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The histone acetyltransferase Gcn5 and the multiple personalities of CK2 kinase define new roles in stress response and chromatin function

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The thesis of Jeremy Chung-Huan Chen is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego

2017

DEDICATION

I would like to thank Lorraine Pillus and all of the members of the Pillus lab for their support and willingness to always offer advice. I am truly grateful for Emily Petty for taking the time to answer all my questions and helping me view everything in a more positive light.

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LIST OF ABBREVIATIONS

DDR- DNA damage response

HAT – histone acetyltransferase

HDAC – histone deacetylase

H2AS129ph- phosphorylation

$Cka1^{Holo}$ - Cka1 containing holoenzyme

$Cka2^{Holo}$ - Cka2 containing holoenzyme

$Cka1\text{-}Cka2^{Holo}$ - Cka1-Cka2 containing holoenzyme

DUB – deubiquitination

NHEJ – non-homologous end joining

HR- homologous repair

MMR- mismatch repair

NER – nucleotide excision repair

dsDNA – double stranded DNA

MS – mass spectrometry

MMS – methyl methanesulfonate

HU – hydroxyurea

6AU – 6-azauracil

5-FOA – 5-fluorouracil

RNR – ribonucleotide reductase

ABSTRACT OF THE THESIS

The histone acetyltransferase Gcn5 and the multiple personalities of CK2 kinase define new roles in stress response and chromatin function

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Master of Science in Biology

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Professor Lorraine Pillus, Chair

Post-translational histone modifications have a critical role in regulating transcription, the cell cycle, and stress responses. These histone modifications can occur in complex patterns depending on the activity of multiple chromatin modifiers. Histone acetyltransferases (HAT) are one of the classes of chromatin modifiers important for altering chromatin structure and activating transcription.

Gcn5 is a highly conserved catalytic subunit found in multiple HAT complexes that regulate gene expression. In addition to HAT activity, phosphorylation of histones and non-histone proteins by protein kinases is also important. Kinases are necessary for signaling pathways such as a rapid response to stress. Here we define previously unsuspected interactions between Gcn5 and different isoforms of the protein kinase CK2, in *Saccharomyces cerevisiae.* We show that although CK2 has hundreds of targets *in vivo*, distinctions can be made between the individual catalytic subunits encoded by *CKA1* and *CKA2.* This distinction was found in cells lacking Gcn5. Loss of *CKA1* and *CKA2* suppresses *gcn5*Δ mutant sensitivity to elevated temperatures and DNA damage induced by hydroxyurea, respectively. Suppression is lost when either *CKB1* or *CKB2* are also deleted. Loss of *CKA1,* but not *CKA2* partially recovers the decrease in H3K9,K14ac in *gcn5*Δ cells. A directed screen of tyrosine residues of core histones demonstrated novel roles of multiple residues, but left unanswered key questions into the interactions seen between Gcn5 and CK2. Together, this work has shown distinct functions of the CK2 isoforms in their roles in specific stress responses and interactions with Gcn5.

Chapter 1. Introduction

In eukaryotic cells, chromatin is the complex of DNA packaged with highly conserved basic core histone proteins. The accessibility of genes is dependent on the chromatin architecture, which is influenced by dynamic post-translational histone modifications. Post-translational modifications are covalent modifications of the protein after protein biosynthesis. Thus, histone modifications can alter many cellular processes such as cell cycle progression, DNA damage response (DDR) pathways, DNA replication, DNA-protein interaction, and transcriptional regulation.

The chromatin complex's fundamental unit, the nucleosome, allows DNA to be packaged tightly by having 147 base pairs of DNA wrapped around a histone octamer (Figure 1-1). Each histone octamer contains two copies of each highly conserved histone protein: H2A, H2B, H3, and H4. The very basic histone N-terminal tails protrude from the nucleosome core structure and can contact adjacent nucleosomes. Modifications of histone tails can regulate chromatin structure and recruit enzymes [reviewed in (Bannister and Kouzarides 2011)]. Common modifications are acetylation, methylation, and phosphorylation which can affect histone-histone, histone-DNA, and protein-DNA interactions [reviewed in (Tessarz and Kouzarides 2014)]. Interestingly, not all modifications seem to have an effect on gene regulation. In fact, understanding each modification individually does not always explain the phenotypes that occur. Some modifications may rely on other histone residues. For example, in some cases

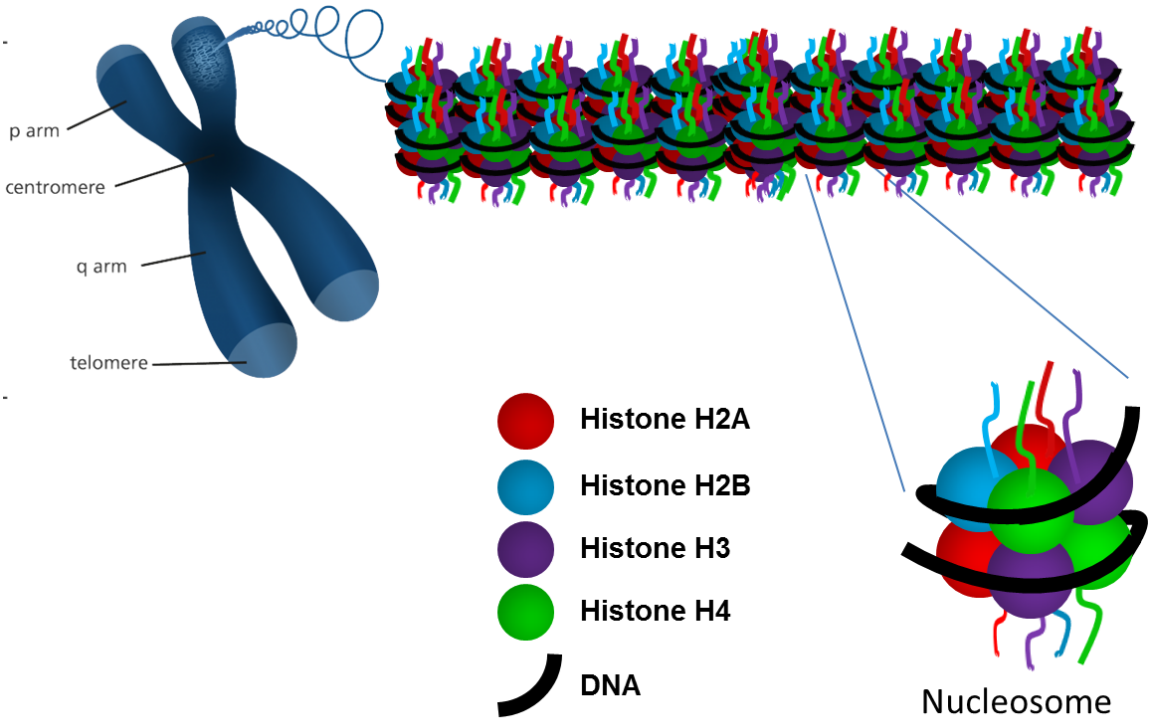


Figure 1-1. DNA is compacted into chromatin. DNA is organized into chromatin inside the nucleus. The nucleosome is the basic unit of chromatin. Each nucleosome is composed of a histone octamer with 147 base pairs of DNA wrapped around it. The histone octamer is composed of two histone H2A-H2B dimers and two histone H3-H4 dimers. The unstructured histone tails extend from the histone and core and are subject to post-translational modifications. Credits: Chromosome from Genome Research Limited; Nucleosome modified from S. Tomlinson 2012.

methylation at one histone residue requires acetylation on another histone residue to effect transcription [reviewed in (Rando 2012)]. The study of histone modifications has therefore become increasingly complex. There is still much unknown about the regulation of histone modifications as well as the effects of histone modifications on downstream events.

Accessibility to DNA is an important factor in regulating gene expression. Lysine acetylation, is well understood and neutralizes the positive charge of its amino group. Removing the positive charge from lysine residues within histone tails can cause the negatively charged DNA to bind less tightly, and allow greater access to the DNA molecule (Figure 1-2). Therefore, acetylation causes a more open state and removal of the acetyl group, or deacetylation causes a more closed and condensed state making the deacetylated nucleosomes act as a physical barrier. The correlation and importance of histone acetylation and increased transcription was found more than 50 years ago (Allfrey 1964). Since then, there have been many studies experimenting with lysine residues, acetyltransferases, and deacetylases. Multiple evolutionarily conserved histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes have already been discovered such as Gcn5 and Sir2, respectively. In most eukaryotes, many of these enzymes have been identified as transcriptional co-regulators and found to target promoter regions [reviewed in (Kurdistani and Grunstein 2003)]. Moreover, HATs and HDACs have roles in transcription, DNA replication, DNA repair, and heterochromatin formation and maintenance [reviewed in (Kurdistani and Grunstein 2003)]. Further study of HAT complexes has shown their

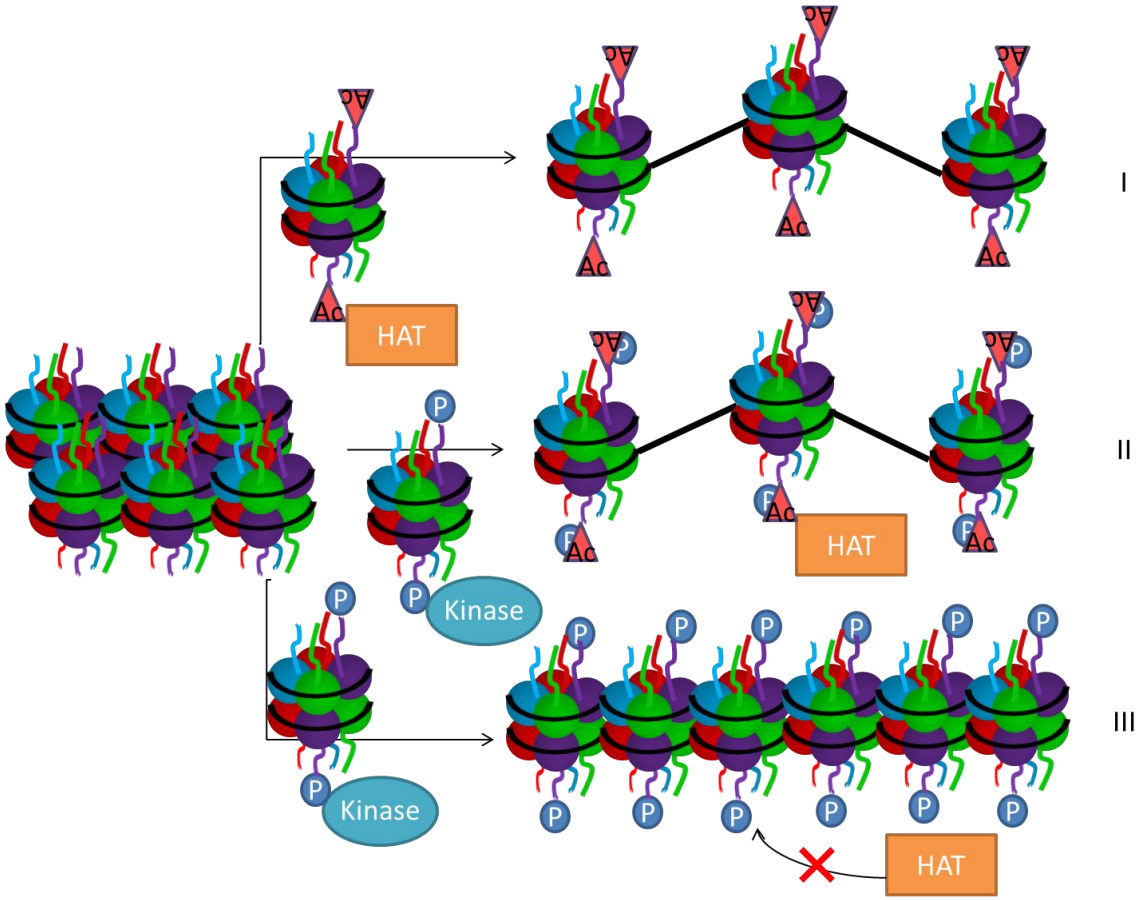


Figure 1-2. Dynamics of histone acetylation and phosphorylation. I) Histone acetyltransferases (HATs) transfer acetyl groups from Acetyl CoA to lysine residues on histone tails to neutralize the positive charge of the lysine residue. This loosens the interactions between the negatively charged DNA and the histone which can lead to the open conformation of chromatin. II) Phosphorylation at specific histone residues by a kinase can facilitate HAT activity also causing a more open conformation. III) In contrast, Phosphorylation at specific histone residues by a kinase can also block HAT activity, preventing histone residues from being acetylated.

importance in genome stability, as well as their interactions with other types of histone modifications [reviewed in (Lee and Workman 2007)].

Among the histone acetyltransferases the focus here is on Gcn5 and its complexes. The structure and function of Gcn5 have been widely studied in yeast. On its own, recombinant Gcn5 can acetylate free cytoplasmic histones, but not nucleosomal histones. HAT activity by Gcn5 on nucleosomes requires it to be a part of multi-subunit high-molecular weight complexes (Grant et al. 1997). These complexes preferentially acetylate histone H3 tails, as well as histones H2B. Histone H3 Lysine 9 (H3K9), K14, K18, K23, and K27, and H2B K11 and K16 are several known histone targets of Gcn5 (Suka et al. 2001, Lee and Workman 2007). Some of these modifications, such as K14 acetylation, are linked to transcriptional activation (Lo, et al. 2000). Gcn5 also functions as a coactivator at specific promoters and is important for genome-wide basal level acetylation (Waterborg 2000). The basal level acetylation may increase the affinity and associations of different factors and transcription machinery for transcriptional activation (Imoberdorf 2006).

Gcn5 is part of three HAT complexes in budding yeast, ADA, SAGA, and SLIK/SALSA (Figure 1-3) (Grant et al. 1997 and Pray-Grant MG, et al. 2002). Multiple HAT complexes can modify the same histone residues, however the subunits in each complex allow each HAT to modify their preferred target. For example, SAGA can modify both histone H3K9 and to a lesser extent H3K14, but the NuA3 complex, with a different HAT, will preferentially modify H3K14 (John et al. 2000). This argues the importance of both the non-catalytic subunits and

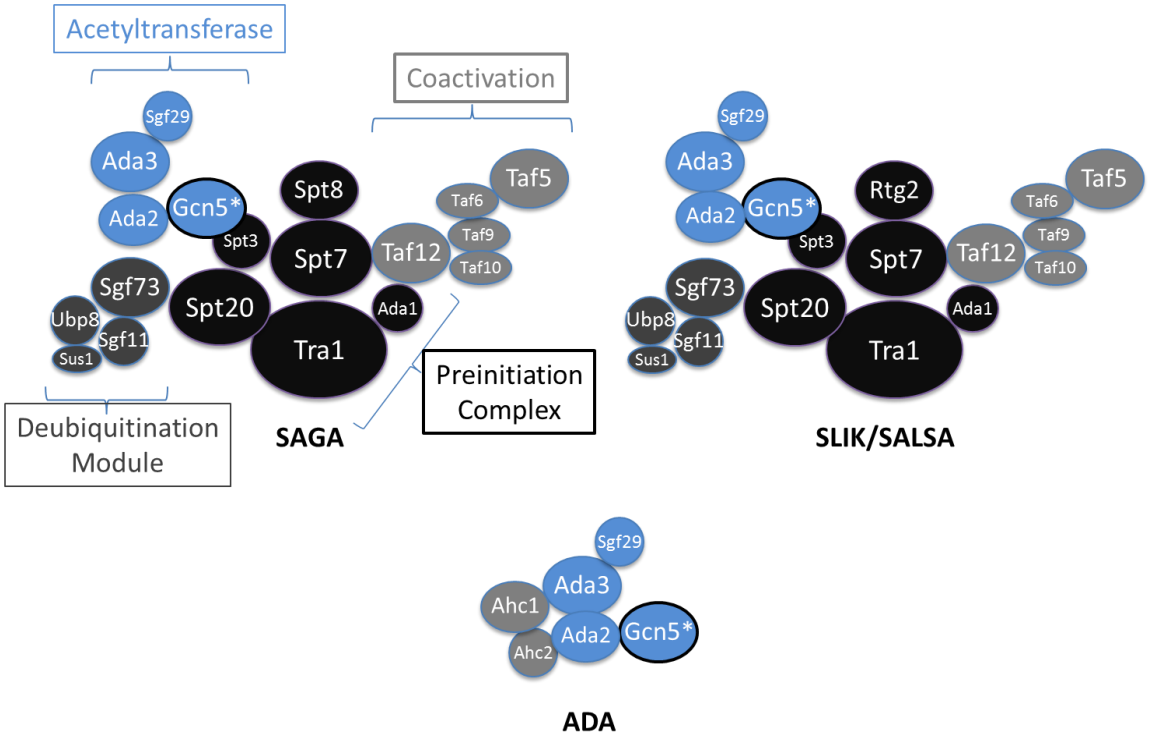


Figure 1-3. Gcn5-containing complexes: SAGA, SLIK/SALSA, and ADA. SAGA, composed of 19 subunits, can be divided into functional modules. In addition to transcription related functions such as coactivation and assembly of the preinitiation complex that recruits RNA polymerase II, SAGA also has a deubiquitination module (DUB) that targets histone H2B. Finally, the histone acetyltransferase module's catalytic subunit is Gcn5. The SLIK/SALSA complex contains the same proteins as SAGA, except it contains a truncated version of Spt7, and Rtg2 in place of Spt8. The ADA complex contains the acetyltransferase module with two additional structural subunits: Ahc1 and Ahc2. Modified from Lee et al. 2011.

HATs in each complex for identifying their proper targets within specific regions within the genome [reviewed in (Lee and Workman 2007)]. At last count, SAGA is a 19 subunit HAT complex that is highly conserved between yeast, flies, and mammals [reviewed in (Spedale et al. 2012)]. SAGA not only has HAT activity, but it also contains a deubiquitination (DUB) module that is specific for H2B ubiquitination (Powell et al. 2004). SAGA is also involved in transcriptional activation and cell cycle progression through the G2/M phase (Durant and Pugh 2006, Howe et al. 2001). Moreover, Gcn5 plays a key role in regulating the transcriptional response to different stresses and nutritional changes including high temperature, osmotic stress, nitrogen starvation, change in carbon source, and DNA damage (Huisinga and Pugh 2004).

In addition to acetylation, phosphorylation is also an important reversible post-translational modification. Phosphorylation can affect the function of a protein in many ways from changing its activity, stabilizing it or marking it for destruction, to facilitating subcellular localization, and modifying protein interactions [reviewed in (Cohen 2002)]. Phosphorylation is essential to many aspects of cellular life. Protein kinases phosphorylate proteins by catalyzing the transfer of the γ-phosphate of ATP or GTP to a serine, threonine, or tyrosine residue [reviewed in (Johnson 2009)]. In particular, kinases and phosphorylation are important for the DDR pathways. Protein kinases not only help the cell respond to stress and changes in environment through phosphorylation, but can also directly affect broader genetic response by specifically targeting histones for phosphorylation.

Histone phosphorylation occurs during transcriptional regulation, chromatin compaction, and DDR [reviewed in (Rossetto 2012)]. H2AS129 phosphorylation (H2AS129ph) is involved in both non-homologous end joining (NHEJ) and homologous recombination (HR) repair pathways (Redon C et al. 2003). It facilitates the recruitment of the NuA4 acetyltransferase complex at double stranded breaks, leading to the delay in cell cycle progression, thereby allowing the cell to repair any damaged DNA (Downs JA et al. 2004). Histone phosphorylation is essential for the cell's ability to adapt to its environment. In addition to H2AS129ph, H4S1ph is an example of the importance of histone phosphorylation for transcriptional activation (Utley et al. 2005).

Individually, HATs and protein kinases are necessary for many cellular functions. Cross-talk between phosphorylation and acetylation is also important for chromatin regulation, transcriptional activation, and DDR pathways. The combinatorial effects between histone modifications can change what specific regions of the genome protein complexes may recognize and the likelihood of other histone modifications [reviewed in (Suganuma and Workman 2011)]. Histone phosphorylation can have either a positive or negative effect on histone acetylation (Figure 1-2). For example, induced by genotoxic stress, the enzyme CK2 (Casein Kinase 2) phosphorylates H4S1 which causes a decrease in H4 acetylation, therefore regulating transcription (Utley, et al. 2005). This response can allow nucleosomes to stabilize during DNA repair and tightly regulate gene expression during specific cellular events (Utley, et al. 2005).

CK2 was one of the first described protein kinases and is arguably the most widely studied serine/threonine/tyrosine protein kinase. Studying CK2 and its effects on other modifications is important in understanding the relationships between combinatorial modifications. There are about ~6000 different proteins in *S. cerevisiae* with an average length of ~450 amino acids. Of these, ~17.7% are Ser (~8.9%), Thr (~5.7%), or Tyr (~3.1%) residues. Thus, each protein kinase must distinguish its one or more phosphorylation target sites from a sea of about 477,900 phosphorylatable residues [Lodish et al. 2000, Gaur 2013, reviewed in (Ubersax and Ferrell 2007)]. To distinguish between all of the possible targets and the actual targets protein kinases recognize certain consensus sequences. For example, CK2 recognizes the peptide sequences S/T-x-x-E/D/pS and S-x-x-pS that account for about 100 out of the over 300 known CK2 substrates that are involved in cell survival, gene expression, protein synthesis, signaling, and metabolism (Meggio and Pinna 2003). CK2 is constitutively active and can utilize both ATP and GTP as phosphate donors supporting its role in multiple cellular functions (Pinna 1990). In yeast, the CK2 holoenzyme is composed of four possible subunits: two catalytic subunits (Cka1 and Cka2) and two regulatory subunits (Ckb1 and Ckb2) (Figure 1-4) (Glover 1998). In addition to CK2 holoenzyme activity, the individual catalytic subunits ($Cka1^{Free}$ and $Cka2^{Free}$) are active independently of the regulatory subunits, and the loss of both catalytic subunits results in loss of viability (Abramczyk et al. 2003, Padmanabha 1990). There are only three possible isoforms for the holoenzyme because the Ckb1-Ckb2 heterodimer is necessary for holoenzyme activity (Domanska et al. 2005,

Kubinski et al. 2006). The Cka1 containing holoenzyme ($Cka1^{Holo}$), Cka2 containing holoenzyme ($Cka2^{Holo}$), and Cka1-Cka2 containing holoenzyme ($Cka1\text{-}Cka2^{Holo}$) have different affinities for different targets, and understanding their specificities is still an area being explored.

Recently, CK2, predominantly known to phosphorylate Ser/Thr residues, was discovered to phosphorylate histone H2A on tyrosine residue 58 in *S. cerevisiae* and the corresponding residue Y57 in murine and human cells. This residue is deeply conserved and its function emphasizes the significance of CK2 tyrosine kinase activity, which requires further study. Phosphorylation at H2AY58 by CK2 regulates transcriptional elongation and affects multiple histone marks such has H3K4me3 and H2B ubiquitination which is linked to the SAGA DUB module (Basnet et al. 2014). Furthermore, CK2 has been implicated in multiple DNA damage repair pathways: mismatch repair (MMR), nucleotide excision repair (NER), base excision repair (BER), NHEJ, HR, as well as cellular signaling and chromatin structure [reviewed in (Montenarh 2016)]. For example, phosphorylation by CK2 of mammalian XRCC4, a member of the XRCC protein family with important roles in DNA repair is necessary for its interaction with the polynucleotide kinase (PNK) which is responsible for DNA repair in the NHEJ pathway (Parker et al. 2003).

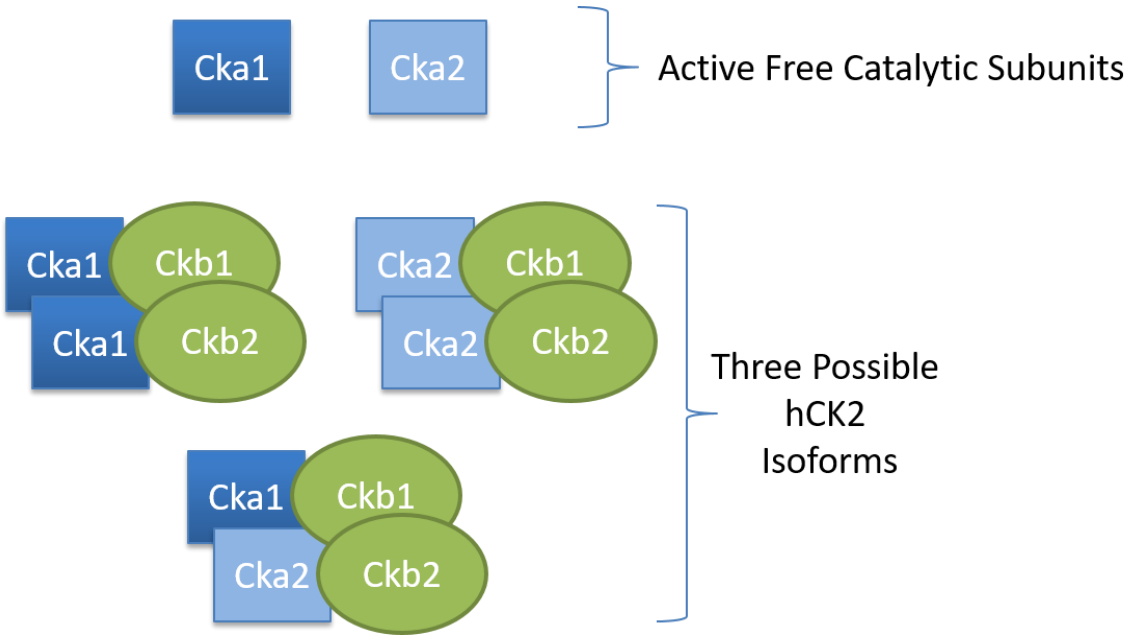


Figure 1-4. Active forms of CK2. CK2 is a kinase with many substrates and many roles in cellular functions. This can be due to the activity of its multiple isoforms. The free catalytic subunits of CK2 are functionally active. It is also believed that both regulatory subunits are required for the holoenzyme structure. Therefore, there can only be three possible CK2 holoenzyme isoforms.

The yeast homologue of XRCC4, Lif1, is also involved in double-stranded DNA (dsDNA) break repair similar to XRCC4 and was captured in mass spectrometry (MS) in association with Cka2 (Herrmann et al. 1998, Ho et al 2002). I hypothesize that CK2 in yeast is also involved in the NHEJ pathway to repair dsDNA breaks.

Some evidence, including physical interactions of Cka2 and Gcn5 via MS suggests that these proteins interact (Lee et al 2011). The awesome power of yeast genetics allows us to easily manipulate their genome to rapidly answer questions of basic biology. There are currently many tools available for *S. cerevisiae* in constructing and testing various gene deletions. Not only are deletions easier to generate in yeast than other model organisms, but non-null and homozygous null mutants can easily be created and recovered. Yeast can grow and divide as haploids, facilitating strain construction. To understand the different functions of new deletions we can perform many distinct assays and select the phenotypes we find interesting. In addition to testing single deletions, genetic networks and interactions between multiple gene products can be elucidated through mutant analysis [reviewed in (Forsburg 2001)]. Mutant analysis can be described as the rescue or exacerbation of growth of the original mutant's phenotype by the addition of another mutation or increased gene dosage (Prelich 1999). This gives evidence that the mutated genes interact. In particular, to uncover novel functional or genetic interactions between CK2 and Gcn5-associated HAT complexes, mutant analysis can be used.

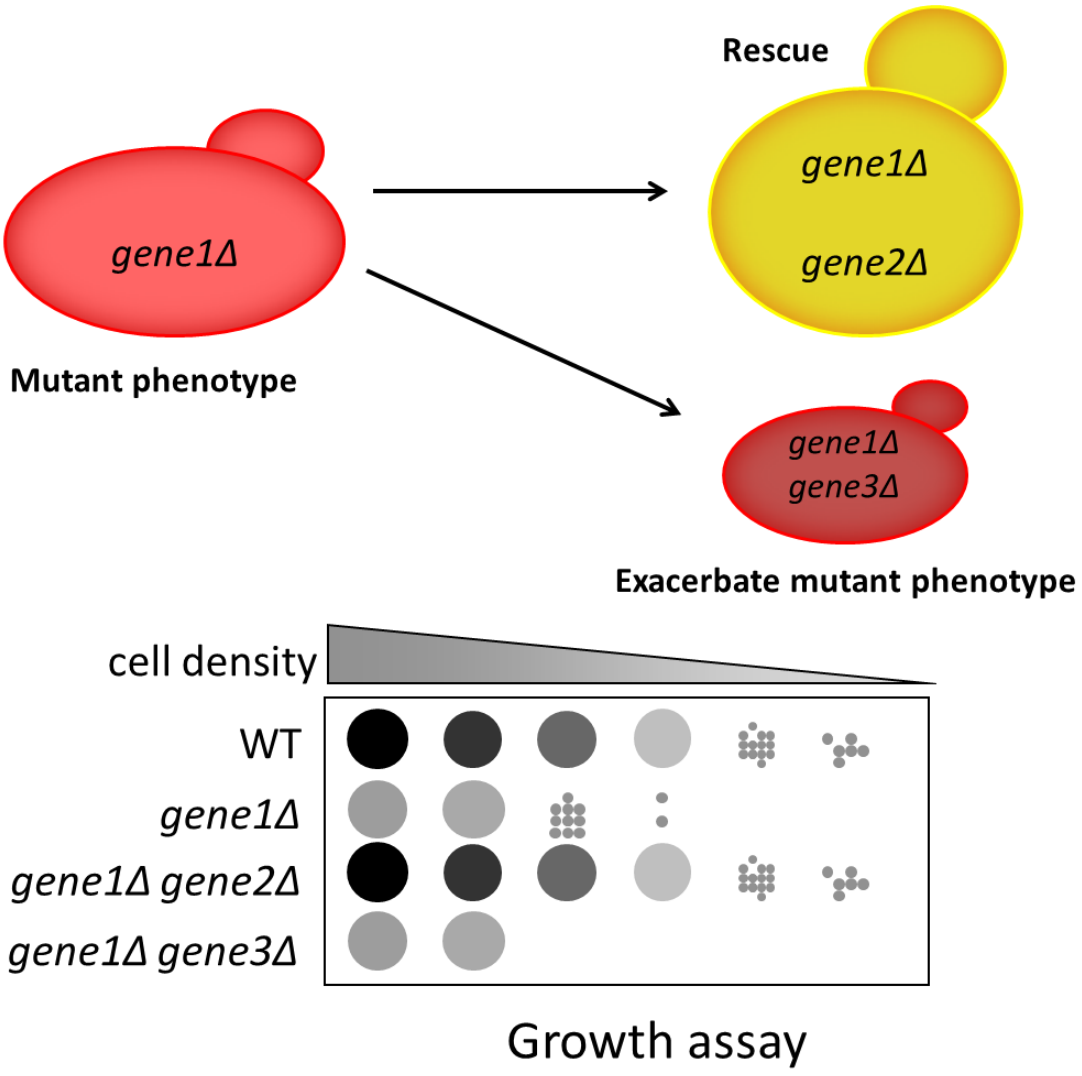


Figure 1-5. Mutant Analysis. Mutant phenotypes associated with the loss of function of one gene can be restored to almost wild-type growth through loss of another gene. Conversely, loss of function of one gene can exacerbate the growth caused by the loss of function of another gene. Both results, can establish a functional relationship between the two genes and can imply involvement in parallel or overlapping pathways in the cell. The initial changes can be tested in a growth assay, observing the changes in cell density.

To test whether different isoforms of CK2 interact with Gcn5-associated HATs, multiple CK2 deletions were constructed with *gcn5*Δ strains (Table 1-1). The focus of this research and the following chapters came as a result of a screen of these strains against different stresses. In Chapter 2, *cka1*Δ is found to be a suppressor of *gcn5*Δ high temperature sensitivity. More evidence of CK2 and Gcn5 interactions was found in the *cka2*Δ *gcn5*Δ mutant. Loss of *CKA2* suppressed *gcn5*Δ sensitivity to hydroxyurea (HU), but not methyl methanesulfonate (MMS). This provided evidence that different CK2 isoforms interact with Gcn5 in specific pathways. Loss of either or both regulatory subunits eliminates the *cka1*Δ and *cka2*Δ suppression phenotype. The mechanism in which these proteins interact was further studied by exploring histone protein and histone modification levels (Chapter 3). Furthermore, a histone tyrosine screen revealed H2AY40 as a residue involved in the *cka2Δ gcn5Δ* suppression phenotype (Chapter 4). More evidence is given in the appendix (Appendix 1). The possibilities and implications of the results presented will be discussed in Chapter 5. The interactions suggested in this project contribute to our understanding between histone modifications and the enzymes that catalyze them.

Table 1-1. CK2 and Gcn5 strain construction for uncovering interactions. Various combinations of CK2 and *GCN5* knockouts were constructed. The strains marked with a black checkmark could not be constructed. Double catalytic knockouts are known to be synthetically lethal. The *cka1*Δ *ckb1*Δ *gcn5*Δ mutant would not sporulate, so no haploids could be recovered. All the strains that were constructed have both mating types available, except for *cka2*Δ *ckb1*Δ (only *MAT*α was recovered).

Strain	Construction
cka1Δ	✓
cka2Δ	✓
ckb1Δ	✓
ckb2Δ	✓
cka1Δ cka2Δ	✓
cka1Δ ckb1Δ	✓
cka1Δ ckb2Δ	✓
cka2Δ ckb1Δ	✓
cka2Δ ckb2Δ	✓
ckb1Δ ckb2Δ	✓
cka1Δ ckb1Δ ckb2Δ	✓
cka2Δ ckb1Δ ckb2Δ	✓

Strain	Construction
gcn5Δ	✓
cka1Δ gcn5Δ	✓
cka2Δ gcn5Δ	✓
ckb1Δ gcn5Δ	✓
ckb2Δ gcn5Δ	✓
cka1Δ cka2Δ gcn5Δ	✓
ckb1Δ ckb2Δ gcn5Δ	✓
cka1Δ ckb1Δ gcn5Δ	✓
cka1Δ ckb2Δ gcn5Δ	✓
cka2Δ ckb1Δ gcn5Δ	✓
cka2Δ ckb2Δ gcn5Δ	✓
cka1Δ ckb1Δ ckb2Δ gcn5Δ	✓
cka2Δ ckb1Δ ckb2Δ gcn5Δ	✓

Chapter 2. The dissection of CK2 and Gcn5 using mutant analysis

Introduction

Gcn5, the catalytic subunit of multiple HAT complexes, including SAGA, ADA, and SLIK/SALSA has a marked preference for acetylating histone H3 tails (Lee and Workman 2007). Gcn5 HAT activity is important for transcriptional activation and the regulation of multiple stress responses (Waterborg 2000, Durant and Pugh 2006, Huisinga and Pugh 2004). Among other phenotypes, the loss of *GCN5* causes sensitivity to elevated temperature and DNA damage [reviewed in (Burgess and Zhang 2010)]. Similarly, the protein kinase CK2 plays key roles in cell survival through regulation of gene expression and multiple stress responses (Meggio and Pinna 2003). The CK2 holoenzyme is composed of two catalytic and two regulatory subunits. In budding yeast, *CKA1* and *CKA2* encode catalytic subunits and *CKB1* and *CKB2* encode regulatory CK2 subunits that can combine to form different holoenzyme isoforms which have distinguishable properties (Glover 1998, Domanska et al. 2005). The specific isoform targeting each CK2 substrate is still largely unknown. The inhibitors, TBBz (4,5,6,7-tetrabromo-1*H*-benzimidazole) and TBBt (4,5,6,7-tetrabromo-1*H*-benzotriazole) block CK2 holoenzyme and $Cka2^{Free}$ activity, respectively (Damanska et al. 2005). However, not all CK2 isoforms have a known inhibitor. Therefore, I used a genetic approach to better understand the distinct functions and interactions of the CK2 subunits in budding yeast by constructing the strains listed in Table 1-1.

Individually, Gcn5 and CK2 are involved in many cellular functions, but whether they function together positively or antagonistically is still unknown. Both Cka2 and known CK2 substrates have been identified in complex with Gcn5, suggesting that these enzymes are connected (Lee et al. 2011, Basnet et al. 2014). Therefore, I sought to uncover novel functional or genetic interactions between CK2 and Gcn5-associated HAT complexes using mutational analysis (Figure 1-5). This is the first in depth *in vivo* dissection of CK2 function. The twenty-five strains constructed in Table 1-1 were used to assess interactions between these two enzyme-encoding genes as well as interplay between CK2 subunits. After their construction, I then assessed growth of these strains under heat stress, distinct types of DNA damage induction, and transcriptional elongation stress to evaluate their response to these challenges. Improved growth would suggest antagonistic behavior between the genes whereas reduced growth would suggest a positive relationship. The growth assays were designed to distinguish between the pathways in which the enzyme-encoding genes may interact with one another.

In response to heat shock, *S. cerevisiae* undergoes changes in gene expression that repress the capacity to synthesize most proteins, while inducing cytoprotective genes encoding multiple heat shock proteins [reviewed in (Verghese et al. 2012)]. The two classes of heat shock proteins, Hsp70 and Hsp90, act as trans-acting repressors of the gene encoding the Hsf1 transcription factor and are necessary to inhibit Hsf1 at normal growth temperatures (Voellmy and Boellmann 2007). The primary transcription factors activated in response to

heat shock are Hsf1 and Msn2/4, and high levels of Hsf1 activity increase thermotolerance (Giaever et al. 2002, Harris et al. 2001). Hsf1 has many phosphorylation sites and can be regulated by multiple proteins. It has been shown that deleting genes that regulate Hsf1 increases thermotolerance. Interestingly, CK2 acts quite dynamically in regulating Hsf1. Depending on the stress CK2 activates or inhibits Hsf1 activity by phosphorylating distinct sites (Soncin et al. 2003, Cho et al. 2014). Thus, the role of CK2 in the heat response remains to be resolved.

Moreover, in response to DNA damage, *S. cerevisiae* regulates DNA replication and arrests the cell cycle prior to anaphase. A kinase signaling cascade involving Mec1, Rad53, Chk1, and Dun1 inhibits Bfa1, a GTPase-activating protein (GAP), activity by inhibiting its activator, Cdc5 (Sanchez et al. 1999). Inhibiting the GAP activity after the signal response due to DNA damage prevents the cell from entering anaphase (Hu et al. 2001). In addition to arresting the cell cycle during the DNA damage response to allow for repair, Mec1 and Rad53 also play critical roles in the recovery from DNA replication blocks (Desany et al. 1998). The cell is able to specifically respond to both DNA replicative stress and damaging agents that cause double stranded breaks. In contrast, there are many genes that are commonly upregulated in response to a variety of environmental stresses. A major contributor to the regulation and transcriptional activation of these genes is SAGA, a Gcn5-associated complex (Huisinga and Pugh 2004). The actions of SAGA are induced during high stress conditions which activate important stress response genes (Huisinga and Pugh

2004). CK2 is also involved in multiple pathways important for DNA damage repair: cellular signaling, MMR, NER, NHEJ, HR, and BER [reviewed in (Montenarh 2016)]. Responding both globally and specifically to different stresses is important to the health and survival of the cell.

Results and Discussion

It was necessary to dissect the functional interactions between the CK2 subunits themselves before testing the interactions between Gcn5 and CK2. These experiments were designed to test the possibility that individual CK2 subunit functions could be more complex than previously reported. In humans, CK2 contains only one regulatory subunit, CK2β, which was found to modulate the activity of other protein kinases such as A-Raf and Chk1, thereby adding to its complexity [reviewed in (Bibby and Litchfield 2005)]. To study the interactions the strains were tested against stresses for which *gcn5*Δ mutants were known to be sensitive to such as, elevated temperatures and DNA damage (Vernarecci et al. 2008, Petty et al. 2016). The strains to be tested were all grown to saturation and plated in a 5-fold serial dilution on pre-warmed plates at the indicated temperatures. The individual, double, and triple mutants of CK2 were previously not known to be sensitive to high heat. Also, the *cka1*Δ *cka2*Δ double mutant was previously reported to be synthetically lethal (Padmanabha 1990) which I confirmed, thus its analysis was not pursued. None of the individual CK2 deletions were sensitive to heat stress at 37°C (Figure 2-1). In contrast to the single mutants, both *cka1*Δ *ckb2*Δ and *cka1*Δ *ckb1*Δ *ckb2*Δ mutant strains are sensitive to elevated temperatures (Figure 2-1). The temperature sensitive

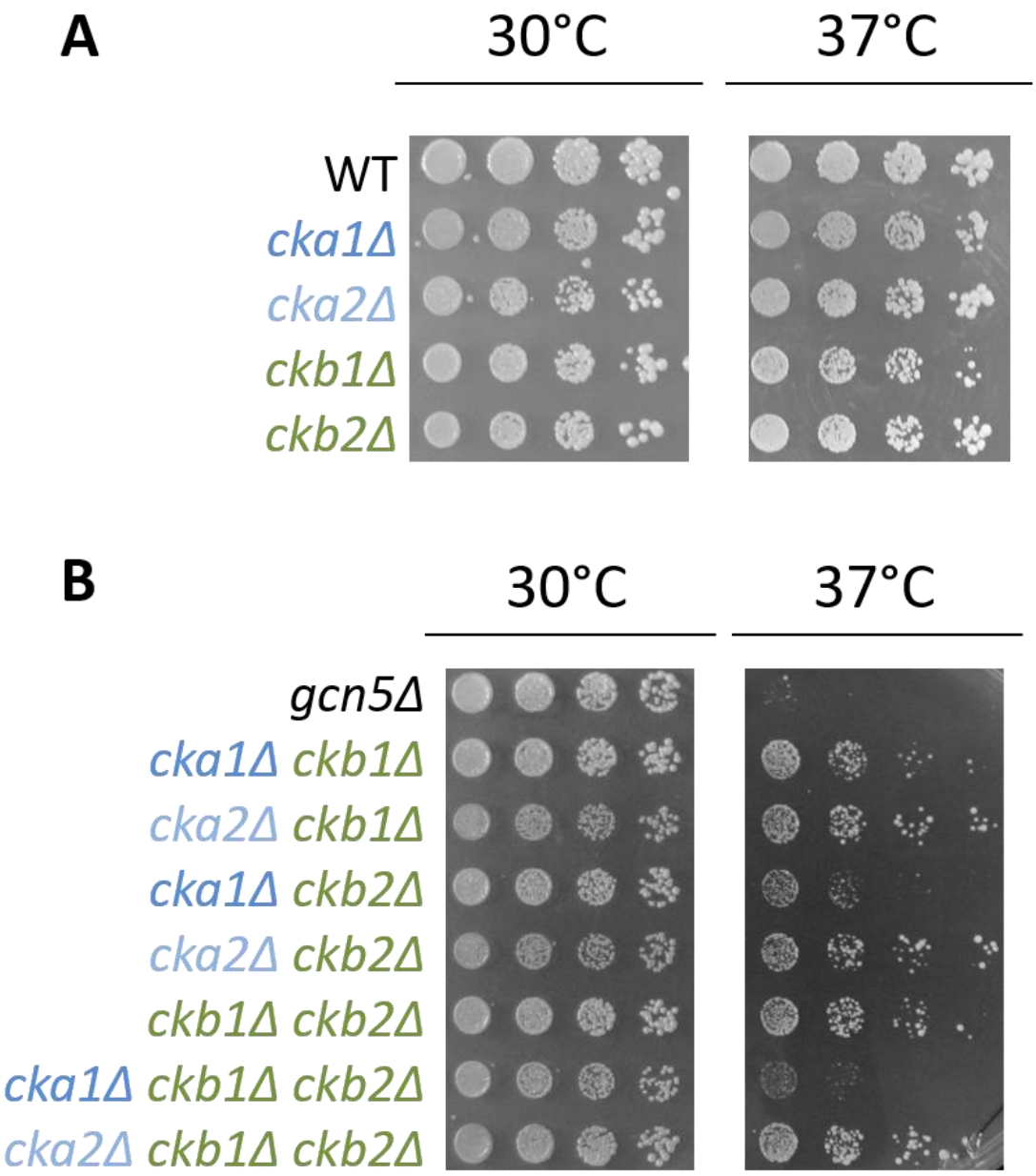


Figure 2-1. Several CK2 mutants, are newly found to be sensitive to elevated temperatures. Strains were grown to saturation and then plated on pre-warmed media in 5 fold dilutions. Cells were incubated for 3 days. A) WT (LPY5), *cka1*Δ (LPY21663), *cka2*Δ (LPY21665), *ckb1*Δ (LPY21784), *ckb2*Δ(LPY21757) were tested, no new phenotypes were observed. B) The *gcn5*Δ (LPY13320), *cka1*Δ *ckb1*Δ(LPY21827), *cka2*Δ *ckb1*Δ (LPY21889), *cka1*Δ *ckb2*Δ (LPY21779), *cka2*Δ *ckb2*Δ (LPY 21786), *ckb1*Δ *ckb2*Δ (LPY 21825), *cka1*Δ *ckb1*Δ *ckb2*Δ (LPY21883), *cka2*Δ *ckb1*Δ *ckb2*Δ(LPY21952) strains were evaluated, and *cka1*Δ *ckb2*Δ and *cka1*Δ *ckb1*Δ *ckb2*Δ mutants were found to be sensitive to elevated temperatures.

phenotype is specific to the loss of both *CKA1* and *CKB2* together, since individual *CKA1* and *CKB2* mutants grow normally at elevated temperatures. In contrast, loss of both *CKA1* and the other regulatory subunit gene, *CKB1*, together does not cause a temperature sensitive phenotype.

Also, the *cka2*Δ *ckb1*Δ *ckb2*Δ triple mutant and the *ckb1*Δ *ckb2*Δ double mutant are not sensitive to elevated temperatures, suggesting that the sensitivity of the *cka1*Δ *ckb2*Δ mutant is not due to the loss of the CK2 holoenzyme, but may instead be due to the loss of the free catalytic activity. Only the loss of the Cka1^{Free} activity coupled with the loss of Ckb2 caused a temperature sensitive phenotype. This suggests that the loss of Ckb2 specific regulatory activity may influence the activity of other proteins and in combination with the loss of Cka1^{Free} catalytic activity, cause temperature sensitivity.

With evaluation of the CK2 subunits complete, in order to test the interactions between CK2 and Gcn5, a combination of deletions was also tested at elevated temperatures. Loss of *CKA1* specifically rescued *gcn5*Δ temperature sensitivity (Figure 2-2). Loss of all other CK2 subunits in combination with *gcn5*Δ leads to either exacerbation of the *gcn5*Δ mutant temperature sensitivity or similar growth. The additional deletion of *CKB2* to the *cka1*Δ *gcn5*Δ mutant interferes with its suppression of *gcn5*Δ heat sensitivity. The importance of the *CKA1* deletion in *gcn5*Δ growth rescue was confirmed using covering plasmids in *cka1*Δ *gcn5*Δ and *cka1*Δ *ckb2*Δ *gcn5*Δ mutants (Figure S1). The *cka1*Δ *gcn5*Δ mutants containing a wild-type copy plasmid of *CKA1* were sensitive to temperature and the *cka1*Δ *ckb2*Δ *gcn5*Δ mutants containing a wild-type copy

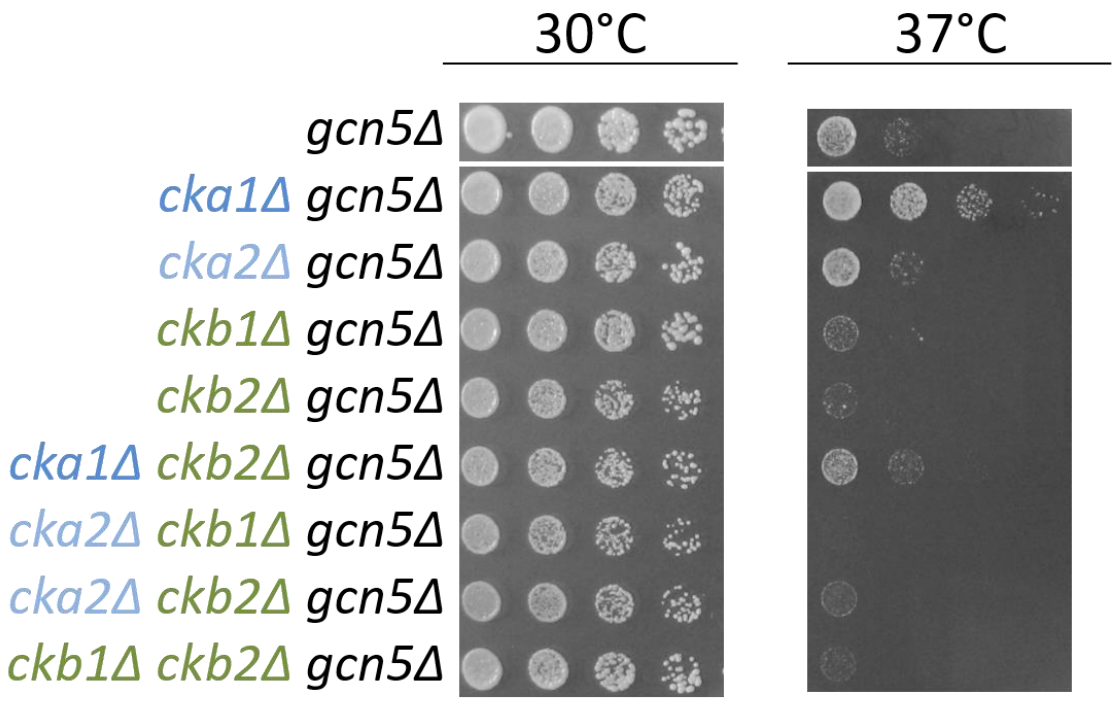


Figure 2-2. Different CK2 subunits dynamically affect growth of *gcn5*Δ at elevated temperatures. Strains were grown to saturation and then plated on pre-warmed media in 5 fold dilutions. Cells were incubated for 3 days. The strains tested were *gcn5*Δ (LPY13320), *cka1*Δ *gcn5*Δ (LPY22313), *cka2*Δ *gcn5*Δ (LPY21671), *ckb1*Δ *gcn5*Δ (LPY21823), *ckb2*Δ *gcn5*Δ (LPY21777), *cka1*Δ *ckb2*Δ *gcn5*Δ (LPY22315), *cka2*Δ *ckb1*Δ *gcn5*Δ (LPY22317), *cka2*Δ *ckb2*Δ *gcn5*Δ (LPY21956), *ckb1*Δ *ckb2*Δ *gcn5*Δ (LPY21881). The majority of the mutants tested exacerbate *gcn5Δ* temperature sensitivity. However, loss of *CKA1* specifically rescues growth of *gcn5Δ* at elevated temperatures.

plasmid of *CKB2* grew better under temperature stress.

Both Gcn5 and CK2 contribute to the DNA damage responses. The strains tested above (Fig. 2-1 and 2-2) were also tested for their response to distinct DNA damaging conditions. Challengest with both MMS and HU were used in these assays.

MMS is an alkylating agent that adds a methyl group to both guanine and adenine causing base mispairing and replication blocks, respectively (Beranek, D.T. 1990). In yeast and mammals, base excision repair is necessary to repair MMS-induced heat-labile DNA damage (Lundin et al. 2005). Mutations that cause defects in base excision repair or recognition of damage caused by MMS will be sensitive to it. Confirming previous studies, the single mutants: *cka1*Δ*,* *ckb1*Δ*,* and *ckb2*Δ were sensitive to MMS (Figure 2-3)(Kapitzky et al. 2010, Svensson et al. 2011). At higher concentrations of MMS all CK2 double and triple mutants were found to be sensitive, including *cka2*Δ double and triple mutants. This is in contrast to the *cka2*Δ single mutant which was not sensitive to MMS and grew comparably to the WT control (Figure 2-3). This observation implies that the $Cka1^{Holo}$ is sufficient in responding to DNA base mispairings, but the $Cka2^{Holo}$ is not. Moreover, the double and triple *cka2*Δ mutants were even more sensitive than the other CK2 mutants at lower concentrations of MMS (Figure 2-3). This suggests that loss of either or both regulatory subunits is enough to cause sensitivity. Not only are the regulatory subunits important for the holoenzyme structure and response to MMS, but the regulatory deletion strains show that the holoenzyme, specifically $Cka1^{Holo}$ is necessary for the response

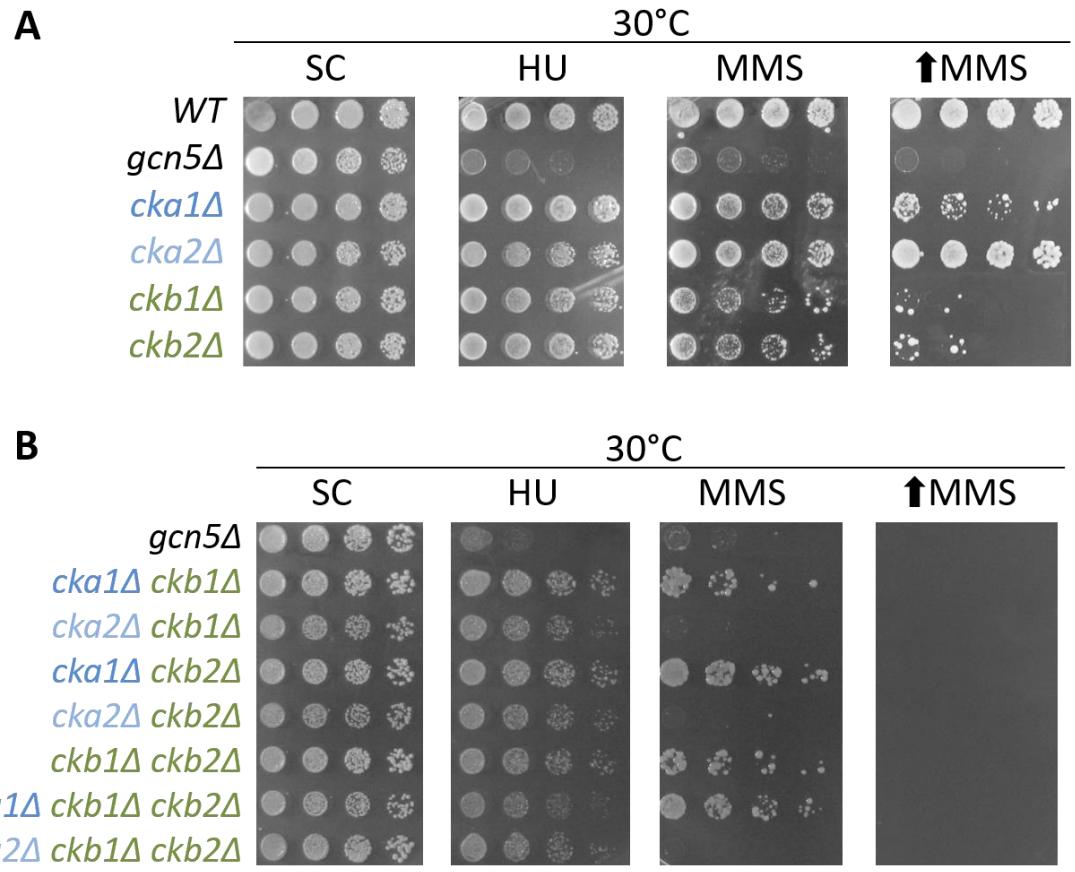


Figure 2-3. Defining roles of CK2 subunits in DNA damage response. Strains from Figure 2-1 were grown to saturation then plated on synthetic complete (SC), 0.1M Hydroxyurea (HU), 0.01% methyl methanesulfonate (MMS), and 0.015% MMS pre-warmed plates in 5 fold dilutions. Cells were incubated for 3 days. A) Individual *cka1*Δ, *ckb1*Δ, and *ckb2*Δ mutants are sensitive to MMS. No CK2 mutant was sensitive to HU. B) CK2 multi-subunit mutants have exacerbated MMS sensitivity, especially when the *cka2*Δ mutant is combined with the loss of either or both regulatory subunits (*CKB1 or CKB2)*

to DNA damage induced mismatch mutations.

HU, an inhibitor of ribonucleotide reductase, was also used to induce DNA damage. HU blocks DNA replication by depleting dNTP levels, and prolonged exposure leads to DNA breaks (Ahmet Koc et al. 2004). In my assays, none of the single mutants were sensitive to HU, although, both *cka2*Δ and *ckb2*Δ single mutants were previously identified in a genome-wide screen in a different genetic background to be sensitive to HU (Figure 2-3) (Kapitzky et al. 2010). Also, none of the CK2 double or triple mutants were sensitive to HU. However, the combined *gcn5*Δ and CK2 mutant strains exposed to the same DNA damage reagents revealed that loss of *CKA2* suppresses *gcn5*Δ sensitivity to HU, but not MMS (Figure 2-4).

In addition, loss of either CK2 regulatory subunit prevents the suppression phenotype observed in the *cka2*Δ *gcn5*Δ mutant. It is possible that the observed rescue in growth when exposed to HU could be due to increased activation of the DNA damage response due to higher levels of $Cka1^{Holo}$ or due to the loss of inhibition by $Cka2^{Holo}$. The interactions between CK2 and Gcn5 are complex because the combined deletion of the CK2 genes and *GCN5* caused similar, exacerbation, and rescue in the *gcn5*Δ slow growth phenotype. The promotion of growth seen in *cka2*Δ *gcn5*Δ strains when exposed to HU and the importance of a regulatory subunit were confirmed (Figure S1). The *cka2*Δ *gcn5*Δ mutant containing a wild-type *CKA2* plasmid grew similarly to *gcn5*Δ and multiple *cka2*Δ *ckb1*Δ *gcn5*Δ strains grew worse than *gcn5*Δ in response to HU.

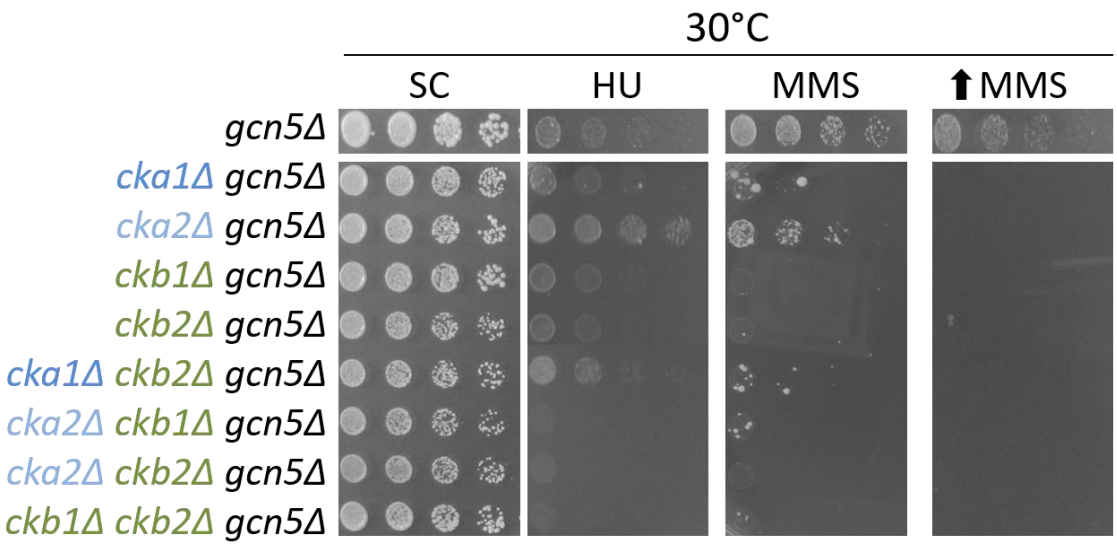


Figure 2-4. Different CK2 subunits affect growth of *gcn5*Δ upon DNA stress. . Strains were grown to saturation and 5-fold dilutions were plated on synthetic complete (SC) without drug, or containing 0.1M Hydroxyurea (HU), 0.01% methyl methanesulfonate (MMS), or 0.015% MMS. Plates were incubated for 3 days at 30ºC before imaging. The strains tested were *gcn5*Δ (LPY13320), *cka1*Δ *gcn5*Δ (LPY21667), *cka2*Δ *gcn5*Δ (LPY21671), *ckb1*Δ *gcn5*Δ (LPY21823), *ckb2*Δ *gcn5*Δ (LPY21777), *cka1*Δ *ckb2*Δ *gcn5*Δ (LPY22315), *cka2*Δ *ckb1*Δ *gcn5*Δ (LPY22317), *cka2*Δ *ckb2*Δ *gcn5*Δ (LPY21956), *ckb1*Δ *ckb2*Δ *gcn5*Δ (LPY21881).

Both CK2 and Gcn5 are involved in transcriptional elongation. 6-azauracil (6AU), a pyrimidine analog, is known to deplete intracellular GTP levels and is commonly used as a first-pass method of evaluating deficiencies in transcriptional elongation (Exinger and Lacroute 1992). To test 6AU sensitivity, additional strain construction was necessary because the W303 strain background has a *ura3-1* mutation, and strains lacking the *URA3* gene are constitutively sensitive to 6AU. In order to test the CK2 and *GCN5* mutant strains they were first transformed to *URA3* using the marker swap plasmid M4758 from the Stillman lab (Voth et al 2003). As expected, *gcn5*Δ mutants were extremely sensitive to 6AU (Figure 2-5) (Ginsburg et al. 2009). Individual *cka2*Δ mutants have not previously been tested against 6AU, but it was not unexpected to observe slower growth in the *cka2*Δ mutant because of CK2's involvement in phosphorylating H2AY58, a residue important for transcription (Basnet et al. 2014). Together, the *cka2*Δ *gcn5*Δ mutant grew similarly to the *gcn5*Δ mutant. They were both extremely sensitive to 6AU. This suggests that the suppression of *gcn5*Δ sensitivities by the CK2 mutant does not involve its roles in transcriptional elongation.

The majority of the previously reported characterizations of CK2 activity have not determined which specific CK2 isoform is performing the function. The role of CK2 in response to multiple stresses is more complex than the activity of a single enzyme. This work demonstrates that multiple CK2 isoforms and individual subunits have different roles in multiple response pathways.

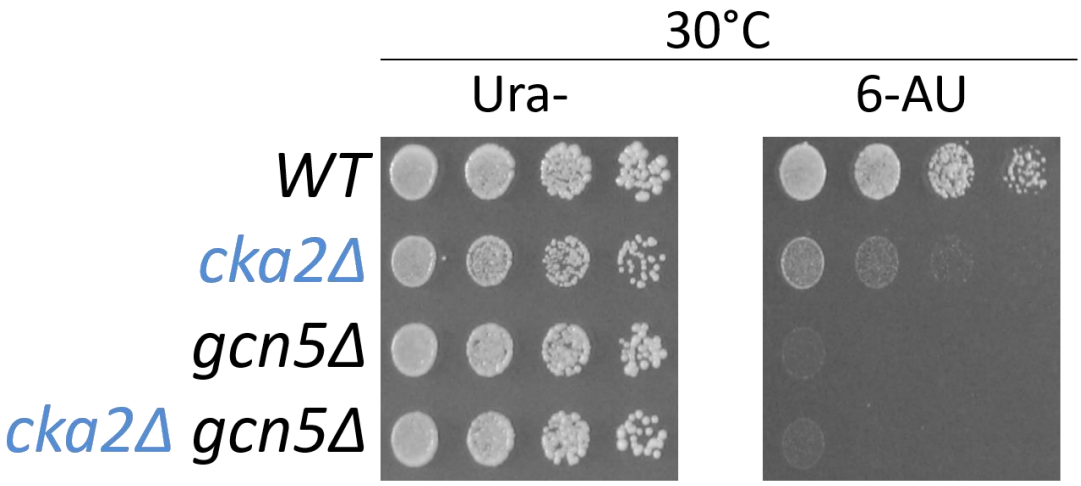


Figure 2-5. CK2 and Gcn5 sensitivity to 6-azauracil (6AU). Strains were grown to saturation and then plated on SC-Ura plates lacking or containing 100µg/mL 6AU. Plates were incubated for 3 days. The strains tested were *WT* (LPY2533), *cka2*Δ (LPY22199), *gcn5*Δ (LPY22296), *cka2*Δ *gcn5*Δ (LPY22246). All W303 yeast strain backgrounds have the *URA3* gene mutated, and mutants deficient in any of the *URA* genes are more sensitive to 6AU (Exinger and Lacroute 1992). To use 6AU to identify transcriptional elongation deficiencies, the strains tested previously were marker-swapped to render the cells wild type for *URA3.* Both CK2 and Gcn5 have roles in transcriptional elongation. As shown here, *gcn5*Δ is most sensitive and loss of *CKA2* also causes sensitivity to 6AU.

It is important to recognize the isoform specificity to better understand the role of CK2 and how it regulates many pathways. Some of the CK2 single mutants were sensitive to MMS, and none were sensitive to heat. However, some CK2 double and triple mutants were sensitive to heat and all were sensitive to MMS. This suggests that different CK2 isoforms have distinguishable roles in cellular responses to these environmental and genotoxic stresses. More evidence of their distinct roles was seen in the *cka1*Δ *gcn5*Δ and *cka2*Δ *gcn5*Δ mutants rescue in growth against only elevated temperatures and HU, respectively. This shows that CK2 is important for stress response, and that different CK2 isoforms function in two stress response pathways to which *GCN5* contributes. Exploring the changes of the targets modified by these enzymes in Chapter 3 can provide evidence on how CK2 and *GCN5* may be functioning in the different stress response pathways.

Chapter 3. Global histone modifications provide insight into CK2 and Gcn5 interactions

Introduction

In eukaryotic cells, DNA is packed into chromatin and its fundamental subunit, the nucleosome, contains a histone octamer that is important for multiple aspects of biological regulation. The histones have basic N- and C-terminal tails that are heavily modified to regulate chromatin structure and recruit enzymes [reviewed in (Bannister and Kouzarides 2011)]. Not only are the tails modified, but the core domains in the nucleosome may also be modified to regulate chromatin structure [reviewed in (Mersfelder and Parthun 2006)]. Multiple histone modifications may influence each other. In transcriptional activation, for example, histone acetylation at one residue may be necessary for methylation to occur at another histone residue [reviewed in (Rando 2012)]. There are many well-characterized individual modifications, but understanding the dynamics between multiple histone modifications requires more research.

Loss of key chromatin modifiers can cause the depletion of important histone modifications that are necessary for histone-DNA or protein-DNA interactions. For example, pharmacological inhibition of CK2 blocks H2AY58 phosphorylation, and also reduces H2BK123 monoubiquitination levels. Additionally, mutating the H2AY58 residue to phenylalanine causes a decrease in trimethylation of H3K4 and H3K79 (H3K4me3 and H3K79me3) (Basnet et al.

2014). These histone marks are all associated with active transcription [reviewed in (Kouzarides 2007)].

Several histone marks are associated with the stress response and DNA damage repair. H3 acetylation by SAGA induces multiple stress response genes (Huisinga and Pugh 2004). Both H2A129 and H4S1 phosphorylation are important for different stages of the DNA damage response [reviewed in (Rossetto 2012)]. H2A129 phosphorylation facilitates the recruitment of different chromatin modifiers to DNA breaks repaired by non-homologous end joining and homologous repair (Redon C et al. 2003). Conversely, H4S1 phosphorylation by CK2 prevents recruitment of chromatin modifiers, thereby interfering with acetylation (Utley RT et al. 2005). These marks act as signals for repair and signals for protection, allowing the cell to properly respond to any damage. Changes in these histone marks could thus hinder or improve the cell's response to different stresses.

Results and Discussion

The genetic interactions revealed in Ch. 2 could be explained by global changes in key histone modifications. The histone modifications discussed previously, and other known histone modifications that have been well characterized can be used as global markers for particular cellular functions. For instance, Gcn5 is one of two enzymes in yeast known to target H3K9 and H3K14 for acetylation, and loss of *GCN5* causes depletion in H3K9, K14 acetylation (H3K9, K14ac) (Lee and Workman 2007). Gcn5 catalyzed acetylation of H3K9,

K14 at promoters increases transcription and at sites of DNA damage, it promotes repair. Similarly, inhibition of CK2 decreases H2AY58 phosphorylation levels which also affect H3K4me3 and H3K79me3 transcriptional marker levels (Basnet et al. 2014).

A standard method of measuring protein modification levels is through immunoblotting with isoform-specific and control immunoreagents. The procedure I employed is detailed in Appendix 2, Materials and Methods. The global acetylation levels of the Gcn5 targets, H3K9 and H3K14, were measured as markers for changes in transcription and DNA damage repair. Also, the global H3K4me3 and H3K79me3 levels were measured to analyze changes in transcription in relation to H2AY58 phosphorylation. However, H2AY58 phosphorylation levels were not measured in the current studies. The mark occurs in low abundance and requires an enrichment immunoprecipitation to be detected, and the antiserum for its detection is not robust.

As revealed by my genetic analysis in Chapter 2, each CK2 subunit is involved in both distinct and similar cellular functions. Therefore, I evaluated global effects on selected histone modifications under normal growth and suppressing conditions in the *gcn5*Δ *cka1*Δ*/cka2*Δ double mutants compared to controls. I began with testing the *cka1*Δ *gcn5*Δ strain at optimal growth and under high temperature stress. Briefly, WT, *cka1*Δ*, gcn5*Δ, and *cka1*Δ *gcn5*Δ strains were grown to early log-phase, incubated at different temperatures, either 30ºC or 37°C and then collected for protein lysate preparation. The H3K9, K14 acetylation levels were slightly elevated in the *cka1*Δ *gcn5*Δ mutant compared to

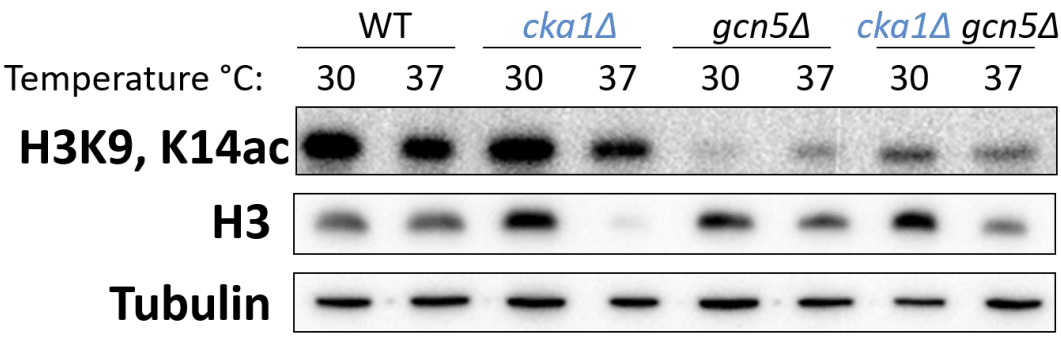


Figure 3-1. Partial rescue of histone acetylation through loss of *CKA1* during temperature stress. The strains WT (LPY5), *cka1*Δ (LPY21663), *gcn5*Δ (LPY13320), *cka1*Δ *gcn5*Δ (LPY22313) were grown overnight and then diluted to 0.15 ODs and incubated at 30ºC or 37ºC. The cells were collected during exponential growth at ~1OD and then lysed and a protein immunoblot was performed using the protocols presented in Appendix 2, Materials and Methods. Images were captured using the ProteinSimple FluorChem E system, using a 40 second exposure.

the *gcn5*Δ mutant (Figure 3-1). However, the H3K9, K14 levels were not restored to WT levels and the non-modified H3 levels decreased upon temperature stress. This may suggest that loss of *CKA1* increases the ability of another histone acetyltransferase such as Sas3 to target those acetylation sites, or decreases activity of corresponding deacetylases. In future studies, additional histone marks will be measured to determine how CK2 and Gcn5 are interacting, whether they are indirect or direct interactions.

Next, I determined whether the *cka2*Δ *gcn5*Δ mutant affected growth and histone modifications under normal growth and in response to acute HU treatment. The WT, *cka2*Δ*, gcn5*Δ*,* and *cka2*Δ *gcn5*Δ strains were collected during exponential growth under non-stress growth conditions in synthetic complete media. The known histone targets involved with Gcn5 and CK2 were tested. During normal growth no histone modifications were affected (Figure 3-2). H3K9 and H3K14 acetylation levels were not rescued in the *cka2*Δ *gcn5*Δ mutant. The normal levels of H3K4me3 and H3K79me3 suggest that active transcription in *gcn5*Δ mutants was relatively normal under non-stress conditions. This raises the possibility that H2AY58 phosphorylation may not require *CKA2* or that the other CK2 isoforms can compensate for the loss of Cka2 catalytic activity.

An explanation to why the loss of *CKA2* suppresses *gcn5*Δ sensitivity to HU may be through the rescue of H3 acetylation or other histone marks. Uncovering these changes could help elucidate the interaction between CK2 and Gcn5.

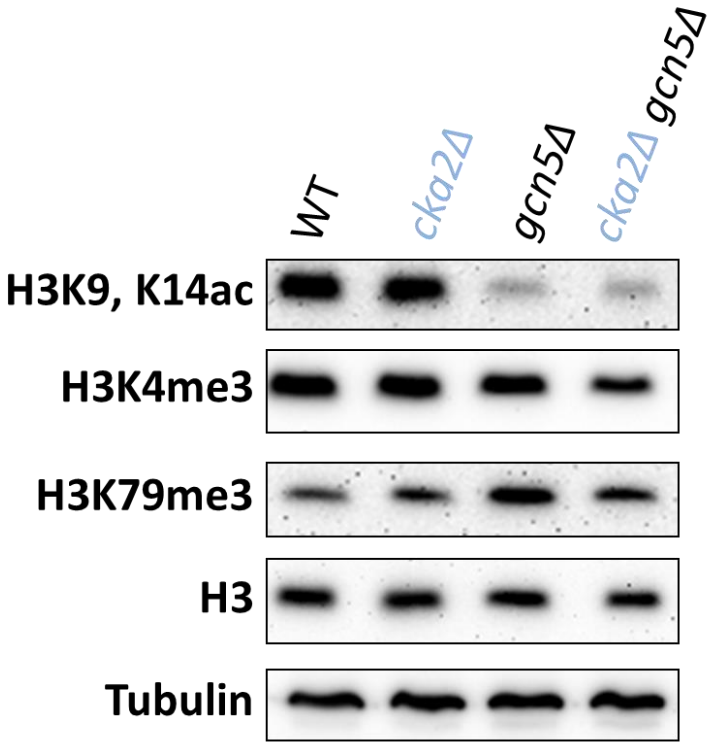


Figure 3-2. Loss of *CKA2* does not affect multiple histone marks during non-stress conditions. The strains WT (LPY5), *cka2*Δ (LPY21665), *gcn5*Δ (LPY13320), *cka2*Δ *gcn5*Δ (LPY21671) were grown overnight and then diluted to 0.15 ODs. They were collected during exponential growth at ~1 OD and then lysed and protein immunobloting was performed using the protocols in Appendix 2, Materials and Methods. Images were captured using the ProteinSimple FluorChem E system, using a 40 second exposure.

Samples prepared upon HU treatment were also evaluated. During exponential growth the cells were treated with HU for two hours and then collected. However, instead of rescuing histone H3K9, K14ac levels, the *cka2*Δ *gcn5*Δ mutant had decreased or similar levels of acetylation during HU treatment (Figure 3-3). The loss of H3K9, K14ac levels may be due to the decreased levels in histone H3 observed in the blot. Other modifications, including H2AS129 phosphorylation, H3K4me3, H2BK123 monoubiquitination, and H2AY58 phosphorylation were evaluated. However, these results were inconclusive or inconsistent and must be carefully revisited before drawing conclusions (Figure S3)

The immunoblot evidence suggests that CK2 is not involved in regulating H3 acetylation levels under DNA damage conditions, but may be important during the heat stress response. The interactions between CK2 and Gcn5 require more data to be understood. The H2AS129 and H4S1 phosphorylation marks are of interest with regard to DNA damage repair, whereas the transcriptional marks, H3K4me3, H3K79me3, and H2BK123Ub1, are important for understanding their relationship under all conditions. Evaluating how these marks and others known to be involved with CK2 or Gcn5 such as H2AY58 can help elucidate their common functionality. Furthermore, understanding not only the relationship between CK2 and Gcn5, but also the relationship between the different CK2 subunits can provide more knowledge of how the cell responds to different conditions such as DNA damage and heat stress. In addition to evaluating the histone modification levels to determine functionality, each individual histone

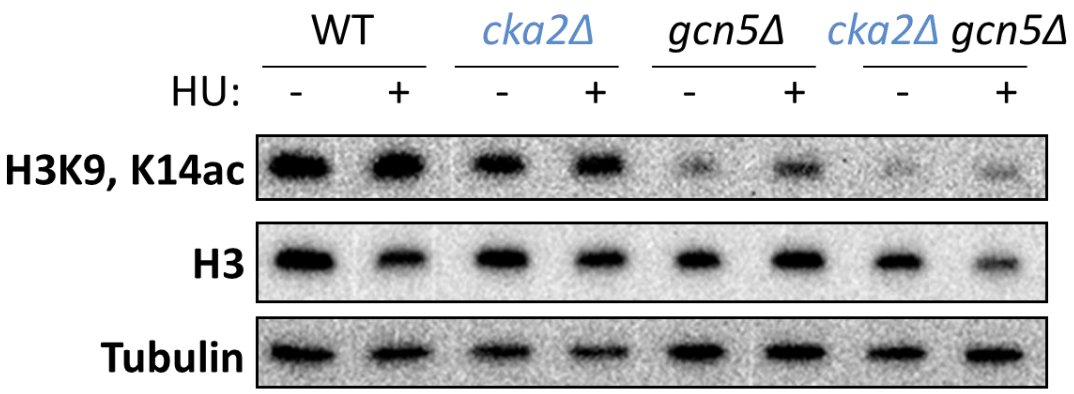


Figure 3-3. Histone acetylation not rescued by loss of *CKA2* upon HU treatment. The strains WT (LPY5), *cka2*Δ (LPY21665), *gcn5*Δ (LPY13320), *cka2*Δ *gcn5*Δ (LPY21671) were grown overnight and then diluted to 0.1 OD. After growing to 0.4-0.5 ODs SC (vehicle for HU) or HU was added to a concentration of 0.1M. The cells were collected after 2 hours of HU treatment then lysed and a protein immunoblot was performed using the protocols in Appendix 2, Materials and Methods. Images were captured using the ProteinSimple FluorChem E system, using the 40 second exposure.

residue can also be tested to provide evidence for the relationship between these enzymes. In the next chapter, the function of each histone tyrosine residue will be investigated.

Summary

The immunoblotting analysis in Figure 3-1 was repeated 3 times across independent lysate collections. Comparing the blots quantitatively showed large variability in each background. Also after further repeating the analysis in Figure 3-1, the *cka1Δ gcn5Δ* mutant no longer showed increased acetylation levels. This could be due to the lysates being seven months old, and despite storage at -80°C the protein modifications may have degraded. I believe that this experiment still needs to be optimized to conclusively define the cell's response to heat response at a molecular protein level. Preliminary analysis of H2AS129ph, H3K4me3, H2BK120ub1, and H2Y58ph levels are presented in Appendix 1.

Chapter 4. Histone tyrosine residue mutant analysis

Introduction

CK2 was historically known primarily as a Ser/Thr protein kinase, but recently was discovered to phosphorylate histone H2A on tyrosine residue 58 (H2AY58) in *S. cerevisiae* as well as tyrosine residues in mammalian cells (Basnet et al. 2014, Vilk et al. 2008). Post-translational histone modifications are important for regulating chromatin structure, recruiting enzymes, and signaling in chromatin regulation pathways [reviewed in (Bannister and Kouzarides 2011), (Fischle et al. 2003)]. Common modifications such as acetylation, methylation, and phosphorylation can affect histone-histone, histone-DNA, and protein-DNA interactions [reviewed in (Tessarz and Kouzarides 2014)]. Specifically, histone phosphorylation takes part in the DDR pathways, transcriptional regulation, and chromatin compaction [reviewed in (Rossetto et al. 2012)]. Many canonical histone phosphorylation sites from multiple organisms are catalogued at PhosphoSite Plus or SGD for *S. cerevisiae* (Hornbeck et al. 2012, Cherry et al. 2012), and many of these sites remain to be fully characterized. Both CK2 and Gcn5 are functionally known to be involved in dynamic histone modifications. Based on CK2's recently discovered function in phosphorylation of histone H2AY58, I hypothesized that CK2 targets other highly conserved histone tyrosine residues (Figure 4-1). Further, additional sites of CK2 histone phosphorylation may affect *gcn5*Δ suppression phenotypes observed in *cka1*Δ *gcn5*Δ and *cka2*Δ *gcn5*Δ mutants.

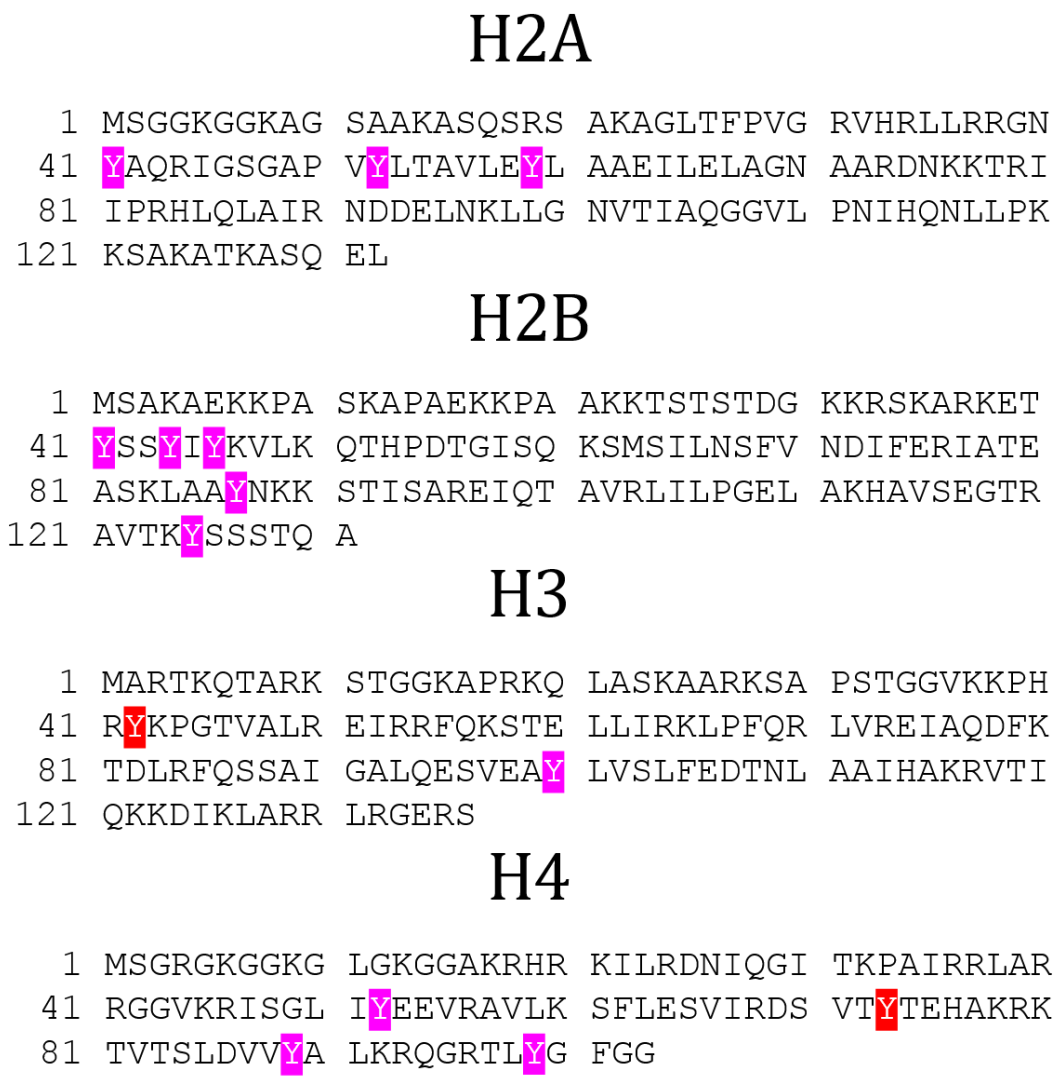


Figure 4-1. Screening conserved histone tyrosine residues. The fourteen histone tyrosine residues in *S. cerevisiae* are conserved in humans. Humans have fifteen histone tyrosine residues, an extra H2A.X tyrosine residue 142 [reviewed in (Singh and Gunjan 2011)]. Twelve of the fourteen histone tyrosine residues were tested (highlighted in pink) for their effects on the CK2-Gcn5 interactions. Two essential residues H3Y41 and H4Y72 remain to be tested (highlighted in red).

Altering the dynamics of histone modifications can change transcriptional activation, the recruitment of protein complexes to nucleosomes, and the response to different stresses. Using mutational analysis by modifying an individual amino acid residue can determine if it has a functional role in a particular process. Indeed, a plasmid library in which each amino acid of the canonical histones was mutated to alanine was created for this purpose (Nakanishi et al. 2008). I utilized this Scanning Histone Mutagenesis with Alanine (SHIMA) mutant library to evaluate functions of the histone tyrosine residues in the Gcn5-CK2 functional interaction (Nakanishi et al. 2008). This screen was performed by transforming the SHIMA library plasmids into “histone shuffle” strains as diagrammed in Figure 4-2. These strains had either both H2A-H2B (*HTA1-HTB1/HTA2-HTB2*) genes deleted or both H3-H4 (*HHT1-HHF1/HHT2-HHF2*) genes deleted with one copy of the other pair of histone genes present. Since the histone genes are essential for viability, each histone mutant strain at the outset contains a *URA3*-CEN marked plasmid containing a single copy of the canonical wild-type histone genes (*HTA1-HTB1-HHT2-HHF2)*. After transformation with a mutant SHIMA plasmid, the wild-type plasmid would no longer be necessary if the residue is not required for viability. 5-fluoroorotic acid (5-FOA), a compound that is decarboxylated by the enzyme encoded by *URA3* into 5-fluorouracil which is toxic to the cell, was used in media to select against cells that retained the wild-type *URA3* plasmid (Boeke et al. 1984). Mutant strains that are not viable after 5-FOA treatment suggest that the mutated residue is essential for normal growth. In contrast, mutant strains that survive 5-FOA

treatment can be further tested against different stresses to understand the genetic interactions. This “histone shuffle” strategy was used to construct histone mutant strains that only expressed the mutant histone (Figure 4-2).

Mutating the tyrosine residue to alanine or phenylalanine prevents phosphorylation. However, alanine can significantly change the protein structure, whereas phenylalanine contains an aromatic ring similar to tyrosine, therefore is a more structurally conservative substitution (Figure 4-3). Using the “histone shuffle” strategy, plasmids from the SHIMA library were used to screen tyrosine residues mutated to alanine. When available, mutant plasmids containing tyrosine to phenylalanine mutations were used. The histone shuffle strains were grown to saturation and 5-fold serial dilutions were performed with a starting A_{600} O.D. of 5.

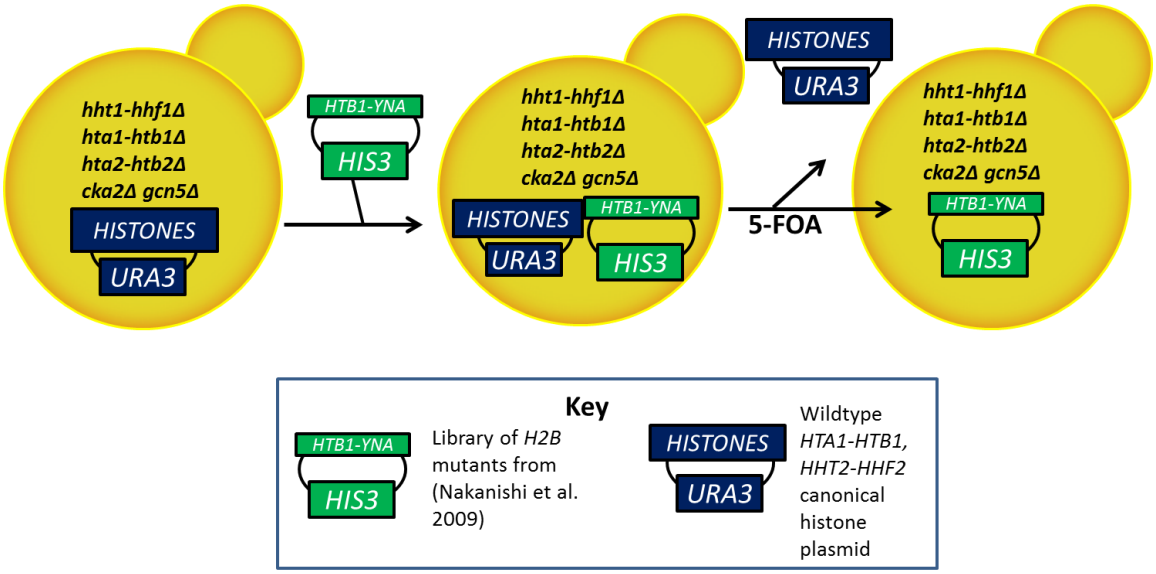


Figure 4-2. Histone shuffle strategy. A single copy of each canonical histone gene is necessary for cell survival. The H2A-H2B and H3-H4 shuffle strains contain multiple deletions as shown and are maintained with a canonical histone covering plasmid (pLP2212). Shuffle strains are then transformed with a second plasmid carrying a mutation in one of the histone residues. To test the effect of that mutant residue on cell growth and stress response, the covering plasmid is first eliminated by *URA3* negative selection with 5-FOA. Therefore only the cells containing the mutant plasmid will remain viable, if the mutated residue is not itself essential for viability.

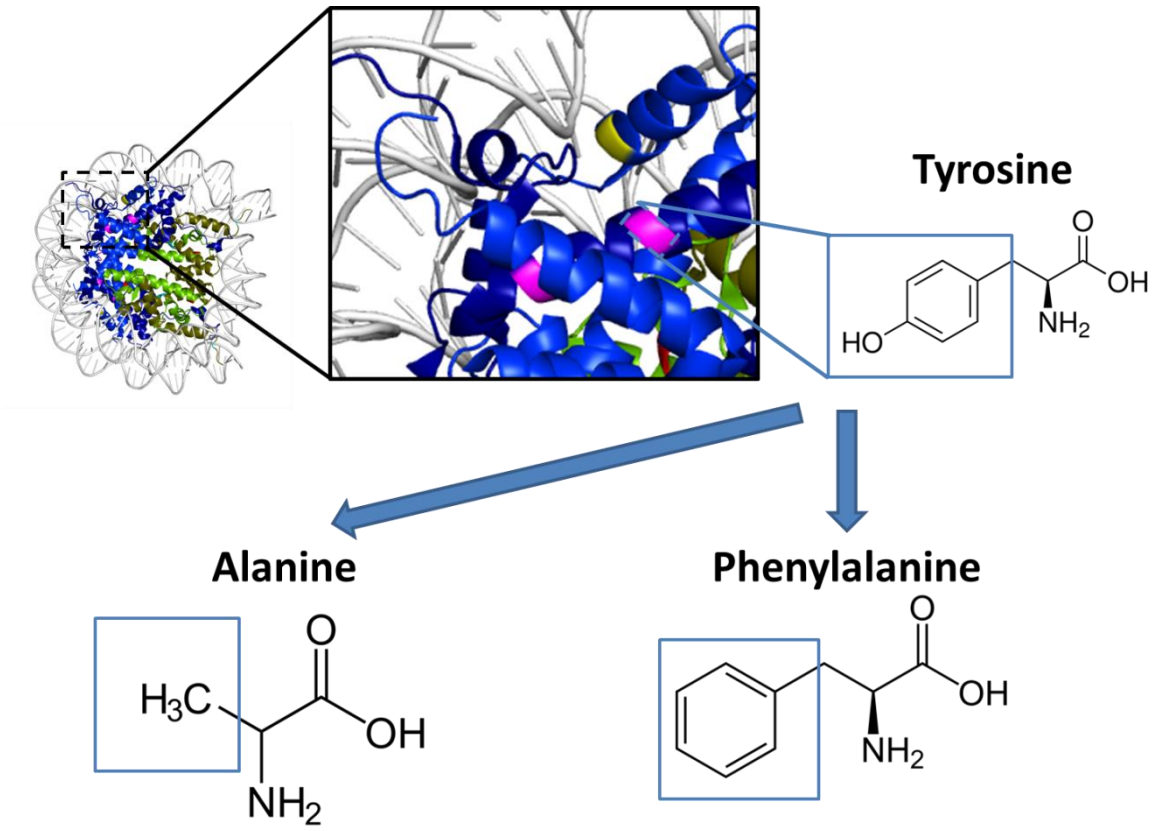


Figure 4-3. Histone residue mutation effects. Different site mutants may suggest different functional roles for a specific protein residue. Tyrosine can be phosphorylated at its hydroxyl group. When tyrosine is mutated to either alanine or phenylalanine its capacity to be phosphorylated is lost. However, phenylalanine has an aromatic ring similar to tyrosine's R-group whereas alanine is very different in structure. Therefore, mutations to alanine that cause a phenotype could suggest that the observed effects may be due to structural changes, or may be due to loss of phosphorylation. Phenylalanine mutations suggest that the observed effects are due to loss of phosphorylation rather than structural changes.

Table 4-1. Summary of histone mutant phenotypes.

Mutation	Strain	Growth at 30°C	HU	MMS	High Temp 37°C
H2A Y51A	WT	+++	+++	+++	+
	*gcn5*Δ	+++	Lethal	+	N/A
	*cka2*Δ *gcn5*Δ	+++	Lethal	+	N/A
H2A Y58F	WT	+++	+	+++	+
	*gcn5*Δ	+	Lethal	Lethal	N/A
	*cka2*Δ *gcn5*Δ	+	Lethal	Lethal	N/A
H2B Y43A	WT	+++	++	N/A	+
	*gcn5*Δ	+++	+	N/A	+
	*cka2*Δ *gcn5*Δ	+++	+	N/A	+
H2B Y43F	WT	+++	+++	N/A	+++
	*gcn5*Δ	+++	++	N/A	+
	*cka2*Δ *gcn5*Δ	+++	+++	N/A	+
H3 Y99A	WT	+++	+++	+++	+++
	*gcn5*Δ	+++	++	+++	N/A
	*cka2*Δ *gcn5*Δ	+++	N/A	++	N/A

Table 4-1. Summary of histone mutant phenotypes (continued)

Mutation	Strain	Growth at 30°C	HU	MMS	High Temp 37°C
H4 Y51A	WT	++	+	+	Lethal
	*gcn5*Δ	Lethal	N/A	N/A	N/A
	*cka2*Δ *gcn5*Δ	Lethal	N/A	N/A	N/A
H4 Y88A	WT	+++	+++	+++	+++
	*gcn5*Δ	+++	Lethal	Lethal	N/A
	*cka2*Δ *gcn5*Δ	+++	Lethal	Lethal	N/A
H4 Y98A	WT	+++	N/A	N/A	N/A
	*gcn5*Δ	Lethal	N/A	N/A	N/A
	*cka2*Δ *gcn5*Δ	Lethal	N/A	N/A	N/A

The growth is indicated by (+) signs for poor growth to (+++) signs for normal growth. No growth is indicated by “Lethal” and data not yet obtained are noted as “N/A”

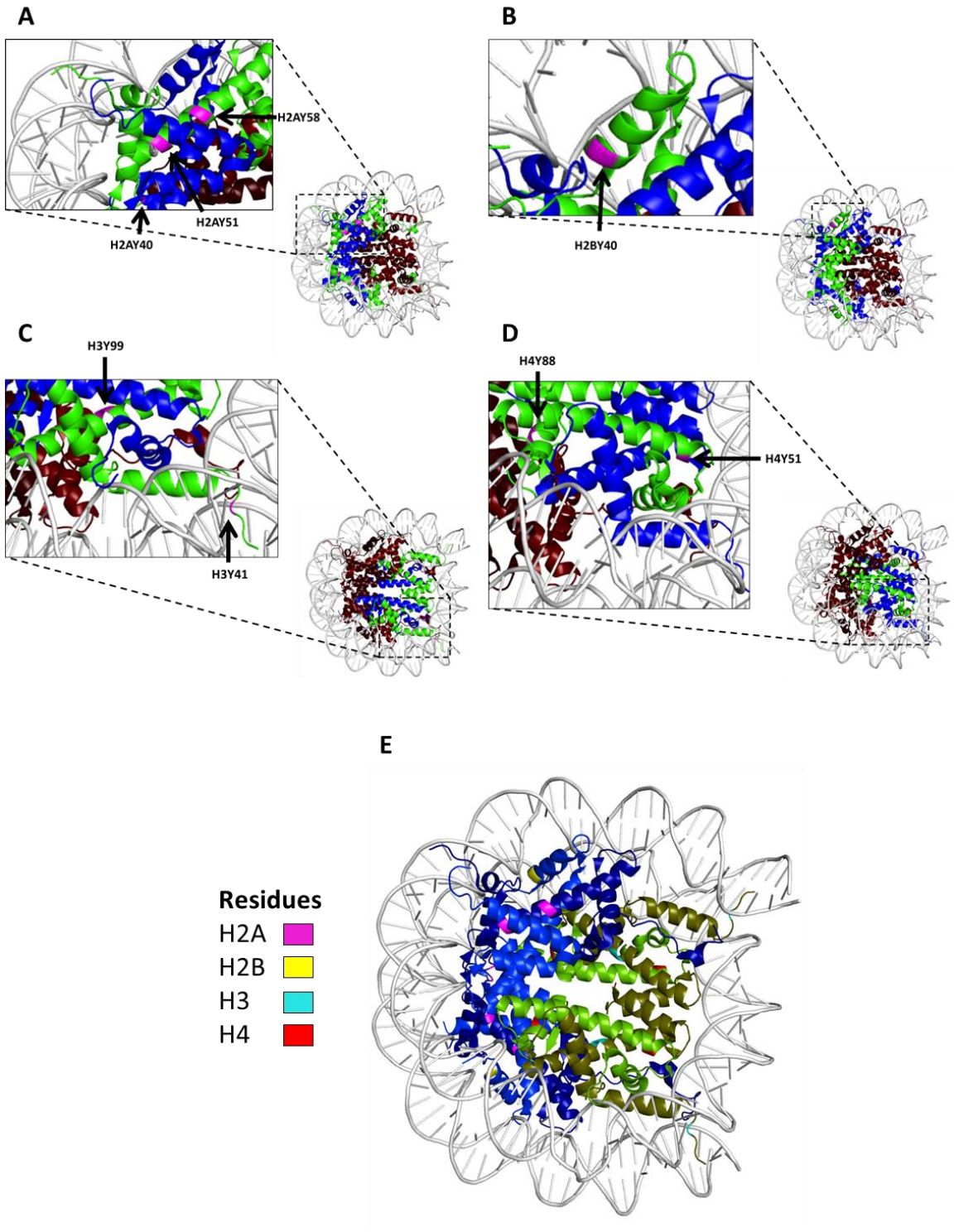
*Previously observed to be synthetically lethal (Nakanishi et al. 2008, Dai et al. 2008)

Results and Discussion

Previously, H2AY58 mutated to alanine (H2AY58A) was observed to be lethal in the histone mutant background (Nakanishi et al. 2008, Dai et al. 2008), but the H2AY58 to phenylalanine (H2AY58F) mutation in the histone mutant background was viable (Basnet et al. 2014). There was some evidence provided that the lethality caused by the H2AY58A mutation may be due to its role in transcriptional elongation. This histone mutation along with other histone mutations that caused a change in phenotype in the WT, *gcn5*Δ, and *cka2*Δ *gcn5*Δ mutant strains tested in this study are summarized in Table 4-1, with the primary data given below. The residues tested, but not listed in the table had no apparent phenotype under the conditions tested. The position of the mutant residues with phenotypes is shown in the molecular model in Figure 4-4.

In the wild-type histone shuffle strain (WT) no growth deficiencies were observed in the histone mutations H2AY40A, H2AY51A, or H2AY58F in non-stress conditions. However, slow growth was observed when exposed to HU, MMS, and elevated temperatures in the WT strain with the H2AY58F mutation (Figure 4-5). Also, the H2AY58F mutation in both the *gcn5*Δ and *cka2*Δ *gcn5*Δ histone mutants caused slow growth when initially selecting against the canonical histone plasmid, during non-stress conditions, and all the tested stresses (Figure 4-5). This suggests that H2AY58 phosphorylation is involved in the DNA damage and elevated temperature response. The slow growth under normal conditions and the increased sensitivity to all stresses in the *gcn5*Δ and *cka2*Δ *gcn5*Δ mutant backgrounds with the H2AY58F mutation could be caused by the loss of

Figure 4-4. The positions of the histone tyrosine residues of interest important for elucidating function. Using Polyview-3D (Porollo and Meller 2007) and the yeast nucleosome structure (White et al. 2001), the location of each tyrosine residue was noted. For panels A-D: The histones of interest were colored green. The complementary histone for the dimer was colored blue (H2A with H2B & H3 with H4). The other histone dimer was colored in red. Residues of interest are labeled and colored magenta. A) Histone H2A (green) with residues: Y40, Y51, Y58. B) Histone H2B (green) with residue Y40. C) Histone H3 (green) with residues: Y41, Y99. D) Histone H4 (green) with residue: Y51, Y88. D) Histone residues and their locations relative to the other residues noted. H2A (dark blue) residues: Y40, Y51, Y58 (magenta); H2B (light blue) residue: Y43 (yellow); H3 (dark green) residues: Y41, Y99 (cyan); H4 (light green) residues: Y51, Y88 (red).


A
H2AY58
H2AY51
H2AY40
B
H2BY40
C
H3Y99
H3Y41
D
H4Y88
H4Y51
E
Residues
H2A
H2B
H3
H4

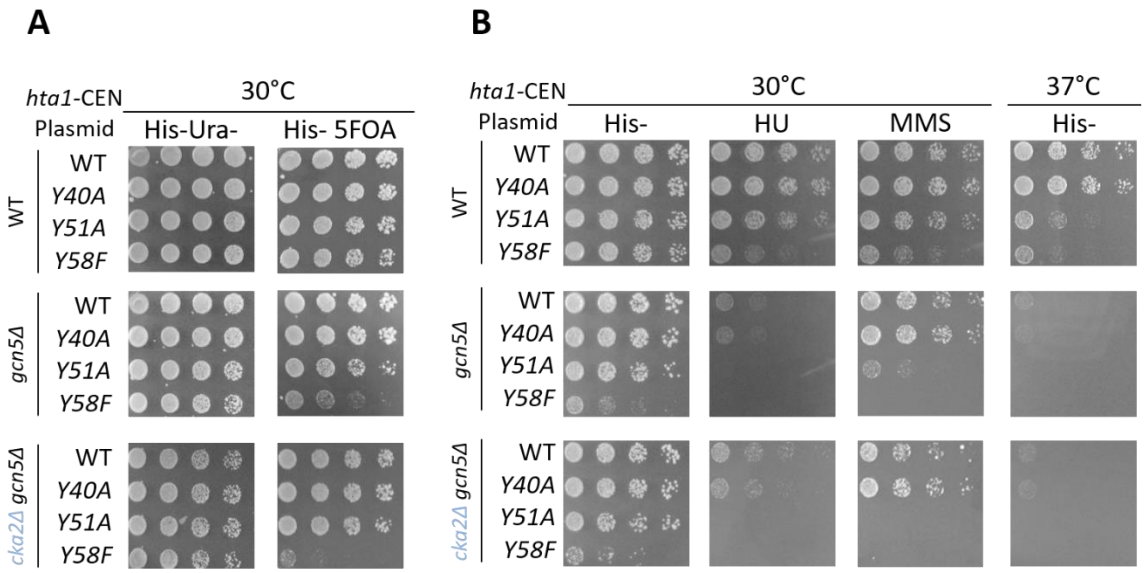


Figure 4-5. New roles for H2A tyrosine residues. The WT (LPY20647), *gcn5*Δ (LPY20679), *cka2*Δ *gcn5*Δ (LPY21961) histone shuffle strains were transformed with a plasmid with either the WT H2A-H2B (pLP 2492), mutant H2AY40A (pLP2881), mutant H2AY51A (pLP2882), or mutant H2AY58F (pLP3202) plasmid. These strains were all grown to saturation. A) 5 fold dilutions were made starting at A_{600} O.D. of 5 and the cells were plated on His-Ura-SC media and His-SC 0.5X 5-FOA plates. Cells were incubated for 4 days. B) The strains grown on the 5-FOA plates were re-streaked on His-SC plates to allow for growth recovery after drug treatment. These cells were then grown to saturation and 2.5 fold dilutions were made starting at A_{600} O.D. of 5 and the cells were plated on pre-warmed His-SC media plates, or His-SC media plates containing 0.75M HU or 0.0075% MMS. Cells were incubated for 4 days before imaging.

H2AY58's contributions to transcription, which may exacerbate their mutant phenotypes that already include deficiency in transcription (Figure 2-5) (Basnet et al. 2014).

Previously, the H2AY51 residue was identified to be in an inaccessible region within the nucleosome, and important for the H2A-H2B/H3-H4 dimerization surface "handshake" motif for nucleosome structure (Figure 4-4) [reviewed in (Singh and Gunjan 2011)]. The WT histone shuffle strain containing the H2AY51A mutation was only sensitive to elevated temperature. In addition to temperature sensitivity, the *gcn5*Δ and *cka2*Δ *gcn5*Δ histone shuffle strains with the H2AY51A mutation also showed increased sensitivity to HU and MMS. The tyrosine to alanine mutation could have caused a change in histone structure that affected DNA-histone, DNA-protein, or protein-histone interactions. During responses to heat shock or DNA damage, it is possible that the nucleosome changes structure, thereby allowing H2AY51 to be accessible and modified or the residue may be important for the nucleosome structure. The mutated residue does not affect normal growth in any strains, suggesting that its role is specific for the stress response. Moreover, the residue may be important to other parallel response pathways not involving *GCN5* because it exacerbates the *gcn5*Δ mutant sensitivity to all stresses tested.

Neither *gcn5*Δ nor *cka2*Δ *gcn5*Δ histone shuffle strains containing the WT histone plasmid grew at elevated temperatures, so no comparisons could be drawn. The H2AY51 and H2AY58 residues may also be important for the interaction, but the slow growth observed may be due to the *gcn5*Δ mutant

sensitivity and not linked for the rescue in growth seen in the *cka2*Δ *gcn5*Δ mutant. These H2A residues are important in uncovering the interactions between these two genes. Further experiments are needed to determine whether it is the structure or lack of phosphorylation at H2AY51 that may cause defects in response to DNA damage but not normal growth. These residues may be involved in a *GCN5*-independent stress response explaining the rescue in growth by the loss of *CKA2*. These observed mutant residue phenotypes are examples of how dynamic and important histone residues can be for growth, seen differently in each mutant background.

Screening of the histone H2B tyrosine residues was performed using the same method (Figure 4-1). The H2B tyrosine mutations did not affect the growth of the three strains under non-stress conditions (Figure 4-6). However, when exposed to HU and high temperature the H2BY43A mutation caused slow growth in all strains. It was expected that the H2BY43A mutant sensitivity was due to loss of phosphorylation and not the change in structure because the other H2B tyrosine residues H2BY40 and H2BY45 which are nearly adjacent to H2BY43 had no growth defects. Contrary to initial predictions, the H2BY43F mutations in all three strains grew similarly to the strains containing the WT plasmid. This suggests that the sensitivity to stress observed for H2BY43A was due to changes in structural integrity rather than loss of phosphorylation. H2BY43 may be important to the DNA-histone interaction based on its proximity to DNA (Figure 4-4). None of the other H2B tyrosine residue mutations caused any noticeable

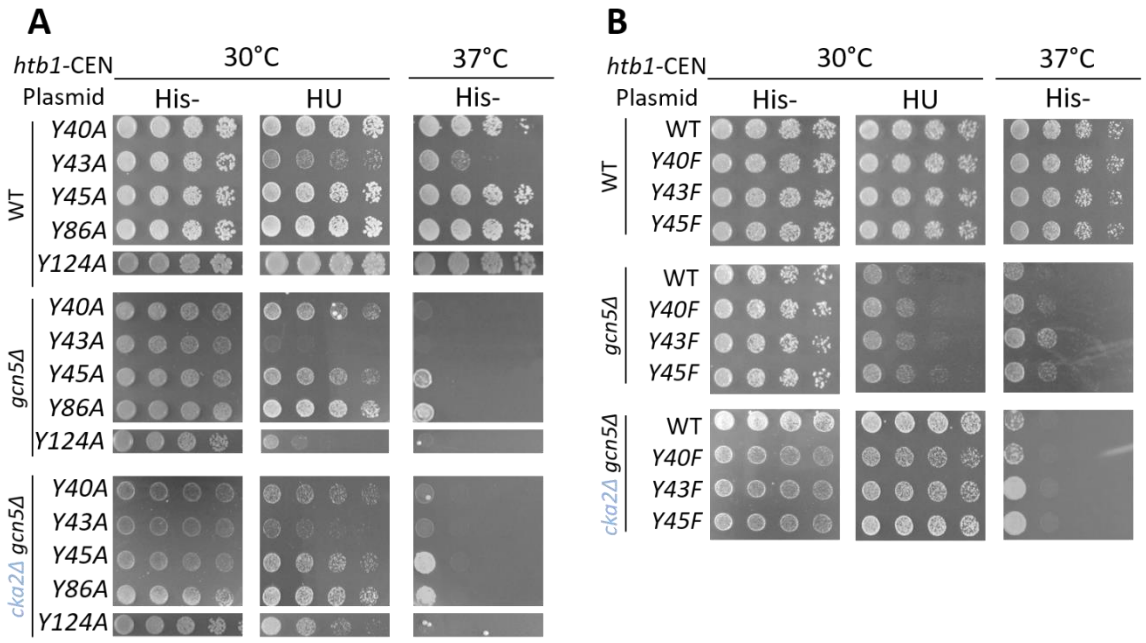


Figure 4-6. Histone H2BY43A sensitivity is lost when H2BY43 is mutated to phenylalanine. The WT (LPY14461), *gcn5*Δ (LPY16434), *cka2*Δ *gcn5*Δ (LPY21961) histone shuffle strains were transformed with a H2A-H2B WT plasmid (pLP2492), or mutant plasmids: H2BY40A (pLP3369), H2BY43A (pLP3370), H2BY45A (pLP3375), H2BY86A (pLP3371), H2BY124A (pLP 2887), H2BY40F (pLP3250), H2BY43F (pLP3251), H2BY45F (pLP3252). The transformed strains were also plated on 5-FOA plates to select against the WT covering plasmid. These strains showed no initial growth defects on 5-FOA (not shown). After recovering growth on His-SC plates the strains were grown to saturation and plated after a 5 fold dilution starting at A_{600} O.D. of 5. The cells were plated on His-SC plates lacking or containing 0.75M HU at 30°C or 37°C. Cells were incubated for 4 days before imaging. The H2BY124A mutants were plated separately.

defects. This is an example of how a specific histone mutation to either alanine or phenylalanine is useful for identifying the residue's function.

Finally, both histone H3 tyrosine residues were tested as well as three out of the four histone H4 tyrosine residues. Multiple H3 and H4 tyrosine residues were earlier found to be lethal in the s288C and GRF167 yeast backgrounds (Dai et al. 2008). In humans, H3Y41 is phosphorylated and in yeast a tyrosine mutation to either alanine or glutamic acid is synthetically lethal in WT (Dawson et al. 2009, Dai et al. 2008, Nakanishi et al. 2008).

In contrast, I found that the H3Y41A mutation did not cause any growth defects in the three histone shuffle strains (Figure 4-7). It appears that this is likely due to a quality control problem in the library. In my follow-up sequencing validation, the H3Y41A mutant plasmid was demonstrated to instead encode H3R40A. The correct mutation must be tested in future studies.

Beyond histone H3, the H4Y51A mutation caused the WT histone shuffle strain to grow more slowly under non-stress conditions as well as at elevated temperatures and upon exposure to HU (Figure 4-8). The importance of H4Y51 was clear in both *gcn5*Δ and *cka2*Δ *gcn5*Δ histone shuffle strains containing the mutated residue; neither grew when colonies from 5-FOA were picked, then struck onto a plate with fresh medium (Figure 4-7). Based on the position of H4Y51 in the nucleosome it is a possible candidate for phosphorylation and important for the structural integrity of the nucleosome (Figure 4-4) [reviewed in (Singh and Gunjan 2011)]. The evidence here suggests that H4Y51 is important in normal cell metabolism and the stress response because the H4Y51A

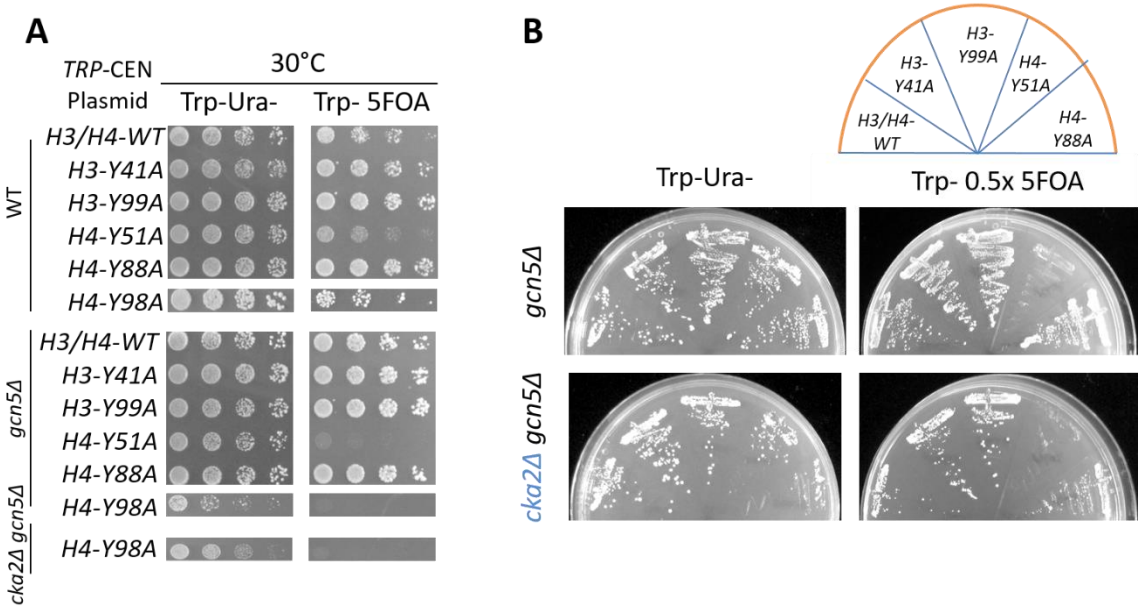


Figure 4-7. Histone H4 tyrosine residues are critical for growth. The WT (LPY12231), *gcn5*Δ (LPY16291), *cka2*Δ *gcn5*Δ (LPY22025) histone shuffles were transformed with a H3-H4 WT plasmid (pLP1490), or mutant plasmids: H3Y41A (pLP3372), H3Y99A (pLP3373), H4Y51A (pLP3374), H4Y88A (pLP3376), H4Y98A (pLP3406). A) The transformed strains were grown to saturation and plated after a 5 fold dilution starting at A_{600} O.D. of 5 on Trp-Ura- and Trp- 5-FOA plates. The plates were incubated for 4 days. The *cka2*Δ *gcn5*Δ histone shuffle strain did not grow well in the dilution assay; little or no growth was detected on the Trp- 5-FOA plates. The histone shuffle strains with H4Y98A mutated was plated separately. B) To get a sense of the growth of the *cka2*Δ *gcn5*Δ histone shuffle it was instead struck on the plates and compared to the *gcn5*Δ histone shuffle strain. Streaking colonies is less quantitative, but it is apparent that the *cka2*Δ *gcn5*Δ histone shuffle strain is sick on 5-FOA plates with the WT plasmid and most mutant plasmids, but is still viable. Both *gcn5*Δ and *cka2*Δ *gcn5*Δ histone shuffle strains with the H3Y51A mutant plasmid were lethal. The strains would not grow when struck from the 5-FOA plates to Trp- plates.

mutation caused reduced growth of the WT histone shuffle strain in non-stress as well as stressful conditions. Neither *gcn5*Δ nor *cka2*Δ *gcn5*Δ histone mutant strains could grow with the H4Y51A mutation further supporting its critical role. The functional role of H4Y51 will be determined through more mutational studies.

The *cka2*Δ *gcn5*Δ H3 and H4 histone shuffle strains were extremely sick and grew very slowly when selecting against the *URA3* plasmid using 5-FOA (Figure 4-6). For this reason, only a few mutant residues were further tested against different stresses in the *cka2*Δ *gcn5*Δ histone shuffle background. No growth could be compared in either HU or elevated temperature stress because the *cka2*Δ *gcn5*Δ histone mutant containing the WT histone plasmid did not grow. The H4Y88A mutation exacerbated the *gcn5*Δ and *cka2*Δ *gcn5*Δ histone mutant's growth sensitivity to MMS (Figure 4-8). The residue may be part of a parallel response pathway to the *GCN5*-dependent stress response because the WT strain is unaffected by this mutation. The residue is located within the nucleosome and may be important for the H3-H4 tetramer interface or the stress response (Figure 4-4). The mutation to alanine could disrupt nucleosomal structural integrity making it less stable and more susceptible to DNA damaging agents or the mutation may prevent the signaling of the proper repair mechanisms for base mispairing. Both possibilities are consistent with the data and have not yet been resolved

Histone modification function is not restricted to chromatin-bound histones. For example, H3Y99 is accessible for modification in free H3, but this accessibility may be lost when incorporated into the nucleosome. This allows H3-

Y99 to be phosphorylated and targets the free histone for degradation (Singh et al. 2009). H3Y99 itself is located in a part of the nucleosome important for H3-H4 heterodimerization and seems to be important for ring stacking stabilization in the nucleosome (Singh et al. 2009). The modifications of all other residues may also have dual roles depending on whether the histone molecule bearing it is chromatin bound, monomeric, dimeric, or chaperone-bound. Recently, the histone levels of the *gcn5*Δ mutants have been noted to be significantly less than WT histone levels, meaning histone H3 levels are lower in *gcn5*Δ mutants (Petty et al. 2016, Eriksson et al. 2012). Both the H3Y41A and H3Y99A mutations slightly improve the growth of the *gcn5*Δ histone shuffle strained when compared to the WT plasmid containing strain (Figure 4-8). The H3Y99A mutation does not show any defects in the WT histone shuffle strain, suggesting that it is not essential for the cell. Preventing free histone H3 from being phosphorylated and targeted for degradation caused by the tyrosine to alanine mutation may allow a slight recovery of histone H3 levels in *gcn5*Δ mutants allowing a slight improvement in growth. This may be due to the importance of the many histone H3 tail residues for transcription and the incorporation of histone H3 into the nucleosome for continued growth.

The initial characterization of the core histone tyrosine residues revealed many interesting results. However, only the surface of understanding the functions of these residues has been uncovered. There are many more questions that need to be asked about the important roles of the residues identified here. Further studies on the histone mutants that had an observed phenotype can be

performed. Understanding their roles may provide insight into how the cell responds to stress and can uncover novel proteins that interact with these residues for proper response and regulation

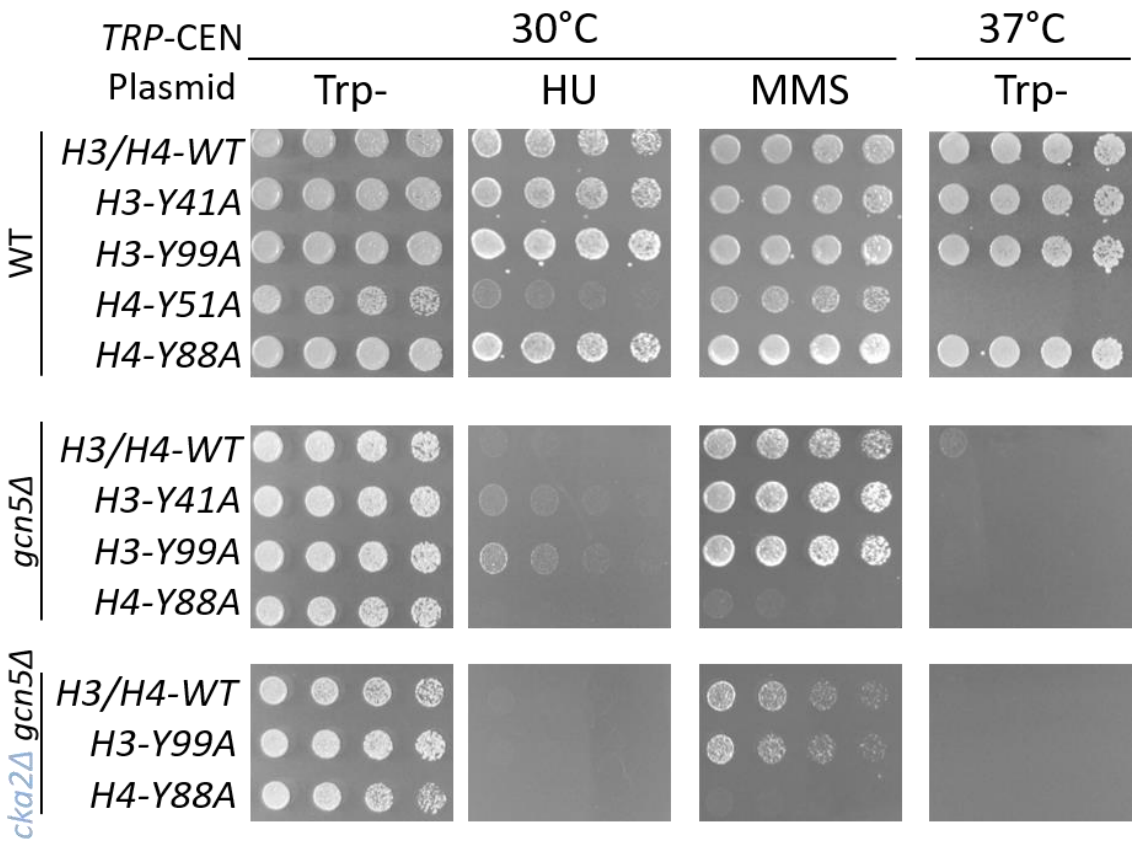


Figure 4-8. Histone H3 and H4 tyrosine residues involved in stress response. The strains from Figure 4-7 were grown to saturation and plated after a 2.5-fold dilution starting at A_{600} O.D. of 5 on 0.75M HU, 0.0075% MMS, and Trp-SC plates at 30°C or 37°C. The plates were incubated for 3 days. Neither *gcn5*Δ *nor cka2*Δ *gcn5*Δ histone shuffle strains containing the H4Y51A mutant plasmid were viable. However, the *cka2*Δ *gcn5*Δ histone shuffle strain containing H3Y41A was viable, but was not tested in this experiment.

Chapter 5. Discussion and future Directions

Post translation modifications on histones are important for regulation of chromatin structure, enzyme recruitment, and rapid response to changes in the environment [reviewed in (Bannister and Kouzarides 2011, Tessarz and Kouzarides 2014)]. The nucleosome acts as a physical barrier, preventing other proteins from accessing DNA. Histone modifications such as acetylation can loosen the barrier making the DNA more accessible by transcriptional machinery. Such modifications can also change the nucleosome structure, promoting the recruitment of proteins in response to stress or other cellular activities. For example, SAGA, a Gcn5-associated complex, is responsible for the activation of multiple genes in the response to stress by modifying chromatin (Huisinga and Pugh 2004). These modifications allow for a rapid response to changes in the environment.

During stressful conditions cells need to properly adapt and regulate cellular functions. The cell can respond to significant stresses caused by temperature shifts or DNA damage by repressing most of transcription and arresting the cell cycle to allow time for stress response proteins to repair or protect the cell from further damage [reviewed in (Verghese et al. 2012), Hu et al. 2001]. Among many other stress-response factors, is *GCN5* histone acetyltransferase, whose role is very apparent in *GCN5* null cells' sensitivity to many stresses such as heat, DNA damage, and osmotic stress.

In addition to acetylation by HAT complexes such as SAGA, phosphorylation by protein kinases is also important for stress-response signaling. Depending on the stress, proper signaling by multiple proteins activates various repair pathways. For example, phosphorylation of histone residues such as H2AS129 and H4S1 are important for different steps in DNA damage repair and are known to be involved in multiple DNA repair pathways (Redon C et al. 2003, Utley RT et al. 2005). H2AS129 phosphorylation recruits chromatin modifiers to DNA breaks and H4S1 phosphorylation inhibits acetyltransferase activity during DNA damage repair. Moreover, phosphorylation of non-histone proteins also allows for rapid DNA damage response. In yeast, for a proper replication block in response to DNA damage, the Rad9 checkpoint protein must be phosphorylated to bind Rad53 [reviewed in (Zhou and Elledge 2000)].

Studying the dynamics of how acetyltransferases and kinases interact in response to stress can uncover greater understanding of the stress response regulation. The protein kinase CK2, a major regulator of many cellular functions, targets histones H2A and H4 as well as hundreds of non-histone proteins (Basnet et al. 2014, Utley RT et al. 2005, Meggio and Pinna 2003). H4S1 phosphorylation by CK2 prevents acetylation at H4K8 and H4K12 during DNA damage, presumably to prevent further damage while the cell repairs other areas (Utley RT et al. 2005). This is an example of how histone phosphorylation can interact with other histone modifiers. Similarly, loss of phosphorylation at H2AY58 causes increased activity of the DUB module of the SAGA complex, which may

affect its Gcn5 HAT activity (Basnet et al. 2014). The relationship between both proteins could be important for different responses as seen in other HAT and kinase activities.

In vivo CK2 characterization uncovers specific roles for enzyme isoforms

In this thesis, the functional activity of the CK2 subunits were first dissected using genetic analysis. The functional specificity is still mostly unknown for each CK2 isoform: $Cka1^{Holo}$, $Cka2^{Holo}$, $Cka1\text{-}2^{Holo}$, $Cka1^{Free}$, and $Cka2^{Free}$. The activity of each catalytic subunit was believed to be similar because the *cka1*Δ *cka2*Δ double mutant was synthetically lethal, but the growth of the individual *cka1*Δ and *cka2*Δ mutants was normal (Padmanabha 1990). However, the CK2 isoforms had different sensitivities to multiple inhibitors suggesting that each isoform, including free catalytic subunits, could have distinct affinities for all CK2 substrates (Domańska et al. 2005). The CK2 mutants constructed (Table 1-1) were useful in understanding the activities of individual CK2 subunits as well as the CK2 holoenzyme activity which is lost in the strains lacking either regulatory subunit (Kubinski et al. 2006).

In the first detailed *in vivo* characterization, the CK2 deletion strains were tested in response to heat, HU, and MMS to parse unique from overlapping subunit function. During normal growth the CK2 mutants may function similarly, but I hypothesized that under certain conditions such as environmental stress their functions may be more distinct and specific in activating or inhibiting different pathways.

From earlier reports, individual CK2 mutants were not known to be sensitive to high temperatures, but *cka1*Δ*, ckb1*Δ*,* and *ckb2*Δ single mutants were previously found to be sensitive to MMS (Kapitzky et al. 2010). In this study, the *cka1*Δ *ckb2*Δ and *cka1*Δ *ckb1*Δ *ckb2*Δ mutants were shown to be sensitive to high temperatures (Figure 2-1B). However, the *cka1*Δ *ckb1*Δ double mutant and *cka2*Δ containing mutants grew normally at high temperatures. This suggests that the $Cka1^{Holo}$ or the $Cka1^{Free}$ and the Ckb2 regulatory subunit are involved in the heat response. Only when both *CKA1* and *CKB2* are deleted does the heat sensitive phenotype become apparent. Since neither mutant on its own is sensitive to heat, it suggests that more than one pathway may be involved.

In other eukaryotes, there is only one CK2 regulatory subunit (CK2β) and it functions independently from the $CK2^{Holo}$ regulating c-Mos, Chk1, and A-Raf activity [reviewed in (Bibby and Litchfield 2005)]. CK2β enhances Chk1 activity which is essential for DNA damage-induced G2 arrest (Walworth et al.1993). Similarly, to these findings, I hypothesize that the Ckb2 regulatory subunit in *S. cerevisiae* may regulate additional protein kinases, affecting the response to heat stress. This explains the specific heat sensitive phenotype only observed when both *CKA1 and CKB2* are deleted. Since these genes are involved in the heat shock response, it would also be interesting to see if their overexpression may increase heat tolerance. These findings further support the idea that CK2 activity for specific functions is dependent on the CK2 isoform.

My dissection of CK2 also led to interesting results in regards to the DNA damage response. The strains were exposed to MMS and HU, agents which

cause base mispairing and DNA replicative stress, respectively (Beranek 1990, Ahmet Koc 2004). MMS-induced damage is mainly repaired by the BER pathway (Lundin et al. 2005). In previously reported high-throughput studies, MMS exposure impaired growth in most of the CK2 single mutants, whereas HU exposure impaired growth specifically in mutants lacking *CKA2* or *CKB2* (Kapitzky et al. 2010). However, in my individual characterizations, none of the strains tested were sensitive to HU (Figure 2-3). On the other hand, most of the mutants tested were MMS sensitive, with the notable exception of the single *cka2*Δ mutant*,* but loss of either regulatory subunit in the *cka2Δ* background exacerbates this phenotype (Figure 2-3B). These results suggest that the $Cka1^{Holo}$ is necessary for repairing MMS-induced damage and argues that this holoenzyme is specifically involved in BER. Further, my results suggest that $Cka1^{Free}$ interferes with the response to MMS or is not sufficient in responding to MMS. To sum, this is another example of CK2 isoform specificity.

Previous work showed that specifically the $Cka1^{Holo}$ was required for phosphorylation of the transcriptional repressor Nrg1 responsible for glucose repression (Berkey and Carlson 2006). However, more studies are necessary to understand the full extent of CK2 substrate specificity. For instance, at higher MMS concentrations all CK2 double and triple mutants are completely dead, but growth is only slightly impaired at low MMS concentrations for the *cka1*Δ*, ckb1*Δ*, and ckb2*Δ double and triple mutants. This suggests that the other CK2 isoforms may have affinity for the substrates of the $Cka1^{Holo}$ responsible for responding to MMS-induced damage allowing for a partial response. Even though, a specific

isoform has higher affinity for specific targets, there may always be some redundancy. Studying CK2 with Gcn5, another protein important for the stress response, may help elucidate novel functions and interactions to better understand their roles.

Characterization of *GCN5-CKA1* interaction in response to heat stress

Further dissection of the genetic interaction between CK2 and *GCN5* revealed multiple contrasting effects. Multiple isolates of *cka1*Δ *gcn5*Δ confirmed that loss of *CKA1* rescued growth of the *gcn5*Δ mutants at high temperatures (Figure S1), indicating *GCN5* and *CKA1* functions are normally antagonistic in response to heat stress in wild type cells. Loss of the other CK2 subunits either exacerbated the *gcn5*Δ mutant heat sensitivity phenotype or had no effect on growth (Figure 2-2). The rescue of growth seen in the *cka1*Δ *gcn5*Δ mutant, therefore may be due to the loss of $Cka1^{Holo}$ activity or the increase in the $Cka2^{Holo}$. In either case, the CK2 holoenzyme is critical for growth since loss of the holoenzyme causes greater sensitivity to high temperatures and prevents rescue by loss of Cka1 alone.

To better understand the *GCN5-CKA1* antagonism in the heat response, I observed acetylation of the Gcn5 substrate histone H3. Gcn5 targets histone H3K9 and H3K14 for acetylation, which are important for transcriptional activation of SAGA-regulated genes (Lee and Workman 2007), and in *gcn5*Δ mutants there is a global reduction of H3K9-K14ac. Restoration of H3 acetylation could reduce the heat stress sensitivity of the *gcn5*Δ mutant by restoring heat shock induced gene expression. When the samples from the *cka1*Δ *gcn5*Δ

double mutant are compared to the *gcn5*Δ mutant, loss of *CKA1* partially rescues global H3K9-K14ac levels at normal and high temperatures (Figure 3-3). This could be due to an antagonistic interaction between CK2 and non-HAT activity of Gcn5-associated complexes, other HAT complexes such as Sas3, or HDAC activity. However, there is only a partial rescue in acetylation, suggesting that other factors are also responsible for the suppression of the *gcn5*Δ heat sensitivity phenotype.

Characterization of *GCN5-CKA2* interaction in response to DNA damage

Further distinctions between the CK2 isoforms was seen in the *cka2*Δ *gcn5*Δ double mutant. Rescue of HU sensitivity, but not MMS sensitivity was observed (Figure 2-4). The rescue in growth was lost and the defect even exacerbated in *cka2*Δ *gcn5*Δ mutants with either regulatory subunit deleted. The other double mutants grew similarly to the *gcn5*Δ mutant, whereas all the mutants were more sensitive to MMS. The MMS sensitivity of the individual CK2 mutants could explain the exacerbated phenotype in the *gcn5Δ* combination mutants. However, like the interaction between $Cka1^{Holo}$ and Gcn5 in heat stress response, loss of $Cka2^{Holo}$ improves the *gcn5*Δ mutant response to DNA replicative stress, and the additional loss of either regulatory subunit in these combination mutants exacerbates growth sensitivities. The seemingly similar CK2 isoforms containing either Cka1 or Cka2 are more distinct in functions than previously believed. Each CK2 catalytic mutant only alleviates one *gcn5*Δ mutant phenotype tested, suggesting that they are involved in distinct pathways. In both cases, suppression of *gcn5*Δ mutant phenotypes requires the CK2 regulatory

subunits, and the same possibilities of either losing or gaining activity can explain the rescues in growth.

The rescue of growth by the loss of *CKA2* in the *gcn5*Δ mutant could also be due to changes in levels of different histone modifications. However, the global levels of multiple histone marks are not improved. Under non-stress conditions the global levels of the transcriptional marks H3K4me3 and H3K79me3 are unchanged (Figure 3-1). Global H3K9-K14ac levels decrease in the *cka2*Δ *gcn5*Δ mutant when treated with HU (Figure 3-2). Similarly, H3K4me3 levels are also decreased (Figure S3). These results suggest that loss of *CKA2* may rescue the *gcn5*Δ mutant HU sensitivity through another mechanism. Notably, these modifications may be improved locally at different stress response genes, but global levels are not improved. This is another example of how CK2 isoforms are distinct. The loss of the Cka1^{Holo} isoform may partially improve acetylation levels allowing for increased expression of the stress response genes, but loss of the Cka2^{Holo} isoform does not rescue acetylation levels suggesting that this rescue is indeed through another mechanism. The response of the Cka2^{Holo} may directly affect a pathway parallel to the Gcn5-associated DNA damage stress response.

Histone tyrosine screen reveals new interactions

H2AY58ph by CK2 functions during transcription elongation to regulate the DUB activity of the SAGA complex (Basnet et al. 2014). I hypothesized that CK2 may phosphorylate other histone residues and undertook a mutant screen of 12 of 14 conserved tyrosines to determine their role in the interactions

between CK2 and the Gcn5-associated complexes. Two essential residues, H3Y41 and H4Y72 are currently being tested. All 14 *S. cerevisiae* core histone tyrosines are conserved through humans, where many have been identified as phosphorylated in large-scale proteomic studies [reviewed in (Singh and Gunjan 2011), Hornbeck et al. 2012]. Defects in strains with the tyrosine to alanine mutation suggest that the residue is involved in either the structure or phosphorylation, whereas defects with the tyrosine to phenylalanine mutation suggests that the residue is phosphorylated (Figure 4-3). When available, a tyrosine to phenylalanine mutant plasmid was used, otherwise a tyrosine to alanine mutant plasmid from the SHIMA library was used (Nakanishi et al. 2008). Previously, the histone H2AY58A, H4Y51E, and H4Y88E mutations caused synthetic lethality in different yeast backgrounds (Nakanishi et al. 2008, Dai et al. 2008).

The screen I performed revealed multiple novel interactions in the WT, *gcn5*Δ, and *cka2*Δ *gcn5*Δ histone mutant backgrounds. In the WT background the H2AY58F, H2BY43A, and H4Y51A mutations caused slow growth phenotypes in response to HU and high temperatures. The H2AY51A mutation only caused sensitivity to high temperatures and the H4Y51A mutation also caused slower growth at non-stress conditions. These observations suggest that these residues are involved in the growth of the cell under normal conditions and in response to heat or DNA damage stress (Figure 4-4 & 4-7).

The residues may be necessary for the nucleosomal structure integrity, or important for recruitment of different DNA binding proteins in response to these

stresses. For example, the H2BY43F mutation relieves the slow growth phenotype seen in the H2BY43A mutation strains implying that the structure, rather than its phosphorylation is integral for the stress response (Figure 4-6, Figure 4-5B). Moreover, the slow growth caused by the H4Y51A mutation in the WT background under all conditions argues that this residue is involved in regulating growth or cellular activity. The effect H4Y51A has on growth is apparent in the *gcn5*Δ and *cka2*Δ *gcn5*Δ backgrounds where it causes synthetic lethality. To determine genetically if the effects are likely to be structural or due to phosphorylation the H4Y51F and the other tyrosine to phenylalanine mutations will be generated by performing a plasmid mutagenesis on the WT histone plasmids.

The *gcn5*Δ mutant strain is already sensitive to multiple stresses, while the *cka2*Δ *gcn5*Δ mutant is also sensitive to multiple stresses except for damage caused by HU. The histone H2AY58 residue is phosphorylated by CK2 which is suspected to inhibit the activity of components of the SAGA complex. Loss of phosphorylation of this residue was expected to rescue some *gcn5*Δ mutant growth phenotypes (Pillus lab, unpublished data). However, the histone mutations H2AY51A, H2AY58F, H2BY43A, and H4Y88A all exacerbate the growth of these two mutant backgrounds. The H2AY51A and H4Y88A mutations cause increased DNA damage sensitivity in the mutant backgrounds which was not seen in the WT histone mutant. Mutating these residues may have altered the stress responses in these deficient mutants. The H2AY58F mutation does not rescue any of the *gcn5*Δ mutant phenotypes which suggests that the rescue in

growth of the *gcn5*Δ mutant phenotypes by loss of CK2 activity is not due to CK2 phosphorylation of H2AY58. Instead, loss of H2AY58 phosphorylation exacerbates *gcn5*Δ mutant phenotypes as well as growth under non-stress conditions.

None of the previously mentioned histone residues had a direct effect on the rescue of growth seen in the *cka2*Δ *gcn5*Δ mutant. When treated with HU the H2AY51A, and H2AY58F mutations cause lethality in the *cka2*Δ *gcn5*Δ histone mutant. However, the H2AY51A and H2AY58F mutations also exacerbate *gcn5*Δ mutant sensitivities to HU, thus the cause of the increased sensitivity cannot be distinguished between the two mutant backgrounds. Those two residues could be important to the *cka2*Δ *gcn5*Δ rescue phenotype or the growth deficiencies observed from the mutant residues may be due to the same deficiency in stress response seen in the *gcn5*Δ mutant.

Most of the tyrosine residues tested are known to be phosphorylated in humans (Hornbeck et al 2012). However, their functions are still widely unknown. Studying the different histone residues involved in normal growth and stress responses noted here can help uncover their specific functions. The majority of the tyrosine residues tested are implicated to function in structural integrity or phosphorylation signaling. Further genetic characterization will require the residues to be mutated to phenylalanine to distinguish whether the phenotypes are due to changes in structure or loss of phosphorylation as proposed. Unfortunately, there are currently no methods to mimic tyrosine phosphorylation

in vivo as with serine and threonine residues. Screening of Ser and Thr residues may also reveal histone substrates of CK2 important to the Gcn5-CK2 interaction.

Overall, my results revealed that different CK2 isoforms interact distinctly with Gcn5 in DNA damage and heat response. The *cka1*Δ *gcn5*Δ double mutant showed partial rescue in acetylation in response to heat, whereas the *cka2*Δ *gcn5*Δ double mutant showed no recovery of acetylation in response to HU treatment. This provides evidence that the *cka1*Δ *gcn5*Δ mutant could improve the transcriptional activity during the stress response. The changes to *gcn5*Δ mutant phenotypes caused by the loss of different CK2 subunits can be due to antagonistic activity of CK2 or its parallel role in multiple stress responses.

Possible models and predictions of Gcn5-CK2 interaction

In human cell lines, CK2 localizes to the nuclear matrix upon heat shock and phosphorylates residue T142 on heat shock factor-1 (Hsf1) (Davis et al. 2002, Soncin et al. 2003). Hsf1 is an important regulator of the genes encoding the heat shock proteins, and loss of a CK2 phosphorylation site on Hsf1 prevents activation of the *HSP70* heat shock gene (Soncin et al. 2003). Conversely in yeast, CK2 phosphorylation of Hsf1 at residue S608 represses the Hsf1 ethanol stress response (Cho et al. 2014). Therefore, individual phosphorylation sites of the same protein seem to have distinct outcomes. Similarly, in my study the *cka1*Δ *ckb2*Δ mutant was sensitive to heat, while the *cka1*Δ *gcn5*Δ mutant recovered *gcn5*Δ heat sensitivity suggesting different functions in the response to heat stress (Figure 2-1). The improvement in growth could be due to the loss of an inhibitory phosphorylation on Hsf1 or other heat response proteins. Multiple

additional Hsf1 sites are phosphorylated, but have yet to be characterized and are potential targets of CK2. These include residues S450, S458, S471, and S545 which have been identified in earlier large-scale proteomic studies (Swaney DL, et al. 2013, Albuquerque CP, et al. 2008, Holt LJ, et al. 2009). Growth improvement can also be due to the partial recovery of global acetylation levels which may improve activation of the stress response genes. These possible CK2 targets can either directly or indirectly improve the heat shock gene response (Figure 5-1) These possibilities are all feasible due to the multiple personalities of the CK2 isoforms and their ability to inhibit or activate different stress response proteins.

Further *in silico* data analysis using genetic interaction and visualization information curated in the DRYGIN resource suggested that multiple proteins involved in the heat shock response interact with *CKA1* specifically (Koh J L Y, et al. 2009). DRYGIN identifies double mutants that display synthetically sick interactions as negative genetic interactions, greater fitness as positive genetic interactions, and lists correlated genetic interactions. This resource gives insight into other possible interactions with CK2 that may be involved in the improved growth of *gcn5*Δ mutants. The genes *CDC37, STI1, CTI6*, *HSP40, HSC82* and those encoding the HSP90 family of proteins were correlated or predicted to negatively interact with *CKA1* (Table 5-1). These genes are associated with the heat shock response and important for protein folding. Cdc37 is a substrate of CK2 that is an Hsp90 co-chaperone which participates in protein stabilization and folding (Bandhakavi S. et al. 2003). CK2 phosphorylates Cdc37 at residues S14

and S17 allowing Hsp90 to recruit client kinases, and dephosphorylation by Ppt1 in the Hsp90-Cdc37 complex allows binding with other cochaperones (Vaughan C. K. et al. 2008). These *in silico* data suggests that loss of CK2 phosphorylation causes a diminished response to heat stress. However, loss of $Cka1^{Holo}$ may increase the efficiency or activity of the other heat stress related proteins. *STI1* encodes a protein that regulates the transfer of folded proteins from HSP70 to HSP90, *CTI6* encodes a component of Rpd3L that relieves transcriptional repression and recruits SAGA, and *HSP40* encodes a cochaperone to HSP90 and HSP70.

The role of CK2 in DNA damage repair has been studied intensely. CK2 is involved in multiple processes including cellular signaling MMR, NER, NHEJ, HR, and BER [reviewed in (Montenarh 2016)]. The DNA damaging agent HU causes DNA replicative stress that can lead to stalled replication forks and ultimately DNA breaks that can be repaired by NHEJ or HR (Ahmet Koc 2004). CK2 phosphorylation of histone H4S1 affects the repair efficiency of dsDNA breaks by NHEJ and in humans CK2 phosphorylates residue T233 on XRCC4, a scaffolding protein involved in DSB repair [reviewed in (Montenarh 2016)]. Lif1, the yeast homolog of XRCC4, is also required for NHEJ activity of Dnl4 (Herrmann et al. 1998, Teo S-H, et al. 2000). Similarly, I hypothesize that Lif1 may also be a substrate of CK2 at S383 which is important for Xrs2 interaction for recruitment of the Dnl4 complex to DSB (Matsuzaki K., et al. 2008). Individual CK2 mutants are not sensitive to HU, but in combination with the *GCN5* deletion CK2 mutations can either exacerbate the growth sensitivities to HU or improve

Figure 5-1. Proposed models of CK2 rescue of *gcn5*Δ response to replicative stress. Loss of *CKA1* rescues the heat sensitivity growth defect of the *gcn5*Δ mutant. Whereas loss of *CKA2* rescues the growth defect of the *gcn5*Δ mutant, specifically upon HU treatment, which causes DNA replicative stress or DNA breaks for longer treatments. The data presented here and the literature suggest two possible mechanisms. Further experimentation is required to support either model. A) The $Cka1^{Holo}$ could be inhibitory to the heat shock response and loss of *CKA1* could stop its inhibitory activity. $Cka1^{Holo}$ could target a protein (P1) that involved in activating the heat shock response (solid line). Alternatively, $Cka1^{Holo}$ could also directly phosphorylate a histone residue which activates the heat shock response (dotted line). B) The $Cka2^{Holo}$ could be inhibitory to the DNA damage response and loss of *CKA2* could stop its inhibitory activity. $Cka2^{Holo}$ could target a protein (P2) that is important in activating the DNA damage gene response in parallel to SAGA activity or increase the efficiency of replication hindered by HU (dotted line). $Cka2^{Holo}$ may also directly inhibit a DNA damage response protein (P3) involved in DNA replicative stress response (solid line).

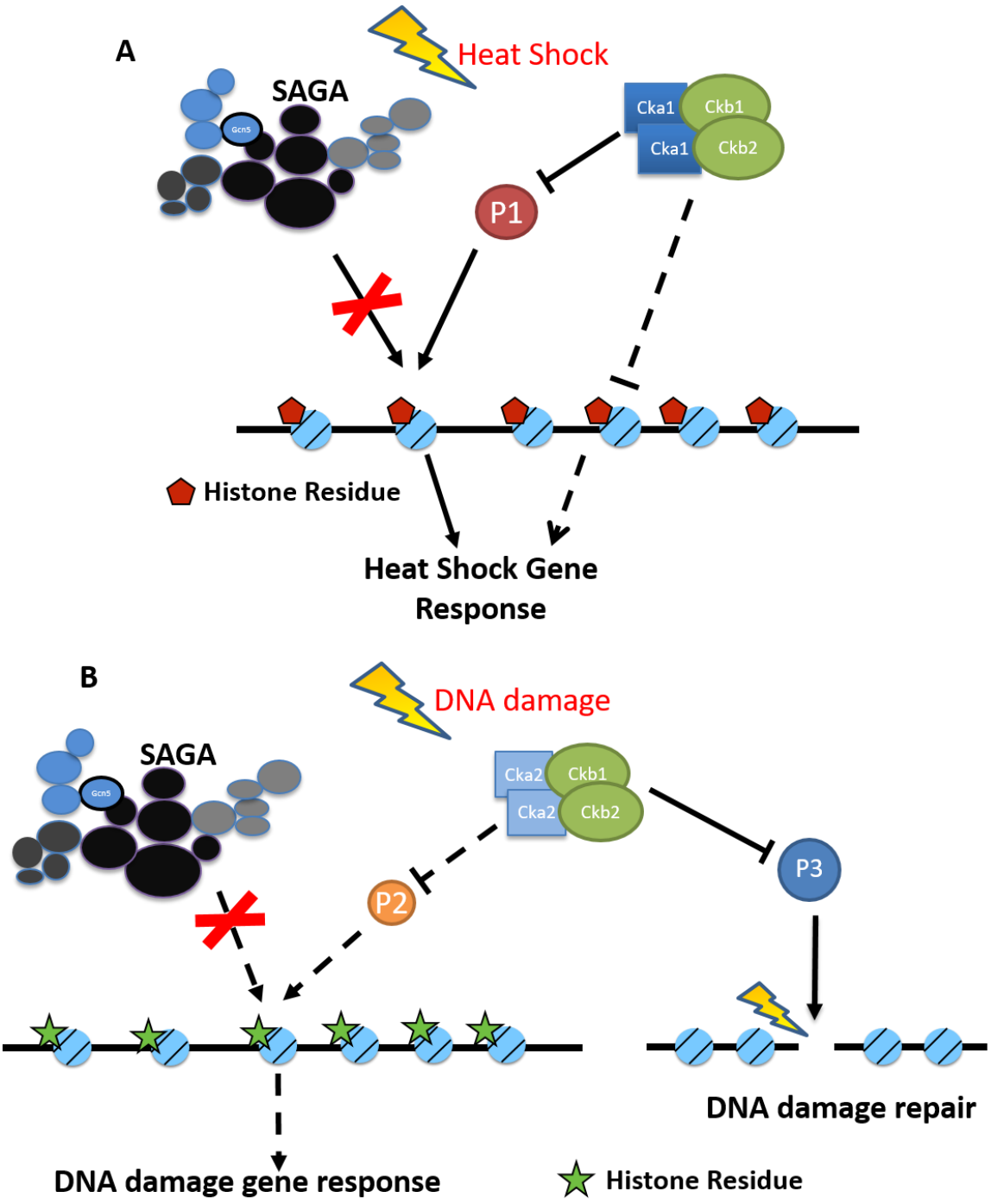

A
Heat Shock
SAGA
Gcn5
Cka1
Ckb1
Cka1
Ckb2
P1
Histone Residue
Heat Shock Gene
Response
B
DNA damage
SAGA
Gcn5
Cka2
Ckb1
Cka2
Ckb2
P2
P3
DNA damage repair
DNA damage gene response
Histone Residue

Table 5-1. *In silico* genetic interactions of *CKA1* derived from DRYGIN

CKA1		
Gene	**Function**	**Correlations (SGA score)**
CDC37	Hsp90 co-chaperone	Phosphorylation sites S14 and S17 are phosphorylated by CK2 (Vaughan CK, et al 2008) (-0.54)
STI1	Hsp90 co-chaperone	(-0.16) (0.23)*
CTI6	relieves transcriptional repression, binds Cyc8p-Tup1p	Affinity capture with both CK2 and Gcn5 (Gavin AC, et al 2006; Han BK and Emr SD 2011) (-0.13)
SIN4	RNA pol II mediator complex subunit	Interacts with *GCN5* (-0.11)
YDJ1	type I Hsp40 co-chaperone	High throughput negative interaction with *GCN5* *(*Costanzo M, et al. 2010) (0.12)*
HSC82	Cytoplasmic chaperone of the Hsp90 family	High throughput synthetic growth defect with CK2 (McClellan AJ, et al. 2007) Affinity Capture with *GCN5* (Lee KK, et al. 2011)(0.24)*
IES2	associates with INO80 chromatin remodeling complex	(0.08)
NAP1	involved in H2A/H2B transport to nucleus,	Nap1 phosphorylated by CK2 (0.08)

*indicates correlation score (not SGA score)

growth as seen in the *cka2*Δ *gcn5*Δ mutant. Unlike the *cka1*Δ *gcn5*Δ mutant, the *cka2*Δ *gcn5*Δ mutant does not rescue acetylation levels nor relieve overall transcriptional activation marker deficiencies (Figure 3-2, Figure 2-5). However, none of the tested histone residues interfered with the *cka2*Δ *gcn5*Δ rescue phenotype. Further screening of possible serine and threonine histone residues may be necessary to determine whether the Gcn5-CK2 interaction directly involves transcriptional activation through histone post-translational modifications. The rescue phenotype could also be due to the direct effects CK2 has on different DNA repair proteins or changes in stress response genes activated by Gcn5-associated complexes or other HATs (Figure 5-1). Neither possibility can be ruled out at this time.

In silico genetic interactions from DRYGIN show *CKA2* correlated with multiple genes related to DNA damage response and the cell cycle. Some genes *CKA2* is correlated with are *RTS1, PPH21, PPH22,* and *MSN1,* while it positively interacts with *RFX1, SGF11, RPD3,* and *SWI3* (Table 5-2). Previously, our lab showed that *RTS1,* the regulatory subunit of PP2A, promoted cell viability and histone gene expression in *gcn5*Δ cells (Petty E. L., et al. 2016). The DRYGIN data may suggest that the *cka2*Δ *gcn5*Δ mutant improved growth may be due to similar interactions. Another possibility is the genetic interaction between *CKA2* and *RFX1.* HU specifically inhibits ribonucleotide reductase (RNR) activity, and *RFX1* is a major transcriptional repressor of DNA-damage-regulated genes including the *RNR* genes (Elledge SJ and Davis RW 1990, Huang M et al. 1998). Phosphorylation of Rfx1 by an unknown kinase prevents the recruitment of the

Tup1-Cyc8 co-repressor complex which induces the DNA damage genes (Huang M et al. 1998). The Tup1-Cyc8 co-repressor has been identified as a repressor-activator, and its phosphorylation or dephosphorylation may be involved in signaling the repressor to function as a coactivator to recruit SAGA and Swi/Snf (Wong and Struhl 2011, Proft and Struhl 2002). I propose that the CK2 kinases may be inhibiting or activating these changes involved in the DNA damage response.

Table 5-2. *In silico* genetic interactions of *CKA2* derived from DRYGIN

CKA2		
Gene	**Function**	**Correlations (SGA score)**
EAF5	NuA4 non-essential subunit	High throughput *CKA2* and *GCN5* (Costanzo M, et al. 2010, Lin YY, et al. 2008) (-0.15)
CDC37	Hsp90 co-chaperone	Phosphorylation sites S14 and S17 are phosphorylated by CK2 (Vaughan CK, et al 2008) (-0.13)
MED1	RNA Pol II mediator complex subunit	Negative genetic interaction with *CKA2* (Sharifpoor S, et al. 2012) Affinity capture with *GCN5* (Lee KK, et al. 2011) (-0.1)
RTS1	B-type regulatory subunit of protein phosphatase 2A	Interacts with *GCN5* (Petty et al. 2016) (0.164)*
PPH22	Catalytic subunit of protein phosphatase 2A	(0.139)*
PPH21	Catalytic subunit of protein phosphatase 2A	(0.133)*
RFX1	Major transcriptional repressor of DNA-damage regulated genes	(0.114)*
MSN1	Transcriptional activator, involved in invertase regulation and glucoamylase expression	(0.102)*
SWI3	SWI/SNF chromatin remodeling complex subunit	Affinity capture with GCN5 (Lee KK, et al. 2011) (0.15)
FAR3	unknown, increases in response to DNA replicative stress	(0.15)
SGF11	SAGA subunit, required for DUB	Affinity capture *GCN5* (Lee KK, et al. 2011) (0.122)

*indicates correlation score (not SGA score)

Table 5-2. *In silico* genetic interactions of *CKA2* derived from DRYGIN (continued)

CKA2		
Gene	**Function**	**Correlations (SGA score)**
HAC1	regulates unfolded protein response, increases in response to replicative stress	(0.11)
SFL1	Transcriptional repressor/activator stress genes/ flocculation	(0.109)
RPD3	HDAC, component of both the Rpd3S and Rpd3L complexes, regulates transcription, silencing, autophagy and other processes	Affinity capture *CKA1* (Gavin AC, et al. 2006) *GCN5* synthetic lethality (Lin YY, et al. 2008) (0.09)
DOA1	WD protein required for ubiquitin-mediated protein degradation, promotes efficient NHEJ	High throughput, negative genetic (Costanzo M, et al. 2010) (0.08)

*indicates correlation score (not SGA score)

Future directions

Mutations to two distinct CK2 isoforms result in the rescue of growth for two *gcn5*Δ stresses. As discussed earlier, these phenotypes can be due to the complete loss of one CK2 isoform, an increase in the other CK2 isoform, or a combination. One genetic strategy to distinguish these possibilities could be an experiment overexpressing either catalytic subunit. For example, if the overexpression of *CKA1* causes improved growth of the *gcn5*Δ mutant in response to HU, then the increased levels of the $Cka1^{Holo}$ rather than the loss of $Cka2^{Holo}$ activity would explain the *cka2*Δ *gcn5*Δ rescue phenotype. Understanding which scenario is true would be the first step. Also the mixed Cka1-$Cka2^{Holo}$ could be responsible for some of these interactions. Initial CK2 catalytic subunit overexpression experiments using plasmids from a genomic plasmid tiling library from Jones et al. 2008 did not support this hypothesis (Figure S2). However, the tiling library plasmids contain multiple genes, so the experiment should be repeated upon subcloning the individual CK2 genes.

Furthermore, the histone tyrosine screen, 6AU experiment, and histone immunoblots experiments performed for the *cka2*Δ *gcn5*Δ mutant should also be performed on the *cka1*Δ *gcn5*Δ mutant to understand its interactions. Tyrosine residues may affect the *cka1*Δ *gcn5*Δ rescue phenotype. During the heat shock response, there is a large increase in transcriptional activity of the heat shock proteins and the *cka1*Δ *gcn5*Δ interaction may be due to improved transcriptional activity which can be tested using the 6AU experiment. The increase in transcriptional activity may be associated with increased levels of the heat shock

proteins. The expression levels or protein levels can be measured to determine whether loss of *CKA1* is affecting them. The strains for completing the proposed screens are currently being constructed.

In addition to studying the *cka1*Δ *gcn5*Δ mutant interaction in more detail, there is still much unknown about the *cka2*Δ *gcn5*Δ interaction. The histone tyrosine shuffle needs to be repeated using the *cka2*Δ histone shuffle background and the *cka2*Δ *gcn5*Δ histone H3/H4 shuffle strain may need to be reconstructed to be able to compare growth. The *cka2*Δ *gcn5*Δ histone H3/H4 shuffle strain already grows poorly before stress testing indicating that there may be background mutations causing the strain to be more sick. It would also be interesting to optimize the histone immunoblots. Phosphorylation levels of the known DNA damage marker H2AS129 were variable, and optimizing this assay would provide insight into the interactions between CK2 and Gcn5. Exploring H4S1 levels, a known CK2 substrate, and non-histone CK2 substrates levels involved in DNA damage repair would also provide more details for the CK2 and Gcn5 interactions.

In vivo analysis of the *in silico* genetic interaction data can also improve our understanding of these interactions. The *cka1*Δ *gcn5*Δ mutant improved growth in response to heat could be due to interactions with *CDC37, HSC82, HSP40, or* other heat stress response genes. Deleting any of these genes would be predicted to exacerbate the growth of the *cka1*Δ *gcn5*Δ mutant if these genes are involved in its improved growth. Similarly, deleting the DNA damage and cell cycle genes expected to interact with *CKA2* may also hinder the growth of the

*cka2*Δ *gcn5*Δ mutant in response to HU. Further strain construction and analysis of these genes and mutants may elucidate the distinct interactions between the CK2 isoforms and *GCN5.*

In addition to strain construction, looking at mRNA and protein expression levels of these targets would provide insight. Loss of either CK2 catalytic subunit may change the mRNA expression and protein levels of other stress response proteins that may be involved in this mechanism. These changes may explain the Gcn5-CK2 interaction and how loss of different isoforms of CK2 rescues the growth of *gcn5*Δ mutants when exposed to HU or elevated temperatures. Furthermore, some stress response factors such as Hsf1, Cdc37, and Rfx1 have phosphorylation sites that are or may be phosphorylated by CK2. Experimenting with these phosphorylation sites is another strategy that can identify the relevant players in this interaction.

Determining which CK2 isoforms are at play, confirming which Gcn5-associated complex, and other proteins involved in this interaction are all important to understanding the different ways the cell can cope with stresses. Both CK2 and Gcn5-associated complexes have been implicated in cancer biology. CK2 is important for cell proliferation and is a potent suppressor of apoptosis and its dysregulation has been correlated with different cancers (Trembley et al. 2009). Altered expression of *GCN5* has also been correlated with aggressive cancers and neurological diseases (Koutelou 2010). There are currently multiple drugs in clinical development that target CK2 (Cozza and Pinna 2015). However, designing drugs to target CK2 in cancer cells may not always

prevent cellular growth. Since CK2 is a major regulator of multiple cellular functions, in combination with other mutations the unwanted effect of improving the growth of the targeted cancer cells may occur.

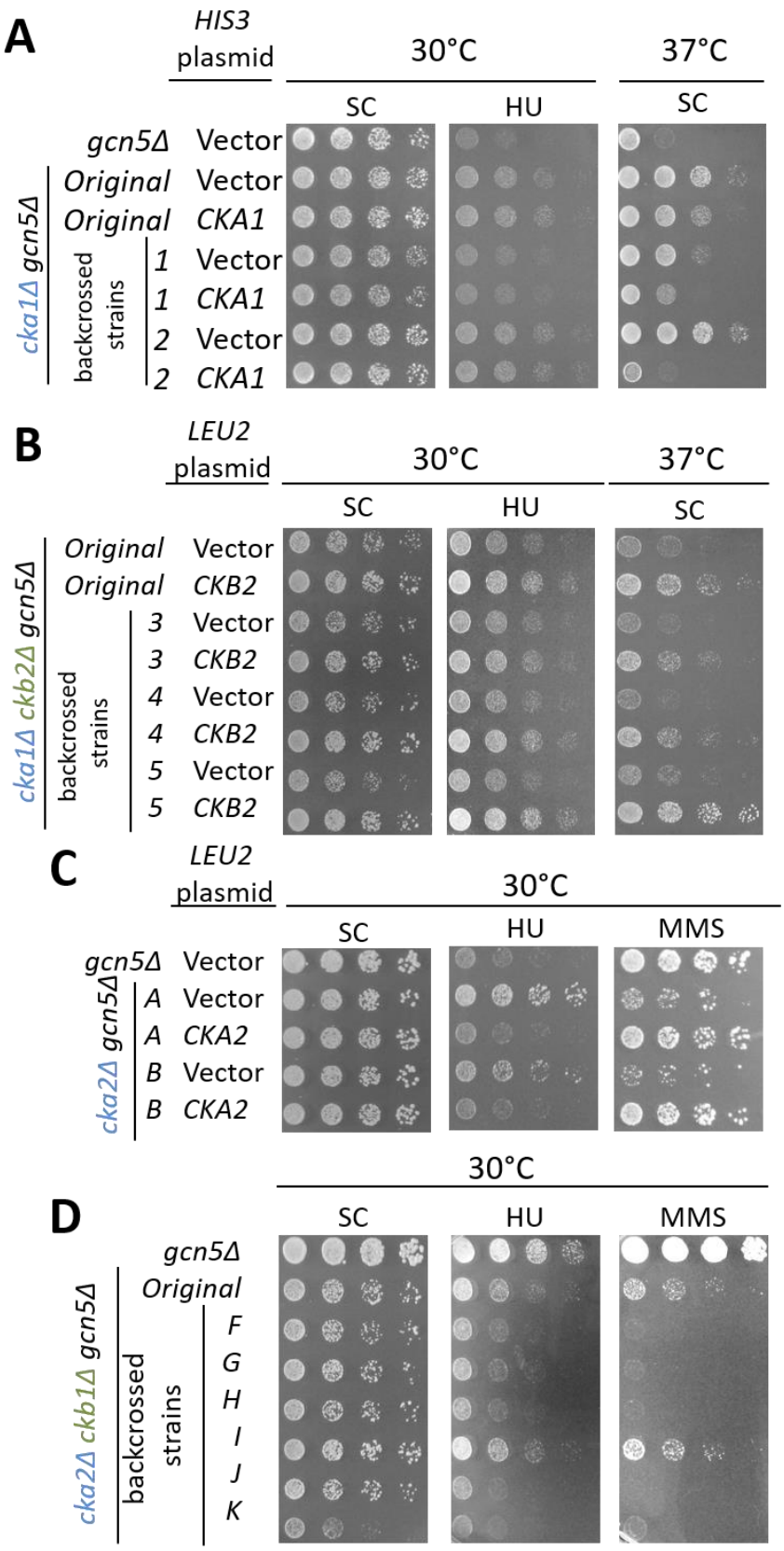
The experimental results presented in this thesis provide a foundation in explaining the complexity of CK2 and how its multiple isoforms specifically contribute to different cellular responses. These results are important for understanding the multitude of processes occurring within the cell.

Appendix 1. Supplemental Figures

The CK2 mutants were difficult to construct. Some of the double and triple mutants had variable growth. Mutants with the same genetic background rescued growth or exacerbated growth for the same stress. To test these strains, additional strains of the same background were constructed by backcrossing strains with either phenotype to WT and reconstructing from the original single mutants. These strains were then transformed with a covering plasmid. The *cka1*Δ *gcn5*Δ and *cka1*Δ *ckb2*Δ *gcn5*Δ mutants were transformed with a *CKA1* and *CKB2* wild-type plasmid, respectively (Figure S1). This experiment confirmed that loss of *CKA1* does rescue and improve the growth of *gcn5*Δ mutants at high temperatures.

The *cka1*Δ *gcn5*Δ*, cka2*Δ *gcn5*Δ*, cka1*Δ *ckb2*Δ *gcn5*Δ*,* and *cka2*Δ *ckb1*Δ *gcn5*Δ mutants were also backcrossed and exposed to DNA damaging reagents. The *cka1*Δ *gcn5*Δ mutant strains that rescued growth in response to temperature did not rescue growth in response to DNA damage (Figures S1). Another mutation may be affecting the strain's response to HU, which may also affect the strains ability to rescue growth in response to heat. The *cka2*Δ *gcn5*Δ mutant rescue in growth was confirmed when a *CKA2* wild-type plasmid suppressed the strain's growth rescue. The confirmation that the additional *ckb1*Δ mutation in the *cka2*Δ *ckb1*Δ *gcn5*Δ stopped the *cka2*Δ *gcn5*Δ mutant's rescue in growth was also supported by multiple strains (Figure S1). Finally, the *cka1*Δ *ckb2*Δ *gcn5*Δ mutant originally showed rescue in growth, but the rescue was due to a suppressor mutation. Another mutation in the background caused the strain to

Figure S1. Confirmation of the suppression and exacerbation of *gcn5*Δ phenotypes. Strains were transformed with an empty vector or the indicated wild-type gene, and then grown to stationary phase in selective media to retain the plasmid. They were plated on synthetic complete plates in 5 fold dilutions. **A)** *gcn5*Δ (LPY13320), original (LPY22077), 1 (LPY22313), and 2 (LPY22314) contained a wild-type *CKA1 HIS3*-CEN plasmid (pLP3392). Previously LPY21667 was tested and showed no rescue in temperature sensitivity. However, after constructing and recovering more *cka1*Δ *gcn5*Δ strains the suppression phenotype was apparent. The original strain that showed the phenotype (LPY22077) was backcrossed to the WT strain (LPY5) to recover LPY22313 and LPY22314. The rescue was still apparent, and covering the *cka1*Δ *gcn5*Δ strains with the WT *CKA1* plasmid stopped the rescue in growth, confirming that the increase in growth is due to the loss of *CKA1* and not any background mutations. Covering with the WT *CKA1* plasmid did not stop the improved growth in these strains when exposed to HU. Another mutation may be driving the improved growth seen in these strains on HU plates. **B)** The original *cka1*Δ *ckb2*Δ *gcn5*Δ strain (LPY21810) was backcrossed with the WT strain (LPY79) to recover strains 3 (LPY22315) and 4. Another *cka1*Δ *ckb2*Δ *gcn5*Δ strain (LPY22121) that showed poor growth was backcrossed with WT (LPY5) to recover strain 5. These strains were transformed with a WT *CKB2 LEU2*-CEN plasmid (pLP3391). All strains with the plasmid grew better at high temperatures and when exposed to HU. **C)** *gcn5*Δ (LPY13320), A (LPY21671), and B (LPY22128) were transformed with a WT *CKA2 LEU2*-CEN (pLP 3196) plasmid. Rescue of growth to *gcn5*Δ HU sensitivity through loss of *CKA2* was stopped when covered with the wild type *CKA2* plasmid. **D)** The original *cka2*Δ *ckb1*Δ *gcn5*Δ strain (LPY21950) was backcrossed with WT (LPY79) and F, G, H, I, J, and K were recovered. These strains support that the triple mutant exacerbates the *gcn5*Δ DNA damage sensitivity.


A
HIS3 plasmid
30°C
37°C
SC
HU
SC
cka1Δ gcn5Δ
gcn5Δ Vector
Original Vector
Original CKA1
backcrossed strains
1 Vector
1 CKA1
2 Vector
2 CKA1
B
LEU2 plasmid
30°C
37°C
SC
HU
SC
cka1Δ ckb2Δ gcn5Δ
Original Vector
Original CKB2
backcrossed strains
3 Vector
3 CKB2
4 Vector
4 CKB2
5 Vector
5 CKB2
C
LEU2 plasmid
30°C
SC
HU
MMS
cka2Δ gcn5Δ
gcn5Δ Vector
A Vector
A CKA2
B Vector
B CKA2
D
30°C
SC
HU
MMS
cka2Δ ckb1Δ gcn5Δ
gcn5Δ
Original
backcrossed strains
F
G
H
I
J
K

grow healthier. For future studies, during strain construction it may be necessary to cover the strains with a WT copy of the deleted gene or genes to prevent any unwanted mutations. Both CK2 and Gcn5 are important for multiple cellular processes including sporulation which may affect strain construction.

Another question was whether the loss of a specific CK2 isoform or the increased levels of the other rescued growth in *gcn5Δ* mutant phenotypes. To identify which was the case *gcn5Δ* mutants were transformed with *CKA1* or *CKA2* overexpression plasmids from a genomic plasmid tiling library from Jones et al. 2008. These overexpression plasmids contained multiple genes in addition to *CKA1* or *CKA2*. The overexpression of those genes may have also affected the observe phenotype. No growth was rescued in this experiment (Figure S2). Cloning only the catalytic subunits into an overexpression plasmid would provide more conclusive evidence of the Gcn5-CK2 interaction.

Evaluating the protein levels of the different mutants was relatively difficult. Histone modifications are dynamic and measuring the levels of different histone marks made this apparent. The protein levels were inconsistent when measuring certain histone modifications. However, the majority of the *cka2*Δ *gcn5*Δ mutant samples had lower levels of the histone marks covered here. The histone modifications linked to H2AY58: H3K4me3, H3K79me3, and H2BK123 monoubiquitination were probed (Basnet et al. 2014). Another histone mark H2AS129ph which is linked to the DNA damage response and replication coupled DNA repair was also tested (Redon C et al. 2003). The preliminary

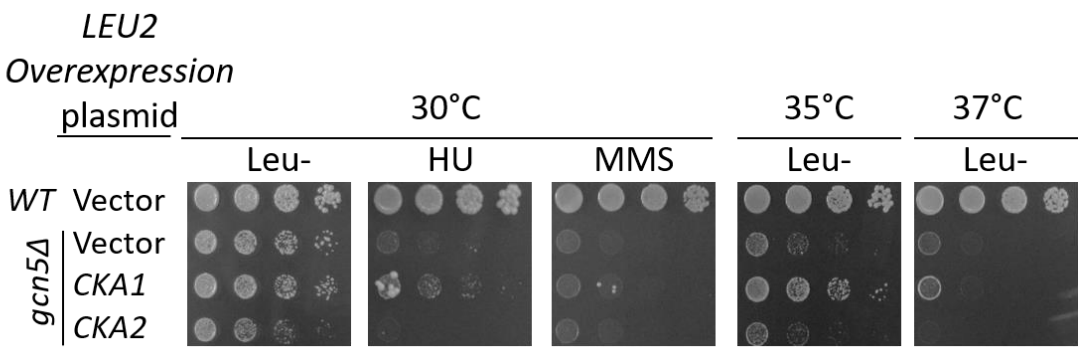


Figure S2. CK2 catalytic subunits overexpression in *gcn5Δ* mutants. Strains were grown to saturation then plated on Leu-, 0.1M HU Leu-, 0.01% MMS Leu-, and pre-warmed plates in 5 fold dilutions WT (LPY5) was transformed with an empty LEU2 overexpression plasmid (pLP 135), and *gcn5Δ* (LPY 13320) was transformed with an empty vector (pLP 135), *CKA1* overexpression, and *CKA2* overexpression plasmid. The *CKA1* overexpression plasmid also contained *NOT3* (truncated), *PRM2, CST6, CAP2,* and *BCY1*(truncated). The *CKA2* overexpression plasmid also contained *YOR059C* (truncated), *SLD7, YOR062C, RPL3, YNG1, CYT1,* and *MSA1* (truncated).

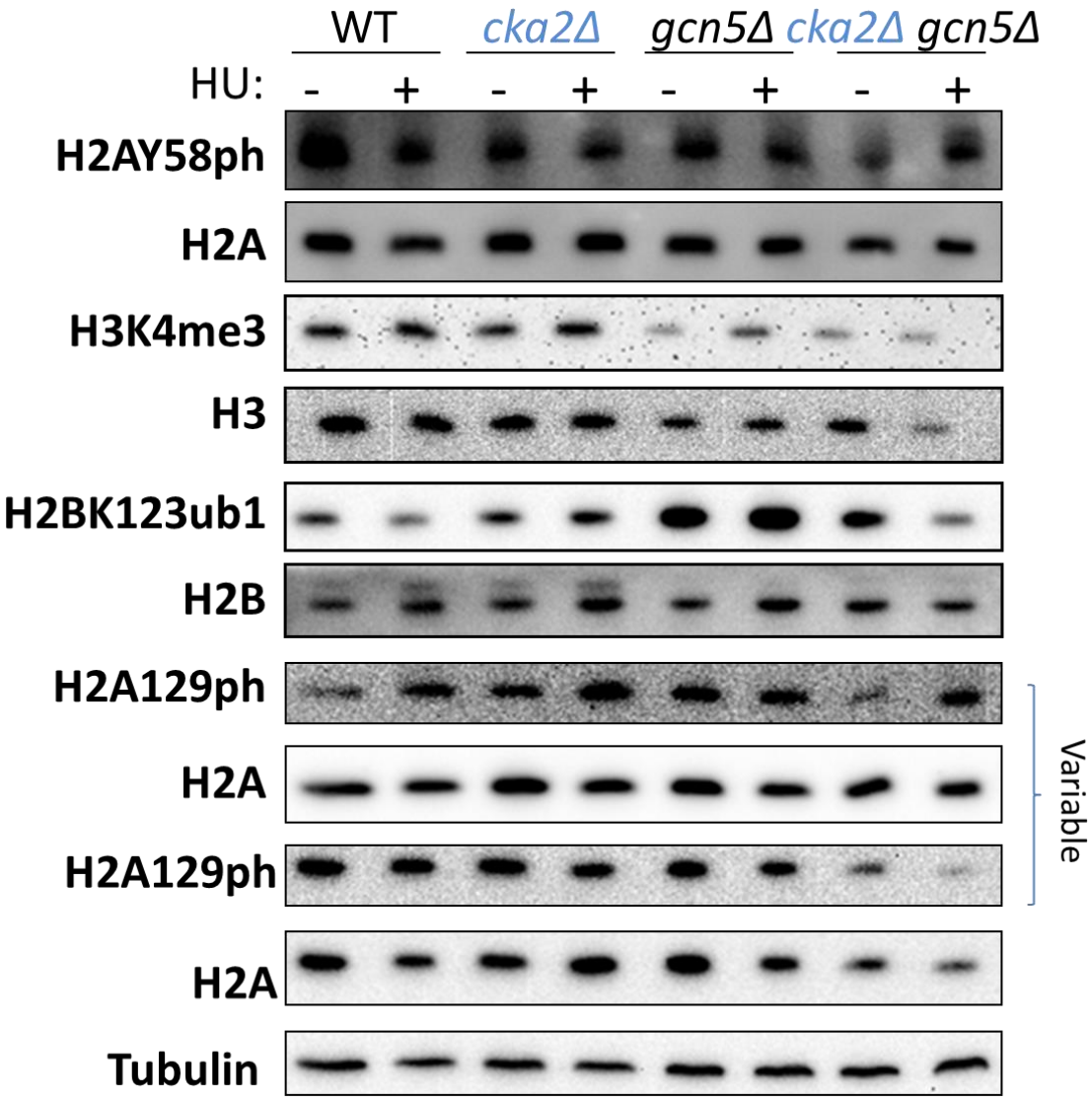
analysis shows changes in these marks, but the results do not reflect the expected levels for improvement in cellular growth and stress response. Levels of phosphorylation on histone H2A serine 129 (H2A129ph) were evaluated. H2AS129ph is known to be involved in multiple DNA damage repair pathways, and is important for recruitment of chromatin modifiers for DNA repair (Redon C et al. 2003). Surprisingly H2AS129 was constitutively phosphorylated before and after exposure to HU in WT, *cka2*Δ*,* and *gcn5*Δ strains (Figure S3). Only the *cka2*Δ *gcn5*Δ mutant strain seemed to have a response to HU exposure, showing an increase in H2AS129 phosphorylation after HU treatment or lower levels overall.

Furthermore, H2AY58 phosphorylation levels were measured. H2AY58 is phosphorylated by CK2, however, the specific CK2 isoform that targets H2AY58 is unknown. During growth under normal conditions, all *cka2*Δ mutants have lower levels of phosphorylated H2Y58 compared to WT. This suggests that the Cka2 holoenzyme or free catalytic Cka2 are important for normal levels of H2AY58ph (Figure 3-2). Curiously, *gcn5*Δ mutant strains also show lower levels of H2AY58 phosphorylation and when combined with the loss of *CKA2* during normal conditions there is significantly less phosphorylated H2AY58. Under HU treatment levels of phosphorylated H2AY58 decreased in WT, *cka2*Δ*,* and slightly in *gcn5*Δ mutant strains. In contrast, the *cka2*Δ *gcn5*Δ mutant had increased levels of H2AY58ph after HU treatment.

Previously, phosphorylation at H2AY58 was expected to affect H3K4me3, H3K79me3 and H2BK123ub1 (monoubiquitination) levels (Basnet et al. 2014).

The expectation was that loss of *CKA2* may result in the similar changes in histone modifications. However, the protein modification levels of H3K4me3 were not significantly different between WT and the *cka2*Δ mutant. There is a decrease in H3 levels in response to HU treatment which may be the reason for lower levels of H3K4me3 in the *cka2*Δ *gcn5*Δ double mutant. Similarly, H2BK123ub1 levels upon HU treatment go down after HU treatment, while *gcn5*Δ mutant monoubiquitination levels increase or stay relatively saturated. Overall the pattern of these transcriptional marks is not very clear, further experiments must be performed. H2AY58 phosphorylation, H3K79me3, and H2BK123ub1 have each only been tested on one set of whole cell lysates due to difficulties with the primary antibodies. More samples needed to be tested to support a stronger conclusion.

Figure S3. Variable global histone modifications upon HU treatment. The strains WT (LPY5), *cka2*Δ (LPY21665), *gcn5*Δ (LPY13320), *cka2*Δ *gcn5*Δ (LPY21671) were grown overnight and then diluted back to 0.1 OD. After growing to 0.4-0.5 ODs SC (vehicle for HU) or HU was added to a concentration of 0.1M. The cells were collected after 2 hours of HU treatment then lysed and a protein immunoblot was performed using the protocols in the “Materials and Methods.” The primary antibodies used were all Rabbit, and the secondary antibodies were all anti-Rabbit. The blocking buffer used was 2% milk in TBS-Tween (TBS-T). The primary antibodies used: H3K4me3 (2% milk TBS-T); H3K79me3 (1:2000 dilution, 2% milk); H3 (1:2500 dilution, 2% milk); H2AS129 phosphorylation (1:5000 dilution, 5% BSA); H2AY58 phosphorylation (1:1000, 5% BSA); H2A (1:5000 dilution, 5% BSA); H2BK123ub1 (1:2000 dilution, 5% BSA); H2B (1:1000 dilution, BSA); tubulin (1:5000 dilution, 3.5% milk). Images were captured using the protein simple FluorChem E system, using the 40 seconds exposure. Note: The H2AY58 phosphorylation antibody only worked once. The primary antibody was incubated for 1 hour at Room temperature then after failing to image, it was incubated overnight at 4°C and probed the next morning.


WT
cka2Δ
gcn5Δ cka2Δ gcn5Δ
HU: - + - + - + - +
H2AY58ph
H2A
H3K4me3
H3
H2BK123ub1
H2B
H2A129ph
H2A
H2A129ph
H2A
Tubulin
Variable

Appendix 2. Materials and methods

General yeast protocol and growth assays. Protocol for the handling of yeast and growth conditions can be found in (Sherman 1991). Yeast cells were grown at 30°C unless otherwise stated. Cell cultures for all dilution assays were normalized to 1.0 OD_{600}/mL and diluted 5 fold unless otherwise stated (histone shuffle strains). Pictures of growth assays were taken after 2-5 days; most commonly at 3 days. Strains used are listed in Table__

Media preparation. Yeast media preparation can be found in (Sherman 1991). Hydroxyurea (HU) plates for DNA damage analysis were prepared by adding filter sterilized HU (aqueous) to SC after autoclaving to a final concentration of 0.1M. Methyl methanesulfonate (MMS) plates for DNA damage analysis was prepared by directly adding MMS to SC after autoclaving to a final concentration of 0.0075%-0.03%. (Note: our older stock of MMS required higher percentage plates). 6-azauracil (6AU) plates for transcriptional elongation analysis were prepared by adding filter sterilized 6AU (aqueous) to SC after autoclaving to a final concentration of 100 µg/mL.

Histone plasmid recovery. This research extensively utilized the SHIMA plasmid library of histone mutants which can be found in Table__ (Nakanishi et al. 2008). Isolating the plasmid from yeast was performed by growing 3 mL of cultures in dropout medium overnight, pelleting, resuspending in 100 µL of STET buffer (8% Sucrose, 50 mM Tris pH8.0, 50mM EDTA, 5% Triton X-100), vortexing with glass beads for 5 minutes. Then 100 µL more of STET was added, vortexed, boiled for 3 minutes. After cooling on ice, samples were centrifuged for

10 minutes at max speed at 4°C (all subsequent centrifugation steps are at 4°C max speed), and supernatants were transferred to fresh tubes with 54 µL of 7M ammonium acetate, and incubated at -20°C for 1 hour. After 10 minutes of centrifugation 100 µL of supernatant was transferred to 200 µL of ice cold ethanol, then centrifuged for 15 minutes. Supernatant was discarded and pelleted, washed in 100 µL 70% ethanol. After 10 minute centrifugation, the supernatant was aspirated. After 10-20 minutes drying, the pellet was resuspended 20 µL mQ water. 10 µL was used to transform competent bacteria for subsequent preparation of plasmid stock DNA.

Bacterial transformation. 100 µL of calcium-competent *E. coli* DH5α cells were added to 1-10 µL DNA and incubated 30 minutes on ice. Cells were heat shocked at 42°C for 90 seconds then recovered on ice for 1-2 minutes. 1 mL of LB medium was added and the cells were incubated for 1-2 hours at 37°C before being plated on selective medium.

Simple transformation of yeast. Yeast were scraped from a plate with a flat toothpick into 1 µg of miniprep plasmid DNA and 10 µL of 10 mg/mL ssDNA. Then 0.5 mL PLATE (81 mL 50% PEG-4000, 1 mL 1M Tris-HCl pH 7.5, 10 mL 1M lithium acetate, 0.2 mL 0.5M EDTA, 9 mL mQ) solution was added followed by vortexing. Transformation mixtures were incubated overnight at room temperature then 50 µL was plated on selective media. The transformants used in this research are listed in Table __. For the shuffling of histone genes to assay individual histone mutations, transformants were struck on 5-FOA to select for loss of the *URA3* marked plasmid bearing the wild type histone genes after being

transformed with the plasmid bearing the mutant histone gene of interest. A single colony was taken from the 5-FOA plate and the same colony was struck on both ura- medium and the appropriate selectable dropout plate to ensure that the plasmid bearing the histone mutation was retained and that the wild type *URA3* plasmid was cured.

Yeast gene deletions. This research utilized the *Saccharomyces* Deletion Consortium (Winzeler et al. 1999) strain collection of *S. cerevisiae* gene deletions. For deletions made in the W303 background, the gene-specific *kan*MX construct was amplified by PCR from the stock strain and then transformed into LPY5. Gene deletions were confirmed by PCR molecular genotyping. Oligos are listed in Table __

Genomic DNA preparation. This method is adapted from (Hoffman and Winston 1987). Cells were grown overnight in 3 mL of YPAD. 1.5 mL were spun down in a microfuge tube and the pellet resuspended in 0.5 mL of mQ water and vortexed. Cells were spun again and the pellet was resuspended in 0.2 mL of breaking buffer (10mM Tris (pH 8.0), 1 mM EDTA, 100 mM NaCl, 2% Triton-X 100, 1% SDS). Glass beads were added to the meniscus and then 0.2 mL of PCI (24phenol: 23 chloroform: 1 isoamyl alcohol) were added. Cells were vortexed at maximum speed for 3 minutes. 0.3mL of TE were added and the tubes were vortexed for 10 seconds. The tubes were spun at top speed in a microcentrifuge for 5 minutes. The top layer was the then transferred to a new tube and 1 mL of ice cold ethanol was added and the tube inverted to mix. The tube was spun for 2 minutes at maximum speed, the supernatant removed and the pellet

resuspended in 0.2 mL of TE. If the genomic DNA (gDNA) was being used for genotyping, this completes the protocol. For using the gDNA for transformations, 5 µL of 10 mg/mL RNAse A were added followed by incubation at 37°C for 15 minutes. 0.2 mL of PCI were added and the tube was spun at maximum for 2 minutes. The top, aqueous layer was again transferred to a new tube and the DNA was precipitated by adding 20 µL 3M NaOAc and 0.5 mL EtOH and incubating at -20°C for 1-2 hours or overnight. The DNA was pelleted at maximum speed in the cold for 10 minutes. The pellet was washed with 0.3 mL of 70% EtOH then spun for 2 minutes at maximum speed. The tube was inverted and the pellet air dried for 5-10 minutes before resuspending in 50 µL TE. Genomic DNA was diluted 1:20 in water before use in PCR.

Whole Cell Lysate protocol. Cells were grown overnight in 3 mL of SC media. The cells were then diluted back to specific ODs (0.1 or 0.15) in 10 mL flasks. After reaching a specific OD or time point the cells were collected. The final ODs were recorded followed by pelleting and resuspending the cells in 1 mL cold PBS. After centrifuging at 4°C at max speed for 5 minutes the supernatant was removed. The cells were resuspended in a PBS + protease inhibitor cocktail(1 mL PBS, 0.5 µL pepstatin, 0.5 µL leupeptin, 1 µL TP, 10 µL B, 10 µL PMSF, 5µL 200mM Na_2VO_4, 10 µL 1M NaF), and the amount of inhibitor cocktail added to each sample depended on their final OD multiplied by 250 µL. Then, glass beads were added to the meniscus and vortexed for 7 minutes switching between 30 seconds of vortexing, and 30 seconds on ice. Next, the amount of 5X SLB added to each sample was based on their final OD multiplied by 62.5 µL.

Then the lysates were boiled for 5 minutes, followed by prepping the tubes for transfer. The tubes were flipped upside down, and using a needle (0.8mm x 40mm) a small hole was made. These tubes were placed inside new tubes and then three quick-spin downs were performed in a centrifuge to transfer the lysates, followed by aliquoting into clean tubes (1OD = 32 µL). Lysates were stored at -80°C.

Protein Immunoblot protocol. Whole cell lysates were boiled for 10 minutes before adding 6 µL (3/16 OD) to 15% SDS-PAGE gels. Then the gels were ran at 70V until the dye ran to the bottom which took about 3.5 hours. Afterwards, the gel was transferred to a nitrocellulose membrane with cold transfer buffer (0.303% Tris, 1.44% Glycine, 15% MeOH) at 4°C at 100V for 1.5-2 hours. The membrane was then stained with 0.5% Ponceau S for less than a minute, followed by a 5 minute wash in TBS-Tween (TBS-T: 945 mL mQ water, 50 mL 20X TBS, 5 mL 10% Tween). After, the blot was blocked in 2% milk or 5% BSA for 1 hour or overnight. Then it was incubated in primary antibody for 1 hour at RT [The primary antibodies used were all Rabbit, and the secondary antibodies were all anti-Rabbit. The blocking buffer used was 2% milk in TBS-Tween (TBS-T) or 5% BSA depending on the primary antibody solution. The primary antibodies used: H3K9,K14acetylation (1:7500 dilution, 2% milk TBS-T); H3K4me3 (2% milk TBS-T); H3K79me3 (1:2000 dilution, 2% milk TBS-T); H3 (1:2500 dilution, 2% milk TBS-T); tubulin (1:5000 dilution, 3.5% milk TBS-T)], followed by three 10 minute washes in TBS-T. The blot was then incubated in

secondary antibody for 1 hour, followed by three washes in TBS-T. Finally, the blot was imaged using simple protein.

Table 7-1. Strains used in this research

Strain	Genotype	Source
LPY5	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL*	Pillus Lab
LPY2533	*MAT*a *ade2-1ochre can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 lysΔ::URA3-(4+2)xUAS-G*	Pillus Lab
LPY10182	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL gcn5Δ::KanMX*	Pillus Lab
LPY12231	*MAT*α *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL hht1-hhf1Δ::kanMX hht2-hhf2Δ::kanMX hta2-htb2Δ::HPH* + pLP2212	Pillus Lab
LPY13320	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL gcn5Δ::NatMX*	Pillus Lab
LPY14461	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL hht1-hhf1Δ::kanMX hta1-htb1Δ::natMX hta2-htb2Δ::HPH* + pLP2212	Pillus Lab
LPY16291	*MAT*α *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL hht1-hhf1Δ:kanMX hht2-hhf2Δ::kanMX hta2-htb2Δ::HPH gcn5Δ::kanMX* + pLP2212	Pillus Lab
LPY16434	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL hht1-hhf1Δ::kanMX hta1-htb1Δ::natMX hta2-htb2Δ::HPH gcn5Δ::kanMX* + pLP2212	Pillus Lab
LPY20647	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL hht1-hhf1Δ::kanMX hta1-htb1Δ::natMX hta2-htb2Δ::HPH barΔ::*+ pLP2212	Pillus Lab
LPY20679	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL hht1-hhf1Δ::kanMX hta1-htb1Δ::natMX hta2-htb2Δ::HPH barΔ:: gcn5Δ::kanMX* + pLP2212	Pillus Lab
LPY21663	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka1Δ::KanMX*	Pillus Lab
LPY21665	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX*	Pillus Lab
LPY21671	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX gcn5Δ::NatMX*	Pillus Lab
LPY21757	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL ckb2Δ::KanMX*	This study
LPY21777	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL ckb2Δ::KanMX gcn5Δ::NatMX*	This study

Table 7-1 Strains used in this research (continued)

LPY21779	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka1Δ::KanMX ckb2Δ::KanMX*	This study
LPY21784	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL ckb1Δ::KanMX*	This study
LPY21786	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX ckb2Δ::KanMX*	This study
LPY21823	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL ckb1Δ::KanMX gcn5Δ::NatMX*	This study
LPY21825	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL ckb1Δ::KanMX ckb2Δ::KanMX*	This study
LPY21827	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka1Δ::KanMX ckb1Δ::KanMX*	This study
LPY21881	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL ckb1Δ::KanMX ckb2Δ::KanMX gcn5Δ::NatMX*	This study
LPY21883	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka1Δ::KanMX ckb1Δ::KanMX ckb2Δ::KanMX*	This study
LPY21889	*MAT*α *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX ckb1Δ::KanMX*	This study
LPY21891	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka1Δ::KanMX ckb1Δ::KanMX ckb2Δ::KanMX gcn5Δ::NatMX*	This study
LPY21951	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX ckb1Δ::KanMX ckb2Δ::KanMX gcn5Δ::NatMX*	This study
LPY21952	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX ckb1Δ::KanMX ckb2Δ::KanMX*	This study
LPY21956	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX ckb2Δ::KanMX gcn5Δ::NatMX*	This study
LPY21961	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL hht1-hhf1Δ::kanMX hta1-htb1Δ::natMX hta2-htb2Δ::HPH cka2Δ::KanMX gcn5Δ::kanMX* + pLP2212	This study
LPY22025	*MAT*α *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL hht1-hhf1Δ:kanMX hht2-hhf2Δ::kanMX hta2-htb2Δ::HPH cka2Δ::KanMX gcn5Δ::kanMX* + pLP2212	This study
LPY22077	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka1Δ::KanMX gcn5Δ::NatMX*	This study
LPY22199*	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX::URA3*	This study

Table 7-1 Strains used in this research (continued)

LPY22200*	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX::URA3*	This study
LPY22246	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX::URA3 gcn5Δ::NatMX*	This study
LPY22247	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX::URA3 gcn5Δ::NatMX*	This study
LPY22313	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka1Δ::KanMX gcn5Δ::NatMX*	This study
LPY22314	*MAT*α *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka1Δ::KanMX gcn5Δ::NatMX*	This study
LPY22315	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka1Δ::KanMX ckb2Δ::KanMX gcn5Δ::NatMX*	This study
LPY22317	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::KanMX ckb1Δ::KanMX gcn5Δ::NatMX*	This study
LPY22246	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ:: URA3 gcn5Δ::NatMX*	This study
LPY22248	*MAT*α *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL cka2Δ::URA3 gcn5Δ::NatMX*	This study
LPY22296**	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL gcn5Δ::URA3*	This study
LPY22297**	*MAT*a *ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 GAL gcn5Δ::URA3*	This study

*21665 *cka2Δ::KanMX* transformed using *kanMX::URA3* marker swap plasmid M4758 (Voth et al 2003), digested with restriction enzyme *Not I* to direct the integration.

***10182 gcn5Δ::KanMX* same as above

Table 7-2 Plasmids used in this research

pLP Number	Gene	Marker/Copy Number	Source
60	Vector	*HIS3*/CEN	Pillus Lab
62	Vector	*LEU2*/CEN	Pillus Lab
1490	*HHT2 HHF2*	*TRP1*/CEN	Roth Dent Lab
2212	*HTA1 HTB1 HHT2 HHF2*	*URA3*/CEN	Mitch Smith
2492	*HTA1 HTB1-FLAG*	*HIS3*/CEN	Shima Library
2881	*hta1-Y40A HTB1*	*HIS3*/CEN	Shima Library
2882	*hta1-Y51A HTB1*	*HIS3*/CEN	Shima Library
2887	*HTA1 htb1-Y124A-FLAG*	*HIS3*/CEN	Shima Library
3196	*CKA2*	*LEU2*/CEN	Pillus Lab
3202	*hta1-Y58F HTB1-FLAG*	*HIS3*/CEN	Pillus Lab
3250	*HTA1 htb1-Y40F-FLAG*	*HIS3*/CEN	Pillus Lab
3251	*HTA1 htb1-Y43F-FLAG*	*HIS3*/CEN	Pillus Lab
3252	*HTA1 htb1-Y45F-FLAG*	*HIS3*/CEN	Pillus Lab
3369	*HTA1 htb1-Y40A-FLAG*	*HIS3*/CEN	Shima Library
3370	*HTA1 htb1-Y43A-FLAG*	*HIS3*/CEN	Shima Library
3371	*HTA1 htb1-Y86A-FLAG*	*HIS3*/CEN	Shima Library
3372	*hht2-Y41A HHF2*	*TRP1*/CEN	Shima Library
3373	*hht2-Y99A HHF2*	*TRP1*/CEN	Shima Library
3374	*HHT2 hhf2-Y51A*	*TRP1*/CEN	Shima Library
3375	*HTA1 htb1-Y45A-FLAG*	*HIS3*/CEN	Shima Library
3376	*HHT2 hhf2-Y88A*	*TRP1*/CEN	Shima Library
3391	*CKB2*	*LEU2*/CEN	This study
3392	*CKA1*	*HIS3*/CEN	This study
3406	*HHT2 hhf2-Y98A*	*TRP1*/CEN	Shima Library

Table 7-3 Primers used in this research

OLP Number	Gene	Use	Sequence
1588	*HHT2*	Sequencing	CGA ATA ACA ACA GCC CAG GCG CG
2163	*CKB1*	Knockout	TGG TAC GTG CAA AAC ACG AT
2164	*CKB1*	Knockout	GCT CCA GGA AAA CAA TTG GA
2165	*CKB2*	Knockout	TGG TCA TTC CAG ATA CGT GGA
2166	*CKB2*	Knockout	TTC GCC TAG GAT TTC ATT TCG
2221	*CKA1*	Cloning	ACA TCT CCT TCT TCT CCC GAC A
2222	*CKA1*	Cloning	ATT TAG CGG CGA ACA CAC AG
2223	*CKB1*	Cloning	TGT GAA GAT GGG CCT GAA AGG
2224	*CKB1*	Cloning	CGC AAT ATC GAT TTG ACG CAA
2225	*CKB2*	Cloning	TTG TTG TCT CCA CGC CAT CA
2226	*CKB2*	Cloning	AGT GTC GCC TGT GAA GAT GCA
2244	*CKA1*	Sequencing	ATG AAA TGC AGG GTA TGG TC
2245	*CKB2*	Sequencing	GTG GAA CAA GAT GAT GTC C
2246	*CKA1*	Mutagenesis	CTA ACG AAA AAG TGC CTC CAA CGA CTT TGC CAT TTC AAA
2247	*CKA1*	Mutagenesis	TTT GAA ATG GCA AAG TCG TTG GAG GCA CTT TTT CGT TAG

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