR* locus (Huang, 2002). Sas3 possesses an evolutionary conserved domain that contains a zinc finger motif and an acetyl-CoA binding motif and mutant analysis has shown that Sas3 needs both of these motifs for its acetyltransferase activity (Takechi and Nakayama, 1999). Sas3 also mediates interaction of the NuA3 complex with Spt16, a subunit of the FACT complex which is involved in transcription elongation and DNA replication. This suggests that NuA3 may be involved in transcription and replication elongation through nucleosomes via its acetyltransferase activity (John et al., 2000).

1.1.2.4 Gcn5:

Gcn5 (General Control Nonderepressible) is the founding member of the Gcn5p-related N-acetyltransferase superfamily and is well characterized both structurally and functionally. Gcn5 composes the catalytic subunit of three distinct chromatin modifying complexes: ADA, SAGA, and SLIK which are involved in transcriptional regulation of numerous genes (Grant et al., 1997; Lee et al., 2000; Sterner et al., 1999). Gcn5 has been shown to modify the N-terminal lysine residues of histones H2B and H3; however, a non-histone acetylation target has also been identified. In 2007, VanDemark and colleagues reported that Gcn5 acetylates the Rsc4 subunit of yeast RSC chromatin remodelling complex on its tandem bromodomain (TBD). TBD domain is essential for cell viability and

involved in binding to K14 of histone H3 (H3K14). Acetylation of Rsc4 at K25 has been shown to inhibit TBD binding to H3K14 thus suppressing the expression of certain genes (VanDemark et al., 2007). As Gcn5 activity is also responsible for H3K14 acetylation, it has been proposed that Gcn5 performs both activating (H3K14ac) and inhibitory (Rsc4 K25ac) modifications to regulate the RSC activity at the site of remodeling (VanDemark et al., 2007).

1.1.2.5 Hat1:

Hat1, identified in 1995 as the first lysine acetyltransferase in yeast (Kleff et al., 1995), was originally considered to be a cytoplasmic acetyltransferase to specifically acetylates K12 of free histone H4. However, a few years later it was reported that this acetyltransferase can also be found in the nucleus to regulate gene silencing through acetylation of H4K12 (Kelly et al., 2000). Hat1 is the catalytic subunit of a small KAT complex formed with tight association with an accessory protein called Hat2. Hat2 protein enhances Hat1 activity by increasing its binding affinity towards free histone H4 (Parthun et al., 1996).

1.1.2.6 Elp3:

Elp3 is the catalytic subunit of the six-subunit Elongator complex and member of GNAT acetyltransferase family. Elongator is a major component of RNA polymerase II holoenzyme which is responsible for transcriptional elongation and has also shown to be involved in chromatin remodeling (Wittschieben et al., 2000). Elp3 possesses acetyltransferase activity preferentially acetylating histone H3 and to a lesser extent histone H4 (Wittschieben et al., 2000). Deletion of Elp3's Acetyl-CoA binding site causes growth

defects and reduced gene activation, indicating that the acetyltransferase activity of Elp3 is essential for its function *in vivo* (Wittschieben et al., 2000).

1.1.2.7 Hpa2:

Among all KATs, Hpa2 is the most recently described acetyltransferase and little information is available about its function. However, based on degree of homology and sequence similarity, Hpa2 has been classified as a member of the GNAT superfamily. Hpa2 acetylates histones H3 and H4 with a preference for K14 of histone H3 (Sterner and Berger, 2000).

1.1.2.8 Eco1:

Eco1 is another member of GNAT superfamily, however unlike other GNAT members no histone targets have been identified for this protein to date. There is an increasing number of evidence that Eco1 is an essential acetyltransferase whose activity targets subunits of the cohesin complex important in both chromosome segregation and DNA damage repair (Heidinger-Pauli et al., 2009; Rolef Ben-Shahar et al., 2008; Sjogren and Nasmyth, 2001; Toth et al., 1999; Unal et al., 2008; Zhang et al., 2008). Sister chromatid cohesion is mediated by cohesin protein complex made up of four subunits, Smc1, Smc3, Scc1, and Scc3. Eco1 and its human counterpart, ESCO1, have been shown to acetylate Smc3 protein at two conserved lysine residues (K105/K106 in human and K112/K113 in yeast) (Zhang et al., 2008) which are key in establishing sister chromatid cohesion during replication and DNA damage repair.

1.1.2.9 Rtt109:

Rtt109 is a lysine acetyltransferase required for acetylation of K56, K9 and K27 on newly synthesized histone H3 which normally occurs during premeiotic and mitotic S phase (Schneider et al., 2006). Several studies have reported the importance of Rtt109-dependent H3K56 acetylation in a number of nuclear processes including DNA replication, DNA damage response, and maintaining genome stability (Driscoll et al., 2007; Han et al., 2007a). Consistent with these observations, *rtt109* null mutants display increased rate of chromosomal rearrangements and are hypersensitive to agents that generate replication stress such as MMS and hydroxyurea. Unlike other yeast KATs, the Rtt109 acetyltransferase activity is controlled by association with histone chaperons, Asf1 and Vps75. Asf1 and Vps75 enhance Rtt109 catalytic action and provide substrate specificity (Berndsen et al., 2008; Han et al., 2007b; Kolonko et al., 2010). Besides its histone H3 substrate, Rtt109 can weakly acetylate its companion Asf1 (Han et al., 2007a). In addition, auto-acetylation of Rtt109 has also been reported by two independent studies where acetylation has been involved in auto-regulating the protein's catalytic activity (Albaugh et al., 2011; Stavropoulos et al., 2008)

1.1.2.10 Spt10

Spt10 is a putative acetyltransferase whose activity has been implicated in H3K56 acetylation (Chang and Winston, 2011; Hess et al., 2004; Neuwald and Landsman, 1997). Despite the fact that no acetyltransferase activity has been reported for Spt10 either *in vitro* or *in vivo*, mutations in Spt10 putative acetyltransferase domain result in phenotypes similar to *spt10Δ* strains, suggesting this domain plays an important role in regulating Spt10 function (Hess et al., 2004). Microarray analysis indicates that Spt10 regulates the

expression of hundreds of genes (Eriksson et al., 2005). Notably, *in vivo* analyses have identified Spt10 at the promoter region of histone genes, suggesting a direct role for Spt10 in transcriptional control (Dollard et al., 1994; Eriksson et al., 2005; Hess et al., 2004). Spt10 is not an essential gene, but its deletion results in significant growth rate and defects in gene regulation (Dollard et al., 1994; Natsoulis et al., 1991; Natsoulis et al., 1994).

1.1.2.11 Taf1

Taf1, an essential TFIID subunit, is involved in RNA polymerase II transcription initiation (Tora, 2002). Much of what is known about Taf1 KAT activity arises from *in vitro* acetyltransferase activity gel assays conducted in higher eukaryotes (Hilton et al., 2005; Mizzen et al., 1996). In yeast Taf1 has been shown to exhibit *in vitro* KAT activity towards histone H3 and H4 (Mizzen et al., 1996); however, its acetyltransferase role *in vivo* has not been confirmed. However, mutation of the putative acetyl-CoA binding domain (Mizzen et al., 1996) of TAF1 results in growth arrest phenotype suggesting the KAT activity is important for Taf1 activity *in vivo* (Dunphy et al., 2000).

1.2 Lysine acetylation- a conserved mechanism to regulate protein function

Lysine acetylation is a reversible and highly abundant PTM that has been associated with a large spectrum of biological processes. At the mechanistic level, addition of an acetyl group on an amino side chain not only neutralizes a positive charge (Figure 1.1) but increases both the overall size and hydrophobicity of the modified peptide. These changes pose a significant impact on protein conformations which in turn can affect protein function in diverse ways.

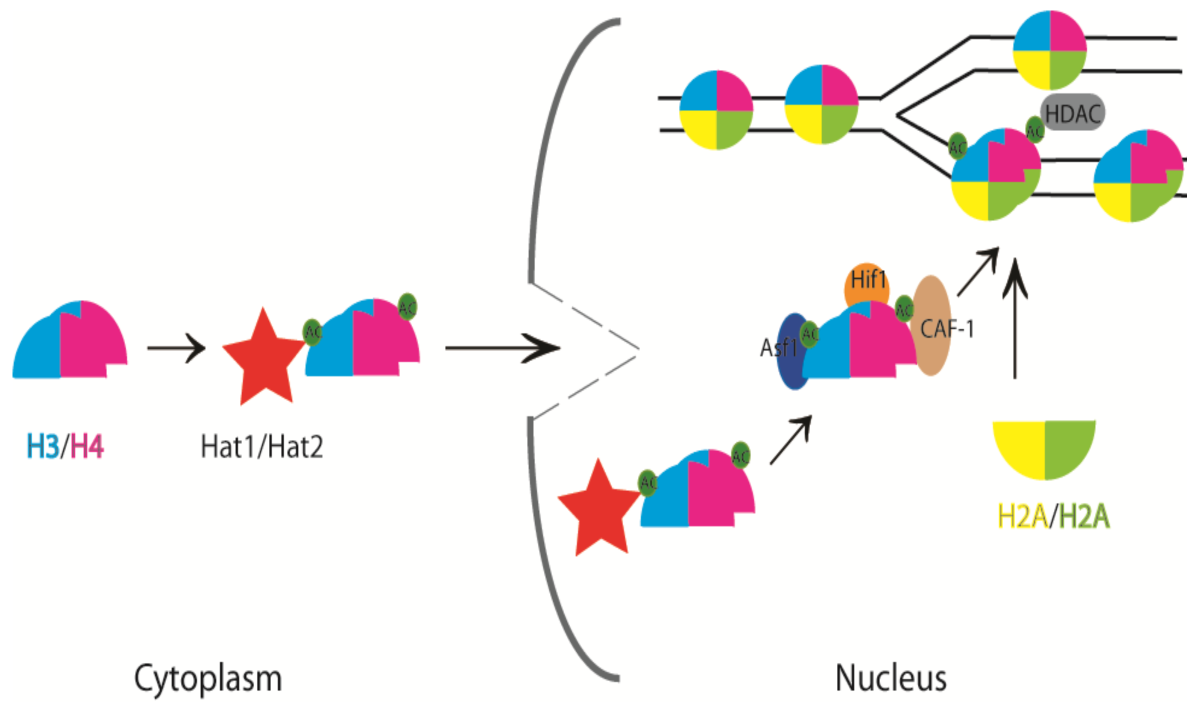
1.2.1 Acetylation of histone proteins

Lysine acetylation was first discovered about 50 years ago on the amino-terminal end of histone proteins (Phillips, 1963). Histones are abundant nuclear proteins and play an important role in packaging genomic DNA into the nucleus. Histone proteins consisting of two H2A-H2B dimers and a H3-H4 tetramer make up the histone core particle around which 146 base pairs of double stranded DNA are wrapped around (Luger et al., 1997). The nucleosome, the complex of DNA and histones, is the building block of chromatin in eukaryotic cells. Chromatin structure is highly dynamic and is essential for almost all DNA-based cellular processes such as replication, transcription, DNA damage repair, silencing, and more (reviewed in Shahbazian and Grunstein, 2007). Within eukaryotic cells, chromatin dynamics are controlled by the action of two different types of protein complexes: chromatin remodelers and histone modifiers. Chromatin remodelers use the energy of ATP hydrolysis to modify nucleosome structure. On the other hand, histone modifiers bring about the change in chromatin structure by covalently modifying histone proteins mainly on their N-terminal tails. Positively charged histone tails, extending from histone core proteins, modulate DNA accessibility within the nucleosome by interacting with negatively charged phosphate groups on the DNA backbone. Histone tails are subject to various PTMs which not only alter DNA-histone interactions, but also regulate chromatin configuration by changing the accessibility of DNA to different proteins such as transcription factors, polymerases and nucleases and/or providing an anchor for binding of accessory proteins important in mediating DNA-templated cellular events (Minard et al., 2009).

Among all known histone PTMs, acetylation is probably the best characterized. In general, histone acetylation is associated with more relaxed and transcriptionally active

chromatin structure (Clayton et al., 2006). Histone acetylation reduces the affinity between histones and DNA, thereby making DNA more accessible to RNA polymerase and facilitating its passage on chromatin. In addition, acetylation may promote transcription by providing docking sites for various binding factors involved in gene activation such as transcription factors and other KATs containing bromodomains, which specifically recognize acetylated regions. Several studies have also provided evidence that site-specific histone acetylation along with other post-translation modifications produce a land mark called the "histone code" which impacts most, if not all, chromatin-templated cellular processes. For instance, MOF catalyzed acetylation of histone H4 at K16 has been associated with DNA double-stranded break repair through recruitment of Rad52 at the damaged site (Sharma et al., 2010). Also, H4 K16 acetylation by Esa1 and Sas2 has been involved in maintaining heterochromatin boundary by opposing the action of Sir silencing complex and blocking the spread of silencing factors into euchromatin regions (Johnson et al., 1992; Shahbazian and Grunstein, 2007). Also, acetylation of all the four lysine residues on the N-terminus of histone H4 (K5, 8, 12, and 16) has been associated with cell cycle progression and the maintenance of genome integrity (Megee et al., 1995). Furthermore, the acetylation pattern on newly synthesized histone proteins is important for nucleosomal assembly. Although not completely understood, it is proposed that the acetylation mark of newly synthesized histones H4 (K5/K56) and H3 (K9/K14) can be recognized by chaperone proteins such as CAF-1, Asf1, and Hif1 which then deposit histone H3-H4 tetramers onto newly replicated DNA along with two histone H2A-H2B dimmers (Figure 1.2) (Shahbazian and Grunstein, 2007).

Figure 1.2: Schematic diagram of nucleosome assembly. Shortly after the synthesis in the cytoplasm, H3 and H4 are acetylated by the action of the Hat1/Hat2 complex. H3/H4 dimer along with the Hat1 complex are then imported into the nucleus where chaperons CAF-1, Asf1, and Hif1 recognize the acetylation pattern on H3/H4 and assemble the tetramer along with two H1A/H2B dimer into the newly replicated DNA. Shortly after assembly, histones are deacetylated by the action of KDACs.


H3/H4
Hat1/Hat2
Asf1
Hif1
CAF-1
HDAC
H2A/H2A
Cytoplasm
Nucleus

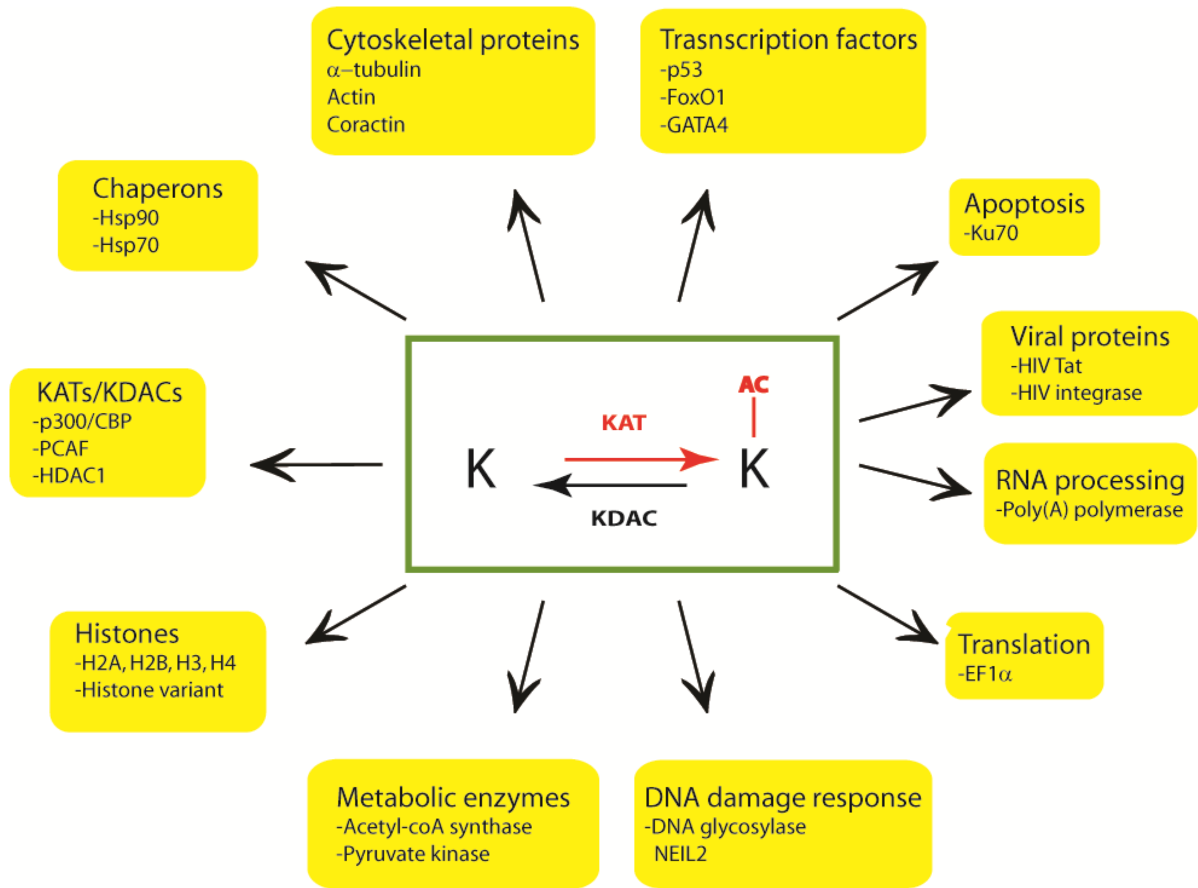
1.2.2 Non-histone acetylation targets:

For years, lysine acetylation was thought to be confined to histones. Because of the intimate connection of histone acetylation and transcription regulation, chromatin associated non-histone proteins such as transcription factors and proteins involved in replication, recombination and DNA damage repair were the first identified non-histone acetylation targets. However, identification of cytoplasmic and mitochondrial localization of some KATs and KDACs suggested that lysine acetylation extends beyond the confines of the nucleus (Close et al., 2010; Schwer et al., 2002). Extensive investigation over the past few years have identified lysine acetylated peptides in a wide range of proteins involved in a variety of biological process (Figure 1.3). Below I describe a few classes of many known non-histone KAT targets.

1.2.2.1 Transcription factors:

The biological significance of lysine acetylation has been well described for a few important mammalian transcriptional regulators including p53 (Gu and Roeder, 1997; Liu et al., 1999; Sakaguchi et al., 1998) and FoxO (reviewed in Calnan and Brunet, 2008). p53 is the best studied transcription factor and a key player in cellular signaling and stress response. Upon DNA damage, p53 acts to both up or down regulate the expression of genes involved in many cellular processes including cell DNA repair, cycle arrest, senescence, and apoptosis (Vousden and Lane, 2007). Multiple lysine residues on the C-terminal regulatory domain of p53 are subject to acetylation by CBP/p300 KAT where these acetylations induce conformational changes, leading to subsequent enhancement of p53 sequence-specific DNA binding activity *in vivo* (Knights et al., 2006; Mujtaba et al., 2004; Sykes et al., 2006).

Figure 1.3. Schematic representation of prevalence of reversible lysine acetylation in diverse cellular processes. Only a few classes and representative proteins are presented in this figure.


Cytoskeletal proteins
α–tubulin
Actin
Coractin
Trasnscription factors
-p53
-FoxO1
-GATA4
Chaperons
-Hsp90
-Hsp70
Apoptosis
-Ku70
Viral proteins
-HIV Tat
-HIV integrase
KATs/KDACs
-p300/CBP
-PCAF
-HDAC1
AC
KAT
K
K
KDAC
RNA processing
-Poly(A) polymerase
Histones
-H2A, H2B, H3, H4
-Histone variant
Translation
-EF1α
Metabolic enzymes
-Acetyl-coA synthase
-Pyruvate kinase
DNA damage response
-DNA glycosylase
NEIL2

In addition, C-terminal acetylation regulates p53 protein stability by inhibiting its ubiquitination-dependent degradation (Albertus et al., 2008; Li et al., 2002).

FoxO transcription factors composed of FoxO1, FoxO3a, FoxO4, and FoxO6 are members of the FOX protein family that play a crucial role regulating the expression of many genes involved in cell growth, proliferation, differentiation, longevity, and metabolism (Salih and Brunet, 2008; van der Horst and Burgering, 2007). The activity and cellular localization of FoxO proteins are tightly regulated by several PTMs such as phosphorylation, acetylation, and ubiquitination imposed by external stimuli such as insulin, insulin-like growth factor, nutrient, and oxidative stress. In particular, in pancreatic β-cells, in response to oxidative stress, FoxO1 undergoes acetylation by p300 and PCAF acetyltransferases which in turn induces its translocation from cytoplasm into the nucleus and prevents its ubiquitin-dependent degradation (Kitamura et al., 2005).

1.2.2.2 Metabolic enzymes:

Earlier studies by Schwer and colleagues identified human mitochondrial matrix protein acetyl-CoA synthetase 2 (AceCS2) as the first substrate for lysine deacetylase SIRT3 (Schwer et al., 2006). AceCS2 is abundant in heart and skeletal muscle and has a critical role in the metabolism of carbon sugar and energy production under low glucose conditions (Sakakibara et al., 2009). Reversible acetylation of K642 located on the active-site region of AceCS2 was shown to act as a simple on/off switch regulating the enzymatic activity of this protein such that constitutively acetylated AceCS2 at K642 display no catalytic activity and cells suffer from energy starvation (Schwer et al., 2006). Similarly, acetylation on enzymes involved in metabolism has been reported by several later studies indicating that lysine acetylation is a prominent PTM on many central metabolic enzymes both in eukaryotic and

prokaryotic systems (Choudhary et al., 2009; Hallows et al., 2011; Lin et al., 2009; Wang et al., 2010; Zhao et al., 2010). Based on these studies, the majority of enzymes participating in glycolysis, gluconeogenesis, tricarboxylic cycle, the urea cycle, fatty acid, and glycogen metabolisms are acetylated and acetylation directly affects the enzymatic activity and/or protein stability of these proteins. Particularly, studies in *salmonella enteric* has demonstrated that the acetylation status of intermediate metabolic enzymes vary in response to different carbon sources, suggesting that similar to human AceCS2, acetylation of these enzymes is involved in coordination of carbon utilization (Wang et al., 2010). Taken together, these data suggest that reversible acetylation of metabolic enzymes is a mechanism conserved from bacteria to human which plays an important role in regulation of metabolism among different species.

1.2.2.3 Cytoskeletal proteins:

Acetylated lysines have been identified in several cytoskeletal and structural proteins. Microtubules, formed by polymerization of αβ-tubulin heterodimers, are important components of the cytoskeleton and are required for faithful segregation of replicated sister chromatids (Wloga and Gaertig, 2010). Acetylation of α-tubulins was reported on the conserved K40 over twenty years ago (Janke and Bulinski, 2011; L'Hernault and Rosenbaum, 1985; LeDizet and Piperno, 1987), a modification that stabilizes microtubule stability and dynamics. Microtubule acetylation has also been found to be necessary for several other biological processes including correct organization of the immune synapse (Serrador et al., 2004) and stimulation of kinesin-1 binding and cargo transport (Reed et al., 2006). Other cytoskeleton proteins such as actin and cortactin are also acetylated *in vivo*. In

this case, acetylation of cortactin at charged patches blocks its association with F-actin, which in turn impairs cell motility (Wu et al., 1991; Zhang et al., 2007).

In summary, lysine acetylation can regulate protein function in a number of ways including altering its stability, localization, protein interaction with other proteins and DNA, as well as directly regulate enzymatic activity.

1.3 Regulation of KAT protein complexes by auto-acetylation

Similar to auto-phosporylation of protein kinases, recent proteomic studies have shown that a large number of KAT enzyme complexes are heavily acetylated *in vivo* (Choudhary et al., 2009; Zhao et al., 2010) suggesting that KAT self-acetylation may be a general property in regulating activity. Although the regulatory mechanism of auto-acetylation has been described for several KATs (Lin et al., 2008; Santos-Rosa et al., 2003; Thompson et al., 2004; Yuan et al., 2011), in the majority of cases, the physiological significance of KAT auto-acetylation has not been explored. In the following few paragraphs, I give a brief overview of a few known self-acetylated human KATs and the role of acetylation in regulating their functions.

1.3.1 p300:

The best studied hyper-acetylated KAT is human p300 whose function is critical for regulation of gene expression and cellular homeostasis in mammalian cells. The intrinsic KAT activity of p300 plays an important role in promoting transcription of several important gene targets involved in a broad range of cellular processes such as p53 (Gu and Roeder, 1997), p73 (Costanzo et al., 2002), steroid hormone response (Wang et al., 2001), NEkB (Greene and Chen, 2004), and GATA (Boyes et al., 1998) through acetylation of histone

proteins. As a result, p300 dysfunction has been implicated in various diseases. The acetylation of p300 has been shown to occur via an intermolecular manner at its acetyltransferase domain, where the acetylation enhances the enzymatic activation of p300 (Thompson et al., 2004). More recently, the yeast homolog of p300, Rtt109, was reported to undergo similar auto-acetylation at active site residue K290 (Albaugh et al., 2011). Biochemical and kinetic analyses by the same group indicates that auto-acetylation at K290 increases the Rtt109 binding affinity for acetyl-CoA and is necessary for its full KAT activity.

1.3.2 PCAF:

Human PCAF is an acetyltransferase that was originally identified as a CBP/p300-interacting protein and has been involved in myogenic differentiation (Puri et al., 1997) and cell cycle progression (Yang et al., 1996). PCAF hyper-acetylation has been reported by two independent studies (Herrera et al., 1997; Santos-Rosa et al., 2003). PCAF acetylation is shown to happen through both auto-acetylation, can either be intra-or intermolecular event, and by the action of another acetyltransferase, p300. Santos-Rosa et al., specifically demonstrated that PCAF is acetylated at both the N-terminal domain and nuclear localization signal (NLS) located at the C-terminus and acetylation at each site results in different *in vivo* consequences. Acetylation of five lysine residues located at the NLS associated with nuclear localization where non-acetylable PCAF mutants (lysine to arginine mutants at NLS) displayed cytoplasmic retention. PCAF acetylation also enhances its catalytic activity, however the exact mechanism behind it is still unclear (Santos-Rosa et al., 2003).

1.3.3 MOF

The MOF, male-absent on the first, is an acetyltransferase that was originally identified in *Drosophila* as a catalytic subunit of dosage-compensation MSL complex. The MOF catalytic activity, through acetylation of K16 of histone H4, has been reported to be essential for embryogenesis, genome stability, and cell cycle progression (Smith et al., 2005; Thomas et al., 2008). More recently, hMOF has been shown to undergo auto acetylation at active site residue K427 via intra-molecular mechanism which lead to increases in the enzymatic activity by maintaining proper loop conformation of the active site for binding an positioning of the substrate lysine (Sun et al., 2011).

1.4 NuA4, an essential KAT in yeast:

The work presented in the second chapter investigates the biological significance of self-acetylation on an essential KAT in *Saccharomyces cerevisae* called NuA4 (Nucleosome Acetyltransferase of H4). NuA4 KAT complex in highly conserved in eukaryotes in particular in human where 12 of the 13 subunits have direct homolog in the orthologous protein complex called Tip60 (Table 1.2). In human, Tip60 plays major roles in wide range of cellular processes, such as transcription, cell cycle control, DNA repair, chromosome stability, and more (reviewed in Sapountzi et al., 2006). Consistent with its diverse function, Tip60 malfunction has been linked to a number of diseases such as several types of cancer, Alzheimer disease, and HIV (Table 1.1). Due to high structural and functional conservation between yeast NuA4 and human Tip60, the ease of genetic and biochemical analysis, and tools available for genome and proteome-wide studies in yeast, NuA4 provides an excellent platform to study Tip60.

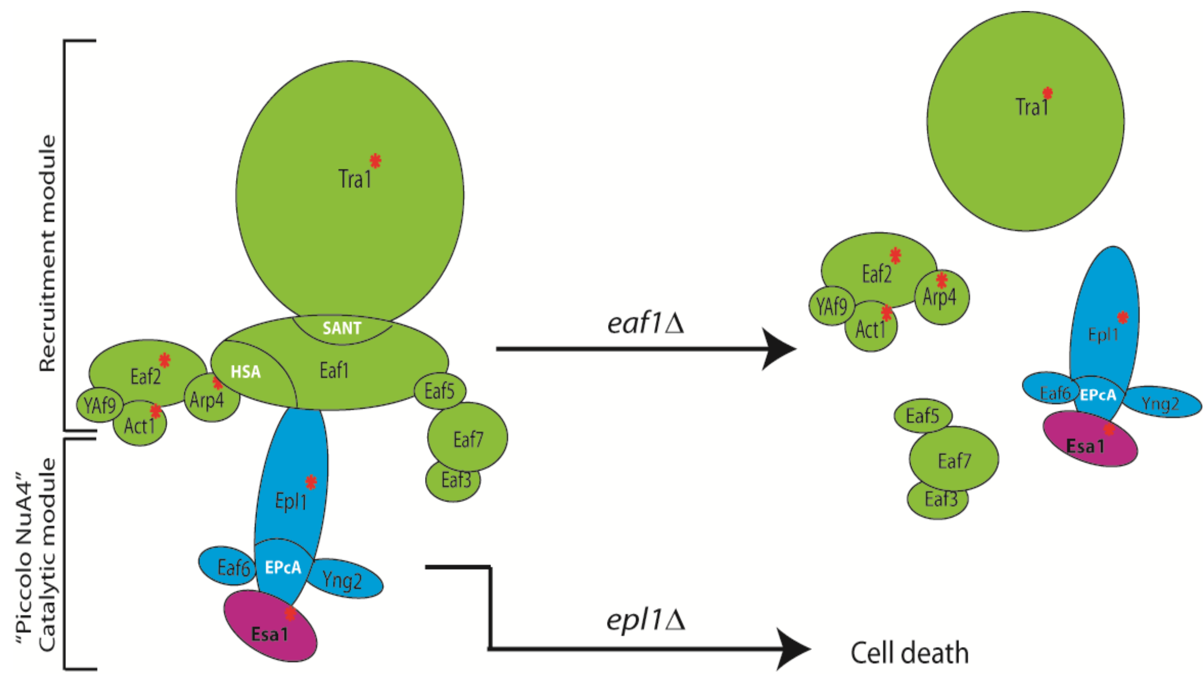
Table 1.2: Conservation of NuA4 subunits across species

s.cervisiae	*D. melanogaster*	*C. elegans*	*S. pombe*	Human
Esa1	dTip60	MYS-1	Mst1	Tip60
Epl1	E(Pc)	EPC-1	Epl1	Epc
Yng2	dIng3		Png1	Ing3
Eaf1	Domino	SSL-1	Vid21	p400
Eaf3	dMrg15		Alp13	MRG15
Eaf6	dEaf6		Eaf6	hEaf6
Eaf7	dMrgB		Eaf7	MrgBP
Swc4	dDMA		Swc4	DMAP1
Arp4	BAP55		Alp5	BAF53a
Eaf5				
Tra1	dTra1	TRR-1	Tra2	TRRAP
Yaf9	dGas41		Yaf9	Gas41
Act1	Act87E		Act1	Actin

1.4.1 NuA4 subunit composition:

Yeast NuA4 is a 13 subunit complex composed of (Figure 1.4) of six essential (Act1, Arp4, Swe4, Epl1, Esa1, and Tra1), and seven are non-essential (Eaf1, Eaf3, Eaf5, Eaf6, Eaf7, and Yng2) proteins (Doyon and cote, 2004). Esa1 comprises the catalytic subunit responsible for acetyltransferase activity. However, on its own Esa1 only acetylates free histones (Allard et al., 1999; Boudreault et al., 2003) with complex formation required for acetylation of nucleosomal histones or chromatin. Analysis of NuA4 subunit composition has identified Esa1 as part of two distinct protein complexes: one which contains all thirteen subunits of NuA4 and a sub-complex made of Esa1, Yng2, Eaf6 and Epl1 called Piccolo NuA4 (picNuA4). In the context of the complete NuA4 complex, Esa1 is recruited to specific chromatin loci for gene regulation, whereas its function within the picNuA4 is responsible for global and non-targeted histone acetylation (Boudreault et al., 2003). Despite a high degree of conservation, the functional role of the individual NuA4 subunits is not completely understood. It is postulated that individual subunits contribute to the regulation of KAT catalytic activity, to the assembly of the native KAT complex, or to the substrate specificity by targeting KAT activity to specific loci through recognition of specific landmarks on other proteins and/or DNA. For instance, Eaf1 is situated at the centre of the complex and serves as a platform upon which the other subunits are assembled (Auger et al., 2008; Mitchell et al., 2008). Epl1 links Yng2 to Esa1 which is shown to be required for targeting Esa1 catalytic activity towards chromatin (Boudreault et al., 2003). Also, upon DNA damage, NuA4 is recruited to the site of damage though its subunit, Arp4 which recognizes the phosphorylated H2A at the site of break (Downs et al., 2004). This interaction is important for subsequent chromatin reconfiguration and efficient DNA repair.

Figure 1.4. Schematic representation of NuA4. Subunits are arranged around the Eaf1 scaffold proteins. The essential subunits are marked with asterisk. The catalytic subunit is colored in purple.


Recruitment module
"Piccolo NuA4" Catalytic module
Tra1
SANT
HSA
Eaf1
Eaf2
YAf9
Act1
Arp4
Eaf5
Eaf7
Eaf3
Epl1
Eaf6
EPcA
Yng2
Esa1
eaf1Δ
epl1Δ
Cell death
essential for cell viability

1.4.2 NuA4 targets and cellular processes:

The NuA4 has been well characterized as a master regulator of gene expression via acetylation of the N-terminal tails of histones H4 (Allard et al., 1999; Smith et al., 1998) and H2A variant Htz1 (Babiarz et al., 2006; Keogh et al., 2006; Millar et al., 2006). Furthermore, phenotypic analysis of NuA4 mutants has linked NuA4 function to myriad of cellular functions such as DNA damage repair (Bird et al., 2002), cell cycle control (Clarke et al., 1999), apoptosis (Sykes et al., 2006), and chromosome stability (Krogan et al., 2004). Though it is possible that NuA4 mediates these functions through its role in transcriptional control by acetylating histones H4 and H2A, the microarray analysis suggests that defect in NuA4 function has minor effects on gene transcription (Lindstrom et al., 2006; Zhang et al., 2004). These observations suggest NuA4 exerts its pleiotropic cellular effect though regulating non-transcriptional mediated pathways and possibly acting on non-histone targets. Along with this hypothesis, the first non-histone NuA4 target was identified by Lin and colleagues where they show that NuA4 can acetylate its own subunit, Yng2, and this acetylation is essential for the protein stability of Yng2 (Lin et al., 2008). More recently, NuA4-dependent acetyltion of the catalytic subunit, Esa1, is also reported to regulate its catalytic activity by modulating the acetyl-CoA binding (Yuan et al., 2011). Also, protein acetylation microarray study has linked NuA4 function to metabolism via acetylation of several enzymes involved in signaling pathways responsive to nutrient availability and energy status in particular gluconeogenesis regulator, phophoenolpyruvate carboxykinase (Pck1) (Lin et al., 2009). NuA4-dependent Pck1 acetylation was shown to be important for its enzymatic activity and the ability of yeast to growth in non-fermentable carbon sources (Lin et al., 2009). More recently, NuA4 genome-wide synthetic dosage lethality screen identified two septin proteins, Shs1 and Cdc10 as NuA4 acetylation targets providing a

novel role for Nua4 in regulating septin dynamics (Mitchell et al., 2011). These types of studies have and are going to expand our knowledge by which NuA4 and therefore its human counterpart may regulate their diverse cellular processes.

1.5 Hypothesis and aims

The diverse role of KATs and their direct involvement in human diseases suggests that modulation of their enzymatic activities may provide a promising therapeutic potential. Further, identification of down-stream targets of the KATs, and discovering fundamental cellular process regulated by KATs could shed light into the development of novel therapeutic means for the treatment of many human diseases. Given the high structural and functional conservation between yeast and human KATs, studies in yeast will greatly enhance our knowledge in understanding the role of acetylation in human diseases.

In chapter 2, the objective of my project was to investigate the role of NuA4 hyper-acetylation on regulating its function. I specifically focused on two key and exclusive NuA4 subunits, Eaf1 and Epl1. Given the key role these two subunits perform within the NuA4 protein complex and identification of acetylated lysine on both subunits, *I hypothesized that Eaf1 and/or Epl1 acetylation plays an important role in regulating NuA4 function potentially by affecting the native complex integrity and/or protein stability and/or catalytic activity*.

In chapter 3, the aim of my project was to build yeast KAT-Synthetic Dosage Lethal (SDL) genetic interaction network to potentially identify cellular pathways and putative targets regulated by KATs. *I hypothesized that KATs mediate their cellular function through*

acetylation of a large number of proteins besides histones and each KAT impacts distinct biological processes.

Chapter 2:

Deciphering the role of Eaf1 and Epl1 acetylation of NuA4 subunits in *Saccharomyces cerevisiae*

2.1. Introduction

The NuA4 (Nucleosome Acetyltransferase of H4), is an essential KAT in budding yeast *Saccharomyces cerevisiae* (Auger et al., 2008; Doyon et al., 2004). The multi-subunit NuA4 complex is highly conserved across species including *Drosophila melanogaster*, *Caenorhabditis elegans*, *Schizosaccharomyces pombe*, and most notably human TIP60 where its function has been implicated in a wide variety of pathologies (Table 1.1). NuA4 was initially identified as a key regulator of gene expression through preferentially acetylating lysine residues on the N-terminal tails of the histones H4 (Allard et al., 1999; Eberharter et al., 1998; Smith et al., 1998) and H2A variant Htz1(Babiarz et al., 2006; Keogh et al., 2006; Millar et al., 2006). However similar to its human counterpart, NuA4 function has also been linked to numerous biological processes, including DNA repair (Bird et al., 2002), genome stability (Krogan et al., 2004), and cell cycle progression (Clarke et al., 1999).

Located at the center of the complex, Eaf1 serves as a platform for native NuA4 complex assembly (Figure 1.4). Eaf1 comprises a SANT domain, HSA domain, a highly charged region, and a C-terminal glutamine rich domain (Figure 1S). Although not much is known about the functional significance of the C-terminal glutamine rich region, the remaining Eaf1 conserved domains provide binding sites for different NuA4 subunits (Auger et al., 2008). Deletion of the HSA and SANT domains result in severe growth defects in various conditions, suggesting these domains play critical role in regulating NuA4 function *in vivo* (Auger et al., 2008). Epl1 is an essential NuA4 subunit and contains a highly conserved enhancer of polycomb A (EPcA) domain necessary for nucleosome interaction (Chittuluru et al., 2011) (Figure 1SA). The EPcA region alone is necessary and

sufficient for cell survival by bridging the Yng2 to the catalytic subunit, Esa1 (Boudreault et al., 2003; Selleck et al., 2005) whereas Epl1 C-terminus acts as a linker to join the catalytic sub-complex to the rest of NuA4 (Figure 1.4). Consistent with their key roles within NuA4 complex, *eaf1* and *epl1* mutants display low levels of histone H4 acetylation and high sensitivity to a wide range of chemical and environmental challenges from DNA damage inducing agents to general stress response activators like caffeine and high temperature (Mitchell et al., 2008; Parsons et al., 1988; Rittberg and Wright, 1989; Sommers et al., 1995; Thomas et al., 1985).

Recent acetylome studies in human cells have discovered acetylation on several subunits of MYST proteins, including seven subunits of Tip60 (Choudhary et al., 2009; Zhao et al., 2010) but the biological importance of these acetylations is not known. Similarly, subunits of yeast NuA4 complex, the yeast homolog of Tip60, are also acetylated and some characterization of the impact of the acetylation state has been discerned. Lin and colleagues determined that Yng2 acetylation on K170 stabilizes the protein and ultimately regulates NuA4 function in DNA-damage repair (Lin et al., 2008). More recently, acetylation of NuA4 catalytic subunit, Esa1, on K262 has been reported to be essential for acetyl-CoA binding and cell viability (Yuan et al., 2011). In addition to these sites our laboratory has identified multiple NuA4 subunits that are acetylated *in vivo* and also *in vitro* by NuA4 (Table 2.1).

To further explore the biological significance of NuA4 hyper-acetylation, I sought to investigate the role of acetylation on Eaf1 and Epl1, two exclusive and important NuA4 subunits. I hypothesized that Eaf1 and Epl1 acetylation regulates NuA4 function through impacting the complex integrity, catalytic activity, or substrate specificity. My data

illustrated that the putative acetylated lysines on Eaf1 and Epl1 impact NuA4 function at elevated temperatures. I also showed that Epl1 acetylation is NuA4-dependent *in vivo* and this acetylation may contribute to regulating Esa1-dependent H4 acetyltransferase activity.

Table 2.1: Acetylated lysine residues on NuA4 subunits

Subunits	NuA4 acetylation sites
Esa1	K82 (1), K97 (3), K135 (15), K232 (6)
Epl1	K16 (1), K39 #(17), K96 (1), K101 (2), K116/K118 (1), K342 (1), K345 #(5), K376/K379 #(8), K395 (1), K426/K429 #(6), K446 (2), K470 #(3), K496 #(5), K512 (1), K569 #(6), K604 (1), K721 (2), K810 (1), K821 (2)
Eaf1	K102 #(8), K280 #(1), K848 #(7)
Yng2	K34 (4), K145 (2), K170*(16), K208 (2)
Arp4	K350 (1)
Eaf7	K343*(2), K381 (1), K399 (2), K409 (1)
Swc4	K345 (1), K350 (2), K570 (3), K574 (1), K575 (1)
Eaf5	K3 (2)
Eaf3	K45 (2), K54 (3)

-NuA4 immunopurification was conducted by Leslie Mitchell. Acetylation sites were identified using LC/MS-MS conducted by Jean-Philippe Lambert and Hu Zhou from the Figey's Lab

-Numbers in brackets illustrate the number of times each residue identified to be acetylated by MS.

-If we could not definitively differentiate between acetylation on two adjacent lysines, both sites are listed (eg. Epl1 K376/379).

* = Sites previously identified by other studies (Lin et al., 2009; Basu et al., 2009).

= Sites selected for analysis in this study.

-Color codes represent: Red: *in vitro* site (heavy acetyl-CoA), Green: *in vivo* sites (light acetyl-CoA), Black: both *in vivo* and *in vitro* sites (light and heavy acetyl-CoA)

2.2. Materials and Methods

2.2.1. Yeast strains and media:

Yeast strains used in this study are listed in Appendix A (Table 1S). Genomic deletion mutants and epitope-tag integrations made for this study were generated using a standard PCR based method as described previously and confirmed by PCR analysis (Longtine et al., 1998). All the strains were grown in Yeast Peptone Dextrose (YPD) unless otherwise stated.

2.2.2 Cloning and generation of acetylation point mutants:

EAF1 and *EPL1* were initially C-terminally epitope-tagged (3HA, three copies of the hemagglutinin epitope) at their endogenous genomic locus using a standard PCR based protocol as described previously (Longtine et al., 1998). After confirming protein expression by Western blot analysis, the full length *EAF1-3HA* and *EPL1-3HA* along with their corresponding promoter region (~500 base pairs upstream of the start codon) were amplified from isolated genomic DNA using Phusion Polymerase (Finnzyme, catalog no. F-530S) with the following primer pairs: OKB559 and OKB568 for *EAF1* and OKB561 and OKB568 for *EPL1* (Appendix B; Table 2S for detailed sequences). Each forward and reverse primer contained a *Hind*III and a *Not*I recognition sequence respectively. Following double digestion with *Hind*III /*NotI* and gel purification (Qiagen, catalog no. 28704), each PCR product was ligated into the pRS415 and pRS416 vectors linearized also with restriction enzymes and treated with calf intestinal phosphatase (NEB, catalog no. M0290). Ligation was carried out overnight at 16°C with T4 DNA ligase (NEB, catalog no. M0202S), and then transformed into competent *E.coli* DH5alpha cells. Final clones were

sequenced, the expression of HA-tagged Eaf1 or Epl1 proteins confirmed by Western blot. Next, the ability of the plasmid-born HA-tagged genes to suppress growth defects of *eaf1Δ* (YKB42) and *epl1Δ* (YKB1972) strains was confirmed (data not shown). Acetylation point mutants mimicking either the acetylated (K → Q) or non-acetylated (K → R) state for candidate acetylated lysine were generated using a site-directed mutagenesis kit (Stratagene, catalog no. 200528) using primers listed in Appendix B. The successful introduction of all point mutations was confirmed by sequencing.

2.2.3 Whole cell-extract and Western blot analysis:

Yeast strains were grown at 25°C in 75mL of YPD to mid-log phase (OD_{600} ~ 0.6-0.9) and collected by centrifugation (3000 rpm, 3 minutes, 4°C), washed in 10mL of ice-cold water, re-suspended in 1mL of water, and transferred into 1.5mL Eppendorf tubes. Cells were pelleted by centrifugation (13200 rpm, 5 seconds, and 4°C), the supernatant was removed by aspiration, and the cell pellets were frozen in dry ice and stored at -80°C. Cell pellets then were re-suspended in an equal volume of Tackett Extraction Buffer (20mM HEPES pH 7.4, 0.1% Tween-20, 2mM MgCl2, 200mM NaCl, Protease Inhibitor Cocktail [Sigma, P-8215]) and an equal volume of acid washed glass beads (Fisher Scientific, 35-535) was added. Cells were lysed through vortexing (6×1 minute vortex with 1 minute incubation on ice in between each pulse) and crude whole cell extract (WCE) was separated from the beads into a new Eppendorf tubes by poking a hole through the bottom of each tube using a 21G1½ (Becton Dickinson, catalog no. 305167) needle heated with a flame, and centrifuging at 1000 rpm for 1 minute at 4°C. The WCE was clarified by centrifugation (15 minutes, 13200 rpm, 4°C) and the supernatant was transferred into fresh1.5mL Eppendorf tubes. Protein concentration for each sample was determined by Bradford Assay (Bio-Rad,

500-0006) and 75µg of each sample was used for Western blot analysis. In brief, an equal volume of 2×loading buffer (100mM Tris pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, 2% 2-mercaptoethanol) and two volumes of 1×loading buffer were added to each sample before boiling at 65°C for 10 min. Proteins were separated on 7.5% SDS polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes using semi-dry transfer apparatus (BioRad, Trans-Blot SD Semi Dry Electrophoretic Transfer Cell, catalog no. 170-3940). Protein transfer was performed for 1 hour using constant milliamps (mA) based on the following calculation: 0.8A × area of membrane × # of gels. The membranes were blocked in 1×Phosphate-buffered saline (PBS) containing 5% nonfat dry milk and 0.1% Tween-20 (1×PBS-T) for 1 hour at room temperature with shaking. Primary antibodies were diluted in 5% milk dissolved in 1×PBS-T, with the exception of anti-acetyl lysine antibody for which the manufacturer's recommendation of 5% BSA in 1×TBS-T was followed. Primary incubation was carried out overnight at 4°C with shaking and washed for 3×10 min with 1×PBS-T before incubation with secondary peroxidase-conjugated antibodies diluted in 5% milk dissolved in 1×PBS-T for 2 hour at room temperature. Membranes were washed 3×10min with 1×PBS-T and developed using ECL Plus Western Blotting Detection System (Amersham BioSciences, RPN2135) as per the manufacturer's instructions. The Following antibodies and dilutions were used in this study: α-HA (Roche, catalog no. 1583816001, 1/1000), anti-TAP (Open Biosystems, catalog no. CAB1001, 1/5000), anti-G6PDH (Sigma, catalog no. A9521, 1/1000), anti-acetyl lysine (cell signaling, catalog no. 9681, 1/500), anti-Acetyl H4 antibody (Millipore, catalog no. 06-8666, 1/1000), peroxidase-conjugated goat anti-rabbit IgG (Chemicon, catalog no. AP307P,

1/5000), and peroxidase-conjugated goat anti-mouse IgG (Bio-Rad, catalog no. 170-6516, 1/5000).

2.2.4 NuA4 PrA-tagged protein purification:

NuA4 was purified from both wild type and mutant strains via one step purification of protein A (PrA; one epitope of the tandem affinity purification [TAP] tag)-tagged of either Eaf5 or Esa1 subunits as described previously (Mitchell et al., 2008). In brief, WCE was extracted from 250mL of mid-log phase (OD_{600} of 0.6-0.9) cultures grown in YPD at 25°C as described above. Ten milligrams of the soluble WCE was incubated with 25µl of magnetic Dynabeads (Dynal Invitrogen,catalouge no. 143-01) crossed-linked to rabbit immunoglobulin G (IgG) (Chemicon, catalouge no. PP64) with end-over-end rotation at 4°C for 2 hours. Subsequently, Dynabeads were collected using a magnet, washed 5 times with 1mL of cold Tackett buffer, and re-suspended in 25µl of 1×loading buffer (50mM Tris pH 6.8, 2% sodium dodecyl sulfate [SDS], 0.1% bromophenol blue, 10% glycerol). PrA-tagged protein and co-purifying proteins were eluted from the beads by heating at 65°C for 10 min. Loading buffer was transferred into a new tube, and 2-β-mercaptoethanol was added to each sample at a final concentration of 200mM. Samples were boiled for 5 min and 20µl was resolved on 7.5% SDS-polyacrylamide gel electrophoresis (PAGE). Proteins were visualized by silver staining.

2.2.5 Dot assays and growth conditions:

Yeast wild-type and mutant strains, grown overnight in yeast peptone dextrose (YPD) at 25°C, were diluted to an OD_{600} of 0.2 in the morning and further grown to mid-log phase (OD_{600} 0.6-0.9) in YPD. Five-fold serial dilutions (OD_{600}= 0.1, 0.02, 0.004, 0.0008,

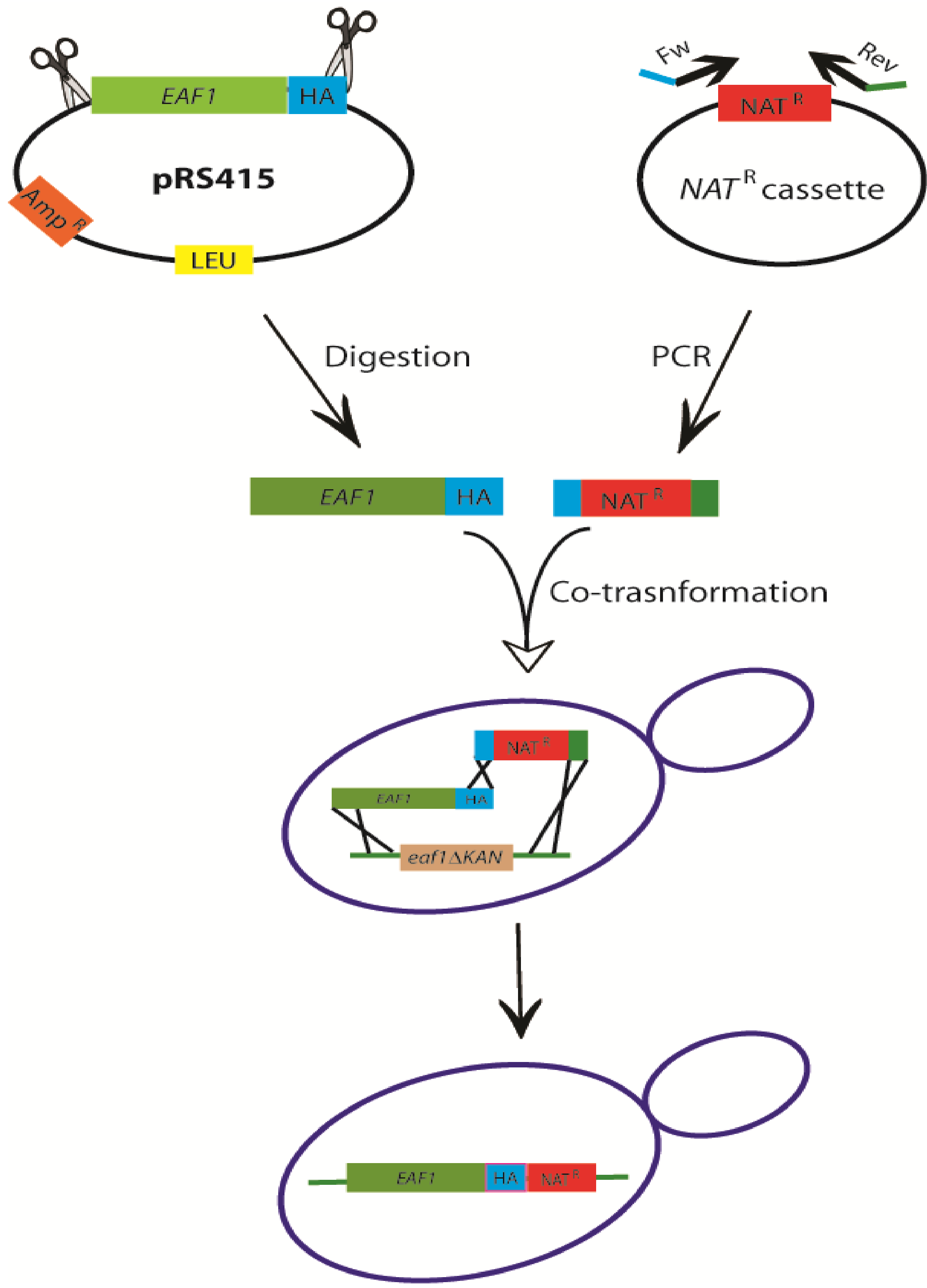
0.00016) of each culture was spotted onto YPD plates containing the following drugs: MMS (0.03%; Auger et al., 2008), Benomyl (15μg/ml; Keogh et al., 2006), Caffeine (3mM; Zhang et al. 2004), HU (100 mM; Keogh et al., 2006). Plates were incubated at 25°C for 3 days and images were collected by epi-white imaging using the Molecular Imager ChemiDoc XRS System (BioRad). For temperature sensitivity assay, cultures were spotted onto YPD plates and incubated at 25°C, 30°C, 33°C, and 37°C for 2-3 days before taking pictures. Dot assay experiments were repeated in triplicate using different isolates of each strain.

2.3 Results

2.3.1 Putative lysine acetylation sites, K102, K280, and K848 on Eaf1 do not affect its protein level in vivo.

Eaf1 is essential for NuA4 complex integrity (Auger et al., 2008; Mitchell et al., 2008) and acetyltransferase activity *in vivo* (Babiarz et al., 2006; Krogan et al., 2004). Using liquid chromatography coupled to mass spectrometry our lab has identified acetylation at three lysine residues (Table 2.1; K102, K280, and K848) on Eaf1 subunit. Although none of these residues were identified as *in vivo* acetylation sites, the *in vitro* KAT assays using radiolabelled acetyl coenzyme A indicated that NuA4 is capable of acetylating these residues (personal communication with L. Mitchell). To investigate the effect of Eaf1 acetylation of NuA4 function, I began by generating Eaf1 acetylation point mutants that fully mimic either the constitutively non-acetylated state (K→R) or the acetylated state (K→Q). The *eaf1-3R* (K102R/280R/848R) and e*af1-3Q* (K102Q/K280Q/K848Q) were generated using a site directed mutagenesis kit (See materials and methods). To resolve the issue arising from the plasmid copy number, I next integrated the acetylation point mutants at their genomic location (Figure 2.1).

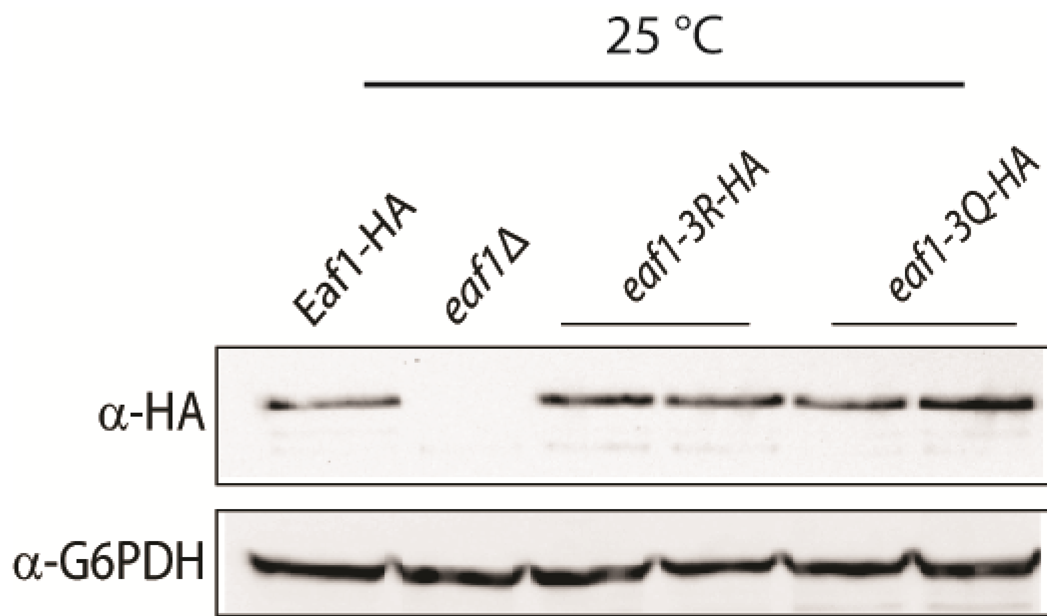
Figure 2.1. Schematic representation of Eaf1 acetylation point mutant integration into the genome. Eaf1 acetylation point mutants were constructed using site-directed mutagenesis kit into pRS415 vector. The constructed mutant genes (*EAF1-3HA* (pKB39); *eaf1-K102R-3HA* (pKB43); *eaf1-K102Q-3HA* (pKB44); *eaf1-K280R-3HA* (pKB45); *eaf1-K280Q-3HA* (pKB46); *eaf1-K848R-3HA* (pKB47); *eaf1-K848Q-3HA* (pKB48); *eaf1-3R(K102/280/848R)-3HA* (pKB187); *eaf1-3R(K102/280/848Q)-3HA* (pKB188)] were excised from the vector using unique restriction enzymes. A NAT cassette (encoding a drug resistance marker gene) is PCR amplified using primers that have 40 base pairs complementary to the HA-tag (for the forward primer) and 3' downstream of the stop codon for *EAF1*. Both PCR products were then transferred into *eaf1ΔKAN* strain and colonies were selected by plating on YPD+NAT and subsequently for G418 (KAN) sensitivity.



EAF1
HA
pRS415
Amp R
LEU
Fw
Rev
NAT R
NAT R cassette
Digestion
PCR
EAF1
HA
NAT R
Co-trasnformation
NAT R
EAF1
HA
eaf1ΔKAN
EAF1
HA
NAT R

Acetylation of Yng2, another NuA4 subunit, at K170 was previously shown to stabilize this protein by preventing its proteosomal degradation (Lin et al., 2008). Therefore I first examined whether Eaf1 acetylation at these residues impacts its protein abundance. Western blot analysis of whole cell extract of *eaf1-3R* (YKB2985) and *eaf1-3Q* (YKB2986) acetylation point mutants shows comparable expression of Eaf1-HA in mutant and wild-type (YKB1688) strains, suggesting that the charge of these putative acetylation sites does not alter its protein level *in vivo* (Figure 2.2).

Figure 2.2: Putative Lysine Acetylation sites at K102, K280 and K848 on Eaf1 do not impact its protein levels *in vivo*. Wild-type Eaf1-HA (YKB1688), *eaf1Δ* (YKB42), as well as mutant *eaf1-3R-HA* (YKB2985) and *eaf1-3Q-HA* (YKB2986) strains expressing genomically integrated HA-tagged Eaf1 were grown to mid-log phase (OD_{600} 0.6-0.9) in YPD media at 30°C. WCEs were collected and probed with anti-HA. Band intensity corresponding to mutant strains was compared to that in the wild-type. Anti-G6PDH was used as loading control.

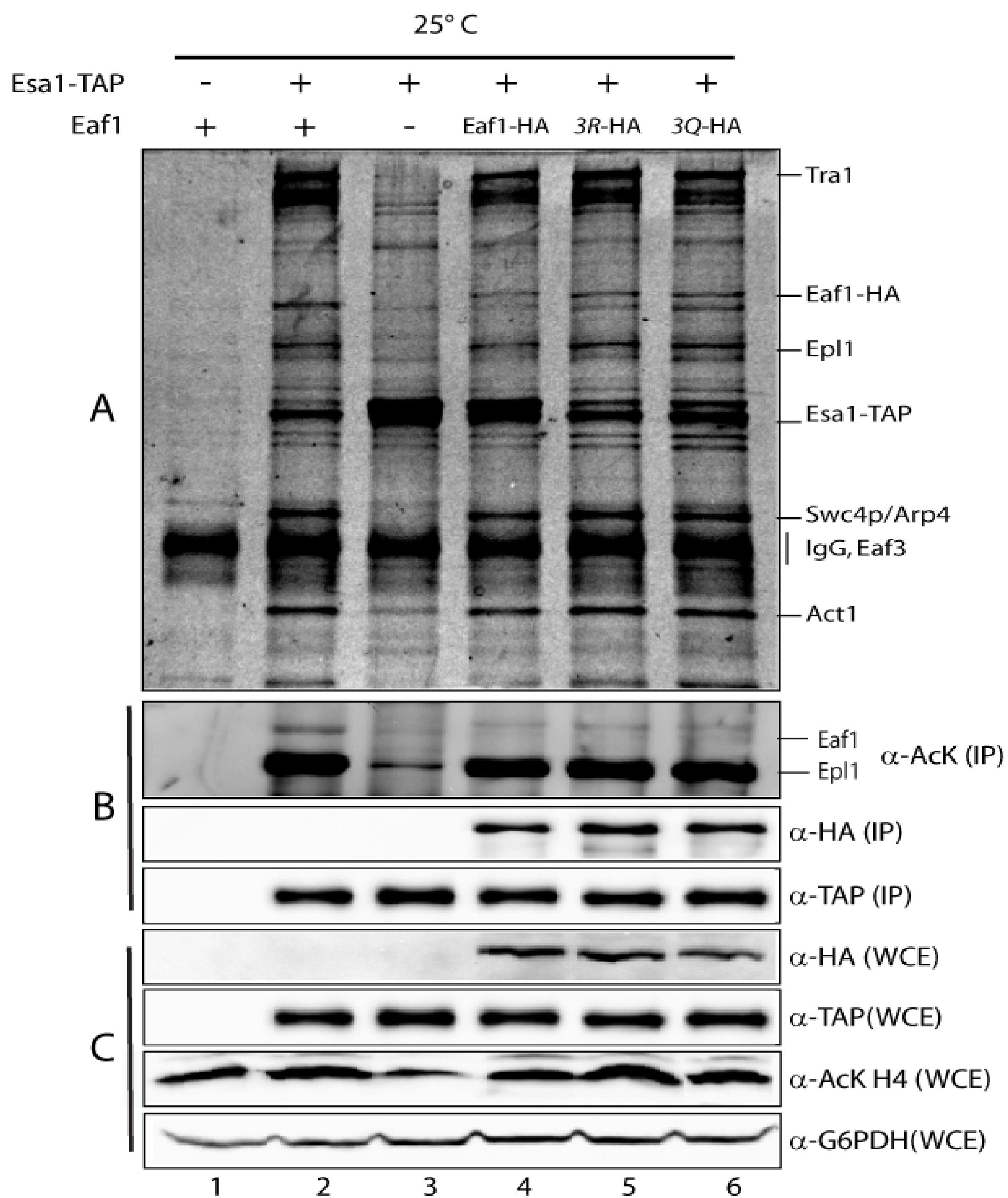

25 °C
Eaf1-HA
eaf1Δ
eaf1-3R-HA
eaf1-3Q-HA
α-HA
α-G6PDH

2.3.2 Lysines 102, 280 and 848 on Eaf1 do not impact NuA4 complex integrity or H4 acetylation.

Centered in the middle of the NuA4 complex, Eaf1 was shown to be required for NuA4 complex assembly (Auger et al., 2008; Mitchell et al., 2008) (Figure 1.4). Given that acetylation was also shown to regulate protein-protein interactions (reviewed in Polevoda and Sherman, 2002), I next checked if the putative acetylation sites on Eaf1 were indeed sites of *in vivo* acetylation and if mutations of these lysine residues impacted the NuA4 complex integrity or catalytic activity. To this end, I purified NuA4 through Esa1-TAP subunit from *eaf1-3R* (YKB2987) and *eaf1-3Q* (YKB 2988) mutant strains as well as control strains (WT untagged, YKB780), (*ESA1-TAP*; YKB440), (*EAF-HA*; YKB3001), and (*eaf1Δ*, YKB855) and assessed the Eaf1 acetylation level using anti-acetyl lysine antibody from Cell Signaling. As shown in Figure 2.3, in control untagged *EAF1* strain (YKB440), a moderate acetylation level is detected on Eaf1 (panel B; lane 2). However, when NuA4 protein complex was purified from strains containing the Eaf1-3HA fusion protein, whether wild-type (lane 4) or mutants *eaf1-3R* (lane 5) and *eaf1-3Q* (lane 6), the detectable acetylation signal was almost abolished. The disappearance of acetylation signal on WT HA-tagged Eaf1 could be reasoned in two ways. First, the presence of HA-tag, although relatively small (~3kDa, 30 amino acids) blocks acetylation of Eaf1 *in vivo*. A similar observation has been reported for septin protein, Shs1 (Mitchell et al., 2011). Secondly, it is possible that addition of the HA-tag disrupts the ability of this antibody to detect acetylation on Eaf1. I repeated this experiment using another anti-acetyl lysine antibody (Upstate; 06-933) and this antibody failed to detect acetylation signal even on untagged Eaf1 (data not shown). Though it is possible that both these antibodies cannot detect acetylation on Eaf1, it is more likely that the *in vitro* acetylation sites we detected on Eaf1 do not occur *in vivo* or only occur on a

minute fraction of total cellular Eaf1. Further, neither the *Eaf1-3Q* nor *Eaf1-3R* impacted NuA4 complex integrity as assessed by silverstain (panel A) or global H4 acetylation levels (panel C; lanes 5 & 6). Together this work indicates that K102, K280 and K848 of Eaf1 do not impact NuA4 complex integrity or KAT activity against H4 at permissive temperature.

Figure 2.3. Putative Eaf1 Lysine Acetylation sites K102, K280 and K848 do not impact detectable acetylation on Eaf1, NuA4 complex integrity, or H4 acetyltransferase activity. The NuA4 complex was affinity purified via Esa1-TAP from strains containing untagged Eaf1 (WT, YKB440, lane 2), *eaf1* null mutant (*eaf1Δ*; YKB 855, lane 3), HA-tagged Eaf1 (YKB3001, lane4), *eaf1-3R* (YKB2987, lane 5), and *eaf1-3Q* (YKB2988, lane 6). Purification was also performed in a strain expressing untagged Esa1 (YKB780, lane 1). All strains were grown to mid-log phase (OD_{600} 0.6-0.9) in YPD media at 25°C. Immunopurified products were resolved on SDS-PAGE, silverstained (panel A) or probed with anti-HA, anti-TAP, and anti-AcK (Cell Sig.) (Panel B). WCEs were collected and probed with anti-HA and anti-TAP to ensure equivalent expression of Eaf1-HA and Esa1-TAP (panel C). NuA4 acetyltransferase activity was measured by assessing histone H4 acetylation as represented by anti-acetyl lysine H4 blot (panel C). Anti-G6PDH blot demonstrates equal protein loading in each lane (panel C). The gel is representative of three purification experiments.

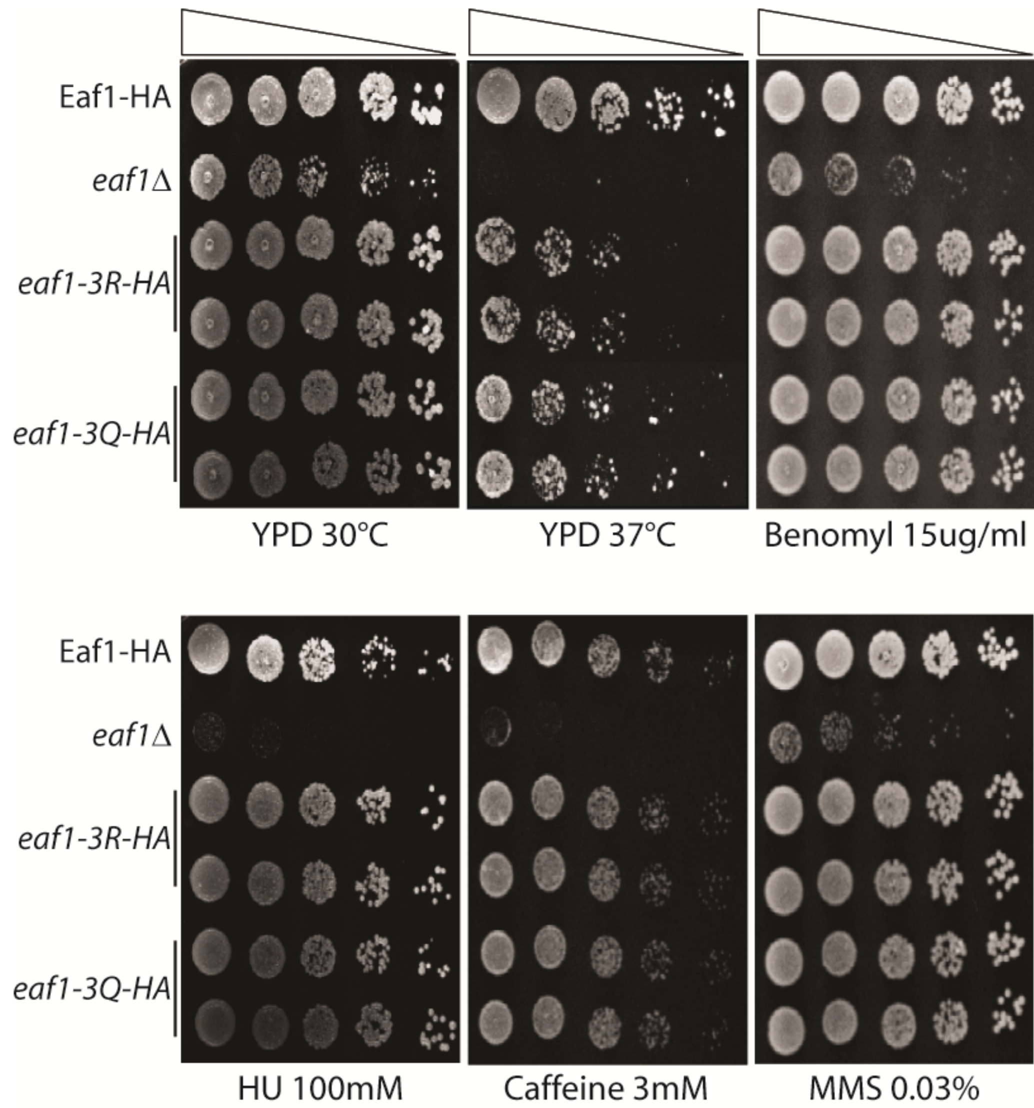


25° C
Esa1-TAP
Eaf1
Eaf1-HA
3R-HA
3Q-HA
Tra1
Eaf1-HA
Epl1
Esa1-TAP
Swc4p/Arp4
IgG, Eaf3
Act1
A
B
C
Eaf1
Epl1
α-AcK (IP)
α-HA (IP)
α-TAP (IP)
α-HA (WCE)
α-TAP(WCE)
α-AcK H4 (WCE)
α-G6PDH(WCE)
1
2
3
4
5
6

2.3.3 Eaf1-3R and Eaf1-3Q point mutants are temperature sensitive.

Despite not impacting NuA4 complex integrity or global H4 acetylation activity under standard culturing conditions, I sought to determine if the lysine sites contribute to Eaf1 function under various stress conditions. I carried out a series of spot assay analysis using *eaf1-3R-HA* (YKB2985) and *eaf1-3Q-HA* (YKB2986) under conditions for which NuA4 has an established role. As shown in Figure 2.4, *eaf1-3R-HA* and *eaf1-3Q-HA* acetylation point mutants exhibit normal growth in the presence of various drugs. However, both mutants display poor growth at elevated temperature of 37°C.

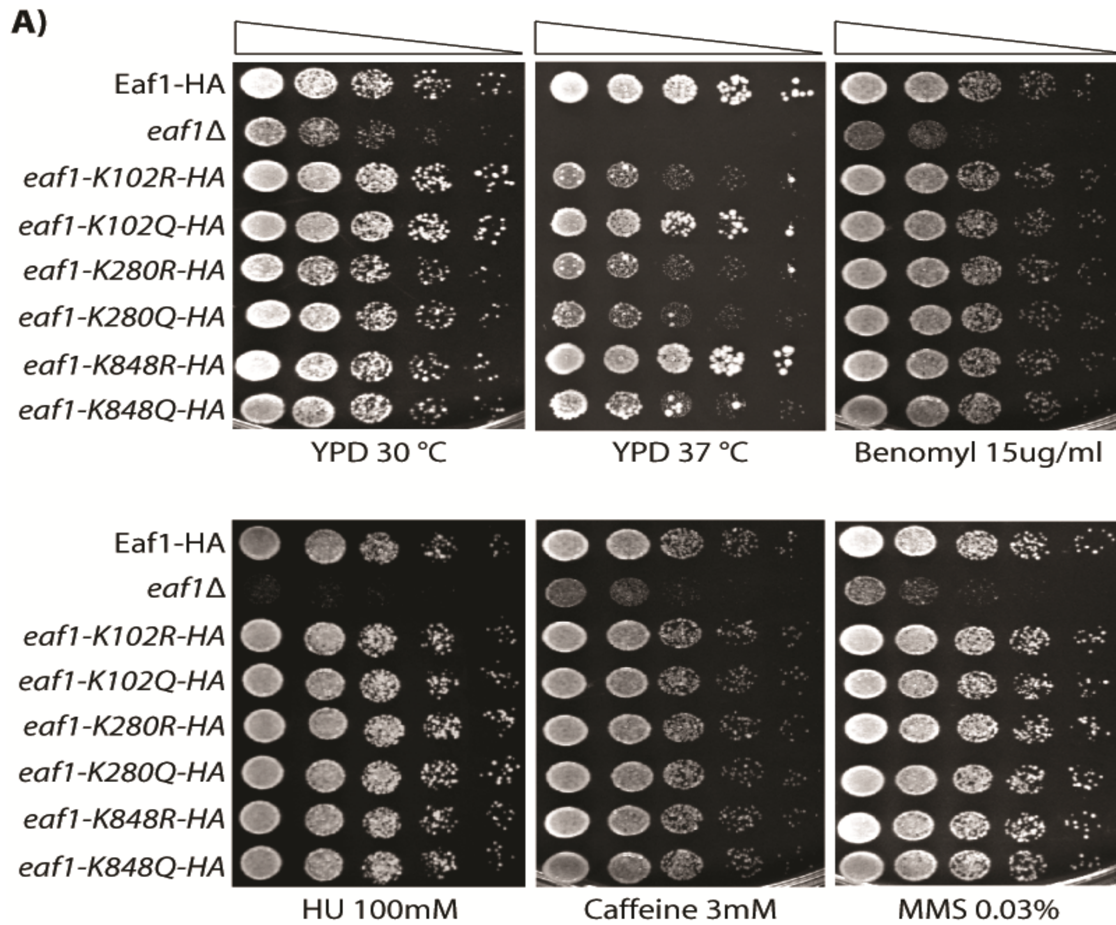
Figure 2.4. *Eaf1-3R-HA* and *Eaf1-3Q-HA* mutants are temperature sensitive. HA-tagged wild-type Eaf1 (YKB1688), *eaf1Δ* (YKB42), *eaf1-3R-HA* (YKB2985) and *eaf1-3Q-HA* (YKB2986) strains were grown to mid-log phase (OD_{600} 0.6-0.9) in YPD media at 30°C. Five-fold serial dilutions of each strain was spotted onto YPD plates containing appropriate drugs and incubated at 30°C for 3 days. For temperature sensitivity assay, YPD plate was incubated at 37°C for 2 days.


Eaf1-HA
eaf1Δ
eaf1-3R-HA
eaf1-3Q-HA
YPD 30°C
YPD 37°C
Benomyl 15ug/ml
Eaf1-HA
eaf1Δ
eaf1-3R-HA
eaf1-3Q-HA
HU 100mM
Caffeine 3mM
MMS 0.03%

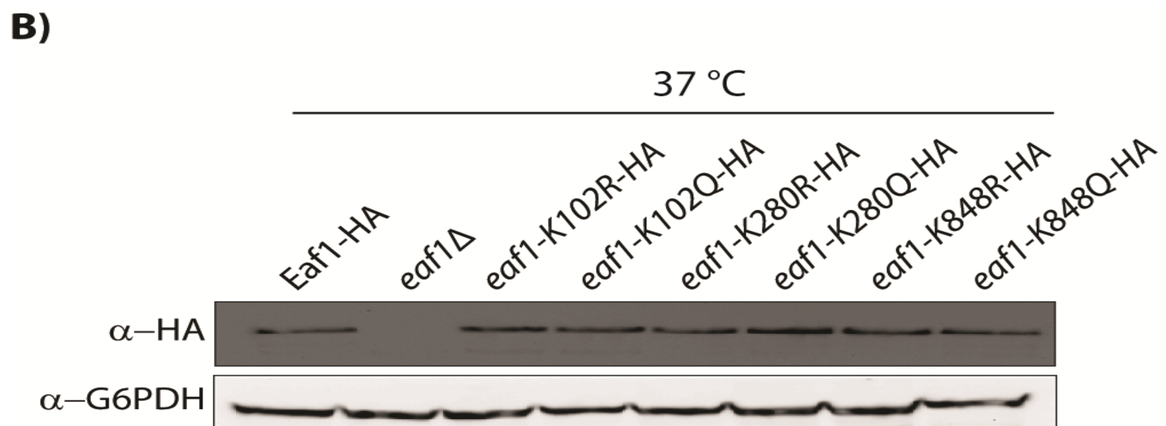
To determine if a specific lysine residue(s) contributes to observed temperature sensitivity phenotype, I repeated the dot assay with Eaf1 single acetylation point mutants (*eaf1-K102R-HA* [YKB2973], *eaf1-K102Q-HA* [YKB2974], *eaf1-K280R-HA* [YKB2975], *eaf1-K280Q-HA* [YKB2976], *eaf1-K848-HA* [YKB2977], and *eaf1-K848Q-HA* [YKB2978]). Similar to triple acetylation point mutants, single acetylation mutants grew normally in all the drug conditions (Figure 2.5A). However, single point mutants (K102R, K280R, K280Q, and K848Q) displayed temperature sensitivity at 37°C similar to the triple acetylation point mutants. These observations indicate that these individual lysine residues attribute to the temperature sensitivity phenotype seen in triple acetylation point mutants and contribute to regulation of proper NuA4 function at high temperatures. Alternatively, these sites may play a role in the heat shock stress response for which NuA4 has an established role in (Lindstrom et al., 2006; Mitchell et al., 2008). To exclude the possibility that the observed growth defects are due to abnormally low Eaf1 protein expression at this growth condition, I investigated the relative abundance of Eaf1-HA of all the single acetylation point mutants grown at 37°C using Western blot of whole cell extracts. As shown in Figure 2.5B, the relative expression of HA-tagged Eaf1 in all the mutants is nearly similar to that in the wild-type indicating that the observed phenotype is not due to a change in Eaf1-HA protein levels in the mutants.

Figure 2.5. Acetylation status of individual residues on Eaf1 contributes to NuA4 temperature sensitivity. (A) HA-tagged wild-type Eaf1 (YKB1688), *eaf1Δ* (YKB42) as well as single acetylation point mutants (*eaf1-K102R-HA* [YKB2973], *eaf1-K102Q-HA* [YKB2974], *eaf1-K280R-HA* [YKB2975], *eaf1-K280Q-HA* [YKB2976], *eaf1-K848-HA* [YKB2977], and *eaf1-K848Q-HA* [YKB2978]) were grown to mid-log phase (OD_{600} 0.6-0.9) in YPD media at 30°C. Five-fold serial dilutions of each strain was spotted onto YPD plates containing appropriate drugs and incubated at 30°C for 3 days. For temperature sensitivity assay, YPD plate was incubated at 37°C for 2 days. (B) WCEs were collected from same strains grown in YPD media at 37°C and probed with anti-HA. Band intensity corresponding to mutant strains was compared to that in the wild-type. Anti-G6PDH was used as loading control.

A)



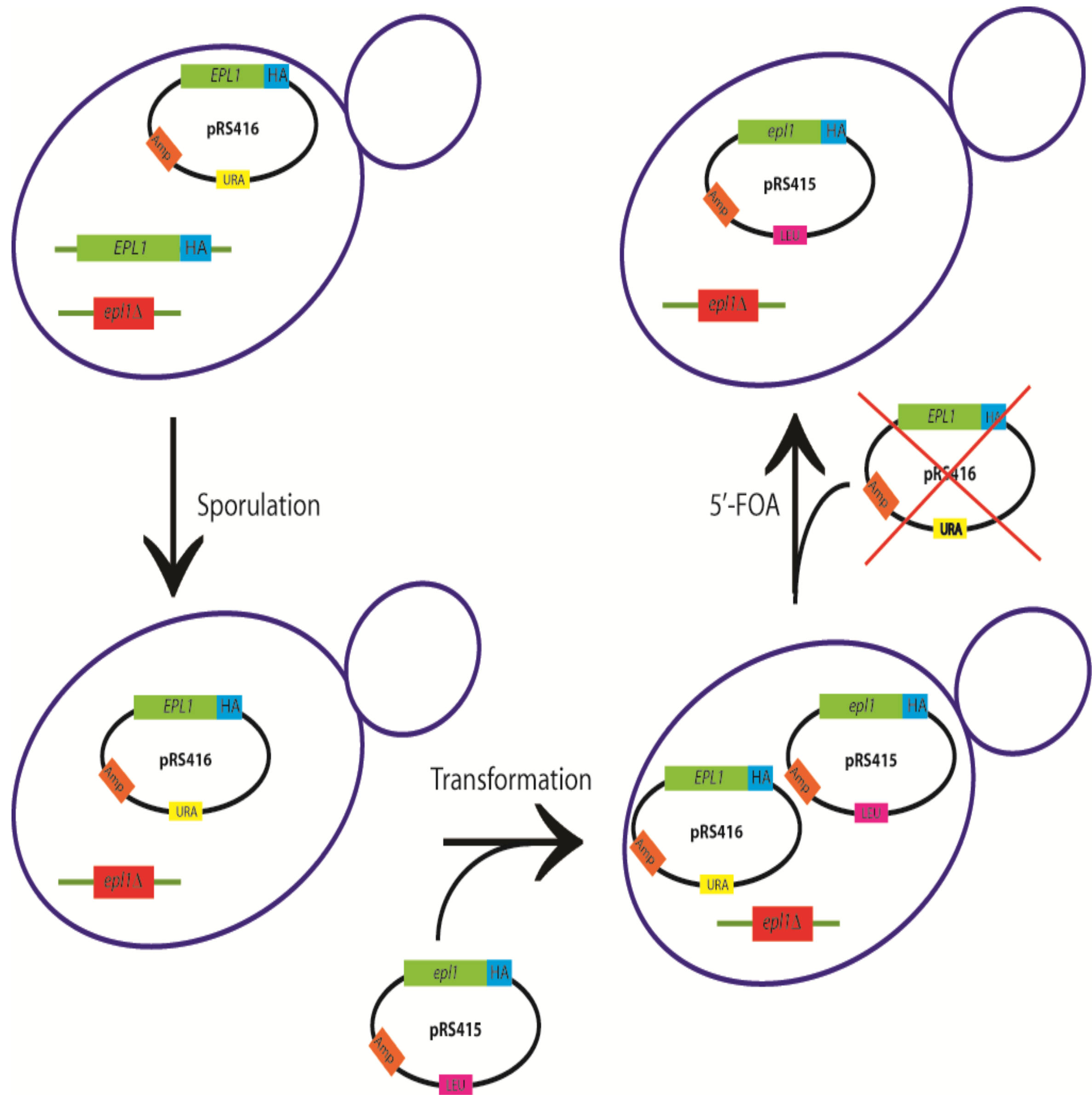
B)



2.3.4 Epl1 acetylation does not affect its protein level in vivo.

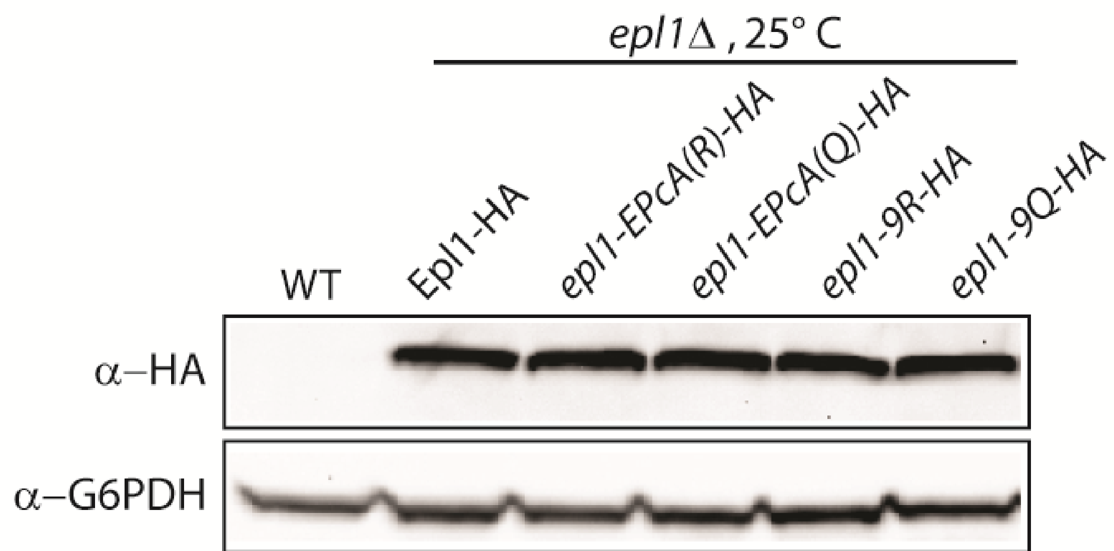
Based on the NuA4 acetylation mass spectrometry data set, Epl1 is the most abundantly acetyl-modified NuA4 subunit (Table 2.1). Due to the large number of acetylated lysine identified, the lysine residues selected for this study were based on the number of times each acetylated site was identified by LC-MS/MS (Table 2.1) and the domain the residue fall in (Appendix C; Figure 1SB). I was particularly interested in acetylation sites within the conserved enhancer of polycomb (EPcA) domain, which serves as a bridge to link Yng2 to the catalytic subunit Esa1 and its expression alone is shown to be sufficient for cell survival (Selleck et al., 2005). I generated (K→R) and (K→Q) acetylation point mutants within either EPcA domain (K39, 345, 376, 379) (*epl1-EpcA(R)-HA* [YKB2877], *epl1-EPcA(Q)-HA* [YKB2878]) or the full length Epl1 (K39, 345, 376, 379, 427, 429, 470, 496, 569)(*epl1-9R-HA* [YKB2879], *epl1-9Q-HA* [YKB2880]) on a *CEN* based plasmid expressing Epl1-HA. Unlike Eaf1, integration of Epl1 acetylation point mutants into the genome was not successful. Hence, all biochemical and phenotypic analysis were performed using *epl1Δ* strain covered by either wild-type *EPL1-HA* or *EPL1-HA* acetylation point mutants expressed from a *CEN* plasmid (Figure 2.6).

Figure 2.6: Schematic representation Epl1 acetylation point mutant construction. HA-tagged wild-type EPL1 cloned into pRS416 (pKB42) vector was introduced into heterozygote Epl1 (YKB2136) and sporulated to select for pRS416-*EPL1-HA* in *epl1ΔKAN* background. The resulting strain was transformed with *epl1* acetylation point mutants constructed on pRS415 vector and generated using site directed mutagenesis [*epl1-EPcA(R)-3HA* (pKB177); *epl1-EPcA(Q)-3HA* (pKB178); *epl1-9R-3HA* (pKB179); *epl1-9Q-3HA* (pKB180); *epl1-K39Q-3HA* (pKB156); *epl1-K345Q-3HA* (pKB158); *epl1-376Q-3HA* (pKB160); *epl1-379Q-3HA* (pKB162)]. Wild-type *EPL1* was eliminated by treating the resulting strain with 5'-FOA to select for final *epl1* acetylation point mutants in *epl1ΔKAN* background.


EPL1
HA
pRS416
Amp
URA
EPL1
HA
epl1Δ
Sporulation
EPL1
HA
pRS416
Amp
URA
epl1Δ
Transformation
epl1
HA
pRS415
Amp
LEU
epl1
HA
pRS415
Amp
LEU
EPL1
HA
pRS416
Amp
URA
epl1Δ
5′-FOA
EPL1
pRS416
Amp
URA
epl1
HA
pRS415
Amp
LEU
epl1Δ

To assess if mutations generated on EPcA and full length Epl1 affect Epl1-HA protein level *in vivo*, I performed Western blot analysis with whole-cell extracts of individual acetylation point mutant as well as control strains. As shown in Figure 2.7, Epl1-HA was expressed at comparable levels to the wild-type in all mutants demonstrating that Epl1 acetylation at these sites does not impact its protein levels *in vivo.*

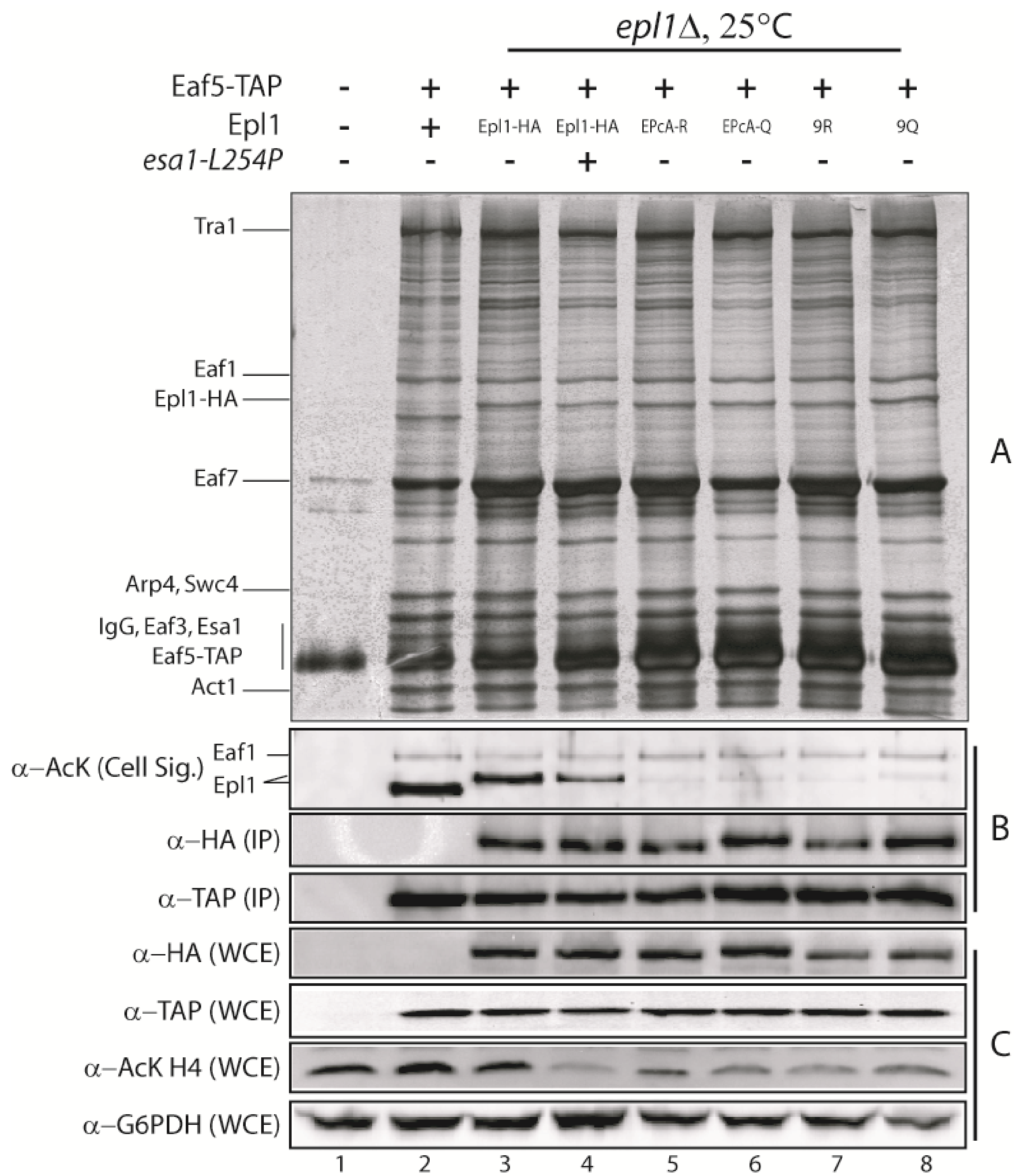
Figure 2.7: Mutation of putative lysine acetylation sites on Epl1 does not impact its protein levels *in vivo*. Wild-type untagged (YKB779), along with *epl1Δ* cells expressing plasmid borne wild-type *EPL1-HA* (YKB1974), *epl1-EPcA(R)-HA* (YKB2877), *epl1-EPcA(Q)-HA* (YKB2878), *epl1-9R-HA* (YKB 2879), and *epl1-9Q-HA* (YKB2880) were grown to mid-log phase (OD_{600} 0.6-0.9) in YPD media at 25°C. WCEs were resolved by SDS-PAGE and Western analysis was performed using anti-HA and anti-G6PDH as loading control.


epl1Δ , 25° C
WT
Epl1-HA
epl1-EPcA(R)-HA
epl1-EPcA(Q)-HA
epl1-9R-HA
epl1-9Q-HA
α–HA
α–G6PDH

2.3.5 Epl1 acetylation is NuA4-dependent in vivo.

To assess the *in vivo* dependency of Epl1 acetylation on NuA4 activity, I first evaluated the acetylation status of Epl1-HA through Western blot analysis in wild-type (YKB2862) or temperature sensitive acetylation deficient *esa1-L254P* strains (YKB2876). The protein encoded by *esa1-L254P* has reduced catalytic activity both *in vivo* and *in vitro* at the permissive temperature of 25°C and is catalytically inactive at the restrictive temperature of 37°C (Clarke et al., 1999). NuA4 was immunopurified through Eaf5-TAP and the acetylation level was assessed using anti-acetyl lysine antibody from Cell Signaling. As shown in Figure 2.8, the acetylation signal on untagged Epl1 (panel B; lane 2) is higher than HA-tagged Epl1 in *ESA1* background (lane 3) suggesting that the presence of the HA-tag can decrease the detection of acetylation by anti-acetyl lysine antibody (similar effect was seen in the case of Eaf1). Though equal amount of Epl1-HA co-purified with Eaf5-TAP, the level of acetylation on Epl1-HA, was higher in the *ESA1* strain compared to that in the *esa1-254P* mutant background at the permissive temperature (lanes 3 vs. 4). Similar results have been shown for an untagged Epl1 (personal communication with L. Mitchell). This suggests that Epl1 acetylation state is at least partially dependent on Esa1 activity *in vivo*.

Figure 2.8: Epl1 acetylation is Esa1-dependent *in vivo* and has no impact on NuA4 complex integrity. NuA4 complex was immunopurified from cells grown at 25°C in YPD through Eaf5-TAP in either a wild type background (lane 2, YKB1042), or an *epl1Δ* background covered by wild type or mutant versions of *EPL1*-HA expressed from a *CEN* plasmid as indicated: (lane 3, *EPL1-HA* YKB2862; lane 4, *esa1-L254P EPL1-HA* YKB2876; lane 5, *epl1-EPcA(R)-HA* YKB 2781; lane 6, *epl1-EPcA(Q)-HA* YKB2782; lane 7, *epl1-9R-HA* YKB2783; and lane 8, *epl1-9R-HA* YKB2784). Immunopurified (IP) products and whole cell extract (WCE) samples were separated on SDS-PAGE (7.5%), silver stained (panel A) or subjected to Western blot analysis (panels B and C). Epl1 acetylation was assessed using anti-acetyl lysine (α-AcK) antibody from cell signaling (Cell Sig). Purification efficiency and expression levels were assessed using anti-TAP and anti-HA antibodies, respectively. Esa1 catalytic activity was assessed by measuring histone H4 acetylation levels (α-AcK H4). Anti-Glyceraldehyde-6-phosphate dehydrogenase (α-G6PDH) was used as a loading control. Figure is representative of four purification experiments.



epl1Δ, 25°C
Eaf5-TAP
Epl1
esa1-L254P
Epl1-HA
Epl1-HA
EPcA-R
EPcA-Q
9R
9Q
Tra1
Eaf1
Epl1-HA
Eaf7
Arp4, Swc4
IgG, Eaf3, Esa1
Eaf5-TAP
Act1
A
α–AcK (Cell Sig.)
Eaf1
Epl1
α–HA (IP)
α–TAP (IP)
B
α–HA (WCE)
α–TAP (WCE)
α–AcK H4 (WCE)
α–G6PDH (WCE)
C
1
2
3
4
5
6
7
8

2.3.6 Epl1 is acetylated within the EPcA, however these sites do not impact NuA4 complex integrity.

Next, I wanted to determine if the Epl1 acetylation sites we detected by mass spectrometry and I selected to mutate in this study were indeed *in vivo* acetylation sites. To do so, the NuA4 complex was purified through Eaf5-TAP in strains expressing the Epl1 acetylation mutants, *epl1-EPcA(R)-HA* (YKB2781), *epl1-EPcA(Q)-HA* (YKB2782), *epl1-9R-HA* (YKB2783), and *epl1-9Q-HA* (YKB2784). I discovered that detectable acetylation signal on all Epl1 mutants was significantly reduced (Figure 2.8; panel B; lanes 5-8). This observation suggests that the Epl1 acetylation sites detectable by Western blot that are co-purified by Eaf5-TAP under these conditions are within the EpcA domain. Further, neither the *Epl1-EpcA-Q/R* nor *Epl1-9Q/R* mutants impacted NuA4 complex integrity as detected by silverstain analysis (Figure 2.8, panel A). This result indicates that these lysine sites in Epl1 do not play a significant role in the complex integrity of NuA4.

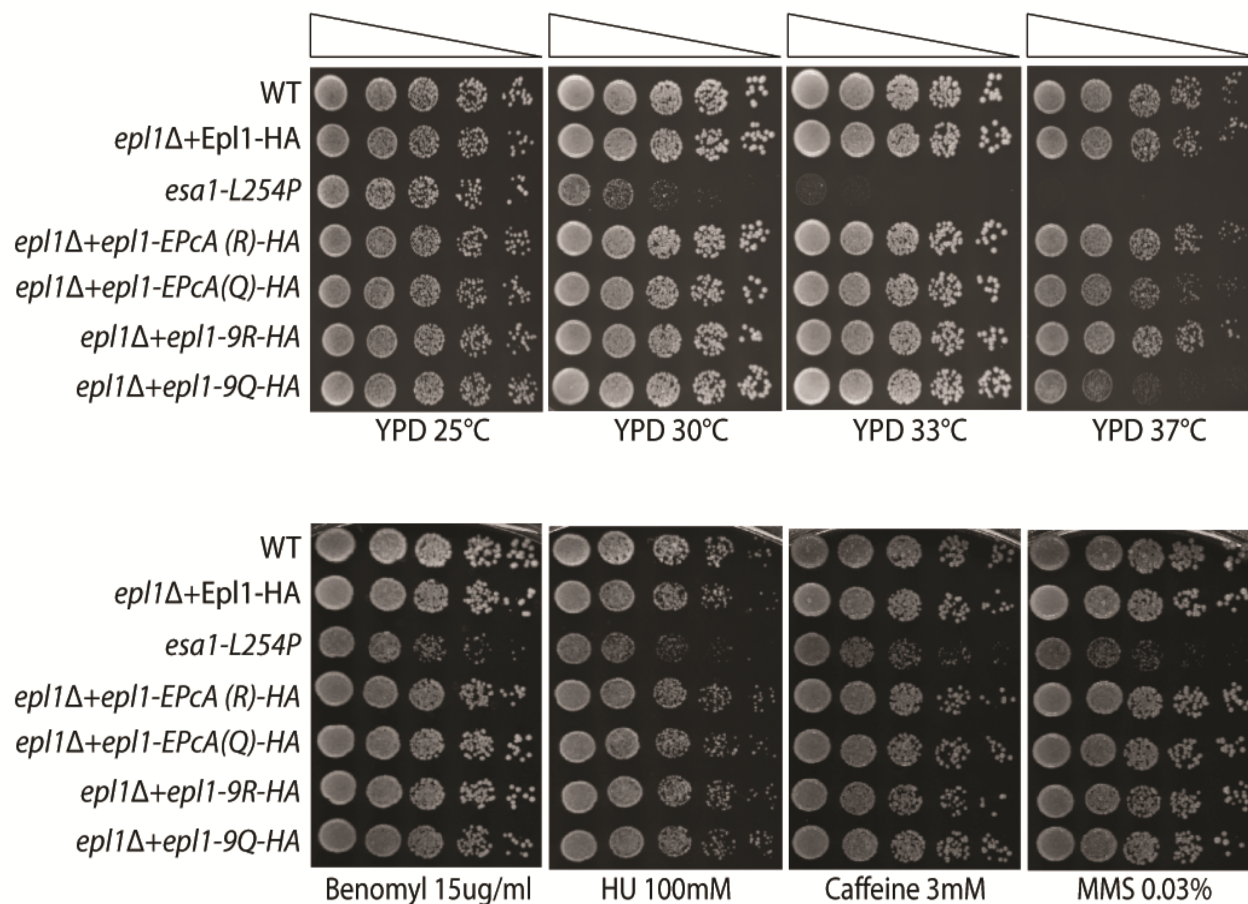
2.3.7 Epl1 acetylation status impacts NuA4 acetylation of H4.

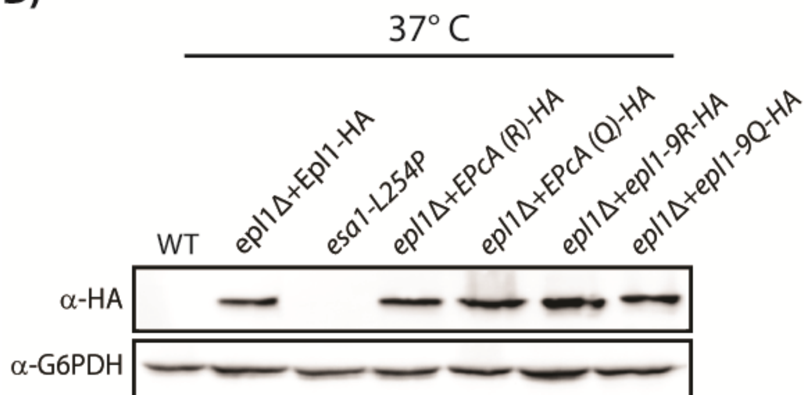
Though the Epl1 lysine mutants did not impact complex integrity, I wanted to determine if the sites impact NuA4 catalytic activity *in vivo*. To test this, I asked if the global levels of histone H4 acetylation is modulated in the Epl1 mutants compared to control strains using Western blot analysis of whole cell extracts (Figure 2.8, panel C). As expected in *esa1-L254P* cells the level of histone H4 acetylation is significantly reduced (lane 4) compared to wild type cells (lanes 1 & 2) or *epl1Δ* cells expressing wild-type Epl1-HA (lane 3). However, the level of Histone H4 acetylation in all the Epl1 acetylation point mutants (lanes 6-8) was considerably lower than the wild-type controls. This result suggests that Epl1 acetylation within the EpcA domain may contribute to the regulation of Esa1

acetyltransferase activity, either within the NuA4 or picNuA4 complexes, *in vivo*. Alternatively, acetylation of the lysine sites may not be regulating function, but rather these lysine sites themselves are key for NuA4 KAT activity. To differentiate between these two possibilities will require not only the identification of the key single lysine mutants within the EPcA region potentially required for NuA4 KAT activity and significantly more detailed investigations.

To further explore the potential impact on the Epl1 mutants on NuA4 function, I performed a series of dot assays to assess growth under stress or drug conditions which require NuA4 function for survival. Surprisingly, my results demonstrated that Epl1 mutants did not display growth defects on any drug plates tested, but mild growth defects were detected at higher temperatures (Figure 2.9A). However, unlike Eaf1 acetylation point mutant, the temperature sensitivity was exclusive to K→Q (mimicking constitutively acetylated lysine) mutants indicating that Epl1 deacetylation maybe important for proper NuA4 functioning at high temperatures. Also, *epl1-9Q-HA* acetylation point mutants exhibited a more pronounced temperature sensitivity compared to that in *epl1-EPcA-Q-HA* suggesting that acetylation of full length Epl1 may have an additive effect on NuA4 temperature sensitivity. Alternatively, these lysines may not be acetylated *in vivo*; however the charged lysines at these sites are essential for protein function at higher temperatures. Western blot analysis of the whole cell extract of the same strains grown at 37°C illustrates relatively equal amounts of Epl1-HA protein expressed in wild-type and mutant strains suggesting that the observed temperature sensitivity phenotype is not due to lower Epl1 protein expression at this growth condition (Figure 2.9B).

Figure 2.9: Epl1 acetylation point mutants show mild temperature sensitivity. (A) Wild-type untagged (lane 1, YKB779), *epl1Δ* covered with wild-type Epl1-HA expressed from a plasmid (lane 2, Epl1-HA YKB1974), temperature sensitive acetylation deficient mutant (lane 3, *esa1-L254P* YKB860) , and *epl1Δ* background covered by mutant versions of *EPL1*-HA expressed from a plasmid as indicated (lane 4, *epl1-EPcA(R)-HA* YKB2877 ; lane 5, *epl1-EPcA(Q)-HA* YKB2878 ; lane 6, *epl1-9R-HA* YKB2879 ; and lane 7, *epl1-9Q-HA* YKB2880) were grown to mid-log phase (OD_{600} 0.6-0.9) in YPD media at 25°C and 5µl of five-fold dilutions of each strain was spotted onto YPD plates containing indicated drugs and incubated at 25°C for 3 days. For temperature sensitivity assay, strain were spotted onto YPD plates and incubated at either 25°C, 30°C, 33°C (for 3 days) and 37°C for 2 days. (B) WCEs were collected from same strains grown in YPD media at 37°C and probed with anti-HA. Band intensity corresponding to mutant strains was compared to that in the wild-type. Anti-G6PDH was used as loading control.


A)
WT
epl1Δ+Epl1-HA
esa1-L254P
epl1Δ+epl1-EPcA (R)-HA
epl1Δ+epl1-EPcA(Q)-HA
epl1Δ+epl1-9R-HA
epl1Δ+epl1-9Q-HA
YPD 25°C
YPD 30°C
YPD 33°C
YPD 37°C
Benomyl 15ug/ml
HU 100mM
Caffeine 3mM
MMS 0.03%


B)
37° C
WT
epl1Δ+Epl1-HA
esa1-L254P
epl1Δ+EPcA (R)-HA
epl1Δ+EPcA (Q)-HA
epl1Δ+epl1-9R-HA
epl1Δ+epl1-9Q-HA
α-HA
α-G6PDH

2.4 Discussion:

The effect of subunit acetylation on function has been described for only two NuA4 subunits; Yng2 (Lin et al., 2008) and more recently Esa1 (Yuan et al., 2011). However, mass spectrometry analysis of NuA4 indicated that many subunits of NuA4 are potentially acetylated. In this study, I assessed the potential effect of acetylation on two subunits of NuA4, Eaf1 and Epl1. Despite the detection of acetylated lysines on both subunits, through this work I was only able to confirm an acetylation signal on only Epl1 subunit. My work shows that acetylation of Epl1, at least partially, depends on NuA4 activity and acetylated residues are within the EPcA domain. Further, my preliminary analysis suggests that potentially acetylation within the EPcA domain of Epl1 may impact NuA4 KAT activity.

2.4.1 Putative acetylation sites of Eaf1 and Epl1 regulate the function of NuA4 under stress conditions.

In an effort to elucidate biological significance of Eaf1 and Epl1 acetylation on NuA4 function, I conducted a series of biochemical assays using various Eaf1 and Epl1 acetylation point mutants. My data from this study suggested although Eaf1 and Epl1 acetylation point mutants do not show major growth defects upon various chemical challenges; they exhibit mild to modest sensitivity to growth at higher temperatures (Figures 2.4, 2.5 & 2.9). The temperature sensitivity of the Eaf1 and Epl1 mutants may be explained by the fact that NuA4 function has been linked to heat shock stress response possibly through an Msn2/Msn4 dependent pathway (Lindstorm et al., 2006; Mitchell et al., 2008). Msn2 and Msn4 are transcription factors that regulate the expression of several stress inducible genes. Under normal growth conditions, NuA4 along with Isw1 and Swr1 chromatin remodelers opposes the Msn2 and Msn4 function (Lindstorm et al., 2006).

However, in the NuA4 mutant background Msn2/Msn4 action up-regulates the expression of stress-induced genes in response to diverse stress signals (Lindstrom et al., 2006). Indeed Mitchell and colleagues reported physical interaction of NuA4 with Msn4. However it is still unknown whether this interaction is direct through one of the subunits or interaction is mediated through other non-NuA4 co-purifying proteins (Mitchell et al., 2008). Along with these data, it is possible that charge alternation due to acetylation or deacetylation may induce conformational changes in overall protein structure of Eaf1 and Epl1 which in turn can alter their interactions with other proteins such as transcription factors. Nevertheless, the exact mechanism by which Eaf1 and Epl1 acetylation regulates NuA4 function at elevated temperature needs more detailed studies.

Mutagenesis analysis of putative acetylation sites, K102, K280, and K848, on Eaf1 subunit illustrated that these residues play an important role in regulating NuA4 activity at elevated temperature (Figure 2.4). Although these lysines were identified as *in vitro* acetylation sites, real evidence that they are actual *in vivo* acetylation sites is still lacking. Typically, the *in vitro* KAT assays are performed by incubation of purified KAT and candidate substrate with radio-labeled version of acetyl coenzyme A (^{3}H, ^{13}C, or ^{14}C) for certain amount of time followed by gel separation and mass spectrometry. Detection of acetylation using this technique, however, can result in spurious KAT enzymatic activity and promiscuous acetylation caused by restrictive, non-physiological experimental conditions, and prolonged incubation time, forcing the KAT to acetylate lysines that are not the real *in vivo* acetylation targets. Nevertheless, being acetylated or not, my data from this study illustrates that K102, K280, and K848 on Eaf1 have a key role in directing NuA4 function at least in elevated temperatures.

2.4.2 Epl1 is a novel acetylation target for NuA4 in vivo.

The capability of NuA4 to auto-acetylate has been described for Yng2 (Lin et al., 2008) and Esa1 (Yuan et al., 2011) subunits. As mentioned previously, however, using *in vitro* KAT assay our lab has discovered that NuA4 is capable of auto-acetylating at multiple subunits (Table 2.1). In this study, using NuA4-PrA-TAP purification and Western blot analysis I have also shown that Epl1 is a novel NuA4 substrate *in vivo* (Figure 2.8). The acetylation signal corresponding to wild type Epl1 is dramatically reduced in an *esa1-L254P* mutant, both at restrictive and non-restrictive temperature, suggesting Epl1 acetylation *in vivo* at least partially depends on normal Esa1 function. My work also suggests that the key acetylation sites are located within the conserved EPcA domain. Analysis of individual single point mutants of EPcA domain did not show any reduction in acetylation signal (Appendix E; Figure 2S, lanes 4-7) suggesting that multiple sites within this region may be acetylated. To date, only a few non-histone acetylation targets have been identified for NuA4. This work introduces Epl1 as a genuine Esa1-dependent acetylation target *in vivo*.

2.4.3 Epl1 acetylation status regulates NuA4 dependent H4 acetyltransferase activity.

NuA4 is the major KAT to acetylate histone H4 (Allard et al., 1999; Smith et al., 1998). Multiple lines of evidence have also shown that the Epl1 subunit is essential for modulating NuA4 activity towards acetylating nucleosomal histones. Also, more recent biochemical analysis illustrates that NuA4 interacts with nucleosomal core protein (NCP) through its Epl1 subunit (Chittuluru et al., 2011). Indeed, Epl1 N-terminus encompassing the EPcA region has been shown to be essential to form a stable complex with NCP. Based on this fact one hypothesis is that acetylation status of EPcA domain may potentially impact the NuA4 interaction with NCP. Therefore, it is possible that reduced H4 acetylation as seen for

K→Q and K→R mutants can be attributed partly to impaired NuA4-NCP interaction. In addition, the EPcA domain of Epl1 links Yng2 to the catalytic subunit Esa1. This link has been shown to create a structure that allows Esa1 to acetylate nucleosomal histone H4 such that deletion of *YNG2* results in similar loss of global histone H4 acetylation. So, another possibility is that mutations in the EPcA domain may impact Yng2 interaction with Epl1 and therefore Esa1, leading to reduced histone H4 acetylation by Esa1. Nevertheless, the true nature of NCP interaction with these mutants and validation of these hypotheses needs further investigation.

2.4.4 Does NuA4 essentiality depend on its Esa1-dependent HAT activity?

An interesting but odd observation during the course of this study was that although acetylation status of EPcA domain of Epl1 had no adverse effect on NuA4 complex integrity (Figure 2.8, panel A) and sensitivity to various chemicals and growth conditions (Figure 2.9A), the HAT activity as assessed by global histone H4 acetylation was significantly reduced in all the mutant strains compared to that in the WT controls (Figure 2.8, panel C). The decrease in H4 acetylation displayed by my *epl1* mutants is similar to that of *esa1-L254P* mutants that do display dramatic sensitivity to all chemical tested (Figure 2.9A). The lack of phenotypes of my *epl1* mutants on the surface is contradictory to its acetylation defects and needs future investigation. One potential explanation is that the anti-Acetyl H4 antibody, despite being used by many other groups, is not adequate for this analysis as this antibody recognizes acetylation on both histone H4 and H2A in yeast (Boudreault et al., 2003) and may cross react with other acetylated proteins. The HAT activity of NuA4 hence could be better determined by measuring the levels of histone H4 acetylation using anti-

hyperacetylated histone H4 antibody which specifically recognizes distinct acetylation on histone H4 (Altaf et al., 2010).

Alternatively, though the essential role of NuA4 in the cell has been linked to the catalytic activity of Esa1 for H4 acetylation (Allard et al., 1999), this may not be fully true. Decker and colleagues have demonstrated that mutation of the catalytic site residues still supports growth though the HAT activity of these mutants was severely impaired in comparison to the WT (Decker et al., 2008). Based on their observations, the authors concluded that although Esa1 catalytic activity is important for the normal physiology, the Esa1 essential role may not only be due to its acetyltransferase activity but its involvement in more than one pathway/function. The increasing evidence for this hypothesis makes it necessary for further biochemical and genetic experiments to reveal additional roles for Esa1 that is essential for cell survival.

Chapter 3:

Building a yeast *KATalogue* of Synthetic Dosage Lethal Interactions

3.1 Introduction:

Several acetylome studies in both prokaryotic and eukaryotic systems have demonstrated that acetylation is a ubiquitous and highly conserved PTM and a major player in protein regulation (Zhao et al., 2010; Wang et al., 2010; Zhang et al., 2009; Choudhary et al., 2009; Kim et al., 2006). Despite many large scale proteomic efforts to identify non-histone KAT targets, lysine acetylation on non-histone proteins is understudied in yeast and to date only few non-histone targets have been identified (Table 1.1). Given that KAT complexes are highly conserved from yeast to human, and are implicated in a wide range of human diseases (Table 1.1 and reviewed in Dekker and Haisma, 2009), the availability of systematic screening techniques in this model organism may provide an important tool for connecting KATs to their biological substrates and to elucidate the pathways governed by lysine acetylation.

Protein acetylation microarray technology has been the only systematic analysis approach which has been employed to date to identify *in vitro* acetylation targets for NuA4, an essential KAT in yeast (Lin et al., 2009). In this approach, a yeast protein microarray containing 5800 proteins was incubated with NuA4 and radiolabelled acetyl-CoA and acetylated proteins were detected by autoradiography. Using this method 91 non-histone acetylated proteins were identified as putative *in vitro* substrates of NuA4. Of the 20 proteins that were selected for further validation, only 13 were confirmed by secondary method as true *in vivo* NuA4 targets including Pck1, a key gluconeogenesis regulator. Though this study dramatically expanded our knowledge on putative substrates for NuA4, it failed to identify known non-histone NuA4 acetylation targets including NuA4 subunits, Yng2 (Lin et al., 2008), Esa1 (Yuan et al., 2011), or other NuA4 subunits (Table 2.1; L.

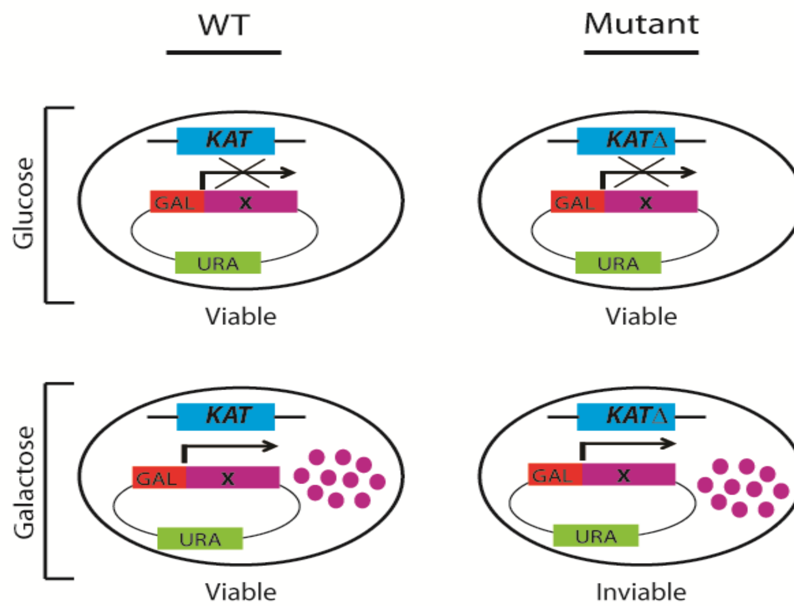
Mitchell unpublished data), and septin proteins (Mitchell et al., 2011). Previous acetylome studies reported that KATs preferentially target protein complexes, suggesting proteins may be required to be in their native complexes in order to be recognized by KATs. Hence, protein acetylation microarrays underestimate the number of targets.

A genome wide screening technique called synthetic dosage lethal (SDL) screen is a powerful alternative approach to probe gene function and identify enzyme-substrate relationship. The SDL is based on the idea that increased level of inappropriately modified protein may have no effect on a wild-type strain but may result in sickness or lethality in a mutant strain (Figure 3.1A). Genome-wide SDL screens exploit systematic genetic analysis (SGA) technology (Tong et al., 2001) to introduce a query mutant of interest into the galactose-inducible over-expression library, which contains ~5200 plasmid-born yeast genes (Sopko et al., 2006). Using SGA technology, double mutants (query deletion mutant containing an over-expression plasmid) are generated, and synthetic dosage sick (SDS) or synthetic dosage lethal (SDL) interactions are identified by slow growth or death of the mutant when over-expression is induced on galactose media (Figure 3.2B). This method has been used successfully in the past to identify known and novel substrates of kinases as well as targets of specific proteolytic pathways (Liu et al., 2009; Sopko et al., 2006; Zou et al., 2009). More recently, the Baetz lab has successfully applied genome-wide SDL profiling method to identify NuA4-dependent acetylation targets. Through SDL screening they discovered a novel link between NuA4 and septin proteins, providing the first evidence that NuA4 function regulates septin dynamics likely through acetylation of septin proteins (Mitchell et al., 2011).

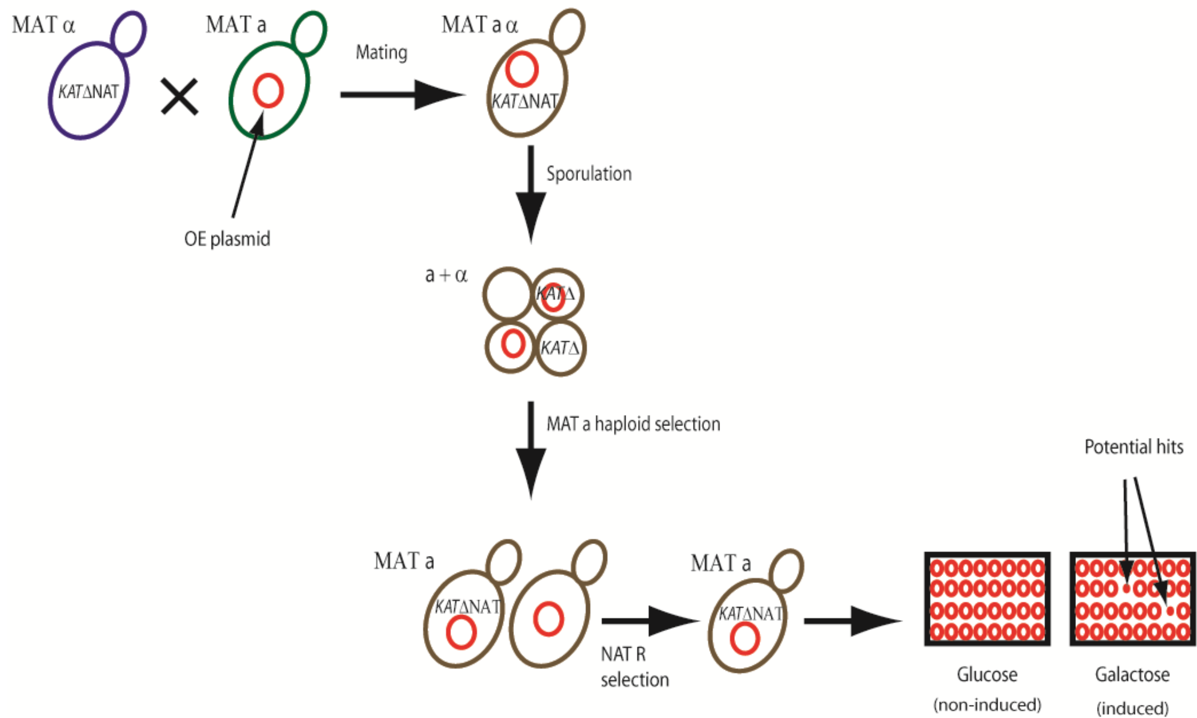
Since the SDL screen has been successful in identifying new pathways and non-histone targets regulated by NuA4 function (Mitchell et al., 2011), I hypothesized that genome-wide SDL screening can also be successfully applied to all non-essential KATs in *S. cerevisiae*. To build the KAT synthetic dosage network, I performed genome-wide SDL analysis for six non-essential yeast KATs; Hat1, Rtt109, Hpa2, Sas3, Sas2, and Elp3. My genetic interaction map revealed the involvement of KATs in a vast number of cellular pathways with each KAT identifying largely distinct genetic interactions indicating that they are mediating their cellular effects through acetylation of a unique set of substrates.

Figure 3.1. Schematic illustration of systematic SDL screening. (A) Conceptual basis for SDL screening. (B) Schematic of SDL-SGA screening. A *MATα* strain carrying a query mutation (*KAT*Δ) is crossed to the ordered array of yeast over-expression library (*MATa*) in which each strain has a unique p*GAL1/10*-GST-6×His-ORF plasmid. Diploids are selected and sporulated, and *MAT*a haploids carrying the query mutation and overexpression plasmid are isolated by selection. Haploids are pinned onto glucose (control) or galactose (experimental) to induce expression from the plasmid based *GAL10* promoter. Colony sizes on galactose plates were compared to those on glucose to identify those strains with a galactose-specific growth defect reflecting sensitivity to over-expression on the plasmid-born gene.

A)



B)



3.2 Materials and methods

3.2.1 Yeast strains and media:

Yeast strains used in this study are listed in Appendix A (Table 1S). The galactose-inducible over-expression array (Sopko et al., 2006) was a generous gift from Brenda Andrews. Genomic deletion and epitope tag integrations made for this study were designed using standard PCR-mediated cassettes as previously described and confirmed by PCR analysis (Longtine et al., 1998).

3.2.2 SDL-SGA query strain construction and scoring:

SDL screening was performed exactly as previously described (Mitchell et al., 2011). Genome-wide SDL screens were performed in triplicates at 25°C using the following query strains: *hat1Δ* (YKB 2611), *rtt109Δ* (YKB 2612), *sas3Δ* (YKB 2613), *elp3Δ* (YKB 2614), *sas2Δ* (YKB 2615), and *hpa2Δ* (YKB 2616). For the final scoring analysis, plate images were acquired using the ChemiDoc XRS Molecular Imaging System two days after pinning onto synthetic medium lacking uracil and containing either 2% glucose or galactose as the sugar source. Images were analyzed using an automated scoring program, ScreenMill (Dittmar et al., 2010) and further curated by visual inspection. SDL interactions that appeared in a list of "toxic gene", whose over-expression alone cause severe fitness defects or death in WT (Sopko et al., 2006), were eliminated from further analysis.

3.2.3 SDL Confirmations:

For each query, SDL, Synthetic Dosage Sick (SDS), or synthetic dosage rescue (SDR) interactions that were identified in at least two screens were confirmed by direct transformation followed by serial spot dilution assays. In short, plasmids were extracted

from the original over-expression array, amplified in bacteria, and purified using PureLink Quick Plasmid Miniprep Kit (Invitrogen, K2100-11). Each over-expression plasmid then was transformed (Gietz and Schiestl, 2007) into the wild-type (YKB1079) strain and the corresponding KAT deletion in a different background; *sas2ΔkanMX* (YKB2990), *sas3ΔkanMX* (YKB2991), *rtt109ΔkanMX* (YKB2992), *hat1ΔkanMX* (YKB2993), *hpa2ΔkanMX* (YKB2994) *, elp3ΔkanMX* (YKB2995).

3.2.4 Serial spot dilution assay to confirm SDL interactions:

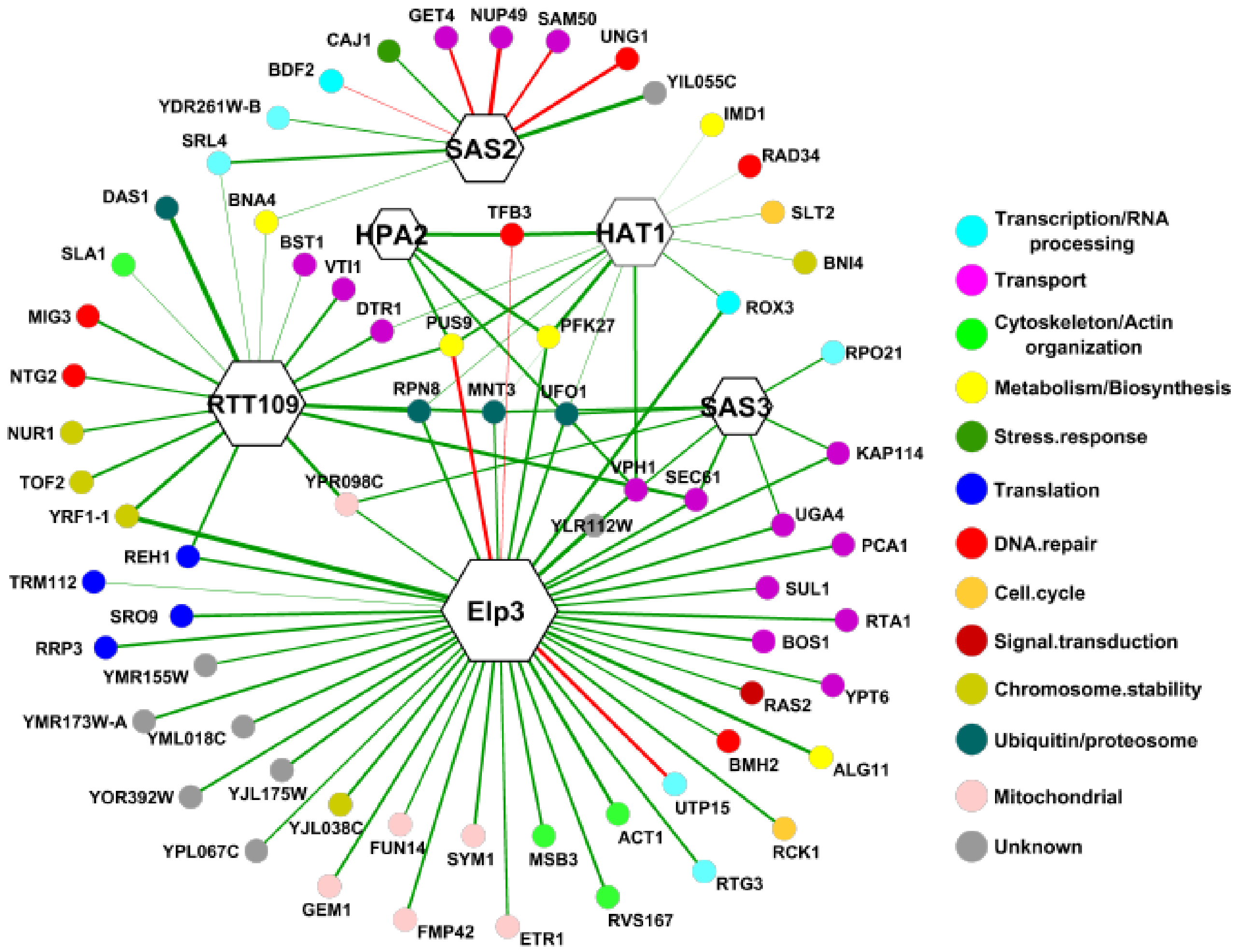
Wild-type and KAT mutant strains, transformed with galactose-inducible over-expression plasmids or an empty vector control (pRS416) were grown to mid-log phase in SD-URA liquid medium at 25°C. The exponentially growing cells were diluted to OD_{600} of 0.1 and ten-fold serial dilutions (OD_{600} = 0.1, 0.01, 0.001, and 0.0001) of each strain were spotted onto SD-URA medium containing 2% of either glucose or galactose. Plates were incubated at 25°C for 48 hours and pictures were collected using the ChemiDoc XRS Molecular Imaging System (BioRad).

3.3 Results

3.3.1 The yeast KAT synthetic dosage lethal network.

To elucidate pathways regulated by yeast lysine acetyltransferase complexes and to discover putative acetylation substrates, I identified SDL interactions for six non-essential yeast KATs. Genome-wide SDL screens were performed in triplicates with six query genes (*hat1Δ, rtt109Δ, sas3Δ, elp3Δ, sas2Δ, and hpa2Δ)* using synthetic genetic array (SGA) technology (Sopko et al., 2006; Tong et al., 2001). Despite several attempts with the *gcn5Δ* query strain, a reproducible genetic-interaction profile could not be obtained. For the six successfully screened KATs, genes whose over-expression caused SDL, SDS, and SDR phenotypes in at least two out of three replicates were confirmed by extraction of the corresponding over-expression plasmid from the array, amplification, and re-transformation into both wild-type and KAT mutant strains in which the interactions were originally identified. Next, dot assay analysis was performed to directly compare the effects of gene over-expression in wild-type to that in the mutant background (see Materials and Methods for more details). The final yeast KAT SDL genetic network encompassed 97 genetic interactions among 74 genes, of which 3% (4/97) were SDL interactions, 8% (8/97) were SDR interactions, and the remaining were SDS interactions (Figure 3.2, Table 3S). The SDL genetic interaction network identified many genes involved in vast cellular processes known to be impacted by acetylation such as transcription, translation, metabolism, DNA damage, transport and more (Figure 3.2).

Figure 3.2: The yeast KAT synthetic dosage lethal interaction network links KAT to diverse and distinct sets of substrates. Genome-wide SDL-SGA screen were performed using query strains for 6 non-essential yeast KATs: *hat1Δ* (YKB 2611), *rtt109Δ* (YKB 2612), *sas3Δ* (YKB 2613), *elp3Δ* (YKB 2614), *sas2Δ* (YKB 2615), *hpa2Δ* (YKB 2616). Query genes are represented within clear hexagonal nodes in larger fonts. The size of the query node correlates with the number of interactions for each query. KAT interacting genes are represented by nodes that are color-coded according to functional annotation as listed in the legend. Green edges indicate genetic interactions either SDS or SDL, and SDR interactions are represented by red line. The thickness of the lines corresponds to the strength on interactions as the stronger the interaction, the thicker the line.


GET4
NUP49
SAM50
CAJ1
UNG1
BDF2
YIL055C
YDR261W-B
IMD1
SAS2
SRL4
RAD34
DAS1
BNA4
TFB3
SLT2
HPA2
HAT1
BST1
SLA1
BNI4
VTI1
MIG3
DTR1
ROX3
PUS9
PFK27
RPO21
NTG2
RTT109
RPN8
MNT3
UFO1
SAS3
NUR1
KAP114
VPH1
TOF2
YPR098C
SEC61
UGA4
YRF1-1
YLR112W
PCA1
REH1
TRM112
SUL1
Elp3
SRO9
RTA1
RRP3
BOS1
YMR155W
YPT6
RAS2
YMR173W-A
YML018C
BMH2
ALG11
YJL175W
YOR392W
UTP15
YJL038C
ACT1
RCK1
FUN14
YPL067C
SYM1
MSB3
RTG3
GEM1
RVS167
FMP42
ETR1
Transcription/RNA processing
Transport
Cytoskeleton/Actin organization
Metabolism/Biosynthesis
Stress.response
Translation
DNA.repair
Cell.cycle
Signal.transduction
Chromosome.stability
Ubiquitin/proteosome
Mitochondrial
Unknown

3.4 Discussion:

The biological function of KATs in yeast has been assessed by several studies using genome-wide synthetic lethal (SL) genetic interaction screening (Costanzo et al., 2010; Hoke et al., 2008; Mitchell et al., 2008; Pan et al., 2006) which generally identifies proteins functioning in parallel rather than direct pathways (Boone et al., 2007). Hence, this method comes short in identifying putative biological targets and direct pathways governed by KAT function. In contrast, genome-wide SDL screens have proved to be an effective method to identify physiological targets by assessing the effect of gene expression in a mutant background as SDL interactions occur between components of the same pathway (Sopko et al., 2006). In this study I performed genome-wide synthetic dosage lethal screen for six non-essential yeast KATs to identify putative downstream targets for yeast lysine acetyltransferases and discover potential novel roles and cellular pathways regulated by this class of enzymes.

3.4.1 Genome-wide SDL screen links KATs to diverse cellular processes.

I completed a genome-wide SDL screen using six non-essential yeast KATs and subsequently directly tested the putative hits on corresponding mutant strains. The constructed SDL genetic interaction map identified 66 genes that when over-expressed caused growth defects and 8 genes that suppress growth defects in the mutant strains (Figure 3.2, Table 3S). My SDL screen map identified genes located in various cellular compartments and implicated in wide variety of cellular pathways for which acetylation has well established roles such as transcription, translation, DNA damage repair, chromosome stability, cytoskeleton, transport, and more (Figure 3.2). Overall, my constructed SDL map confirms previous findings indicating lysine acetylation is a widespread protein modification

and a regulatory mechanism of large number non-histone proteins affecting diverse pathways and process.

3.4.2 Individual KATs have distinctive SDL interaction networks.

Only a few genes when over-expressed impacted multiple KATs, suggesting that each KAT targets a distinct set of substrates and cellular processes *in vivo*. Indeed of the 74 genes identified only 19% (15/74) caused SDL/SDS/SDR with two or more KATs in this screen. I also compared my SDL genetic interaction network to the final hit list of NuA4 SDL map (Mitchell et al., 2011). Of the 74 interacting genes, only 7 genes (9%) overlapped exclusively with NuA4-SDL genetic network indicating the success of SDL in identifying pathways and cellular process specific for each KAT.

Among the screened KATs, the query *elp3Δ* identified the largest number of interactions (46%, 45/97) with majority of genes (20%) identified implicated in transport (Figure 3.2 & Table 3S). These include *VPH1, SEC61, KAP114, UGA4, PCA1, SLU1, RTA1, BOS1, and YPT6*. The importance of lysine acetylation in regulating the activity of proteins involved in vesicular and nucleo-cytoplasmic transport has been described by several independent studies (Albaugh et al., 2011; Choudhary et al., 2009; Hirschey et al., 2009; Sadoul et al., 2011; Schwer et al., 2009). Furthermore, studies in the past report localization of Elp3 into mitochondria and actin patches suggesting possible role for elongator complex in regulating mitochondrial function as well as cell mobility through organization of actin cytoskeleton (Barton et al., 2009; Creppe et al., 2009). Indeed, my SDL genetic interaction network identified several mitochondrial genes as well as few genes involved in actin organization that when over-expressed caused genetic interactions in *ELP3*

mutant. These results suggest that SDL screen was successful in identify cellular processes that may be regulated by Elp3 *in vivo*.

The next highest number of genetic interactions (24%, 18/74) was identified for *rtt109Δ*. Rtt109 function has been implicated in regulation of replication (Han et al., 2007), DNA damage repair (Jessulat et al., 2008), and ultimately genome stability (Scholes et al., 2001). Indeed, Rtt109 deletion mutants display high rate of chromosome loss (Driscoll et al., 2007). Consistent with these observations, *rtt109Δ* display SDL genetic interaction with several genes that either directly or indirectly involved in chromosome stability. These include *NUR1*, *TOF2*, *YRF1-1*, *NTG2*, and *MIG3*. *NUR1* and *TOF2* gene products are directly involved in genome stability through stabilization of rDNA repeats (Huang et al., 2006; Mekhail et al., 2008). *YRF1-1* also encodes for a helicase which contributes to genome stability by induction of homologous recombination and decreasing telomere shortening (Yamada et al., 1998). Ntg2 and Mig3 on the other hand contribute indirectly to genome integrity through their action on DNA damage repair process (Alseth et al., 1999; Dubacq et al., 2004).

Sas2 and Sas3 are closely related yeast genes, both mainly involved in transcription silencing and maintaining heterochromatin boundary by acetylating histones H4 and H3, respectively. Studies report that these two proteins may also have roles in transcription and DNA damage repair (John et al., 2000; Shia et al., 2006). Although my SDL screen did not identify any genes involved in silencing for *SAS2* and *SAS3* query strains, several genes involved in transcription and RNA processing were identified, including *SRL4*, *BDF2*, *CAJ1*, *RPO21*, and *YDR261W-B*. Also, SDL genetic interactions with genes involved in

transport and protein ubiquitination / glycosylation may suggest additional cellular processes regulated by these enzymes *in vivo*.

Hat1 and Hpa2 are the first and latest described yeast KATs, respectively. Both these proteins are among the poorly understood enzymes in terms of their function beside histone acetylation. Through this SDL screening, Hat1 and Hpa2 display genetic interactions with genes involved in metabolism and biosynthesis, DNA damage repair and chromosome stability. These hits may be predictive of cellular process that these two KATs may be involved in and some may even represent true substrates.

3.4.3 Use of SDL for the identification of KAT targets.

Was the SDL screen successful in identifying biological target? Although studies in the past have shown that SDL screens can identify targets of enzymes, the identified genes may not always be the direct targets of the KATs but they could be predictive of cellular processes that KAT may be involved in. As mentioned earlier, though lysine acetylation has been identified as a prevalent PTM in yeast, to date only a handful number of non-histone targets have been identified for a few KATs. Hence, there is no known reference for direct comparison. As SDL screens have been proven to be successful in identifying biological targets in the past and my screen was also able to identify biological pathways known to be impacted by the function of some of the screened KATs such as Elp3 and Rtt109, and as my SDL network is enriched for genes involved in processes for which regulatory effect of acetylation is well established, these indicate that the SDL screen was successful in identifying pathways relevant to KAT function. Furthermore, identifying distinct genetic interaction maps for each KAT is another indication that this SDL screen was successful in

determining specific interactions rather than random interplays. Nevertheless, the validation of this data set requires further studies and analysis.

CHAPTER 4: General Discussion

Studies in higher organisms have reported many non-histone substrates for KATs and KDACs (reviewed in Glozak et al., 2005) suggesting that such substrates may also exist in yeast. In the past few years, genome-wide studies sought to discover such substrates in yeast and unravel their physiological importance by employing several high throughput screening techniques combined with genetics and biochemical assays. In this study, I applied molecular biology, biochemistry, and global screening techniques to investigate the biological significance of KAT regulation by lysine acetylation and to identify potential targets and cellular pathways impacted by KAT function using yeast as a model organism.

4.1 KAT autoacetylation – is it universal?

Enzyme regulation by self-modification has been reported for many enzymes such as auto-phosphorylation of ATM kinase, which induces its activation through intermolecular auto-phosphorylation following DNA damage (Bakkenist and Kastan, 2003)3), auto-methylation of BspRI DNA-methyltransferase, which abolishes its enzymatic activity (Szilak et al., 1994), and auto-acetylation of p300 acetyltransferase, which induces structural changes leading to its increased DNA binding affinity (Thompson et al., 2004). Likewise, self-acetylation of many KATs has also been reported by recent genome-wide proteomic studies (Choudhary et al., 2009; Zhao et al., 2010). Nevertheless, the biological consequences of these acetylations are largely unknown. One possibility is that charge neutralization by acetylation may affect protein interactions, either within the same complex or with other cellular molecules such as proteins, and DNA by providing or masking a binding site. Acetylation may also cross-talk with other PTMs to regulate protein function as reported for p53 where crosstalk between sumoylation and acetylation regulates its binding

to DNA (Wu and Chiang, 2009). Acetylation can also modulate the enzymatic activity as reported for human KAT enzyme p300 where acetylation correlates with increased catalytic activity (Thompson et al., 2004). The evidence that NuA4 self-acetylation may regulates its function comes from studies by Lin et al. and Yuan et al. which have shown subunits acetylation regulates NuA4 function in DNA damage repair and acetyl-CoA binding, respectively (Yuan et al., 2011; Lin et al., 2009). In this study I have shown that Epl1 acetylation is also NuA4 dependent (Figure 2.8; panel B) and has impact on NuA4 function under some stress conditions (Figure 2.9A) as well as regulating its histone H4 acetyltransferase activity (Figure 2.8; panel C).

4.2 Is acetylation the only role of KATs?

Enzymes are mainly classified and named based on the reaction they catalyze. But is it possible that enzyme be involved in other processes besides their known universal role? Proteins performing more than one cellular function referred to as “Moonlighting enzyme” have been described in the past (Jeffery, 2003). Such proteins acquire and maintain a second function, which can be unrelated to the primary function, through evolution and under some selective constraints. Pyruvate carboxylase is an example of moonlighting enzymes. Pyruvate carboxylase, as described by its name, is involved in carboxylation of pyruvate into oxalate. However, in yeast this enzyme is also shown to be essential for proper peroximal import and assembly of alcohol oxidase (Huberts and van der Klei, 2010). The same may also be true for KAT protein complexes. As mentioned previously, site-directed mutagenesis of catalytic site residues of NuA4 catalytic subunit Esa1 although results in significant reduction in HAT activity, is shown to still support cell growth (Decker et al., 2008). Similarly, my results from this study also show that despite significant reduction of histone

H4 acetylation levels in both EPcA(R/Q) and 9(R/Q) *epl1* acetylation point mutants (Figure 2.8; panel C) the strains do not show significance growth defects in various growth conditions to which NuA4 acetyltransferase deficient mutants are sensitive (Figure 2.9A). Taken together, these observations may suggest existence of another yet not explored role(s) for NuA4 which makes this protein complex essential for cell survival.

4.3 Building KAT physical interaction network and integrating with KAT SDL genetic interaction network to identify putative acetylation targets.

Although SGA-SDL screens have been shown to be successful in identifying KAT enzyme targets (Mitchell et al., 2011), additional putative acetylation targets can be discovered through other biochemical approaches that identify physical protein-protein interactions such as combination of large-scale affinity purification with mass spectrometry and *in vitro* protein microarray. In general the idea is that proteins which physically interact with KATs may provide insight into novel physiological roles that these protein complexes play within the cell and a subset of these interactors may be acetylation targets. In this respect, our lab has recently developed a novel method called mChIP-KAT-MS (L. Mitchell unpublished data) which uses modified chromatin immunopurification (mChIP) strategy coupled to *in vitro* KAT assay (to enrich for low abundance acetylated peptide) and mass spectrometry to identify interacting proteins as well as acetylation sites. This method has been successful in identifying NuA4-associated protein network (L. Mitchell unpublished data). Protein microarray is also an effective method to identify *in vitro* protein-protein interactions and has been used in the past to identify non-histone acetylation targets for NuA4 (Lin et al., 2009). Although each method comes with some limitations, the use of complementary datasets had proven to be successful in identifying substrates for yeast

Pho85 kinase (Sopko and Andrews, 2008). Similar types of integrated analyses can be used to build a comprehensive interaction network for yeast KATs to elucidate novel cellular pathways regulated by this class of enzymes and discover putative substrates which may shed light into development of new therapeutics.

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Appendices:

Appendix A:

Table 1S: List of strains used in this study

Strains used in Eaf1 analysis experiment

Strains	Genotype	Sources or References
YKB1688	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 EAF1-HA::kanMX*	This study
YKB42	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX*	(Mitchell et al., 2008)
YKB780	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1*	(Sikorski and Hieter, 1989)
YKB440	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ESA1-TAP::URA3*	Gift from N. Krogan
YKB2973	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K102R-HA::NAT*	This study
YKB2974	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K102Q-HA::NAT*	This study
YKB2975	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K280R-HA::NAT*	This study
YKB2976	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K280Q-HA::NAT*	This study
YKB2977	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K848R-HA::NAT*	This study
YKB2978	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K848Q-HA::NAT*	This study
YKB2979	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K102R-K280R-HA::NAT*	This study
YKB2980	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K102Q-K280Q-HA::NAT*	This study
YKB2981	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K102R-K848R-HA::NAT*	This study
YKB2982	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K102Q-K848Q-HA::NAT*	This study

YKB2983	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K280R-K848R-HA::NAT*	This study
YKB2984	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K280Q-K848Q-HA::NAT*	This study
YKB2985	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K102R-K280R-K848R-HA::NAT*	This study
YKB2986	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX eaf1-K102Q-K280Q-K848Q-HA::NAT*	This study
YKB2987	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX ESA1-TAP::TRP eaf1-K102R-K280R-K848R-HA::NAT*	This study
YKB2988	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 eaf1Δ::kanMX ESA1-TAP::TRP eaf1-K102Q-K280Q-K848Q-HA::NAT*	This study
YKB855	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 ESA1-TAP::TRP eaf1Δ::kanMX*	This study
YKB3001	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1 ESA1-TAP::TRP EAF1-HA::NAT*	This study

Strains used in Epl1 analysis experiment

Strains	**Genotype**	**Sources or References**
YKB779	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1*	(Sikorski and Hieter, 1989)
YKB2136	*MATaα ura3-52 lys2-801 ade2-101 trp1-Δ200 leu2-Δ1*	This study
YKB1972	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN [Epl1-3HA::URA2]*	This study
YKB1974	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN [Epl1-3HA::URA2]*	This study
YKB1042	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 EAF5::TAP::HIS*	Baetz lab
YKB860	*MATa his3-Δ200 leu2-3,112 trp-1Δ1 ura3-52 ade2-101 esa1ΔHIS3 esa1-L245P::URA3*	Baetz lab
YKB2862	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS [Epl1-3HA::LUE2]*	This study

YKB2864	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS [epl1-K39Q-3HA::LEU2]*	This study
YKB2866	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS [epl1-K345Q-3HA::LEU2]*	This study
YKB3060	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS [epl1-K376Q-3HA::LEU2]*	This study
YKB3061	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS [epl1-K379Q-3HA::LEU2]*	This study
YKB2868	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN [epl1-K39Q-3HA::LEU2]*	This study
YKB2870	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN [epl1-K345Q-3HA::LEU2]*	This study
YKB2872	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN [epl1-K376Q-3HA::LEU2]*	This study
YKB2874	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN [epl1-K379Q-3HA::LEU2]*	This study
YKB2876	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS esa1Δ::HIS3 esa1(L254P)::URA3 [Epl1-3HA::LUE2]*	This study
YKB2877	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN [epl1-K39R-K345R-K376R-K379R-3HA::LUE2]*	This study
YKB2878	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN [epl1-K39Q-K345Q-K376Q-K379Q-3HA::LUE2]*	This study
YKB2879	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN[epl1-K39R-K345R-K376R-K379R-K427R-K429R-K470R-K496R-K569R-3HA::LUE2]*	This study
YKB2880	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN [epl1-K39Q-K345Q-K376Q-K379Q-k427Q-K429Q-K470Q-K496Q-K569Q-3HA::LUE2]*	This study
YKB2781	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS [epl1-K39R-K345R-K376R-K379R-3HA::LUE2]*	This study

YKB2782	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS [epl1-K39Q-K345Q-K376Q-K379Q-3HA::LUE2]*	This study
YKB2783	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS [epl1-K39R-K345R-K376R-K379R-k427R-K429R-K470R-K496R-K569R-3HA::LUE2]*	This study
YKB2784	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 epl1ΔKAN EAF5-TAP::HIS [epl1-K39Q-K345Q-K376Q-K379Q-k427Q-K429Q-K470Q-K496Q-K569Q-3HA::LUE2]*	This study

Strains used in KAT-SDL screen

Strains	Genotype	Sources or References
YKB731	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	*Gift from C. Boone*
YKB2611	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 hat1Δ::NAT*	This study
YKB2612	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 rtt109Δ::NAT*	This study
YKB2613	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sas3Δ::NAT*	This study
YKB2614	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 elp3Δ::NAT*	This study
YKB2615	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sas2Δ::NAT*	This study
YKB2616	*MATα can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 hpa2Δ::NAT*	This study

Strains used in KAT-SDL confirmation

Strains	Genotype	Sources or References
YKB1079	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	DMA collection
YKB2990	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sas2Δ::kanMX*	DMA collection
YKB2991	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sas3Δ::kanMX*	DMA collection
YKB2992	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rtt109Δ::kanMX*	DMA collection
YKB2993	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hat1Δ::kanMX*	DMA collection
YKB2994	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hpa2Δ::kanMX*	DMA collection
YKB2995	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 elp3Δ::kanMX*	DMA collection

Appendix B:

Table 2S: List of primers used in this study

Primers used for cloning EAF1 and EPL1

Primer #	Primer Name	Sequence
OKB554	*EAF1 F1*	AAAAACATTGTAATAACATTAGCAAATAACGATAATAAC GCGGATCCCCGGGTTAATTAA
OKB555	*EPL1 R1*	ATGCGTAGAAAGATGTTCGAGAGGTACAAAGTTGTGCTG CGAATTCGAGCTCGTTTAAAC
OKB556	*EPL1 F2*	AGCGAAACAGAACTCTTCTATAACCCAGAAAAATTCATC ACGGATCCCCGCGTTAATTAA
OKB 557	*EPL1 5′*-DETC	ACATTGTACGGCAACTGCGG
OKB558	*EPL1 3′*-DETC	GTTGCCTCGACTTTCGTAG
OKB 559	*EAF1 FL-FW NotI*	GATC**gcggccgc**TTTTGGGCTGCCGAGGACAG
OKB561	*EPL1 FL-FW NotI*	GATC**gcggccgc** GAGCAGCTCATCGCCTATCC
OKB568	*Tag R HindIII*	GATC**aagctt**CGGATCTGCCGGTAGAGGTG
OKB840	*HA+NAT*	GAGGTCGCTCTTATTGACCACACCTCTACCGGCAGATCC
OKB869	*EPL1 NAT MX4 Rev*	GTTAAATGCCAATGCGTAGAAAGATGTTCGAGAGGTACA AAGTTGTGCTGCCAGTATAGCGACCAGCATTCAC

Primers used for Eaf1 mutagenesis

Primer #	Primer Name	Sequence
OKB721	*EAF1 K102R* Sense	GCTGCGACTCCCATAACAAAAAAGAAATTGAGGGATGTA AATTTAATA
OKB722	*EAF1 K102R* Antisense	TATTAAATTTACATCCCTCAATTTCTTTTTTGTTATGGGAG TCGCAGC
OKB723	*EAF1 K102Q* Sense	AGCTGCGACTCCCATAACAAAAAAGAAATTGGAGGATGT AAATTTAATAAA
OKB724	*EAF1 K102Q* Antisense	TTTATTAAATTTACATCCTCCAATTTCTTTTTTGTTATGGG AGTCGCAGCT
OKB725	*EAF1 K280R* Sense	ACGATTATATACCATCGAGAATACCCCAGGCTTTGCC

OKB726	*EAF1 K280R* Antisense	GGCAAAGCCTGGGGTATTCTCGATGGTATATAATCGT
OKB727	*EAF1 K280Q* Sense	GAACGATTATATACCATCGGAAATACCCCAGGCTTTGCC
OKB728	*EAF1 K280Q* Antisense	GGCAAAGCCTGGGGTATTTCCGATGGTATATAATCGTTC
OKB729	*EAF1 K848R* Sense	GTCCAATACCTTCAAATGGCAGATCTTCTTCAAATTTGGC TAG
OKB730	*EAF1 K848R* Antisense	CTAGCCAAATTTGAAGAAGATCTGCCATTTGAAGGTATTG GAC
OKB731	*EAF1 K848Q* Sense	AAGTCCAATACCTTCAAATGGCGAATCTTCTTCAAATTTG GCTAG
OKB732	*EAF1 K848Q* Antisense	CTAGCCAAATTTGAAGAAGATTCGCCATTTGAAGGTATTG GACTT

Primers used for Epl1 mutagensis

Primer #	**Primer Name**	**Sequence**
OKB689	*EPL1 K39-R* sense	TGACGGTCTAGATTCATTCTCAAGAGGCGATTCAGG
OKB690	*EPL1 K39-R* Antisense	CCTGAATCGCCTCTTGAGAATGAATCTAGACCGTCA
OKB691	*EPL1 K39-Q* sense	GGTCTAGATTCATTCTCAGAAGGCGATTCAGGTGCT
OKB692	*EPL1 K39-Q* Antisense	AGCACCTGAATCGCCTTCTGAGAATGAATCTAGACC
OKB693	*EPL1 K345R* Sense	TCAAGAATTGAAAAACGCGAGGGACTTGGCCCTGCT
OKB694	*EPL1 K345R* Antisense	AGCAGGGCCAAGTCCCTCGCGTTTTTCAATTCTTGA
OKB695	*EPL1 K345Q* Sense	TCAAGAATTGAAAAACGCGGAGGACTTGGCCCTGCT
OKB696	*EPL1 K345Q* Antisense	AGCAGGGCCAAGTCCTCCGCGTTTTTCAATTCTTGA
OKB697	*EPL1 K427R* Sense	GCAGCAGCGGCAGCGAGGGCAAAGAACA
OKB698	*EPL1 K427R* Antisense	TGTTCTTTGCCCTCGCTGCCGCTGCTGC
OKB699	*EPL1 K427Q* Sense	GCAGCAGCGGCAGCGGAGGCAAAGAACAATA
OKB700	*Epl1 K427Q* Antisense	TATTGTTCTTTGCCTCCGCTGCCGCTGCTGC

OKB701	*EPL1 K429R* Sense	CAGCGGCAGCGAAGGCAAGGAACAATAAAAGGAATAAC
OKB702	*EPL1 K429R* Antisense	GTTATTCCTTTTATTGTTCCTTGCCTTCGCTGCCGCTG
OKB703	*EPL1 K429Q* Sense	CAGCGGCAGCGAAGGCAGAGAACAATAAAAGGAA
OKB704	*EPL1 K429Q* Antisense	TTCCTTTTATTGTTCTCTGCCTTCGCTGCCGCTG
OKB705	*EPL1 K496R* Sense	CTGTCAAACTACCATCTTCCGAAATTCCTGACATTGTATT GGAAG
OKB706	*EPL1 K496R* Antisense	CTTCCAATACAATGTCAGGAATTTCGGAAGATGGTAGTTT GACAG
OKB707	*EPL1 K496Q* Sense	TTATGTCAAACTACCATCTTCCAGAATTCCTGACATTGTA TTGGAAG
OKB708	*EPL1 K496* Antisense	CTTCCAATACAATGTCAGGAATTCTGGAAGATGGTAGTTT GACATAA
OKB709	*EPL1 K470R* Sense	CCCTTAAGACTGAAAACGGAAGGCAACTCGCGAAAT
OKB710	*EPL1 K470R* Antisense	ATTTCGCGAGTTGCCTTCCGTTTTCAGTCTTAAGGG
OKB711	*EPL1 K470Q* Sense	TGCCCTTAAGACTGAAAACGGAGAGCAACTCGCGA
OKB712	EPL1 K470Q Antisense	TCGCGAGTTGCTCTCCGTTTTCAGTCTTAAGGGCA
OKB713	*EPL1 K569R* Sense	GCATCTATCGCGTCATCCAGGTTTCAGATAGATAGATCT
OKB714	*EPL1 K569R* Antisense	AGATCTATCTATCTGAAACCTGGATGACGCGATAGATGC
OKB715	*EPL1 K569Q* Sense	TGCATCTATCGCGTCATCCGAGTTTCAGATAGATAGATC
OKB716	*EPL1 K569Q* Antisense	GATCTATCTATCTGAAACTCGGATGACGCGATAGATGCA
OKB749	*EPL1 K376R* Sense	TTAAAAATATTCGATCAAAGGGTAAAAATTAGGAATTTG AAAAGATCTTTGAACATTAGTG
OKB750	*EPL1 K376R* Antisense	CACTAATGTTCAAAGATCTTTTCAAATTCCTAATTTTTACC CTTTGATCGAATATTTTTAA
OKB751	*EPL1 K376Q* Sense	TGAATTAAAAATATTCGATCAAAGGGTAAAAATTGAGAA TTTGAAAAGATCTTTGAACATTAG
OKB752	*EPL1 K376Q* Antisense	CTAATGTTCAAAGATCTTTTCAAATTCTCAATTTTTACCCT TTGATCGAATATTTTTAATTCA

OKB753	*EPL1 K379R* Sense	ATCAAAGGGTAAAAATTAAGAATTTGAGAAGATCTTTGA ACATTAGTGGAGAAG
OKB754	*EPL1 K379R* Antisense	CTTCTCCACTAATGTTCAAAGATCTTCTCAAATTCTTAATT TTTACCCTTTGAT
OKB755	*EPL1 K379Q* Sense	GATCAAAGGGTAAAAATTAAGAATTTGGAAAGATCTTTG AACATTAGTGGAGAAG
OKB756	*EPL1 K379Q* Antisense	CTTCTCCACTAATGTTCAAAGATCTTTCCAAATTCTTAATT TTTACCCTTTGATC
OKB857	*EPL1 K427R/429R* Sense	CAGCGGCAGCGAGGGCAAGGAACAATAAAAGGAATAAC
OKB858	*EPL1 K427R/429R* Antisense	GTTATTCCTTTTATTGTTCCTTGCCCTCGCTGCCGCTG
OKB859	*EPL1 K427Q/429Q* Sense	CAGCGGCAGCGGAGGCAGAGAACAATAAAAGGAATAAC
OKB860	*EPL1 K427Q/429Q* Antisense	GTTATTCCTTTTATTGTTCTCTGCCTCCGCTGCCGCTG
OKB861	*EPL1 K376R/379R* sense	CGATCAAAGGGTAAAAATTAGGAATTTGAGAAGATCTTT GAAC
OKB862	*EPL1 K376R/379R* Antisense	GTTCAAAGATCTTCTCAAATTCCTAATTTTTACCCTTTGAT CG
OKB863	*EPL1 K376Q/379Q* Sense	CGATCAAAGGGTAAAAATTGAGAATTTGGAAAGATCTTT GAAC
OKB864	*EPL1 K376Q/379Q* Antisense	GTTCAAAGATCTTTCCAAATTCTCAATTTTTACCCTTTGAT CG

Primers used for generating KAT knockouts used in SDL screen

Primer #	**Primer Name**	**Sequence**
OKB967	*GCN5 Nat-MX4* For	GTGAGCCGCCCAAAAGTCTTCAGTTAACTCAGGTTCGTATTC TACATTAGACATGGAGGCCCAGAATAC CC
OKB968	*GCN5 Nat-MX4* Rev	ATTTATTTCTTCTTCGAAAGGAATAGTAGCGGAAAAGCTTCT TCTACGCACAGTATAGCGACCAGCATTCAC
OKB969	*GCN5 3'*-DETC	CACATCGTCTCGCCGTACTAAAC
OKB970	*HAT1 Nat-MX4* For	GTTTTCAGCAAAATTATGCTTAAGCTATAACTATAGTGAGA ATCAAGAATACATGGAGGCCCAGAATACCC

OKB971	*HAT1 Nat-MX4* Rev	TTTCAGGCTTGTTAAACAAATAAATATGTTATTATATATTTA ATAAACAGCAGTATAGCGACCAGCATTCAC
OKB972	*HAT1 3'*-DETC	CGACAACATAACGGCTTCAACC
OKB973	*HPA2 Nat-MX4* For	GCTCTTAGTATTTTATATGCCAAGAAAGCAAACAGCCCTTTC TGTGTAGCACATGGAGGCCCAGAATACCC
OKB974	*HPA2 Nat-MX4* Rev	TTTAATTTTTTTTTTCTATACATCCATACTACTGAGGTAATT AGTGTTTCAGTATAGCGACCAGCATTCAC
OKB975	*HPA2 3'*-DETC	CTA CAC AAC CTA CGT TAC CGC TG
OKB976	*SPT10 Nat-MX4* For	TAG ACT TCC GCC AAA GTG ATT ATC AAC AAA AAT CGT AAT AAT TAG CTT CAA CAT GGA GGC CCA GAA TAC CC
OKB977	*SPT10 Nat-MX4* Rev	TTTATAGTTTCTAGGGTTGGTGATGTGACCGTCTCTGGCAGA GTCGCAATCAGTATAGCGACCAGCATTCAC
OKB978	*SPT10 3'*-DETC	GAGTTGAGTGCCTTGTTTCC
OKB979	*RTT109 Nat-MX4* For	GAG TTG TCC AGT AGA GTT AAA AGG TCA ATT CAA CCG GTC TTC AAT AAG ACA CAT GGA GGC CCA GAA TAC CC
OKB980	*RTT109 Nat-MX4* For	TCTAAGATCGATGCTACATACGTGTACTAAATAATAAATAT CAATATGTACAGTATAGCGACCAGCATTCAC
OKB981	*RTT109 3'*-DETC	GGCCAACCTGAGCAGTAGAG
OKB982	*SAS2 Nat-MX4* For	ATTGGAGGCTCCTATTTTCTAGTTGCTTTTTGTTTTCACTCGC AAAAAAAACATGGAGGCCCAGAATACCC
OKB983	*SAS2 Nat-MX4* For	TATTCTATCCTGAAATACATATGCCATTAAGTTACATCCTGA ATAGATTCCAGTATAGCGACCAGCATTCAC
OKB984	*SAS2 3'*-DETC	GTTTGGTCCTTGTTCGTCATCG
OKB985	*SAS3 Nat-MX4* For	CTCTCTCTTCTTCCTTCTTCATTAATTAGTCTCCGTATAATT TGCAGATAACATGGAGGCCCAGAATACCC
OKB986	*SAS3 Nat-MX4* For	TTAATAATGTTACATGTATATGCTTATATCCAATATATACCC ATCGCCGCCAGTATAGCGACCAGCATTCAC
OKB987	*SAS3 3'*-DETC	GACCAGCGCAAATGACATACG
OKB988	*ELP3 Nat-MX4* For	AAACAAGTCCTAAAAGCACCTAAGGAAAATCGAAGAACAC CCTGACAAAGACATGGAGGCCCAGAATACCC
OKB989	*ELP3 Nat-MX4* Rev	CTTGGAAAACCGGCCATGTCGGCGGCACATAAAAGTTCTAT TTACCTCAGTATAGCGACCAGCATTCAC
OKB990	*ELP3 3'*-DETC	ACACATGCAGCAGTTACTCC

Appendix C:

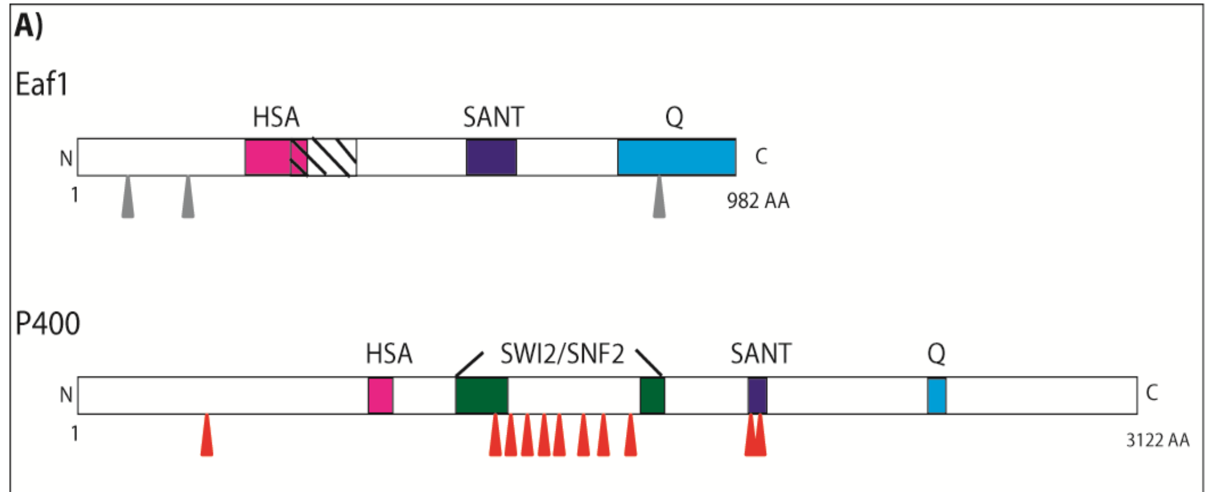
Table 3S: List of plasmids generated in this study

Plasmid	Vector	Insert	Sources
pKB39	pRS415	*EAF1-3HA*	This study
pKB40	pRS416	*EAF1-3HA*	This study
pKB41	pRS415	*EPL1-3HA*	This study
pKB42	pRS416	*EPL1-3HA*	This study
pKB43	pRS415	*eaf1-K102R-3HA*	This study
pKB44	pRS415	*eaf1-K102Q-3HA*	This study
pKB45	pRS415	*eaf1-K280R-3HA*	This study
pKB46	pRS415	*eaf1-K280Q-3HA*	This study
pKB47	pRS415	*eaf1-K848R-3HA*	This study
pKB48	pRS415	*eaf1-K848Q-3HA*	This study
pKB155	pRS415	*epl1-K39R-3HA*	This study
pKB156	pRS415	*epl1-K39Q-3HA*	This study
pKB157	pRS415	*epl1-K345R-3HA*	This study
pKB158	pRS415	*epl1-K345Q-3HA*	This study
pKB159	pRS415	*epl1-K376R-3HA*	This study
pKB160	pRS415	*epl1-K376Q-3HA*	This study
pKB161	pRS415	*epl1-K379R-3HA*	This study
pKB162	pRS415	*epl1-K379Q-3HA*	This study
pKB163	pRS415	*epl1-K376/379R-3HA*	This study
pKB164	pRS415	*epl1-K376/379Q-3HA*	This study
pKB165	pRS415	*epl1-K427R-3HA*	This study
pKB166	pRS415	*epl1-K427Q-3HA*	This study

pKB167	pRS415	*epl1-K429R-3HA*	This study
pKB168	pRS415	*epl1-K429Q-3HA*	This study
pKB169	pRS415	*epl1-K427/k429R-3HA*	This study
pKB170	pRS415	*epl1-K427/429Q-3HA*	This study
pKB171	pRS415	*epl1-K470R-3HA*	This study
pKB172	pRS415	*epl1-K470Q-3HA*	This study
pKB173	pRS415	*epl1-K496R-3HA*	This study
pKB174	pRS415	*epl1-K496Q-3HA*	This study
pKB175	pRS415	*epl1-K569R-3HA*	This study
pKB176	pRS415	*epl1-K569Q-3HA*	This study
pKB177	pRS415	*epl1-K39/345/376/379R-3HA*	This study
pKB178	pRS415	*epl1-K39/345/376/379Q-3HA*	This study
pKB179	pRS415	*epl1-K39/345/376/379/427/429/470/496/569R-3HA*	This study
pKB180	pRS415	*epl1-K39/345/376/379/427/429/470/496/569Q-3HA*	This study
pKB181	pRS415	*eaf11-K102/280R-3HA*	This study
pKB182	pRS415	*eaf11-K102/280Q-3HA*	This study
pKB183	pRS415	*eaf11-K102/848R-3HA*	This study
pKB184	pRS415	*eaf11-K102/848Q-3HA*	This study
pKB185	pRS415	*eaf11-K280/848R-3HA*	This study
pKB186	pRS415	*eaf11-K280/848Q-3HA*	This study
pKB187	pRS415	*eaf11-K102/280/848R-3HA*	This study
pKB188	pRS415	*eaf11-K102/280/848Q-3HA*	This study

Figure 1S: Schematic representation of yeast Eaf1 and Epl1 and their human homologs. (A) Diagram of homology regions of yeast Epl1 compared with human EPC1. (B) Diagram of homology regions of yeast Eaf1 compared with human p400. Colored boxes represent various domains on each protein. Red triangles represent the acetylated sites identified by our lab (for Eaf1 and Epl1) and as provided by phosphositeplus (for EPC1 and p400). Grey triangles represent the acetylated residues investigated in this study.

Appendix D:



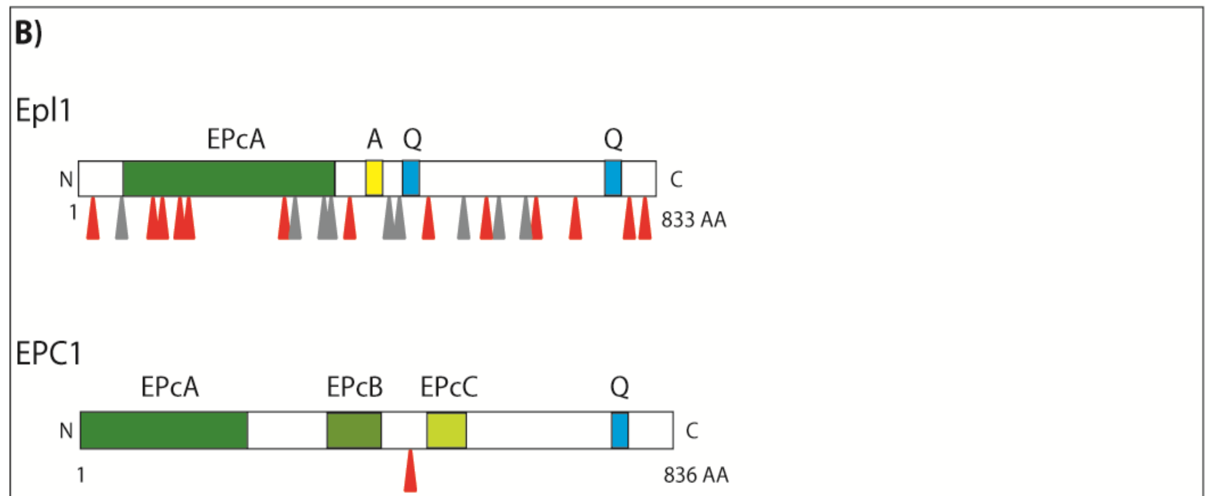
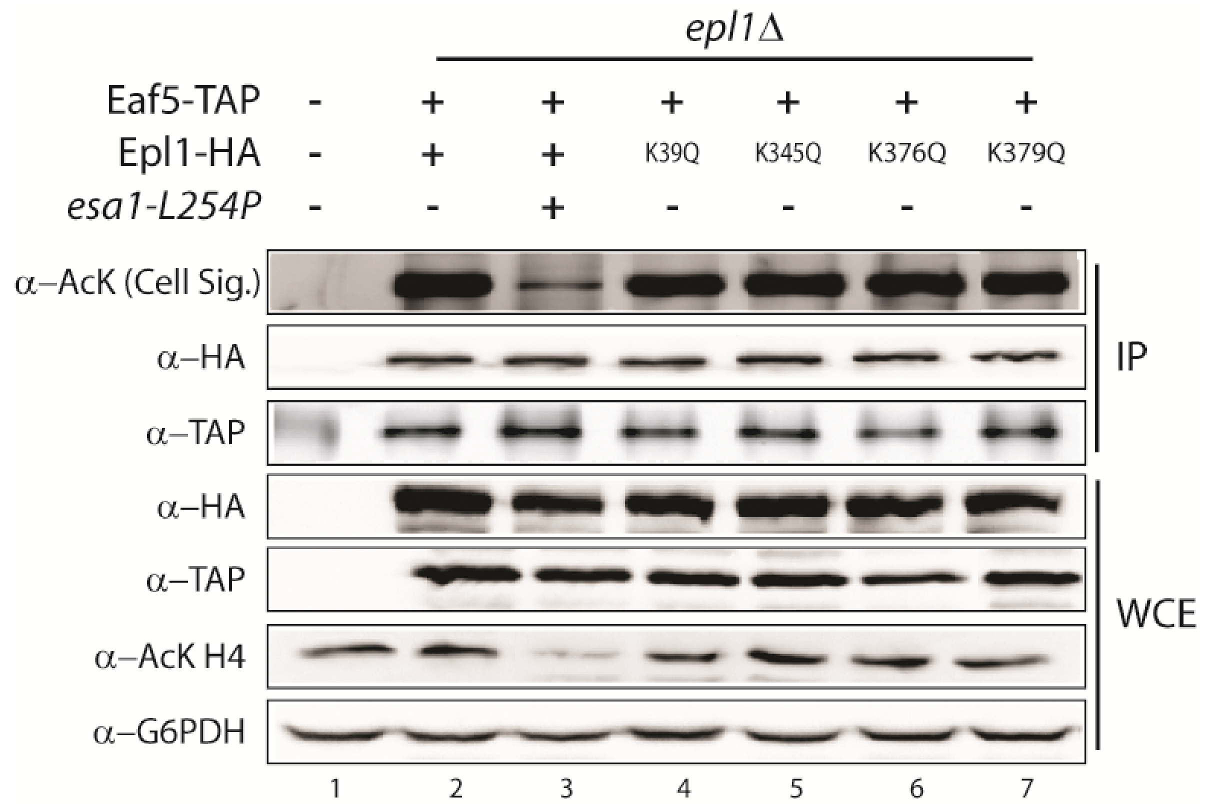


Figure 2S: Single constituvely acetylated *epl1* acetylation point mutants of EPcA domain do not display reduction in acetylation signal and histone H4 acetylation level. Untagged control (lane 1,YKB779) and yeast strains expressing Eaf5-TAP in *epl1Δ* background covered by either wild type (lane 2, YKB2862) or mutant versions of *EPL1*-HA expressed from a *CEN* plasmid as indicated: (lane 3, *esa1-L254P EPL1-HA* YKB2876; lane 4, *epl1-K39Q-HA* YKB2863; lane 5, *epl1-345Q-HA* YKB2864; lane 6, *epl1-376Q-HA* YKB2865; and lane 7, *epl1-379Q-HA* YKB2866 were grown to mid log-phase in YPD at 25 °C. Immunopurified (IP) products and whole cell extract (WCE) samples were separated on SDS-PAGE (7.5%) and subjected to Western blot analysis. Epl1 acetylation was assessed using anti-acetyl lysine (α-Ac K) antibody from cell signaling (Cell Sig). Purification efficiency and expression levels were assessed using anti-TAP (α-TAP) and anti-HA (α-HA) antibodies, respectively. Esa1 catalytic activity was assessed by measuring histone H4 acetylation levels (α-AcK H4). Anti-Glyceraldehyde-6-phosphate dehydrogenase (α-G6PDH) was used as a loading control. Figure is representative of one purification experiment.

Appendix E:



Appendix F:

Table 4S: Summary of KAT SDL scoring

Interacting Genes		Yeast KATs					
		HAT1	Rtt109	SAS3	ELP3	SAS2	HPA2
Systematic Name	Standard Name	YKB (2611)	YKB (2612)	YKB (2613)	YKB (2614)	YKB (2615)	YKB (2616)
Transport							
YBR295W	PCA1				2.5		
YLR378C	SEC61		2	2.5	2.5		
YGR213C	RTA1				2.5		
YLR078C	BOS1				2.5		
YGL241W	KAP114			3	2.5		
YOR270C	VPH1				2.5		2.5
YDL210W	UGA4			3	2.5		
YLR262C	YPT6				3		
YDR099W	BMH2				3		
YBR294W	SUL1				2		
YFL025C	BST1		4				
YBR180W	DTR1	4	2.5				
YMR197C	VTI1		2.5				
YER048C	CAJ1					3	
YGL172W	NUP49					**1**	
Transcription & RNA processing							
YBL093C	ROX3				2		
YBL103C	RTG3				2.5		
YHR065C	RRP3				2.5		
YMR093W	UTP15				**2**		
YER028C	MIG3		2.5				
YPL033C	SRL4		4				
YDR261W-B	YDR261W-B	3.5					
YDL070W	BDF2	**4**					
YDL140C	RPO21			3			
Metabolism & Biosynthesis							
YDL036C	PUS9				**2**		2.5
YNL048W	ALG11				2		
YLR251W	SYM1				2.5		
YBR026C	ETR1				3		
YBL098W	BNA4	4	4				

Cytoskeleton & Actin organization							
YDR388W	RVS167				2.5		
YNL293W	MSB3				2.5		
YFL039C	ACT1				2		
YBL007C	SLA1		4				
Glycosylation/Ubiquitination and proteosome							
YML088W	UFO1	4		2.5	2.5		
YOR261C	RPN8	4	2		2.5		
YJL149W	DAS1		1				
Translation							
YCL037C	SRO9				2.5		
YLR387C	REH1		2.5				
Silencing/condensation							
YKR010C	TOF2		2.5				
Chromosome stability							
YJL038C	YJL038C				2.5		
YDL089W	NUR1		3				
DNA repair							
YDR460W	TFB3				**4**		2
YOL043C	NTG2				3.5		
YDR314C	RAD34	4.5					
YML021C	UNG1					**2**	
Sorting							
YOR164C	*GET4*					**2.5**	
YNL026W	SAM50					**2.5**	
GTPase & Helicase							
YAL048C	GEM1				2.5		
YNL098C	RAS2				3		
YDR545W	YRF1-1		2		1		
Kinase & Methyltrasnferase							
YOL136C	PFK27				2.5		
YGL158W	RCK1				2.5		
YNR046W	TRM112				4		
YHR030C	SLT2	4					
Unknown							
YLR112W	YLR112W			3	2		
YMR221C	FMP42				2.5		
YAL008W	FUN14				3		

YJL175W	YJL175W				2.5		
YOR392W	YOR392W				2.5		
YMR173W-A	YMR173W-A				2.5		
YPR098C	YPR098C		2	3	3		
YMR155W	YMR155W				3		
YML018C	YML018C				2.5		
YPL067C	YPL067C				3		
YAR073W	IMD1	4					
YIL055C	YIL055C					1	

Scoring

All scoring are based on Dot assay analysis.

SDS interactions are shown in blank on the table.
blank - no growth defect
4 - minor growth defect
3 – medium growth defect
2 - major growth defect
1 – nonviable

SDR interactions are highlighted in red on the table.
blank - no growth defect
4 – growth similar to wild type
3 – major growth rescue
2 – medium growth rescue
1 – minor growth rescue

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Education

Sept. 2009- Apr 2012	**Masters of Science (Biochemistry)** Department of Biochemistry, Microbiology and Immunology University of Ottawa Thesis Supervisor: Dr. Kristin Baetz
Jan. 2006 – June 2009	**Bachelor of Science (Honours Biochemistry)** Department of Chemistry University of Waterloo

Publications, Conferences and Presentations:

Paper in press: Apraku D. Donkor, Rogheyeh Pourhanifeh, Zengding Su, Xiauou Tang (2012). "Array of Carbon nanotube needles Transfect Hela cells with Ephrine-B". Manuscript submitted to Nanoletters.

Ottawa Institute of Systems Biology Symposium, Ottawa, ON., Canada (2011). **"Functional analysis of Eaf1 and Epl1 acetylation of NuA4 subunits".** Pourhanifeh, R., Mitchell, L., Zhou, H., Lambert, J.P., and Kristin Baetz. **Oral presentation**

International Yeast Genetics and Molecular Biology Meeting, Vancouver, B.C., Canada (2010). **"Investigation of Eaf1 and Epl1 acetylation of NuA4 subunits".** Pourhanifeh, R., Mitchell, L., Lambert, J.P., Zhou, H., and Kristin Baetz. **Poster Presentation**

Progress in System Biology. **"Investigation of Eaf1 and Epl1 acetylation of NuA4 subunits".** Pourhanifeh, R., Mitchell, L., Lambert, J.P., Zhou, H., and Kristin Baetz. **Poster Presentation**

Academic Honours, Awards, and Scholarships

2010-2011	Canadian Institutes of Health Research (CIHR) Scholarship
2010-2011	Excellence Scholarship, University of Ottawa
2010	Research Travel Award, University of Ottawa
2009-2010	Admission Scholarship, University of Ottawa
2009-2010	Entrance Scholarship, University of Ottawa

2006-2009	Dean's Honours List, University of Waterloo
2006-2009	Excellent Standing, University of Waterloo
2008 Waterloo	Undergraduate Student Research Award (USRA), University of
2007	Gretchen E. Muller Memorial Biochemistry Scholarship, University of Waterloo
2006	Undergraduate Student Research Award (USRA), University of Waterloo
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