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Synthetic Dosage Analysis of the Yeast Nu4A Complex

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Synthetic Dosage Lethal Analysis of the Yeast NuA4 Complex

By

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Submitted in partial fulfillment

of the requirements for the degree of

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ABSTRACT

The NuA4 complex is the only essential lysine acetyltransferase in *Saccharomyces cerevisiae* and is evolutionarily conserved with the human Tip60 complex, which has been implicated in a wide variety of pathologies. NuA4 plays roles in processes that range from DNA repair to gluconeogenesis. To further investigate the cellular processes impacted by and to identify new targets of NuA4, a genome-wide synthetic dosage lethal screen was performed. This analysis produced an extensive network of genetic interactions, and identified a novel link between NuA4 and septin assembly and cytokinesis. I determined that NuA4 mutants have defects in septin structure, which lead to activation of the morphogenesis checkpoint. Further, I determined that NuA4 is working directly in the septin assembly pathway, potentially by regulating the protein levels of the septin Shs1. My study has revealed a new and important role for NuA4 in regulating the cell cycle and chromosome stability.

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List of Abbreviations:

DNA	Deoxyribonucleic Acid
GAP	GTPase Activating Protein
GDI	Guanine Nucleotide Dissociation Inhibitor
GEF	Guanine Nucleotide Exchange Factor
GTP	Guanosine Triphosphate
HAT	Histone Acetyltransferase
HU	Hydroxyurea
KAT	Lysine Acetyltransferase
OEA	Overexpression Array
ORF	Open Reading Frame
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
SD	Synthetic Defined
SDL	Synthetic Dosage Lethal
SDS	Synthetic Dosage Sick
SGA	Synthetic Genetic Array
SL	Synthetic Lethal
SS	Synthetic Sickness
YPD	Yeast Peptone Dextrose

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Chapter 1: INTRODUCTION

Lysine acetyltransferase (KAT) enzyme complexes, traditionally referred to as histone acetyltransferases or HATs, have a well-established role in transcriptional regulation in eukaryotic cells (Thomas and Voss, 2007). The conventional view that KATs only acetylate histone tails has been abolished in light of the fact that acetylation of non-histone target is also critical for mediating many cellular processes (Glozak et al., 2005). Therefore, finding non-histone acetylation targets and exploring the biological reasons behind their modifications is critical to understanding the array of processes important for normal cellular function.

The human Tip60 lysine acetyltransferase complex is important for DNA damage repair and the maintenance of apoptotic competence (Ikura et al., 2000). It has also been implicated in cancer and neurodegeneration, though the exact processes by which this occurs are still undiscovered (Sapountzi et al., 2006). The Tip60 complex is highly conserved structurally and functionally with the budding yeast *Saccharomyces cerevisiae* NuA4 complex, or nucleosome acetyltransferase of histone H4 complex (Auger et al., 2008). Similar to human Tip60, NuA4 has been implicated in numerous biological processes, including DNA repair (Bird et al., 2002), genome stability (Krogan et al., 2004) and cell cycle progression (Clarke et al., 1999). NuA4 is likely regulating many of these key processes through the acetylation of non-histone targets. Therefore, elucidating novel cellular processes regulated by NuA4 and identifying new targets of NuA4 will not only increase our understanding of the only essential KAT in yeast but may well have implications on the study of human diseases such as cancer, whose development and progression is characterized by the loss of genome stability.

In this study, I have utilized a genome-wide synthetic dosage lethal screen to identify new cellular roles for NuA4. This screen dramatically expanded our knowledge of the putative functions of NuA4 *in vivo*, including the exciting discovery linking NuA4 to septin dynamics and cytokinesis.

1.1 Literature Review

I. NuA4, a multifunctional lysine acetyltransferase in yeast

i. The biological significance of acetylation

Post-translational modifications work to regulate a myriad of processes within the cell, from changing the activity of a particular enzyme, to providing a means to recruit effectors to specific locations. Acetylation is a post-translational modification that has been known for over 30 years, and is most commonly known to involve the transfer of an acetyl group from acetyl-CoA to the epsilon-amino group of a lysine residue (Polevoda and Sherman, 2002). Histone acetylation has been the most studied form of this modification, which involves the addition of acetyl groups to the lysine residues found on the N-terminal tails of the various histone subunits that protrude from the core by HATs/KATs (Lee and Workman, 2007). Histones assemble to form an octamer core consisting of histones H2A, H2B, H3, and H4, around which approximately 147 base pairs of DNA wraps, forming the nucleosome structure that make up chromatin. There are several different types of modifications that can occur on different histone residues, each of which play separate roles in chromatin function and the combination of which make up what is termed the "histone code". Histone acetylation works to regulate transcription of genes by two ways: 1) modifying chromatin structure and 2) by serving to attract additional chromatin remodeling proteins (Kouzarides, 2007). Acetylation is thought to neutralize the positive charge on lysine in order to relieve DNA-histone interactions and allow access of transcription factors to promoters regions. Acetylation can also function as a "landing pad" for other factors important in chromatin biology. Histone acetylation can also recruit effectors to sites of DNA damage and mediate DNA damage repair pathways (Wurtele and Verreault, 2006).

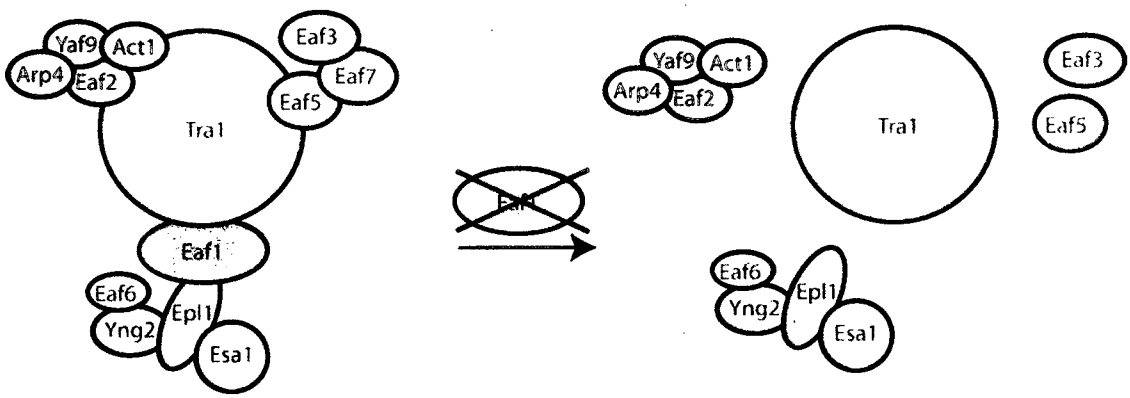
Acetylation of non-histone substrates also plays an important role in numerous cellular functions (Glozak et al., 2005). For example, it has been known that p300/CBP can acetylate the important tumour suppressor p53 at K120 in order to mediate its binding affinity to DNA and to enhance transcription-independent apoptosis (Sykes et al., 2009). Recently, Eco1 was found to acetylate the cohesin subunit Smc3 in order to promote sister chromatid cohesion in budding yeast (Ben-Shahar et al., 2008). Though the biological consequences of acetylation have yet to be fully discovered, it is apparent that this modification is far more significant than originally thought. A recent study sought to determine the human acetylome and found that over 3500 human proteins are acetylated in order to co-regulate cellular processes (Choudhary et al., 2009). This suggests that acetylation of non-histone substrates may be a global mechanism to regulate diverse cellular processes.

ii. NuA4 structure

NuA4 is made up of 13 subunits (Figure 1), six of which are essential for cell viability (Act1, Arp4, Swc4, Epl1, Esa1, and Tra1), and the remaining seven of which are non-essential (Eaf1, Eaf3, Eaf5, Eaf6, Eaf7, Yaf9 and Yng2) (Doyon and Côté, 2004). The catalytic subunit is Esa1 and is the only essential KAT in yeast (Allard et al., 1999). The remaining subunits are believed to regulate Esa1 acetyltransferase activity and recruit the complex to its appropriate targets. Structural studies have elucidated the manner in which the subunits are linked together (Figure 1). A functional subcomplex composed of Esa1/Epl1/Yng2, named Piccolo NuA4, is able to globally acetylate histones (Boudreault et al., 2003). A subcomplex of Eaf3/Eaf5/Eaf7 has also been identified *in vivo* (Auger et al., 2008; Mitchell et al., 2008). Eaf1 is situated at the centre of the complex and serves as a

Figure 1. Schematic of NuA4 and the role of Eaf1 in maintaining complex integrity.
The structure of the 13-subunit NuA4 complex as known to date. Eaf1 is the platform for the assembly of the different functional modules of NuA4. Removal of Eaf1 results in the abolishment of complex integrity, making *eaf1*Δ mutants a useful tool to study NuA4 function *in vivo*.

platform upon which the other subunits assemble, making it critical for complex integrity (Auger et al., 2008; Mitchell et al., 2008). As Eaf1 associates solely with the NuA4 complex (Auger et al., 2008; Mitchell et al., 2008), *eaf1*Δ mutants serve as a powerful tool to study the molecular function of NuA4 *in vivo*.



iii. NuA4 substrates

NuA4 acetylates lysine residues on the N-terminal tails of histones H4 (Allard et al., 1999; Eberharter, John, Grant, Utley, and Workman, 1998; Smith et al., 1998), and the histone H2A variant Htz1 (Babiarz et al., 2006; Keogh et al., 2006; Millar et al., 2006). Defects in NuA4 function have only minor effects on gene transcription (Lindstrom et al., 2006; Zhang et al., 2004), making it likely that NuA4 regulates its host of processes via mechanisms other than histone acetylation. Indeed, NuA4 has been shown to acetylate its own subunit Yng2, and this acetylation is essential for the protein stability of Yng2 (Lin et al., 2008). Even more recently, a study was published detailing the results of a NuA4 acetylation microarray (Lin et al., 2009). A yeast protein microarray containing 5800 proteins was incubated with NuA4 and radiolabelled acetyl-CoA and acetylated proteins were visualized by autoradiography. Nearly 100 non-histone associated proteins were identified as putative *in vitro* substrates of NuA4. However, only a few have been confirmed as true *in vivo* targets of NuA4. In particular, acetylation of phosphoenolpyruvate carboxykinase, Pck1, was found to be important for its enzyme activity and for extension of yeast chronological life span induced by water starvation. These types of studies are rapidly expanding the mechanisms by which NuA4 may regulate several different processes.

II. The cellular functions of NuA4

i. Transcription

Acetylation of histones is almost always related to activation of gene transcription (Kouzarides, 2007). Since NuA4 is able to acetylate histones H4 and Htz1, it inevitably plays a role in activating transcription of certain genes. The catalytic subunit Esa1 has been found to be recruited specifically to the promoters of genes encoding for ribosomal (Reid et al., 2000) and heat-shock proteins (Rohde and Cardenas, 2003). In addition, NuA4 is essential for transcriptional induction of the *PHO5* gene (Nourani et al., 2004) and for optimal induction of the glucose-regulated *SUC2* gene (Geng and Laurent, 2004). Though acetylation is traditionally linked with transcriptional activation, NuA4 also has a role in the repression of stress response genes (see section below and Lindstrom et al., 2006; Mitchell et al., 2008). Although histone H4 acetylation is globally downregulated when NuA4 function is disrupted, only minor transcriptional alterations are seen (Choy and Kron, 2002; Durant and Pugh, 2006; Keogh et al., 2005; Krogan et al., 2004; Masson et al., 2003; Zhang et al., 2004). This suggests that NuA4 is performing many of its roles via mechanisms other than transcriptional regulation.

ii. DNA repair

The human Tip60 complex has been linked to DNA repair of double-strand breaks (Squatrito et al., 2006). Similarly, NuA4 was found to preferentially acetylate nucleosomes near DNA double-strand break sites and play a critical role in repairing these breaks through non-homologous end joining (Bird et al., 2002). The Yng2 subunit is required for replication-coupled repair as *yng2* mutants are highly sensitive to DNA-damaging agents, a sensitivity that can be overcome by restoring H4 acetylation (Choy and Kron, 2002). Rapid

phosphorylation of histone H2A around double-strand breaks was shown to recruit NuA4 via the Arp4 subunit for subsequent chromatin reconfiguration and DNA repair (Downs et al., 2004).

iii. Glucose metabolism

Pck1, or phosphoenolpyruvate carboxykinase, was one the substrates that came out of the NuA4 acetylation microarray that was investigated in detail (Lin et al., 2009). Pck1 is involved in the gluconeogenesis pathway and catalyzes the conversion of oxaloacetate to phosphoenolpyruvate and carbon dioxide. The enzymatic activity of Pck1 was found to be dependent on acetylation by NuA4 and loss of Pck1 activity blocked chronological life-span extension in water starvation. The study went on to look at human Pck1, and found that it was acetylated by the Tip60 complex and that this acetylation appeared to regulate its activity.

iv. Stress response

Msn2 and Msn4 are transcription factors that upregulate genes involved in the stress response through binding to stress response elements at the promoter regions (Boy-Marcotte et al., 1998). Through microarray studies of several mutants, NuA4 was found to play a role in the transcriptional repression of many Msn2/4-dependent stress response genes (Lindstrom et al., 2006). It was also demonstrated that derepression of a subset of Msn2/4 target genes in NuA4 mutants require Msn2 and/or Msn4. In addition, NuA4 was linked to several stress response genes through synthetic lethal screening and subsequent analysis using mass spectrometry and pull-down assays found that NuA4 was able to bind directly to Msn2 and Msn4 (Mitchell et al., 2008). Despite the physical localization to Msn2/4-regulated

genes, chromatin immunoprecipitation experiments indicate that NuA4 is not repressing transcription through acetylation of histones in these promoters.

v. Cell cycle

NuA4 function is required for proper cell cycle progression, as *esa1* mutants were found to arrest after DNA replication but before cytokinesis (Clarke et al., 1999). In that study, it was suggested that this was due to transcriptional events. A later study found that *yng2* mutant cells displayed a mitotic delay that was suppressed by restoring histone H4 acetylation, suggesting that this cell cycle delay was due to reduced H4 acetylation (Choy et al., 2001). More recently, it was found that *eaf1* mutants exhibit slow cell cycle progression through G2/M, and that this was not due to global acetylation changes (Auger et al., 2008). This suggests that the slow cell cycle progression may not be due solely to histone H4 acetylation or transcriptional regulation.

vi. Chromosome stability

Certain NuA4 mutants are highly sensitive to the microtubule-destabilizing drug benomyl (Kobor et al., 2004; Krogan et al., 2004) and exhibit high rates of chromosome loss (Krogan et al., 2004). Though acetylation-deficient Htz1 mutants display genome stability issues, these defects in chromosome stability are minor compared with NuA4 mutants (Keogh et al., 2006). There is also no evidence for the increase of NuA4 localization to kinetochores or an increase in H4 acetylation at kinetochores (Krogan et al., 2004; K. Baetz personal communication), suggesting that another pathway impacting genome stability is regulated by NuA4. Beyond these observations, little is known about the molecular details behind the role of NuA4 in maintaining genomic stability. A possibility to this conundrum

may be that NuA4 has roles in other stages of the cell cycle intimately linked with chromosome stability. Interestingly, in mammalian cells, subunits of the Tip60 complex have been localized to the cytokinesis furrow, however its role there is unknown (Sigala et al., 2005). One intriguing possibility is that NuA4 may contribute to some aspect of cytokinesis, the disruption of which may lead to chromosome instability and defects in G2/M progression.

III. Septin biology

i. Yeast cytokinesis

In budding yeast, cytokinesis is a tightly regulated process that depends on a myriad of signaling cascades and checkpoints to ensure that it proceeds with accuracy. Yeast cells contain a polarized actin cytoskeleton, which is responsible for polarized growth of the daughter bud during the cell cycle (Pruyne and Bretscher, 2000). Upon anaphase, chromosomes segregate and are pushed to opposites poles of the cell via the microtubules protruding from the spindle pole bodies. The spindle position checkpoint ensures that the cell does not exit mitosis until the nucleus is properly positioned in the neck (Bardin et al., 2000). The yeast NoCut pathway ensures that cytokinesis does not occur before all chromosome arms are pulled out of the cleavage plane of the cell (Norden et al., 2006). Cytokinesis in budding yeast is unique in that the division plane is selected at the beginning of the cell cycle (Balasubramanian et al., 2004). During cytokinesis, new cell wall and plasma membrane must be synthesized or transported to the bud neck so that there will be enough material for both the mother and daughter cell. For cytokinesis to occur, an actomyosin contractile ring must be formed from the type II myosin, Myo1, and F-actin in order to pinch the two cells

apart and allow completion of cytokinesis (Bi et al., 1998). In budding yeast, septins play a crucial role in effecting proper cytokinesis.

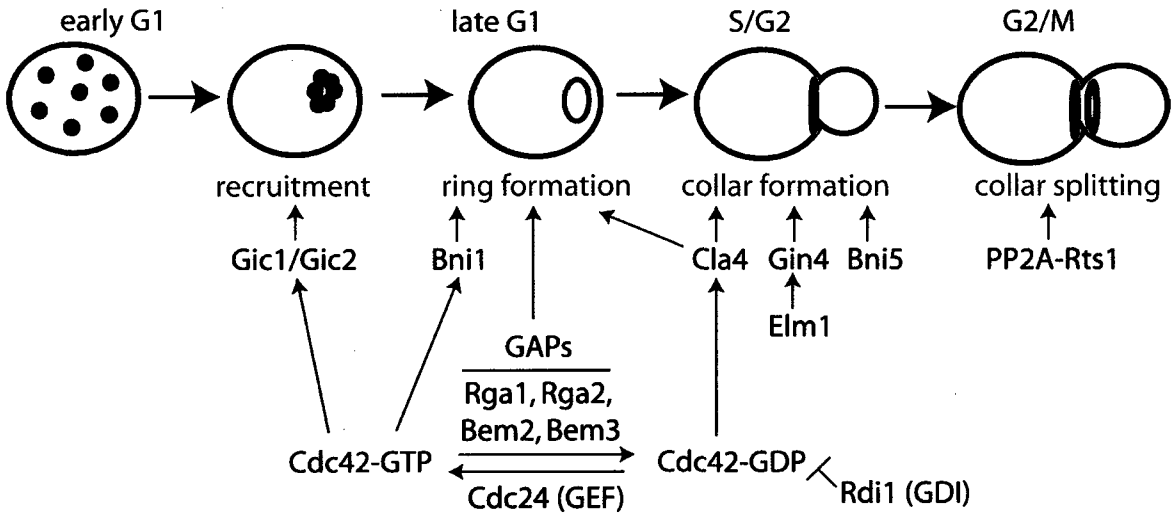
ii. Septins

Cytokinesis is a process that requires tight regulation in order to allow for accurate propagation of genetic material. In budding yeast, five septin subunits (Shs1, Cdc3, Cdc10, Cdc11, and Cdc12) function during cytokinesis in mitosis (Douglas et al., 2005). These filament-forming proteins were originally identified as mutants with cytokinetic defects that were able to progress through the cell cycle (Hartwell, 1971). Septins are GTP-binding proteins conserved from yeast to humans that are organized at the bud neck in a sequential manner (Figure 2). In late G1, septins are highly dynamic and are recruited to the site where the incipient bud will form and immediately arrange into a ring to mark the incipient bud site (Iwase et al., 2006). The septins are then remodeled to form a collar as the bud emerges from the S to M phase of the cell cycle. At this stage, the septins become stabilized structures in which there are no more dynamic exchanges of subunits (Caviston et al., 2003). When cytokinesis occurs, the collar splits into two and separate to the mother and daughter cell. The collar is then disassembled in both cells so that the septins can be reassembled at the site of the new bud at the start of the next cell cycle.

The septin ring is crucial for initiating for two parallel processes required for proper cytokinesis in budding yeast. First, it nucleates the formation of the actomyosin contractile ring needed to pinch the plasma membrane together by recruiting the type II myosin, Myo1 (Bi et al., 1998). Secondly, it reorganizes the actin cables within the cell so that vesicles containing new plasma membrane and cell wall material can be delivered to the bud neck (Kadota et al., 2004). The septin complexes are involved in diverse cellular functions by

Figure 2. Schematic of yeast septin assembly during one cell cycle.
Septin (shown in red) recruitment to the incipient bud site requires Cdc42-GTP and its effectors Gic1 and Gic2. Septin ring assembly requires Cdc42 cycling between GDP- and GTP-bound states through the activity of its GTPase-activating proteins (GAPs) Rga1, Rga2, Bem2, Bem3, its guanine nucleotide exchange factor (GEF) Cdc24, and its guanine nucleotide dissociation inhibitor (GDI) Rdi1. Cdc42 can also act through its effectors Bni1 and Cla4 to regulate septin ring assembly. Septin ring to collar transition requires the Cla4 kinase, the Gin4 kinase (which is activated by Elm1 kinase), Bni5, and GTP binding of the septins. Dephosphorylation of the septins by the PP2A-Rts1 complex promotes collar splitting and septin disassembly. (adapted from Park & Bi, 2007)

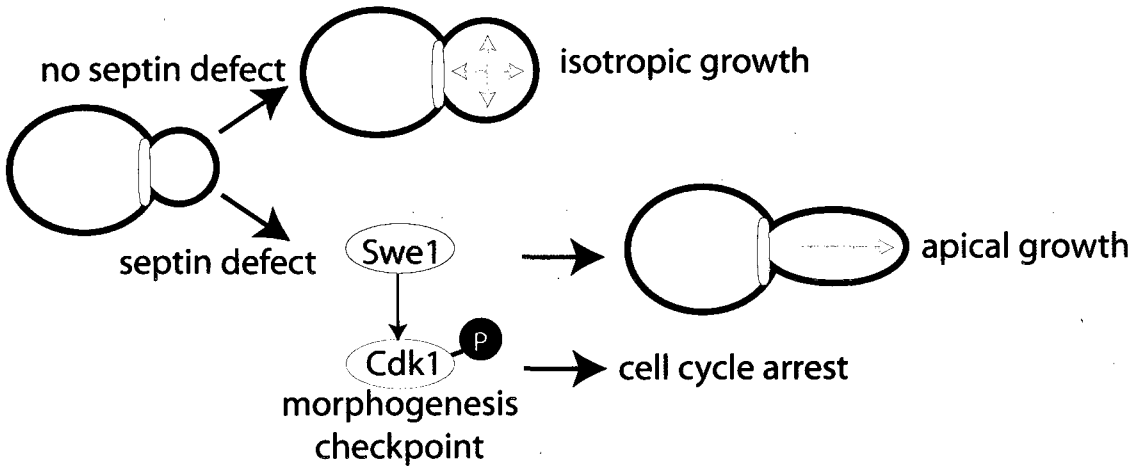
acting as a scaffold for many proteins involved in processes such as bud site selection (Longtine and Bi, 2003), chitin deposition (DeMarini et al., 1997), cytokinesis (Bi et al., 1998), and the morphogenesis checkpoint (Barral et al., 1999).



iii. Septins and the morphogenesis checkpoint

Since proper septin ring assembly is critical for cytokinesis, mechanisms in the cell exist to detect whether the ring is assembled and to delay cell cycle progression if defects are sensed. The morphogenesis checkpoint is responsible for monitoring actin organization (McMillan et al., 1998), septin organization (Longtine et al., 2000), and even the presence of a bud during the cell cycle (Lew and Reed, 1995). This checkpoint is activated upon defects in septin assembly (Figure 3) and though the exact mechanisms are unknown, the main effector of this checkpoint is the kinase Swe1 (Barral et al., 1999; Keaton and Lew, 2006). Septin defects activate Swe1, which then inhibits the cell cycle by phosphorylating a conserved tyrosine on the cyclin-dependent kinase Cdc28 (yeast Cdk1), the master yeast cell cycle regulator required for cells to progress from metaphase to anaphase (Booher et al., 1993). Cells are then arrested in G2/M until the defect is repaired, at which point Swe1 is degraded via a ubiquitin-dependent pathway through interaction with the protein kinase Hsl1 and protein methyltransferase Hsl7 at the bud neck. Localization of Hsl1, Hsl7, and Swe1 to the neck is dependent upon the septins (Barral et al., 1999; Longtine et al., 2000), and therefore septin defects will result in disruption of Hsl1 and Hsl7 localization and continued Swe1 activity. Cdc28 activity is also required for the bud to switch from apical to isotropic growth (Lew and Reed, 1993). Therefore, if Swe1 is activated via septin defects, Cdc28 activity is inhibited and the bud continues to grow apically, resulting in cells with hyper-elongated buds.

Figure 3. Schematic of yeast cytokinesis and the morphogenesis checkpoint.
Defects in septin assembly or structure will result in activation of the Swe1 kinase. Swe1 phosphorylates the master yeast cell cycle regulator Cdc28 to inhibit cell cycle progression until the defect is repaired. Activation of Swe1 also prevents the switch from bud apical to isotropic growth and results in elongated buds.



iv. Regulation of septin dynamics

Septin assembly and dynamics is largely governed by the Rho-family GTPase Cdc42 (Park and Bi, 2007). Cdc42 is involved in actin and septin organization (Longtine and Bi, 2003) and plays a critical role in the establishment of cell polarity (Adams et al., 1990).

Cdc42 is localized to sites of polarized growth and must be in its activated GTP-bound (Cdc42-GTP) state to effect polarization. The three stages of septin assembly, recruitment, ring assembly, and collar formation depend on the presence of activated Cdc42-GTP (Iwase et al., 2006).

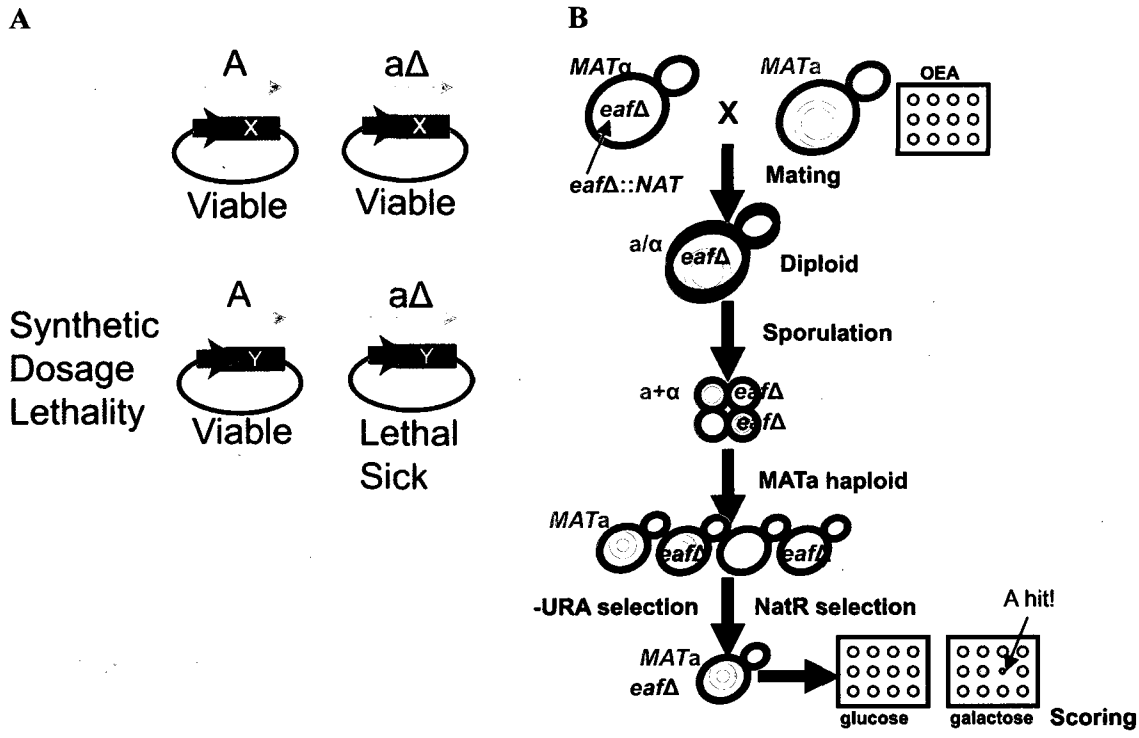
Cdc42 itself is regulated by several different factors that localize it to the septins or control its GTPase activity (Figure 2). The Cdc42 GTPase activating proteins (GAPs) include Rga1, Rga2, Bem2, and Bem3 (Caviston et al., 2003; Gladfelter et al., 2002; Marquitz et al., 2002). Cdc24 acts as a guanine nucleotide exchange factor (GEF) for Cdc42, meaning it can exchange GDP for GTP (Gladfelter et al., 2002). Rdi1 is a guanine nucleotide dissociation inhibitor (GDI) that is able to keep Cdc42 in an inactive cytosolic complex (Tiedje et al., 2008). Gic1 and Gic2 are Cdc42 effectors that are required for septin recruitment during late G1 and bind directly to Cdc12 (Iwase et al., 2006). In addition to Cdc42, other proteins have been implicated in septin assembly. The Gin4 kinase interacts with Cdc3 and is important for proper septin organization (Longtine et al., 1998), and can directly phosphorylate Shs1 (Mortensen et al., 2002). The Elm1 kinase is responsible for Gin4 activation and consequently septin assembly (Asano et al., 2006; Bouquin et al., 2000). Bni1 is a formin required for initial assembly of the septin ring but not for the maintenance of the ring (Kadota et al., 2004). Bni5 is a protein that is responsible for providing stability to the septins, possibly through interaction with Cdc11 (Lee et al., 2002). The Cla4 kinase is able to phosphorylate septins and is required for septin ring and collar formation (Versele and Thorner, 2004). As well, GTP-binding of the septins is also involved in the formation of the septin ring and collar, though the exact mechanisms remain unclear (Versele and Thorner, 2004).

IV. Synthetic dosage lethal screening, a useful tool to elucidate function

i. Synthetic lethal and synthetic dosage lethal screens

In *Saccharomyces cerevisiae*, powerful genome-wide screens can be performed using the synthetic genetic array (SGA) methodology. SGA is a high-throughput technique used to perform genetic analysis of a genome-wide scale using mutant arrays. SGA was originally used to identify synthetic lethal interactions, a situation where individual gene deletions are viable but in combination the two gene deletions produce a fitness defect (Tong et al., 2001). Previous studies have shown that synthetic lethal interactions occur between two genes involved in the same biological pathway (Novick et al., 1989). Another genetic interaction that has been useful in identifying genes involved in the same pathway is synthetic dosage lethality (SDL). SDL is based on the idea that increasing levels of a protein may have no effect on a wildtype yeast strain but may cause sickness or lethality in a mutant strain with reduced activity of an interacting protein (Figure 4A). It has been noted that the genetic networks derived from overexpression and deletion mutant screens are markedly different as highlighted by the lack of overlap between genome-wide SL and SDL screens (Baetz et al., 2006). An overexpression array (OEA) of yeast, each harbouring a plasmid with a galactose-inducible open reading frame, has been constructed (Sopko et al., 2006). SGA technology has been successfully applied to study SDL interactions on a genome-wide scale using this OEA and a query strain containing a knockout of the gene of interest (Figure 4B). In contrast to SL-SGA screens using the deletion mutant array, SDL-SGA screens allow for the screening of both non-essential and essential genes. Genome-wide SDL screens have successfully identified known and novel substrates of kinases (Sopko et al., 2006; Zou et al., 2009). Using the same logic, substrates of other enzymes may be identified using this technique.

Figure 4. Schematic of synthetic dosage lethality and genome-wide SDL analysis.
(A) Schematic of synthetic dosage lethal interaction. A synthetic dosage lethal interaction occurs when overexpression of a gene of interest, Y, has no notable effect on a wild type strain but has reduced viability in a strain carrying a mutation in a gene, A. On the plasmids the red arrow represents the *GAL1* promoter; A is the query gene, X and Y are the overexpressed genes. (B) Schematic of the methodology used for the genome-wide SDL screen. A *MAT*αSGA starting strain carrying a PGAL1-inducible plasmid with the *URA3* selectable marker and the *MFA1-HIS* and *can1*Δis crossed to an ordered deletion mutant array. Diploids are selected on media containing nourseothricin (to select for the deletion mutant carrying the *NAT* marker) and lacking uracil (to select for the *PGAL1*-inducible plasmid), sporulated and *MAT*a haploids carrying the plasmid and the deletion mutant are selected on media lacking histidine and uracil and containing nourseothricin. Haploids are pinned onto media containing galactose to induce the expression of the *GAL1* promoter regulated gene on the plasmid. Colony growth is compared on galactose and glucose to identify mutants whose growth is compromised (SDL or SDS) upon overexpression of the gene on the plasmid.



ii. Synthetic lethal screening of NuA4

Originally, NuA4 was found to have roles in transcriptional regulation, DNA repair, and chromatin remodeling, via acetylation of histones. However, how NuA4 is regulating genome stability or if NuA4 is impacting other cellular functions was largely unknown. In attempt to answer these questions, our lab and others have recently applied genome-wide

synthetic lethal synthetic genetic array (SL-SGA) screens to numerous mutants of NuA4 (Hoke et al., 2008; Lin et al., 2008; Mitchell et al., 2008). The genetic interactions identified from these studies expanded the number of biological processes that NuA4 participates in, including vesicle-mediated transport and the stress response. However, as SL-SGA only screens for non-essential genes, the full gamete of NuA4 biological roles have likely not been fully elucidated. Further, despite the extensive list of genetic interactions, no obvious candidates were identified to explain the role of NuA4 in chromosome stability and slow cell cycle progression. A NuA4 SDL screen may be able to uncover novel genes and potentially pathways regulated by this acetyltransferase.

1.2 Hypothesis

Studies to date have used only synthetic lethal analysis to dissect the roles of NuA4. I hypothesize that a SDL screen using the non-essential subunits of NuA4 will identify new pathways that NuA4 participates in and may provide novel insight into the possible cellular processes or pathways through which NuA4 is contributing to chromosome stability and cell cycle progression.

Objectives of thesis

1. To perform a genome-wide synthetic dosage lethal screen in order to elucidate additional roles of NuA4.
2. To explore the potential molecular mechanisms behind the role of NuA4 in chromosome stability and cell cycle progression using the results of the screen.

Chapter 2: MATERIALS AND METHODS

Yeast strains and media:

The yeast strains used in this study are listed in Appendix A. Deletion strains made for this study were designed using a standard PCR-mediated insertion technique (Longtine et al., 1998) and confirmed by PCR analysis. Cells were grown in standard yeast peptone dextrose (YPD) or synthetic defined (SD) medium supplemented with amino acids (Abelson et al., 2004), unless otherwise described.

Synthetic dosage lethal screens:

Genome-wide Synthetic Dosage Lethal SGA screens were conducted in triplicate at 25°C using a RoToR HDA robot (Singer Instruments, UK) as previously described (Sopko et al., 2006). In detail, *MAT*α haploid query strains (*eaf1*Δ, YKB622; *eaf3*Δ, YKB995; *eaf5*Δ, YKB852; *eaf6*Δ, YKB623; and *eaf7*Δ, YKB853) linked to a nourseothricin-resistance marker that confers resistance to the antibiotic nourseothricin, and containing *MFA1pr-HIS3* and *can1*Δ reporters were crossed to the *MAT*a yeast overexpression array (gift of Brenda Andrews, University of Toronto), an ordered array of ~ 5300 haploid yeast strains pinned in duplicate, each containing a uracil-resistant *pGAL1/10*-GST-6xHis-*ORF* plasmid. The mating was performed on YPD plates containing extra adenine. Diploid selection was achieved through pinning on medium containing cloNAT (Werner BioAgents, 5.0000) and lacking the amino acid uracil. Diploids were sporulated for 10 days at room temperature on medium containing reduced levels of carbon and nitrogen to produce four possible haploid meiotic spore progeny. Spores were transferred to synthetic medium lacking histidine, which allowed for selective germination of *MAT*a meiotic progeny (because these cells express the *MFA1pr-HIS3* reporter), lacking uracil to select for progeny carrying the plasmid, and

containing canavanine (Sigma, C1625) (to select for *can1*Δ haploid mutant cells with canavanine resistance). This step was done twice. The haploid meiotic progeny were transferred to medium containing cloNAT lacking the amino acid uracil to specifically select for only the gene-deletion single mutant meiotic progeny carrying the plasmid. After replica pinning onto galactose media to induce overexpression of the *GAL1*-inducible array, colonies were grown for 2 days at 25°C and scored for a phenotype of interest (slow growth or lethality) by both computational analysis with AlphaEase and visual inspection. The phenotypes of wildtype cells upon overexpression of each yeast gene were determined in a previous study that pinned this OEA onto glucose and galactose plates (Sopko et al., 2006). The list of genes toxic to wildtype cells was compared to the results generated from the NuA4 SGA-SDL analysis in order to remove the genes that caused lethality in both wildtype and mutant strains.

Confirmation of SDL-SGA screen:

Putative interactions that were identified a minimum of two out of three replicates were confirmed by spot dilution assays. The plasmid containing the overexpression gene was extracted from the overexpression array (Sopko et al., 2006) and the plasmid was amplified in bacteria and purified using the PureLink Quick Plasmid Miniprep Kit (Invitrogen, K2100-11). The plasmid of interest was directly transformed into the *MAT*a NuA4 mutant strain of interest (*eaf1*Δ*kanMX*, YKB44; *eaf3*Δ*kanMX*, YKB1162; *eaf5*Δ*kanMX*, YKB658; *eaf6*Δ*kanMX*, YKB504; and *eaf7*Δ*kanMX*, YKB530) containing resistance to kanamycin (G418) and into a *MAT*a wildtype strain (YKB779) using traditional yeast transformation methods (Gietz and Schiestl, 2007). Mutant strains distinct from the original queries were

used to eliminate any possibility of background specific interactions. A vector control (pRS416) was also transformed into each strain. The growth of the strains was assessed by replica spotting serial dilutions of the strains (dot assays – see below) onto SC-URA plates containing either glucose or galactose and incubating at 25°C for at least three days. The fitness of the deletion mutant strain was compared to that of the wildtype strain upon overexpression of the gene on the plasmid in order to confirm the putative SDL interaction. The resulting list of genes that exhibited positive SDL interactions with at least one of the NuA4 mutant strains was compiled. Each overexpression gene confirmed in one NuA4 mutant was tested against the remaining NuA4 mutants. In this case, the interactions were tested by streak tests. In addition, the confirmed overexpression genes were tested in the two remaining non-essential NuA4 subunits that were not put through SGA-SDL screening (*yng2ΔkanMX*, YKB494; *yaf9ΔkanMX*, YKB464). The growth of these strains was assessed by streaking onto glucose and galactose plates.

Dot assays:

Ten-fold serial dilutions (OD_{600}= 0.1, 0.01, 0.001, 0.0001) of the appropriate strains were grown to mid-log phase in YPD at 25°C and plated onto appropriate media. For the dot assay confirmations of the SDL screen, the strains were replica spotted onto media lacking uracil and containing either glucose or galactose. For all other dot assay analyses, the strains were replica spotted as needed onto YPD plates and incubated at different temperatures (25°C, 30°C, 34°C, and 37°C). Unless otherwise indicated, plates were incubated at 25°C for 3 days before epi-white imaging using the Molecular Imager ChemiDoc XRS System (BioRad).

Iodine staining:

Strains were grown to mid-log phase (OD_{600} ~ 0.6-0.8) in YPD at 25°C before diluting to an OD_{600} of 0.2 and spotting onto YPD media. Plates were incubated at 25°C for 3 days before exposing them to iodine crystals for three minutes so that the iodine vapours could stain the glycogen dark red. Plates were then scanned for a colour image (Epson Perfection 2580).

Bud morphology analysis:

Cells were grown at 25°C in 20 mL YPD to mid-log phase (OD_{600} ~ 0.6-0.8) and 1 mL was collected by centrifugation and fixed in 4% paraformaldehyde for 15 minutes. The cells were washed once with KPO_4/sorbitol solution (1M sorbitol, 0.1M KPO_4) and resuspended in 100 µl of KPO_4/sorbitol. 5 µl of cells were placed on a glass slide and sealed with a cover slip. The cells were viewed under bright field light using a Leica CTR 6500 (Leica Microsystems, Heidelberg, Germany) microscope at 63x magnification, images were acquired using Volocity Acquisition Software (Quorum, Guelph, ON, Canada) and a minimum of 100 cells was counted and scored for morphology. The experiment was repeated three times.

Calcofluor staining:

Cells were grown at 25°C in 20 mL YPD to mid-log phase (OD_{600} ~ 0.6-0.8) and 1 mL was collected by centrifugation and fixed in 4% paraformaldehyde for 15 minutes. The cells were washed once with KPO_4/sorbitol solution and resuspended in 100 µl of KPO_4/sorbitol. 10 µl of 1 mg/mL calcofluor (Fluorescent Brightener 28, Sigma F3543-1G) was added to the cells and the mixture was incubated at room temperature in the dark for 30

minutes. The cells were pelleted at 13200 rpm for 30 seconds and washed twice with water and resuspended in 100 µl of KPO_4/sorbitol. 5 µl of cells were placed on a glass slide and sealed with a cover slip. The cells were viewed under blue fluorescence (Excitation 380 nm, Emission 465 nm) at 63x magnification and images were acquired using a Leica CTR 6500 (Leica Microsystems, Heidelberg, Germany) using Volocity Acquisition Software (Quorum, Guelph, ON, Canada). A minimum of 100 cells was scored for chitin localization.

Cdc11-GFP localization:

Cells were grown at 25°C in YPD supplemented with 40 mg/L adenine to mid-log phase (OD_{600} ~ 0.6-0.8) and 1 mL was collected by centrifugation and fixed in 4% paraformaldehyde for 15 minutes. The cells were washed once with KPO_4/sorbitol solution and resuspended in 100 µl of KPO_4/sorbitol. The cells were sonicated for 15 seconds at a 3W power output level before placing 1 µl on a glass slide and sealing with a cover slip. The cells were viewed under green fluorescence (Excitation 490 nm, Emission 535 nm) and brightfield at 63x magnification and z-stacks were acquired using a Leica CTR 6500 (Leica Microsystems, Heidelberg, Germany) and Volocity Acquisition Software (Quorum, Guelph, ON, Canada). The z-stacks were compressed into a single plane prior to scoring, and a minimum of 100 cells was scored for septin and bud morphology.

Septin-tagged protein extractions and Western blot analysis:

Cells were grown at 25°C in 75 mL of YPD to mid-log phase (OD_{600} ~ 0.6-0.8) and collected by centrifugation (3000 rpm, 3 minutes, 4°C), washed in 10 mL water, resuspended in 1 mL of water and transferred in 750 µl aliquots to 1.5 mL Eppendorf tubes. Cells were pelleted by centrifugation (13200 rpm, 30 seconds, 4°C), the supernatant was removed by

aspiration, and the cell pellets were frozen in dry ice and stored at -80°C until they were ready to be harvested. To harvest cells, an equal volume of Tackett Extraction Buffer (20mM HEPES pH 7.4, 0.1% Tween-20, 2mM $MgCl_2$, 200mM NaCl, Protease Inhibitor Cocktail (Sigma, P-8215)) was added and the pellets were thawed before adding an equal volume of acid washed glass beads (Fisher Scientific, 35-535). Cells were lysed through vortexing (6 x 1 minute vortex with 1 minute incubation on ice in between vortexing). The beads were separated from the whole cell extract (WCE) by making a hole through the bottom of each 1.5 mL Eppendorf tube using a 21G1½ needle heated with a flame, and centrifuging at 1000 rpm for 1 minute at 4°C to collect the WCE through the needle hole into a fresh 1.5 mL Eppendorf tube. The WCE was clarified by centrifugation (15 minutes, 13200 rpm, 4°C) and the supernatant was transferred to fresh tubes. Protein concentration was determined by Bradford Assay (Bio-Rad, 500-0006) and 200 µg of each sample was used for Western Blot Analysis. In brief, an equal volume of 2x loading buffer (100mM Tris pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, 2% 2-mercaptoethanol) was added to each sample before boiling at 65°C for 10 min. Proteins were separated by SDS polyacrylamide gel electrophoresis on 8% gels and electrophoretically transferred onto nitrocellulose membranes using semi-dry transfer (BioRad). The members were blocked in Phosphate-buffered saline (PBS) containing 5% nonfat dry milk and 0.1% Tween 20 (PBS-T), for 1 hour at room temperature with shaking, or at 4°C overnight. Primary and secondary antibodies were diluted in the blocking solution and incubated with the membranes at room temperature for 1 hr with shaking. After incubation with each antibody, the membranes were washed with shaking in triplicate with PBS-T for 10 minutes at room temperature. The primary antibodies used in these studies were: α-HA (Covance, MMS-101P) (1:1000), α-Myc (Roche,

11667149) (1:1000), and α-actin (Novus Biologicals, 600-505) (1:1500). Secondary antibodies used in these studies were peroxidase-conjugated goat α-rabbit IgG (Chemicon, AP307P) (1:5000) and peroxidase-conjugated goat α-mouse IgG (BioRad, 170-6516) (1:5000). Membranes were developed using ECL Plus Western Blotting Detection System (Amersham BioSciences, RPN2135) according to the manufacturer's instructions.

Cell cycle blocks:

Cells were grown at 25°C in 200 mL of YPD to OD_{600} ~ 0.4 before separating into four cultures of 50 mL each. The following conditions were employed to induce cell cycle arrests: G1 arrest using 10 μg/μL α-mating factor (Sigma, T6901); S-phase arrest using 200 μM hydroxyurea (Sigma, H8627); G2 arrest using 15 μg/μL nocodazole (Sigma, M1404). One culture was not induced with any reagent and used as a control. The culture were shaken at 25°C for 3 hrs before harvesting as described previously and analyzed by Western blotting. To assess the blocks, 1 mL of cells were taken and fixed with paraformaldehyde and bud morphology was visualized using light microscopy.

Chapter 3: RESULTS

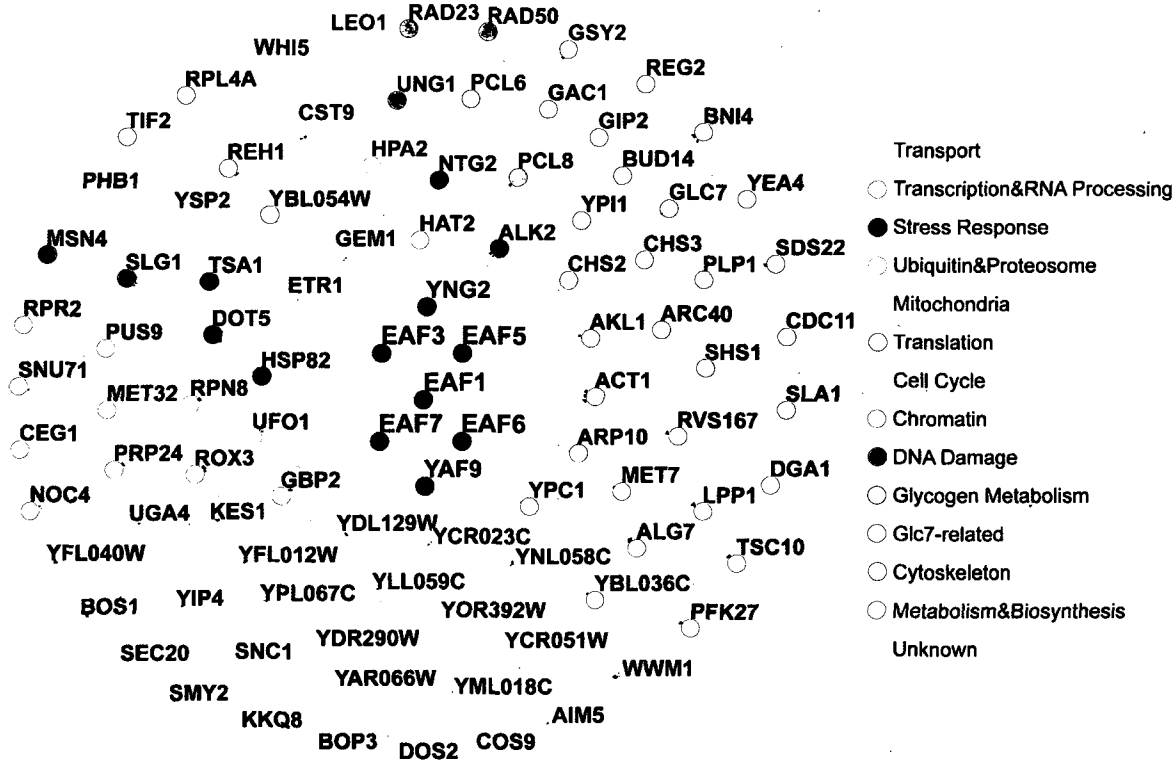
Synthetic dosage lethal genetic interaction map elucidates novel cellular processes that NuA4 impacts

In an effort to elucidate additional pathways impacted by NuA4, with the help of technician Ying Fong, I performed a series of genome-wide synthetic dosage lethal screens to identify genes that cause lethality or slow growth when overexpressed in NuA4 mutants. Screens were performed in triplicate for five non-essential NuA4 genes (*eaf1*Δ, *eaf3*Δ, *eaf5*Δ, *eaf6*Δ, and *eaf7*Δ). Putative genetic interactions that were identified in least two out of three replicates were confirmed by extraction of the plasmid for the array, amplification of the plasmid, and retransformation of the overexpression plasmid into both mutant and wildtype strains. Next, dot assay analysis was performed to directly compare the effects of overexpression of the gene in a wildtype cell to that in the mutant (see Materials and Methods for more details). The putative genetic interactions that had been previously reported to cause lethality in wildtype cells (Sopko et al., 2006) were eliminated from the putative list of interactions and were not confirmed. The final confirmed list of genes that exhibited SDL and SDS (synthetic dosage sick) interactions with at least one of the non-essential NuA4 genes was compiled and directly tested against all NuA4 deletion mutants, including *yaf9*Δ and *yng2*Δ, queries we had previous shown not to be amenable for SGA screening (Mitchell et al., 2008). Hence, in the final data set, each overexpression plasmid identified in the high throughput robotic-based screen has been directly tested in each of the seven non-essential NuA4 mutants.

These screens uncovered 182 interactions among 89 genes (Figure 5A), of which 23% (41/182) were SDL interactions and the remainder were SDS interactions. The query *eaf1*Δ identified the largest number of interactions, 59. This was expected as the Eaf1 subunit

Figure 5. Genetic interactions identified from SDL screening with NuA4 subunits.
(A) SDL network for the non-essential subunits of NuA4. Genome-wide SDL-SGA screens were performed using query strains for five non-essential NuA4 subunits: the *eaf1*Δ (YKB622), *eaf3*Δ (YKB993), *eaf5*Δ (YKB852), *eaf6*Δ (YKB623), and *eaf7*Δ (YKB853) strains. All identified SDL interactions were directly tested in *yng2*Δ, and *yaf9*Δ mutants. Genes are represented as nodes, and interactions are represented as edges. The genes are grouped and colour coded according to their GO biological process. (B) Grid diagram showing overlap between NuA4 query genes. (C) Venn diagram showing the overlap between hits that appeared in the SDL screen performed here and hits that appeared in SL screens from previous studies (Hoke, Guzzo, Andrews, & Brandl, 2008; Lin et al., 2008; Mitchell et al., 2008; Pan et al., 2006).

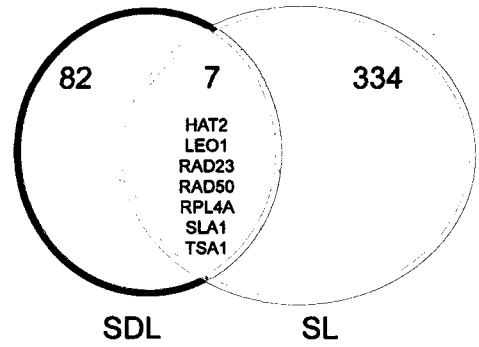
A



B

	EAF1	EAF3	EAF5	EAF6	EAF7	YAF9	YNG2
EAF1	59	5	16	2	16	6	22
EAF3		6	3	2	4	1	2
EAF5			20	4	12	7	12
EAF6				19	6	3	7
EAF7					33	5	14
YAF9						10	7
YNG2							32

C



largely acts to maintain NuA4 complex integrity and similarly large number of genetic interactions were shown for the SL-SGA screen (Mitchell et al., 2008). When compared to a compilation of the list of genes that were obtained from SL analyses of non-essential NuA4 mutants (Hoke et al., 2008; Lin et al., 2008; Mitchell et al., 2008; Pan et al., 2006), there were only seven common hits (Figure 5C). This underscores the fact that in general, deletion mutant or loss of function phenotypes differs from those overexpression or gain of function phenotypes, resulting in largely distinct genetic interaction networks.

Since genetic interactions predict functional relationships, the NuA4 genetic interaction map identified many genes that encode proteins implicated in cellular processes previously associated with this complex, including chromatin structure, transcription, DNA repair, and chromosome stability, as determined by their gene ontology (GO) annotations (Figure 5A). In addition to supporting these well-characterized roles for NuA4, the genetic interaction map identified genes implicated in a wide variety of functions, including glycogen metabolism and septin biology.

Synthetic dosage lethal interactions between NuA4 and glycogen-related genes may be due to its regulation of the Msn2 and Msn4 stress response transcription factors

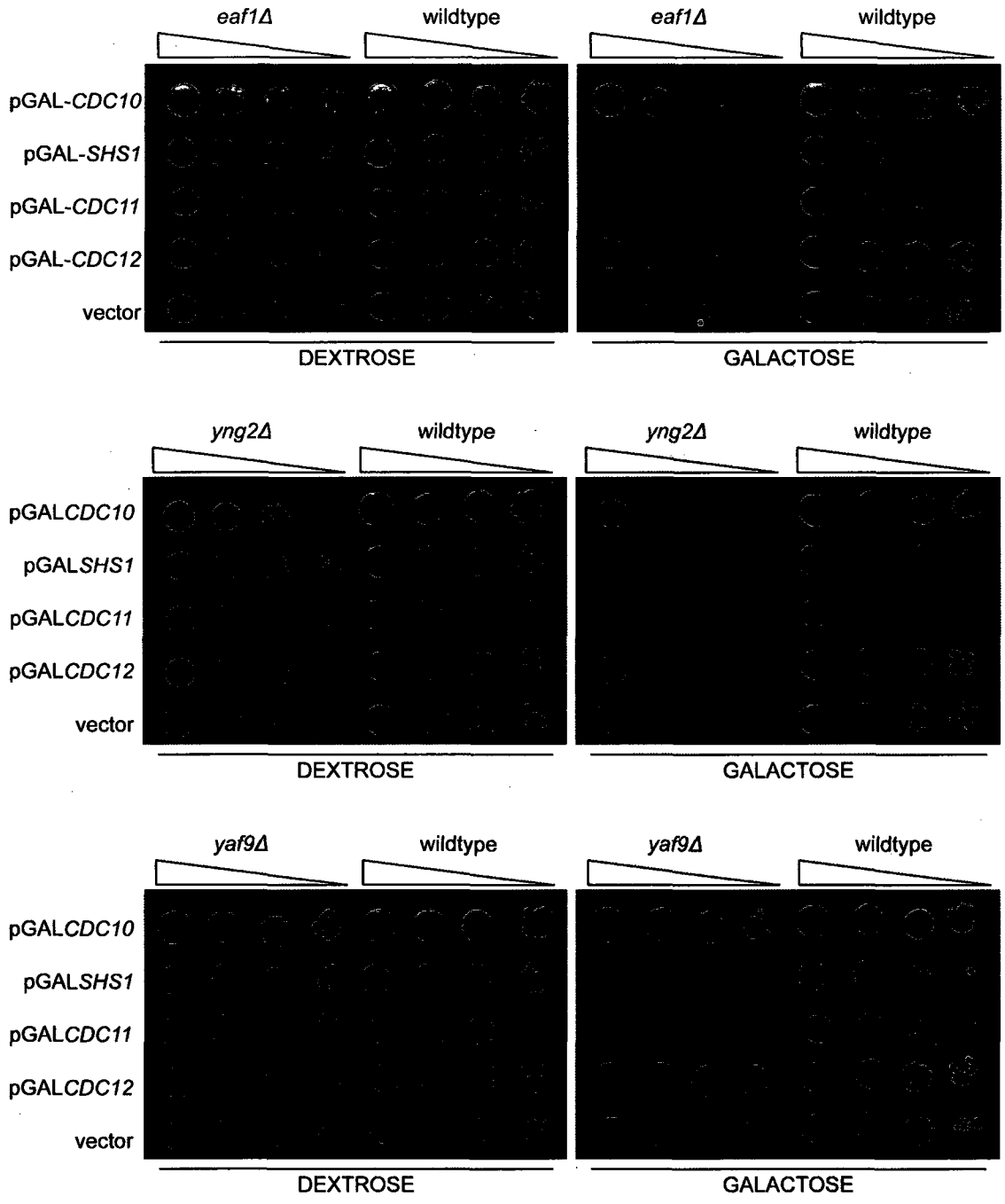
Several glycogen-related genes appeared in the NuA4 SDL screen (Figure 5A), including *GAC1*, *GIP2*, *GSY2*, *PCL6*, and *PCL8*. A potential reason for this enrichment of glycogen-related genes may be due to the role of NuA4 in repressing the Msn2 and Msn4 stress response transcription factors (Mitchell et al., 2008). Msn2 and Msn4 dimerize and bind to stress response elements to upregulate several genes including *GSY2*, which encodes for glycogen synthase 2, an enzyme that catalyzes the polymerization of glucose into glycogen for long-term storage (Boy-Marcotte et al., 1998). Since the absence of Eaf1 abolishes NuA4 complex integrity, *eaf1*Δ strains may exhibit an increase in glycogen accumulation due to a lack of Msn2/Msn4 repression by NuA4. To test this, *eaf1*Δ and *eaf1*Δ *msn2*Δ *msn4*Δ strains were exposed to iodine vapour, which is able to bind glycogen and stain yeast a red-brown colour (Figure 6). As expected, *eaf1*Δ strains showed a marked increase in glycogen accumulation, as shown by the dark red-brown staining. This accumulation was abolished by subsequently deleting *MSN2* and *MSN4*, which suggests that *eaf1*Δ mutants contain high amounts of glycogen potentially due to increased transcription of *GSY2* or additional Msn2/4 target genes. Since *eaf1*Δ mutants store unusually high amounts of glycogen, any additional disruption in glycogen metabolism via overexpression of certain glycogen-related genes may exacerbate the growth of *eaf1*Δ strains.

Figure 6. Deletion of stress response transcription factors suppresses glycogen accumulation in *eaf1* mutants. Three different isolates of *eaf1*Δ (YKB44) and *eaf1*Δ *msn2*Δ *msn4*Δ (YKB1097) strains were grown on YPD medium at 25°C for three days. The strains were exposed to iodine vapour for three minutes and then scanned for a colour image.

Synthetic dosage lethal interactions suggest a role for NuA4 in regulating septin function

One surprising feature of the NuA4 SDL genetic network was the identification of numerous genes implicated in various aspects of cytokinesis. Remarkably, overexpression of the septin genes *CDC11* and *SHS1* resulting in sickness in six out of the seven NuA4 mutants tested. *SHS1* exhibited SDL interactions with all but *eaf6*Δ, while *CDC11* exhibited SDL interactions with all but *eaf3*Δ. No other gene was identified that, when overexpressed, resulted in sickness in this many NuA4 subunits. To both verify and further investigate the connection between NuA4 and septins, I tested whether two additional yeast mitotic septins, *CDC10* and *CDC12*, would have the same effect (Figure 7). Unfortunately, sequence analysis revealed that the fifth mitotic septin *CDC3* was not correct in the overexpression array and therefore I did not test for its effects upon overexpression in the NuA4 mutant strains. In contrast to overexpression of *SHS1* and *CDC11*, overexpression of the septin subunits *CDC10* and *CDC12* did not cause SDL or SDS phenotypes. The observation that several NuA4 subunits show SDL interactions with yeast septins suggests that NuA4 may be playing a role in septin assembly or function.

Figure 7. Overexpression of *CDC11* and *SHS1*, but not *CDC10* or *CDC12*, cause synthetic growth defects in NuA4 mutants. The yeast strains WT (YKB779), *eaf1*ΔNAT (YKB1375), *yng2ΔkanMX* (YKB494), *yaf9ΔkanMX* (YKB464) were transformed with the plasmids *pGAL1-CDC10*, *pGAL1-SHS1*, *pGAL1-CDC11*, *pGAL1-CDC12*, and *pGAL1-PRS426* as indicated. These strains were serially diluted 10-fold, replica spotted onto medium containing glucose and galactose and grown at 25°C for 3 days.


eaf1Δ
wildtype
eaf1Δ
wildtype
pGAL-CDC10
pGAL-SHS1
pGAL-CDC11
pGAL-CDC12
vector
DEXTROSE
GALACTOSE
yng2Δ
wildtype
yng2Δ
wildtype
pGALCDC10
pGALSHS1
pGALCDC11
pGALCDC12
vector
DEXTROSE
GALACTOSE
yaf9Δ
wildtype
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eaf1 mutants activate the morphogenesis checkpoint via the Swe1 kinase

Defects in septin assembly activate the morphogenesis checkpoint via the Swe1 kinase, which ultimately results in cells with elongated bud morphology due to continued apical bud growth (Keaton and Lew, 2006). Genetic experiments were performed to determine whether NuA4 mutants interact with *swe1*Δ mutants. Deletion of S*WE1* was found to partially rescue the temperature sensitivity of an *eaf1*Δ strain (Figure 8A). This suggests that the temperature sensitive and slow growth phenotype of *eaf1*Δ cells may be partially attributed to activation of the Swe1-dependent morphogenesis checkpoint. As slow or defective progression through the cell cycle has previously been shown to cause temperature sensitivity (Hartwell et al., 1970), deletion of Swe1 and the abolishment of the morphogenesis checkpoint may allow *eaf1*Δ cells to progress more quickly through the cell cycle and allow for better growth at higher temperatures.

To look at this in more detail, microscopy was used to analyze the bud morphology of the single and double mutant strains used in the spot dilution assays (Figure 8B). The cells were grown to mid-log phase prior to analysis and the experiment was repeated in triplicate. Both wildtype and *swe1*Δ cells had similar distributions of cell morphologies with almost no cells displaying elongated bud phenotypes. As previously shown, *eaf1*Δ cells have a G2/M delay (Auger et al., 2008), and almost 30% of *eaf1*Δ cells exhibited elongated buds. This elongated bud phenotype was dramatically reduced in an *eaf1*Δ *swe1*Δ strain. However, the G2/M delay of *eaf1*Δ cells was not decreased by deletion of *SWE1*. This suggests that the cell cycle delay of *eaf1*Δ cells is not due to activation of Swe1. The rescue of temperature sensitivity and elongated bud morphology of *eaf1*Δ cells by deletion of *SWE1* supports the

Figure 8. Swe1 deletion rescues *eaf1*Δ temperature sensitivity and elongated bud morphology. (A) Yeast strains with the indicated genotypes (WT, YKB779; *eaf1*Δ*kanMX*, YKB44; *swe1*Δ*kanMX*, YKB1807; *eaf1*Δ*kanMX swe1*Δ*kanMX*, YKB1266) were serially diluted 10-fold, replica spotted onto YPD plates and grown at the indicated temperatures for 3 days. (B) Yeast strains with the indicated genotypes (same as above) were grown in YPD to log-phase and fixed in paraformaldehyde before visualization using bright-field microscopy. A minimum of 100 cells was scored and the experiment was performed in triplicate using three different isolates per strain. Legend to the right describes the bud morphology. The error bars represent standard deviation.

hypothesis that NuA4 mutants activate the morphogenesis checkpoint, potentially due to defects in septin morphology.

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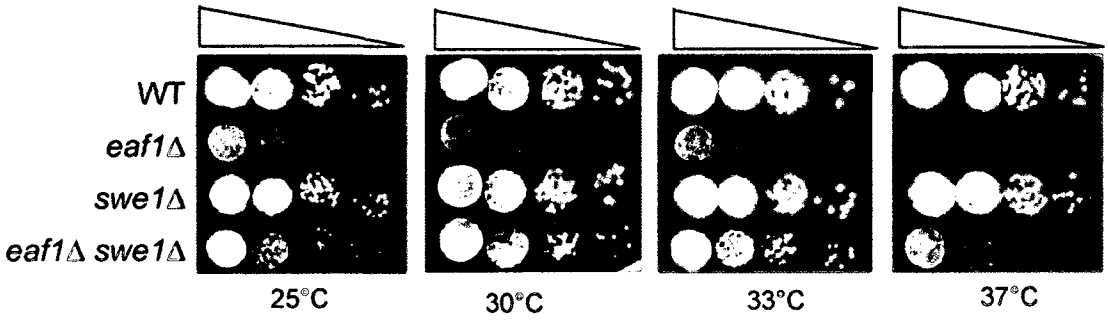

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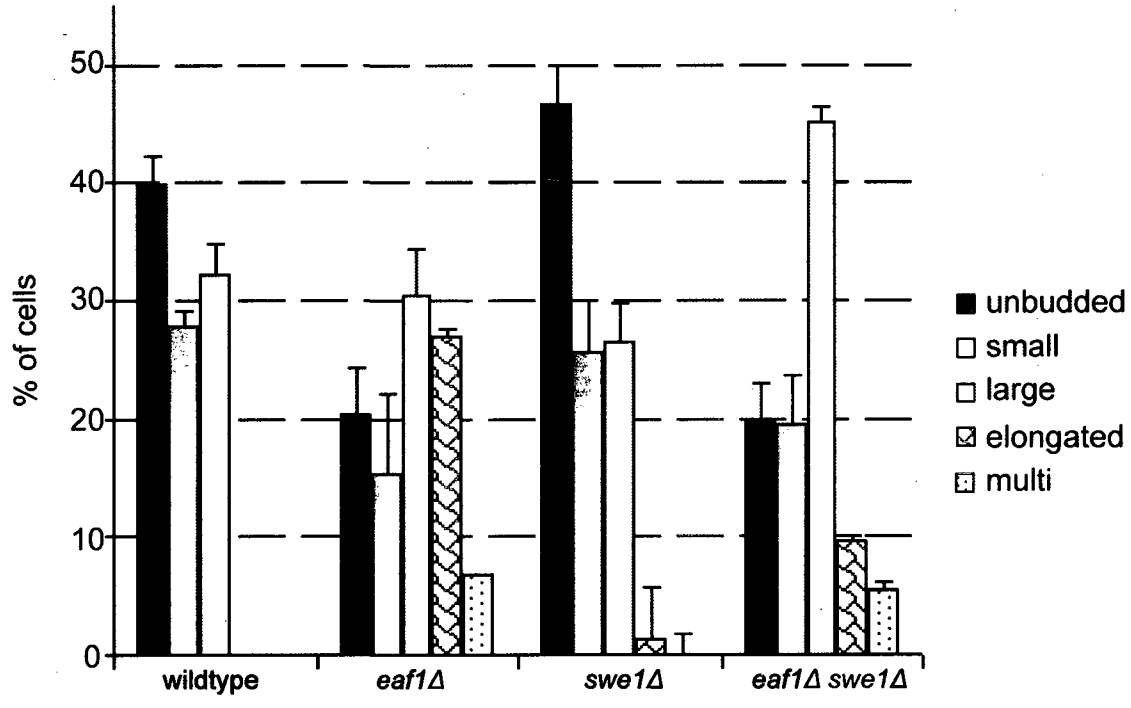

eaf1 mutants exhibit defects in chitin deposition

One important role for septins is the repolarization of the actin cables towards the bud neck, which allows for the vesicle transport of key cell wall and plasma membrane components to the site of cytokinesis. The localization of chitin synthase to the bud neck for the synthesis and deposition of chitin specifically at the site of cytokinesis is dependent on septins (DeMarini et al., 1997). Mutants with defects in septin dynamics normally display defects in chitin deposition at the bud neck (DeMarini et al., 1997). To further investigate whether NuA4 had a potential role in septin organization, I looked at chitin distribution in large-budded wildtype and *eaf1*Δ cells. Calcofluor white was used to stain the chitin in the cells before visualizing under fluorescence microscopy (Figure 9) (Elorza et al., 1983). As expected, greater than 95% of budded wildtype cells displayed concentrated chitin at the bud neck (Norden et al., 2006). In *eaf1*Δ cells, 61% of cells displayed chitin that was distributed to both the mother and daughter cells with no concentration at the bud neck. This indicates that chitin synthase deposition is mislocalized or defective in *eaf1*Δ cells, which may stem from a defect in septin assembly and subsequent disordered actin cables.

Figure 9. *eaf1* mutants exhibit mislocalized chitin deposition. (A) Cells with the indicated genotypes (WT, YKB779; *eaf1*Δ*kanMX*, YKB44) were grown to log-phase at 25°C in YPD, fixed with paraformaldehyde, and stained with calcofluor white before visualization under fluorescence microscopy. The wildtype cells are representative of images of concentrated chitin at the bud neck, while the *eaf1*Δ cells illustrate the diffuse staining (left three panels) or inappropriate chitin deposition in elongated buds. (B) Quantification of cells with concentrated and diffuse chitin deposition in wildtype and *eaf1*Δ strains. A minimum of 100 cells was scored and the experiment was performed in triplicate. The error bars represent standard deviation.

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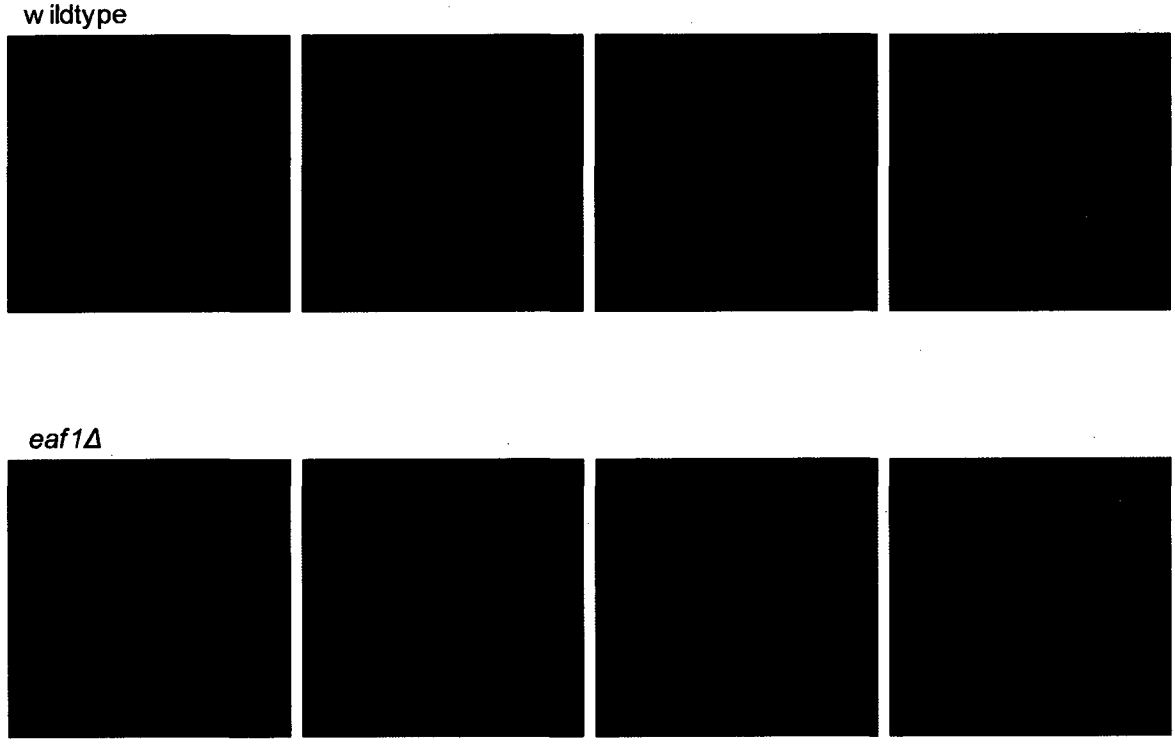
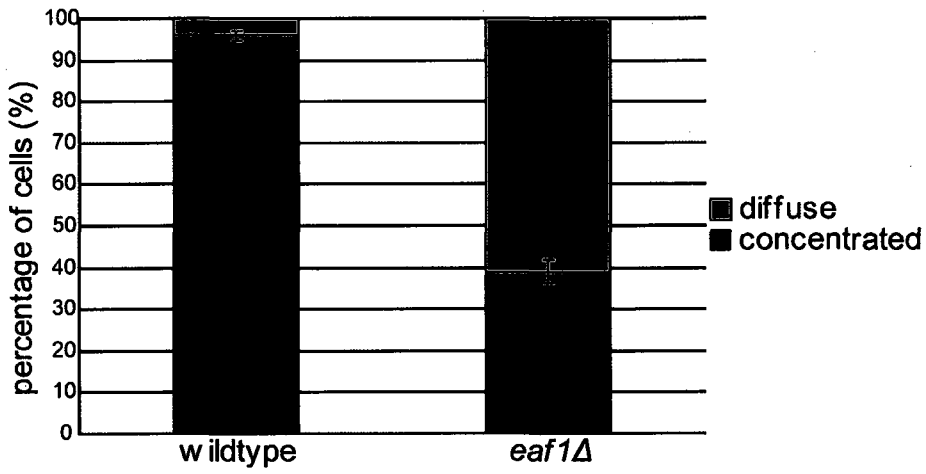

wildtype
eaf1Δ

B


100
90
80
70
60
50
40
30
20
10
0
percentage of cells (%)
wildtype
eaf1Δ
diffuse
concentrated

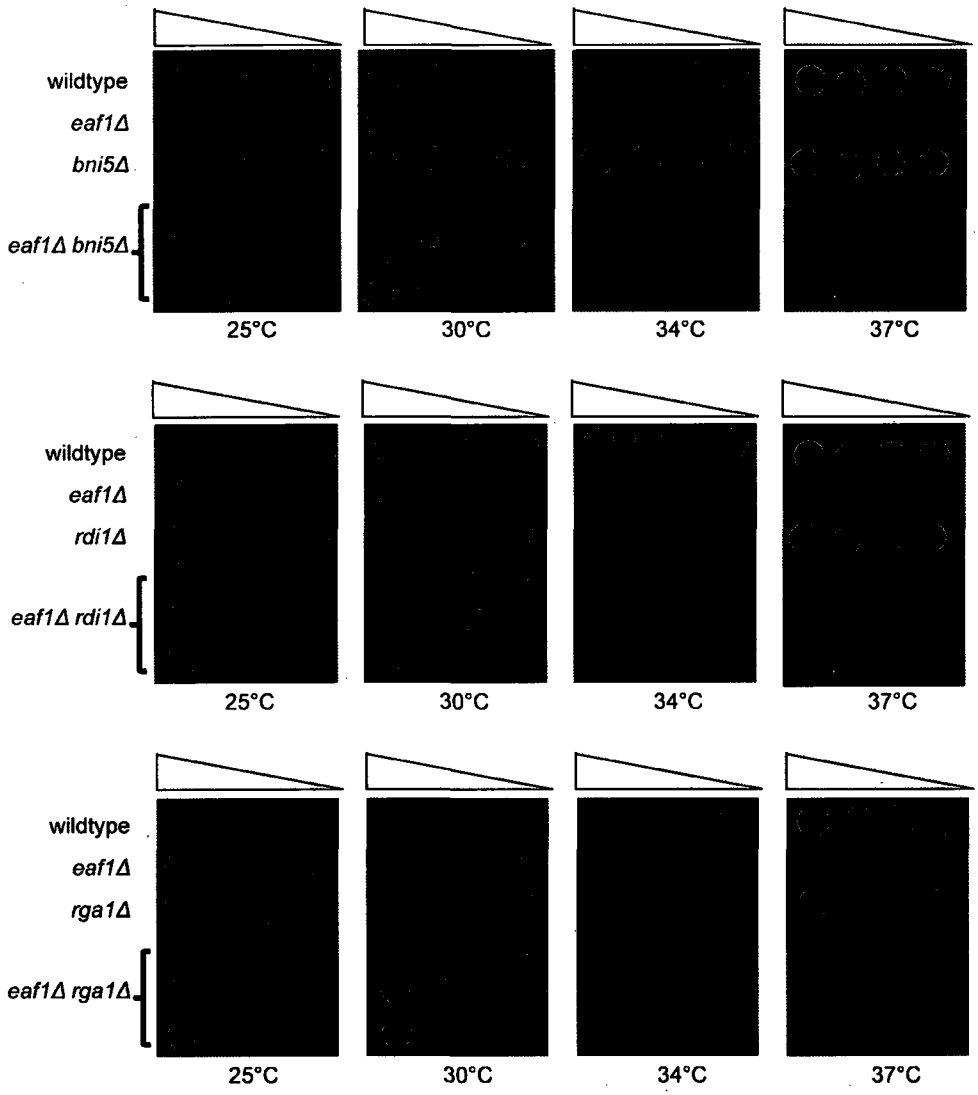
NuA4 may function directly in the septin collar formation pathway

Work performed by Leslie Mitchell, a PhD student in our laboratory, determined that NuA4 mutants have a defect in septin assembly. Using Cdc11-GFP to visualize septin dynamics in live cells, Leslie observed that greater than 25% of *eaf1*Δ cells had defects in establishing the septin collar. I was interested in determining whether NuA4 was functioning in a parallel pathway or within the known septin assembly pathway (Figure 2). If NuA4 was functioning directly in the known pathway, I would predict that combining septin regulator mutants with *eaf1*Δ would not exacerbate the abnormal septin morphologies. In contrast, if NuA4 was functioning in a parallel pathway, I would predict that deletion of the septin regulators would exacerbate the abnormal septin morphologies observed in *eaf1*Δ cells. To do this, I constructed single and double knockout strains of several genes encoding for septin assembly regulators in combination with *eaf1*Δ in order to test for any genetic interactions by spot dilution assays at different temperatures (Figure 10 and Table 1). Only deletions of *BNI5*, *RDI1*, and *RGA1* exhibited very mild synthetic sick interactions with deletion of *EAF1* (Figure 10).

Table 1. Genetic interactions between Eaf1 and septin assembly regulators.

Gene	Genetic interaction with *EAF1*
BEM3	None
BNI1	None
BNI5	Very mild
ELM1	None
GIC1	None
RDI1	Very mild
RGA1	Very mild

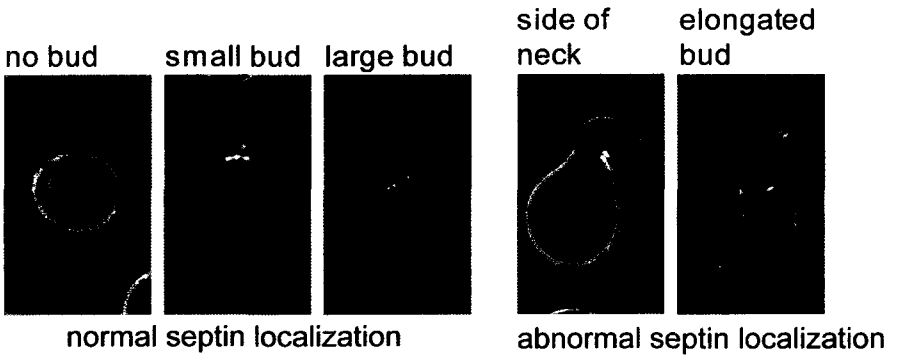
Figure 10. Eaf1 exhibits mild genetic interactions with septin regulators Bni5, Rdi1, and Rga1. Cells with the indicated genotypes (WT, YKB779; *eaf1ΔkanMX*, YKB44; *bni5ΔTRP*, YKB1410; *bni5ΔTRP eaf1ΔkanMX*, YKB1477; *rdi1ΔTRP*, YKB1413; *rdi1ΔTRP eaf1ΔkanMX*, YKB1478; *rga1ΔTRP*, YKB1414; *rga1ΔTRP eaf1ΔkanMX*, YKB1479) were serially diluted 10-fold, replica spotted onto YPD plates and grown at the indicated temperatures.



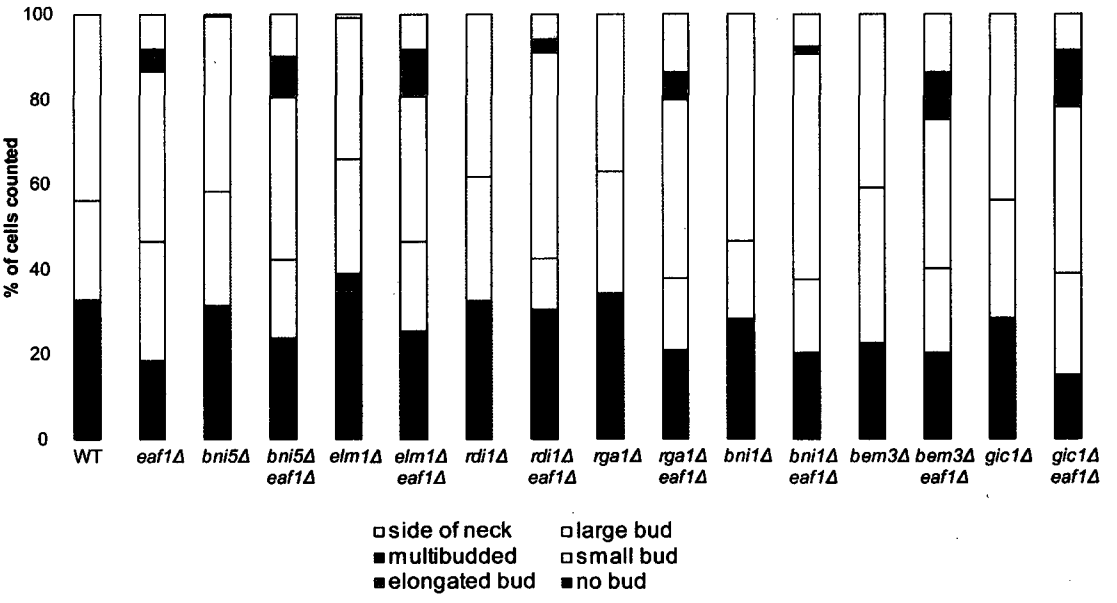
To explore this in more detail, I constructed strains with the same gene knockouts with a Cdc11-GFP fusion protein present in order to observe septin morphology of the cells using fluorescence microscopy (Figure 11A). In agreement with the genetics, there were no dramatic increases in septin abnormalities in the double knockout strains (Figure 11B). This suggests that NuA4 may be functioning directly within the septin assembly pathway.

Figure 11. *eaf1* mutants do not have synthetic lethal phenotypes with genes encoding septin regulators. Cells containing *CDC11-GFP::HIS* with the indicated genotypes (WT, YKB1428, *eaf1ΔkanMX*, YKB1430; *bni5ΔTRP*, YKB1482; *bni5ΔTRP eaf1ΔkanMX*, YKB1483; *elm1ΔTRP*, YKB1484; *elm1ΔTRP eaf1ΔkanMX*, YKB1485; *rdi1ΔTRP*, YKB1486; *rdi1ΔTRP eaf1ΔkanMX*, YKB1487; *rga1ΔTRP*, YKB1488; *rga1ΔTRP eaf1ΔkanMX*, YKB1489; *bni1ΔTRP*, YKB1490; *bni1ΔTRP eaf1ΔkanMX*, YKB1491; *bem3ΔTRP*, YKB1492; *bem3ΔTRP eaf1ΔkanMX*, YKB1493; *gic1ΔTRP*, YKB1494; *gic1ΔTRP eaf1ΔkanMX*, YKB1495) were grown to log phase, fixed, and at least 100 cells were scored according to morphology. (A) Representative images of septin morphologies used in scoring. (B) Quantitative analysis of septin morphologies with the indicated genotypes.

A


no bud
small bud
large bud
side of neck
elongated bud
normal septin localization
abnormal septin localization

B

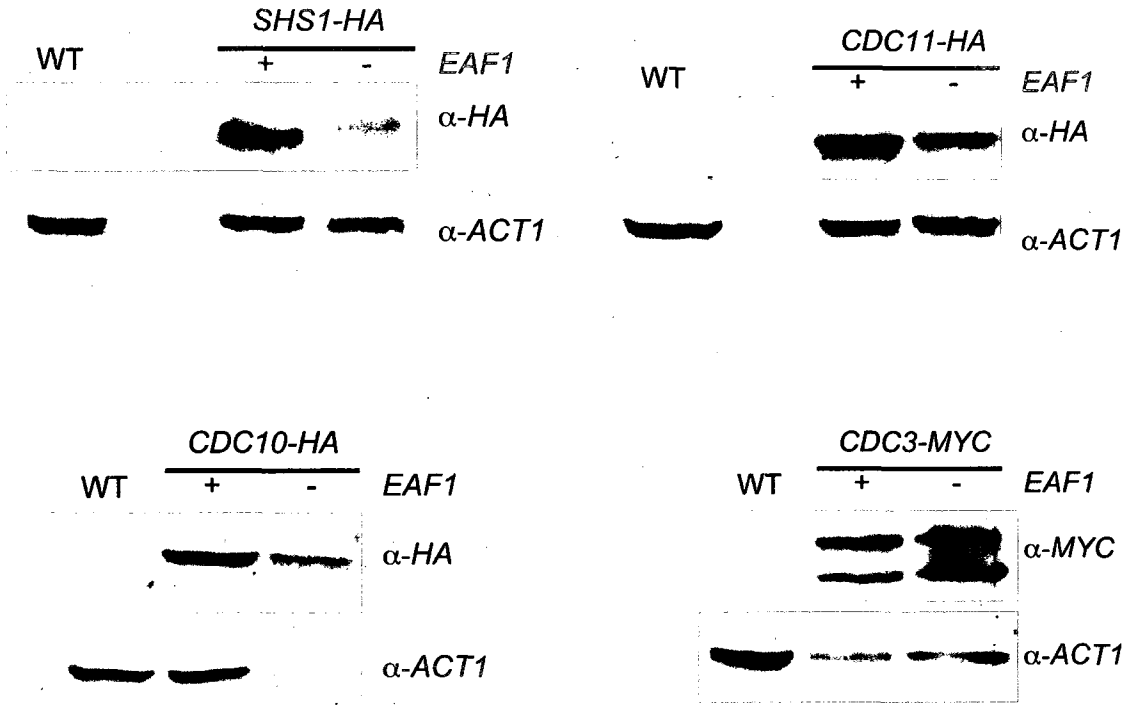

100
80
60
40
20
0
% of cells counted
WT
eaf1Δ
bni5Δ
bni5Δ eaf1Δ
elm1Δ
elm1Δ eaf1Δ
rdi1Δ
rdi1Δ eaf1Δ
rga1Δ
rga1Δ eaf1Δ
bni1Δ
bni1Δ eaf1Δ
bem3Δ
bem3Δ eaf1Δ
gic1Δ
gic1Δ eaf1Δ
side of neck
multibudded
elongated bud
large bud
small bud
no bud

NuA4 may be directly regulating septin stability

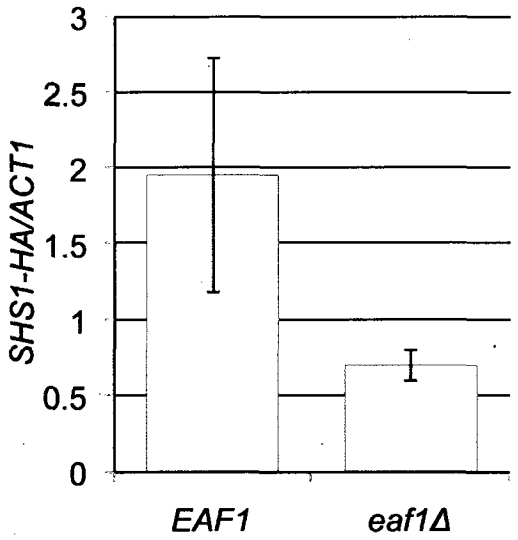
Microarray studies of numerous NuA4 mutants have not revealed changes in the transcripts of septin genes or any genes with established roles in regulating septin dynamics (Choy and Kron, 2002; Durant and Pugh, 2006; Keogh et al., 2005; Krogan et al., 2004; Lindstrom et al., 2006; Masson et al., 2003; Zhang et al., 2004), which suggests that NuA4 may regulate septin collar assembly through acetylation of a non-histone target. Indeed, through a variety of techniques, the Baetz lab has determined that the septin proteins Cdc3, Cdc10, Cdc12 and Shs1 are acetylated *in vivo* and can be acetylated by NuA4 *in vitro* (Baetz Lab, unpublished data). NuA4 acetylation of Yng2 is required to maintain its protein stability (Lin et al., 2008). Therefore, I was interested in exploring whether NuA4 may be regulating septin protein stability through acetylation. To test this hypothesis, I used conventional Western blotting techniques to determine whether deletion of *EAF1* affected septin protein levels. Shs1, Cdc10, and Cdc11 were tagged with HA, while Cdc3 was tagged with c-myc at the C-termini to allow for easy detection with the appropriate antibodies. A Cdc12-tagged strain could not be constructed. The experiment was performed in triplicate, actin was used as a loading control, and bands were quantified using Quantity One software (BioRad). From these experiments, it was found that Shs1-HA protein levels were significantly decreased in *eaf1*Δ cells (Figure 12A and B) while Cdc10-HA, Cdc11-HA and Cdc3-myc protein levels do not appear to be affected (Figure 12A). This suggests that NuA4 may be regulating the protein levels of Shs1.

Figure 12. *eaf1* mutants have lower levels of Shs1. (A) Whole cell extracts were prepared from exponentially growing cells with the following genotypes: WT (YKB779), *SHS1-HA::kanMX* (YKB1498), *SHS1-HA::kanMX eaf1ΔNAT* (YKB1563), *CDC11-HA::kanMX* (YKB1457), Cdc11-HA *eaf1ΔNAT* (YKB1509), *CDC10-HA::kanMX* (YKB1556), *CDC10-HA::kanMX eaf1ΔNAT* (YKB1692), *CDC3-MYC::TRP* (YKB1427), and *CDC3-MYC::TRP eaf1ΔNAT* (YKB1475). Samples were normalized by Bradford assay and subjected to western blot analysis with anti-HA, anti-Myc, or anti-actin antibodies, as indicated on the right side of the panels. (B) Graphical representation of Shs1-HA/actin density ratios as analyzed by Quantity One software. Data are the mean (±standard deviation) of three independent isolates.

A

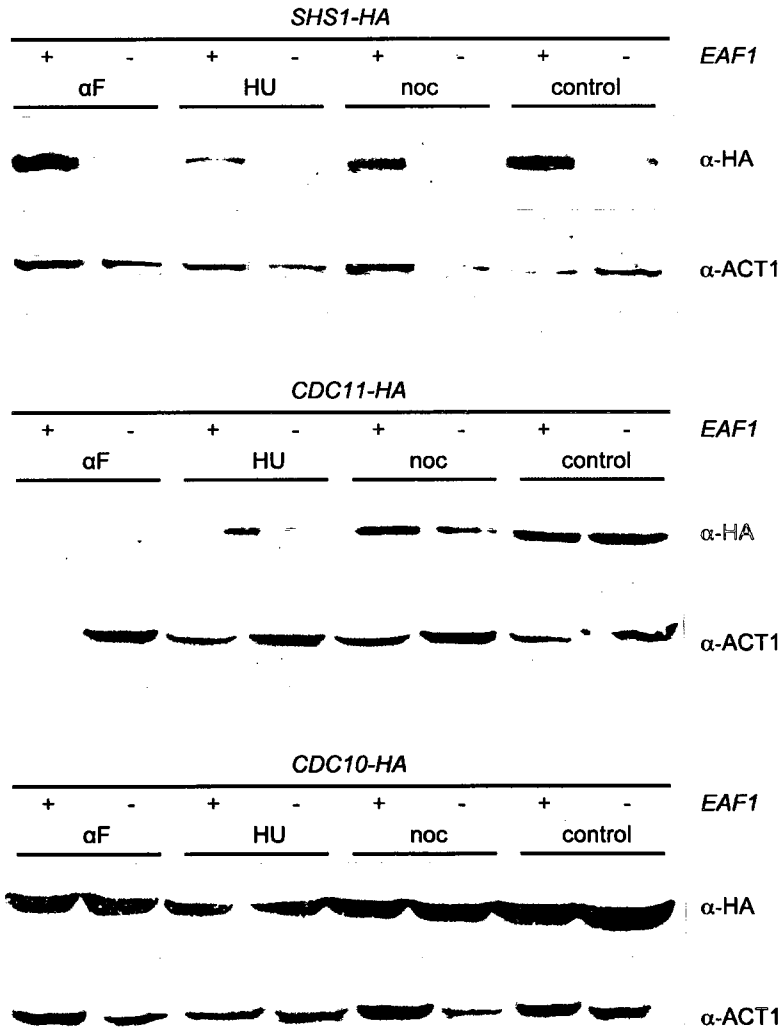

SHS1-HA
WT
+
-
EAF1
α-HA
α-ACT1
CDC11-HA
WT
+
-
EAF1
α-HA
α-ACT1
CDC10-HA
WT
+
-
EAF1
α-HA
α-ACT1
CDC3-MYC
WT
+
-
EAF1
α-MYC
α-ACT1

B


SHS1-HA/ACT1
3
2.5
2
1.5
1
0.5
0
EAF1
eaf1Δ

Since septins are regulated in a cell-cycle dependent manner by Cdc28-mediated cell cycle signals, GTP binding, and Cla4 phosphorylation (Cid et al., 2001; Gladfelter et al., 2005; Longtine and Bi, 2003), the regulation of septin levels by NuA4 may also be cell cycle dependent. In order to test this, I performed cell cycle blocks on three septin-tagged strains: alpha factor for G1; hydroxyurea for S; and nocodazole for G2/M. Western blotting was used to analyze protein levels of Shs1-HA, Cdc11-HA, and Cdc10-HA (Figure 13). Shs1-HA, Cdc10-HA and Cdc11-HA protein levels displayed no differences between cell cycle blocks with or without NuA4. For this reason, the Cdc3-myc strains were not used in these cell cycle block experiments. The regulation of Shs1-HA protein levels by NuA4 does not appear to be cell cycle regulated.

Figure 13. NuA4 function does not impact septin protein levels at various cell cycle stages. Cells with the indicated genotypes (*SHS1-HA::kanMX*, YKB1498; *SHS1-HA::kanMX eaf1ΔNAT*, YKB1563; *CDC11-HA::kanMX*, YKB1457; Cdc11-HA *eaf1ΔNAT*, YKB1509; *CDC10-HA::kanMX*, YKB1556; *CDC10-HA::kanMX eaf1ΔNAT*, YKB1692) were grown to log-phase in YPD before blocking for 3 hours with the indicated reagents (αF, alpha factor; HU, hydroxyurea; noc, nocodazole), and preparing whole cell extracts. Samples were normalized by Bradford assay and subjected to western blot analysis with anti-HA, anti-Myc, or anti-actin antibodies, as indicated on the right side of the panels.


SHS1-HA
+ - + - + - + -
αF HU noc control
EAF1
α-HA
α-ACT1
CDC11-HA
+ - + - + - + -
αF HU noc control
EAF1
α-HA
α-ACT1
CDC10-HA
+ - + - + - + -
αF HU noc control
EAF1
α-HA
α-ACT1

Chapter 4: DISCUSSION

In this study I performed a genome-wide synthetic dosage lethal screen in an attempt to elucidate potential mechanisms behind the role of NuA4 in chromosome stability and cell cycle progression. My screens provided a novel link between NuA4 and cytokinesis – a step of the cell cycle that is tightly controlled to assure genome stability. Further, my secondary analysis confirmed that NuA4 is involved in the septin assembly pathway.

Genome-wide SDL screen reveals diverse cellular roles for NuA4

I performed a genome-wide SDL screen using five non-essential subunits of NuA4 and subsequently directly tested all putative hits against all seven NuA4 non-essential deletion mutants (Figure 5A and Appendix B). The genome-wide SDL screen identified 89 genes that when overexpressed caused growth defects or death in one or more NuA4 deletion mutants. Many lines of evidence indicate that this screen was successful. Importantly, the SDL screen identified genes implicated in cellular processes that NuA4 has established roles in such as such as chromosome stability, transcription, chromatin remodeling, DNA repair, vesicle-mediated transport and the stress response (Doyon and Côté, 2004; Mitchell et al., 2008). This suggests that like SL screens, the SDL screen was successful in identifying pathways relevant to NuA4 function.

Another indicator that the SDL screen was successful is that the number of interactions per NuA4 query directly correlates with the role and importance of the subunit in NuA4 function. *eaf1*Δ exhibited the highest number of SDL interactions, 59, which was expected as it encodes for the structural platform for the NuA4 complex (Figure 1 and Auger et al., 2008; Mitchell et al., 2008). This was similar to what was observed in the NuA4 SL

screen using the same five non-essential mutants, where *eaf1*Δ cells exhibited synthetic sick or lethal genetic interactions with 148 or the 204 deletion mutants in the NuA4 SL genetic network (Mitchell et al., 2008). Hierarchical clustering analysis revealed that the SL genetic profiles of *EAF5*, *EAF7*, and *EAF3* cluster together (Mitchell et al., 2008) and in my SDL screen, *EAF5* and *EAF7* had the highest level of overlap. This is indicative of Eaf5-Eaf7-Eaf working as a subcomplex (Auger et al., 2008; Mitchell et al., 2008). The similarities in the trends seen in both the SL and SDL dataset provide support for the validity and quality of the results from this screen.

Few genome-wide SDL screens have been published to date, and the dataset from these studies were compared to the dataset from my NuA4 SDL screen to determine if there are any trends. I would predict that there should be few common hits among query genes encoding for proteins involved in different cellular processes. Pho85 is a cyclin-dependent kinase that regulates numerous biological functions from cell cycle control to glycogen metabolism via phosphorylation. A Pho85 SDL screen identified 65 genetic interactions (Sopko et al., 2006) of which three were also identified in the NuA4 SDL. Another study performed two genome-wide SDL screens using strains with either both the Cdk1 G1 cyclins *CLN1* and *CLN2* (*cln1*Δ *cln2*Δ) deleted or both the Pho85 G1 cyclins *PCL1* and *PCL2* (*pcl1*Δ *pcl2*Δ) deleted (Zou et al., 2009). Of the 30 and three SDL interactions identified in the respective two cyclin screens, only five were found to be common with the NuA4 SDL screen. Despite NuA4, Pho85, and the cyclins all being implicated in cell cycle progression, their SDL datasets are remarkably distinct, suggesting that genome-wide SDL does identify unique interaction profiles. Further, as it has been shown that deletion and overexpression of genes normally result in markedly different phenotypes due to the differences between loss-of-function and hypermorphic effects (Sopko et al., 2006), I would predict that the NuA4

SDL and SL screens should be distinct. My SDL dataset was compared to a compilation of datasets from previous SL screens performed with the same five non-essential subunits of NuA4 (Hoke et al., 2008; Lin et al., 2008; Mitchell et al., 2008; Pan et al., 2006) in order to determine whether there were any common genes to further validate the results of this screen. Only 7 genes were found in both the SL screens and the SDL screen performed in this study: *HAT2, LEO1, RAD23, RAD50, RPL4A, SLA1,* and *TSA1* (Figure 5C). The limited overlap between the screens mirrors that seen in previous studies (Zou et al., 2009) and indicates that genome-wide SDL screens may be a useful tool for finding novel genetic interactions and potential targets.

A novel role for NuA4 in septin dynamics

My SDL study identified a novel link between NuA4 and septins, the critical players in yeast cytokinesis. Specifically, two of the genes encoding for septins, *SHS1* and *CDC11*, caused slow growth or lethality in most NuA4 non-essential mutants (Figure 7). The strong phenotypes suggested that NuA4 might play a role in regulating septin dynamics and progression through the cell cycle. Indeed, we have shown that *eaf1*Δ cells have a defect in establishing the septin collar (Figure 11 and Leslie Mitchell), which is likely the cause of the activation of the morphogenesis checkpoint and hyper-elongated buds (Figure 8). Though deletion of *SWE1* partially rescued the growth and temperature sensitivity seen in *eaf1*Δ strains (Figure 8A) and dramatically reduced the elongated neck morphology characteristic of *eaf1*Δ cells (Figure 8B), it did not decrease the high proportion of *eaf1*Δ cells found in the G2/M phase of the cell cycle (Figure 8B). This suggests that while NuA4 plays an important role in regulating septin dynamics, NuA4 is regulating additional pathways or targets that are required to traverse the G2/M stage of the cell cycle.

How is NuA4 regulating septin dynamics? The assembly of the septin ring involves GTP loading and hydrolysis by the Rho-GTPase Cdc42 (Gladfelter et al., 2002). Cdc42 itself is regulated by a host of GTPase-activating proteins (GAPs) and other factors (Figure 2). In addition to Cdc42, there are other regulators that directly interact with the septins and regulate their assembly. Genetic studies did not show any strong interactions between Eaf1 and the septin regulators (Figure 10, Table 1), suggesting that NuA4 is functioning directly within the septin assembly pathway. When Cdc11-GFP morphology was analyzed in these strains, there did not appear to be any major differences in the proportion of cells with abnormal septin morphology between *eaf1*Δ and double mutant strains (Figure 11B). As no additive effects were detected, this experiment was performed once. The relationship between septin regulators appears to be more complicated than originally thought, as some septin regulator mutants that function within a single pathway display additive of synergistic effects when combined (Gladfelter et al., 2004). This suggested that there is a significant amount of cross talk between parallel pathways that regulate septin assembly. Therefore, NuA4 may be acting directly on the septins or on any one of the septin regulators to maintain proper septin assembly.

Indeed, our lab has shown that septin proteins are acetylated *in vivo* and that NuA4 can acetylate the septins *in vitro* (data not shown). Further, a recent large-scale proteomic study has shown that acetylation of septins is conserved in man (Choudhary et al., 2009). Since NuA4 acetylation has been known to regulate stability of its own subunit Yng2 (Lin et al., 2008), it is possible that NuA4 is regulating septin stability in the same way. To assess whether NuA4 is involved in regulating septin stability, Western blots were used to quantify the amounts of tagged septins in wildtype and *eaf1*Δ strains in log phase (Figure 12A) or at various stages of the cell cycle (Figure 13). Only levels of Shs1-HA appeared to be

significantly affected by NuA4 (Figure 12B). As microarray studies do not reveal changing septin transcript levels in NuA4 mutants (Choy and Kron, 2002; Durant and Pugh, 2006; Keogh et al., 2005; Krogan et al., 2004; Masson et al., 2003; Zhang et al., 2004), it suggests that NuA4 acetylation of Shs1 may regulate its protein stability. Presently, point mutants at the identified Shs1 acetylation sites are being made to test this hypothesis.

Could decreased levels of Shs1 account for the septin defects seen in *eaf1*Δ cells? Shs1 is not essential in most strain backgrounds nor do mutants display abnormal septin collars or elongated buds (Iwase et al., 2007). Shs1 does not appear to be required for assembly of the septin complex in wildtype cells, making it unlikely that decreased levels of this subunit is the explanation for all the septin defects seen in *eaf1*Δ cells (Iwase et al., 2007). The remaining mitotic septins (Cdc3, Cdc10, Cdc11, and Cdc12) are essential in budding yeast and form the basic octameric rod structures that assemble to form septin filaments during cytokinesis (Bertin et al., 2008). Due to their similar primary structures, it has been hypothesized that Shs1 can replace Cdc11 in the rod structure to generate septin filaments that are able to recruit unique proteins important for yeast cell morphogenesis (Iwase et al., 2007). The reason that this may be the case is because Shs1 subjected to more post-translational modifications such as phosphorylation and sumoylation than the other septin subunits and some of these modifications have been shown to impact septin dynamics (Dobbelaerer et al., 2003). Recently, a study demonstrated that Shs1 is also regulated by G1 cyclin-dependent kinases (Egelhofer et al., 2008). Phosphorylation of Shs1 by these kinases decreased its interaction with the Gin4 kinase, which is required for septin dynamics during G2/M. Therefore, it is possible that acetylation of Shs1 might be regulating its interactions with the other septins and proteins. Another possibility is that acetylation of Shs1 may cross

talk with the other post-translational modifications, either enhancing or decreasing the amount of phosphorylation or sumoylation.

Alternatively, acetylation of the other septin proteins may be contributing the role of NuA4 in septin dynamics. Though NuA4 function is not regulating their protein levels, acetylation may be regulating structure dynamics or interactions with other proteins. As all yeast septins have been shown to be heavily modified by phosphorylation and/or sumoylation (Johnson and Gupta, 2001; Versele and Thorner, 2004), acetylation may regulate these modifications or vice versa. Further study will be required to determine the biological significance of the conserved septin acetylation sites. Another possibility to explain the role of NuA4 in septin dynamics is that NuA4 maybe acetylating other proteins of the septin assembly pathway. Recently, protein array studies found that Bni5 was a putative *in vitro* substrate of NuA4 (Lin et al., 2009). Bni5 is a septin regulator that interacts with Cdc11 and is required for establishment of the septin collar by an unknown mechanism (Lee et al., 2002). Lee et al reported that *bni5*Δ cells form long, multi-nucleated bud chains where the cytoplasm is still connected as the septum has not formed and septin proteins are forming discrete bars that run parallel down the length of the bud neck. It is important to note that I did not detect these phenotypes in a *bni5*Δ in our strain background (Figure 11B). Further, the reported phenotypes for *bni5*Δ mutants have not been detected in NuA4 mutants, which suggest that Bni5 may not be the key target of NuA4 in regulating septin function. Alternatively, defective acetylation of Bni5 may not cause the same phenotypes as a *bni5*Δ strain. Though it has yet to be shown as an *in vivo* target of NuA4, Bni5 along with other regulators of septin dynamics may be targets of NuA4 and mediate the role of NuA4 in septin dynamics. Systematically assessing the acetylation state of bud neck associated

proteins and septin regulators may provide a means to understanding the role of NuA4 in septin dynamics.

Conclusions

The objective of this study was to identify cellular processes requiring NuA4 using a synthetic dosage lethal screen. The results suggest a link between NuA4 and yeast cytokinesis via involvement in the septin assembly pathway. The link between NuA4 and these processes may provide an explanation to its known role in maintaining chromosome stability and in cell cycle progression. The NuA4 SDL genetic interaction map provides only a starting point for elucidating additional mechanisms behind NuA4 function in the cell. In addition, my septin work clearly shows a novel role for NuA4 during the cell cycle and opens a new area of research – septin acetylation.

Future Directions

My NuA4 SDL interaction map provided a base from which to explore several facets of NuA4 function. There are several other pathways that have yet to be probed and dissected to further enrich the roles of this multifunctional lysine acetyltransferase. Below I discuss three avenues of research that I am most interested in or have preliminary data for.

NuA4, GLC7 and spindle dynamics

Another intriguing result from the NuA4 SDL screen was that Glc7 and several of its regulators were found to exhibit SDL interactions with NuA4 mutants, namely *EAF1*. Glc7 is a phosphatase that through the interaction with its various regulators, impacts a myriad of biological processes, including promoting exit from the spindle checkpoint by dephosphorylating Ipl1 kinase kinetochore targets (Pinsky et al., 2009). *SDS22* and *YPI1* are

genes that exhibited SDL interactions with *EAF1*, and both are Glc7 regulators that help localize it to the nucleus of the cell and promote its spindle checkpoint role (Bharucha et al., 2008). The link between NuA4 and Glc7 could potentially explain why NuA4 plays such a critical role in maintaining chromosome stability, as proper spindle checkpoint function is required for accurate propagation of chromosomes (Keogh et al., 2006; Krogan et al., 2004). Genetic experiments using Ipl1 and Glc7 mutants showed mild interactions with *EAF1*, (Appendix B Figure 1 and Table 1), but further study would be needed to determine the role of NuA4 in the spindle checkpoint.

NuA4, Glc7 and glycogen

Glc7 also plays roles in glycogen metabolism. Gac1 is a Glc7 regulator that is able to allow Glc7 to control the phosphorylation state of glycogen synthase, Gsy2 (Wu et al., 2001). Both *GAC1* and *GSY2* were identified in the SDL screen, but this may not be due solely to NuA4 regulation of Glc7 function. Indeed, NuA4 may be able to regulate transcription of *GSY2* through the binding and repression of the Msn2 and Msn4 stress response transcription factors, which promote the transcription of *GSY2* (Boy-Marcotte et al., 1998). It was hypothesized that deletion of *EAF1* would cause hyperaccumulation of glycogen due to derepression of the Msn2/4 transcription factors, and this was indeed the case as indicated by iodine staining of an *eaf1*Δ strain (Figure 6). This excess accumulation of glycogen was abolished by a subsequent deletion of both Msn2/4 transcription factors, suggesting that it was due to increased transcription of *GSY2*. This may also provide an explanation for the several glycogen-related genes that appeared in the NuA4 SDL screen; perturbations in glycogen levels would exacerbate the already-present excessive amounts of glycogen present in *eaf1*Δ strains. To determine whether this is the case, a *gsy2*Δ *eaf1*Δ strain could be constructed which should exhibit less glycogen accumulation, and the plasmids

containing the glycogen-related genes can be transformed into this strain to determine whether they would still cause slow growth or lethality when overexpressed. NuA4 may also be regulating glycogen metabolism through direct acetylation of glycogen-related targets, as Gsy2 was identified as a putative *in vitro* NuA4 substrate (Lin et al., 2009).

NuA4, chitin and protein trafficking

Yeast septins reorganize the actin cables within the cell during cytokinesis so that plasma membrane and cell wall materials can be transported to the bud neck (Gladfelter et al., 2005). In addition, proper septin organization is critical for the localization of chitin synthase 3, Chs3, the main enzyme responsible for the synthesis of chitin, which is the major component of the yeast cell wall (DeMarini et al., 1997). *CHS3* was a hit in the NuA4 SDL screen as well as *BNI4*, the gene encoding for a Glc7 regulator that is also responsible for tethering Chs3 to the bud neck (Kozubowski et al., 2003). In order to further examine the role of NuA4 in septin function, chitin localization was visualized in wildtype and *eaf1*Δ cells by calcofluor white stain (Figure 9A). In wildtype cells, the majority of the chitin is deposited at the bud neck during cytokinesis, which is what would be expected during normal cytokinesis. In *eaf1*Δ cells, however, the chitin was diffusely localized throughout both mother and daughter cells in over 50% of scored cells, which suggests that Chs3 is mislocalized, potentially due to a septin malfunction. Alternately, NuA4 may be regulating chitin deposition through acetylation of other targets. Chitin synthase 5, Chs5, was identified as a putative *in vitro* substrate of NuA4 (Lin et al., 2009). Chs5 is a component of the exomer complex that is required for the proper transport of Chs3 from the Golgi network to the plasma membrane (Sanchatjate and Schekman, 2006; Santos et al., 1997). One possibility is that NuA4 acetylation of Chs5 regulates its protein transport function; hence mutants of NuA4 may result in mislocalization of Chs3 and consequently diffuse chitin deposition.

Indeed, while only 20-30% of *eaf1*Δ mutants display defects in septin morphology, greater than 50% of mutant cells display diffuse chitin deposition. This suggests that the role of NuA4 in chitin deposition may indeed not be solely due to septin dynamics. Intriguingly, a role for NuA4 in protein transport has already been established (Mitchell et al., 2008). Combined, these studies suggest that NuA4 may potentially play a pivotal role in protein trafficking. It will be interesting to determine if defects in protein trafficking are the secondary result of septin dynamics and actin cytoskeleton, or if NuA4 has direct targets in these pathways, such as Chs5.

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Appendix A. Yeast strains used in this study

NuA4 mutant strains mated with OEA

Strain	Auxotrophies	Reference
YKB731	*MATα can1Δ::STE2pr-Sp-his5 lyp1Δ his 3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+*	Gift from C. Boone
YKB622	*MATα can1Δ::STE2pr-Sp-his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+, eaf1Δ::NAT*	(Krogan et al., 2004)
YKB995	*MATα can1Δ::STE2pr-Sp-his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+, eaf3Δ::NAT*	(Mitchell et al., 2008)
YKB852	*MATα can1Δ::STE2pr-Sp-his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+, eaf5Δ::NAT*	(Krogan et al., 2004)
YKB623	*MATα can1Δ::STE2pr-Sp-his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+, eaf6Δ::NAT*	Gift from J. Greenblatt
YKB853	*MATα can1Δ::STE2pr-Sp-his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+, eaf7Δ::NAT*	(Krogan et al., 2004)

NuA4 mutant strains used for confirmations

Strain	Auxotrophies	Reference
YKB779	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	(Sikorski and Hieter, 1989)
YKB44	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, eaf1Δ::kanMX*	(Mitchell et al., 2008)
YKB1162	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, eaf3Δ::kanMX*	This study
YKB658	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, eaf5Δ::kanMX*	(Mitchell et al., 2008)
YKB504	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, eaf6Δ::kanMX*	This study
YKB530	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, eaf7Δ::kanMX*	This study
YKB494	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, yng2Δ::kanMX*	(Mitchell et al., 2008)
YKB464	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, yaf9Δ::kanMX*	(Mitchell et al., 2008)

Msn2/4 mutant strain

Strain	Auxotrophies	Reference
YKB1097	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 msn2Δ::TRP msn4Δ::kanMX eaf1Δ::kanMX*	(Mitchell et al., 2008)

Septin SDL strains

Strain	Auxotrophies	Reference
YKB1808	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 pGAL1-CDC10*	This study
YKB1809	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 pGAL1-SHS1*	This study

YKB1810	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 pGAL1-CDC11*	This study
YKB1811	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 pGAL1-CDC12*	This study
YKB1812	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 pRS426*	This study
YKB1813	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf1Δ::NAT pGAL1-CDC10*	This study
YKB1814	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf1Δ::NAT pGAL1-SHS1*	This study
YKB1815	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf1Δ::NAT pGAL1-CDC11*	This study
YKB1816	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf1Δ::NAT pGAL1-CDC12*	This study
YKB1817	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf1Δ::NAT pRS426*	This study
YKB1818	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yng2Δ::kanMX pGAL1-CDC10*	This study
YKB1819	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yng2Δ::kanMX pGAL1-SHS1*	This study
YKB1820	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yng2Δ::kanMX pGAL1-CDC11*	This study
YKB1821	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yng2Δ::kanMX pGAL1-CDC12*	This study
YKB1822	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yng2Δ::kanMX pRS426*	This study
YKB1823	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yaf9Δ::kanMX pGAL1-CDC10*	This study
YKB1824	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yaf9Δ::kanMX pGAL1-SHS1*	This study
YKB1825	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yaf9Δ::kanMX pGAL1-CDC11*	This study
YKB1826	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yaf9Δ::kanMX pGAL1-CDC12*	This study
YKB1827	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 yaf9Δ::kanMX pRS426*	This study
YKB1828	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf3Δ::kanMX pGAL1-CDC10*	This study
YKB1829	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf3Δ::kanMX pGAL1-SHS1*	This study
YKB1830	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf3Δ::kanMX pGAL1-CDC11*	This study
YKB1831	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf3Δ::kanMX pGAL1-CDC12*	This study
YKB1832	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf3Δ::kanMX pRS426*	This study
YKB1833	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf5Δ::kanMX pGAL1-CDC10*	This study
YKB1834	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf5Δ::kanMX pGAL1-SHS1*	This study
YKB1835	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf5Δ::kanMX pGAL1-CDC11*	This study

YKB1836	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf5Δ::kanMX pGAL1-CDC12*	This study
YKB1837	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf5Δ::kanMX pRS426*	This study
YKB1838	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf6Δ::kanMX pGAL1-CDC10*	This study
YKB1839	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf6Δ::kanMX pGAL1-SHS1*	This study
YKB1840	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf6Δ::kanMX pGAL1-CDC11*	This study
YKB1841	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf6Δ::kanMX pGAL1-CDC12*	This study
YKB1842	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf6Δ::kanMX pRS426*	This study
YKB1843	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf7Δ::kanMX pGAL1-CDC10*	This study
YKB1844	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf7Δ::kanMX pGAL1-SHS1*	This study
YKB1845	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf7Δ::kanMX pGAL1-CDC11*	This study
YKB1846	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf7Δ::kanMX pGAL1-CDC12*	This study
YKB1847	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 eaf7Δ::kanMX pRS426*	This study

Swe1 strains

Strain	Auxotrophies	Reference
YKB1807	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, swe1Δ::kanMX*	From DMA library
YKB1266	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, swe1Δ::kanMX eaf1Δ::kanMX*	This study

septin regulator knockouts

Strain	Auxotrophies	Reference
YKB1410	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, bni5Δ::TRP*	This study
YKB1477	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, bni5Δ::TRP eaf1Δ::kanMX*	This study
YKB1412	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, elm1Δ::TRP*	This study
YKB1476	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, elm1Δ::TRP eaf1Δ::kanMX*	This study
YKB1413	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, rdi1Δ::TRP*	This study
YKB1478	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, rdi1Δ::TRP eaf1Δ::kanMX*	This study
YKB1414	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, rga1Δ::TRP*	This study
YKB1479	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1,*	This study

	rga1Δ::TRP eaf1Δ::kanMX	
YKB1416	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, bni1Δ::TRP*	This study
YKB1480	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, bni1Δ::TRP eaf1Δ::kanMX*	This study
YKB1417	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, bem3Δ::TRP*	This study
YKB1496	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, bem3Δ::TRP eaf1Δ::kanMX*	This study
YKB1418	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, gic1Δ::TRP*	This study
YKB1481	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, gic2Δ::TRP eaf1Δ::kanMX*	This study

Cdc11-GFP strains and septin regulator knockouts

Strain	**Auxotrophies**	**Reference**
YKB1428	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS*	This study
YKB1430	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS eaf1Δ::kanMX*	This study
YKB1482	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS bni5Δ::TRP*	This study
YKB1483	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS bni5Δ::TRP eaf1Δ::kanMX*	This study
YKB1484	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS elm1Δ::TRP*	This study
YKB1485	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS elm1Δ::TRP eaf1Δ::kanMX*	This study
YKB1486	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS rdi1Δ::TRP*	This study
YKB1487	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS rdi1Δ::TRP eaf1Δ::kanMX*	This study
YKB1488	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS rga1Δ::TRP*	This study
YKB1489	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS rga1Δ::TRP eaf1Δ::kanMX*	This study
YKB1490	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS bni1Δ::TRP*	This study
YKB1491	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS bni1Δ::TRP eaf1Δ::kanMX*	This study
YKB1492	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS bem3Δ::TRP*	This study
YKB1493	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS bem3Δ::TRP eaf1Δ::kanMX*	This study
YKB1494	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS gic1Δ::TRP*	This study
YKB1495	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-GFP::HIS gic2Δ::TRP eaf1Δ::kanMX*	This study

Septin-tagged strains

Strain	Auxotrophies	Reference
YKB1498	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, SHS1-HA::kanMX*	This study
YKB1563	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, SHS1-HA::kanMX eaf1Δ::NAT*	This study
YKB1457	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-HA::kanMX*	This study
YKB1509	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC11-HA::kanMX eaf1Δ::NAT*	This study
YKB1556	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC10-HA::kanMX*	This study
YKB1692	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC10-HA::kanMX eaf1Δ::NAT*	This study
YKB1427	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC3-MYC::TRP*	This study
YKB1475	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, CDC3-MYC::TRP eaf1Δ::kanMX*	This study

Glc7 and Ipl1 strains

Strain	Auxotrophies	Reference
YKB1351	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, ipl1-321*	This study, (Biggins et al., 1999)
YKB1358	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, ipl1-321 eaf1Δ::kanMX*	This study
YKB1352	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, ipl1ΔkanMX::ipl1-315::LEU2*	This study, (Kotwaliwale, Frei, Stern, and Biggins, 2007)
YKB1360	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, ipl1ΔKanMX::ipl1-315::LEU2 eaf1Δ::kanMX*	This study
YKB1353	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, glc7-10::TRP*	This study, (Sassoon et al., 1999)
YKB1362	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1, glc7-10::TRP eaf1Δ::kanMX*	This study

Appendix B. Summary of NuA4 SDL scoring

Interacting Gene		NuA4 Subunit							
		WT	EAF1	EAF3	EAF5	EAF6	EAF7	YAF9	YNG2
Systematic Name	Standard Name	YKB 779	YKB 44	YKB 1162	YKB 658	YKB 504	YKB 530	YKB 464	YKB 494
Chromatin									
YOR123C	*LEO1*					2	1		
YPR193C	*HPA2*		0						
YEL056W	*HAT2*						1		
Chromosome Segregation and Stability									
Glc7-related									
YER133W	*GLC7*		0						
YBR050C	*REG2*		1		3		3		
YKL193C	*SDS22*		0			2.5			S+
YFR003C	*YPI1*		1						
YNL233W	*BNI4*	3	1				1		
YAR014C	*BUD14*	3*	0						
YER054C	*GIP2*	3	1	3		3	2		
YOR178C	*GAC1*		1				4		
Glycogen Metabolism									
YER059W	*PCL6*		1						
YLR258W	*GSY2*		1						
YPL219W	*PCL8*		2						
DNA Damage									
YML021C	*UNG1*	3	1						
YOL043C	*NTG2*	4	1						
YBL009W	*ALK2*	*	0+		0		1.5		S+
YEL037C	*RAD23*					2			
YNL250W	*RAD50*		2						
Cytoskeleton									
YDR106W	*ARP10*		0						
YFL039C	*ACT1*	2	0+		0			S+	S+
YBR234C	*ARC40*		2						
YDR388W	*RVS167*			S+			2		
YBR059C	*AKL1*	2.5*	1		2.5		1		S+
YBL007C	*SLA1*		0		0	S+			
YDR183W	*PLP1*		2						
YOL136C	*PFK27*		0		1.5			S+	
YJR076C	*CDC11*	3	0		2	1	2	2	0
YDL225W	*SHS1*	3	0	2	1		2	2	0
YBR038W	*CHS2*		1						
YBR023C	*CHS3*		2						

Transport									
YLR078C	*BOS1*		0						S+
YDR498C	*SEC20*		2						
YGL198W	*YIP4*		2	S+					
YPL145C	*KES1*	*			0.5		1		0+
YBR172C	*SMY2*	*	S+		0		1.5		S+
YAL031C	*SNC1*	3*	1		1		1.5		S+
YFL040W	YFL040W	*			1				S+
YDL210W	*UGA4*	3				1.5			S+
Ubiquitin & Proteosome									
YML088W	*UFO1*	3.5				2			S+
YOR261C	*RPN8*	3.5	0+	1	0	1	0		0+
Cell Cycle									
YOR083W	*WHI5*		0						
YLR394W	*CST9*		0					S+	S+
Mitochondria									
YGR132C	*PHB1*					2			
YBR026C	*ETR1*		0						S+
YAL048C	*GEM1*						0		S+
YDR326C	*YSP2*		2		2.5		1.5		
Transcription & RNA Processing									
YMR268C	*PRP24*	3	1						S+
YGR013W	*SNU71*					1	1.5	S+	
YCL011C	*GBP2*		0+			2.5			
YBL093C	*ROX3*		S+				0		S+
YGL130W	*CEG1*					2.5			S+
YPR144C	*NOC4*		0+		1				
YIR015W	*RPR2*		1						
YDL036C	*PUS9*	3	0	1	1.5				
YDR253C	*MET32*					2			
Translation									
YBR031W	*RPL4A*						1.5		
YJL138C	*TIF2*						2		
YBL054W	YBL054W		S+				2		
YLR387C	*REH1*	3	1						S+
Stress Response									
YML028W	*TSA1*		1						
YIL010W	*DOT5*					2.5		S+	S+
YKL062W	*MSN4*	*			2	2			
YPL240C	*HSP82*						2		
YOR008C	*SLG1*	*			1		2	S+	S+
Metabolism & Biosynthesis									
YBR265W	*TSC10*						2		S+

YOR245C	*DGA1*						2		
YDR503C	*LPP1*		0						S+
YOR241W	*MET7*		2						
YBR243C	*ALG7*	3	0						S+
YBL036C	YBL036C						2		
YBR183W	*YPC1*						1.5		
Unknown									
YBR262C	*AIM5*						2		
YDR290W	YDR290W		0						
YOR392W	YOR392W						1		
YFL012W	YFL012W		2						S+
YLL059C	YLL059C		2			S+			
YAR066W	YAR066W		0				1		S+
YFL010C	*WWM1*		0		0			S+	S+
YPL067C	YPL067C						1		0+
YCR051W	YCR051W					2			
YDL129W	YDL129W	*			1.5		1.5	S+	
YNL058C	YNL058C	3	0						S+
YDR068W	*DOS2*						2		
YML018C	YML018C		1						S+
YNL042W	*BOP3*					2	1		
YKL219W	*COS9*		2						
YCR023C	YCR023C		0+				2		
YKL168C	*KKQ8*	2	0						

Legend

*	caused slow growth in wildtype as determined by Sopko et al., 2006
S+	slow growth by streak test
0+	inviable by streak test

Scoring

blank - no growth defect
3 - minor growth defect
2 - medium growth defect
1 - major growth defect
0 - inviable

Appendix C. Glc7/Ipl1 genetic experiments

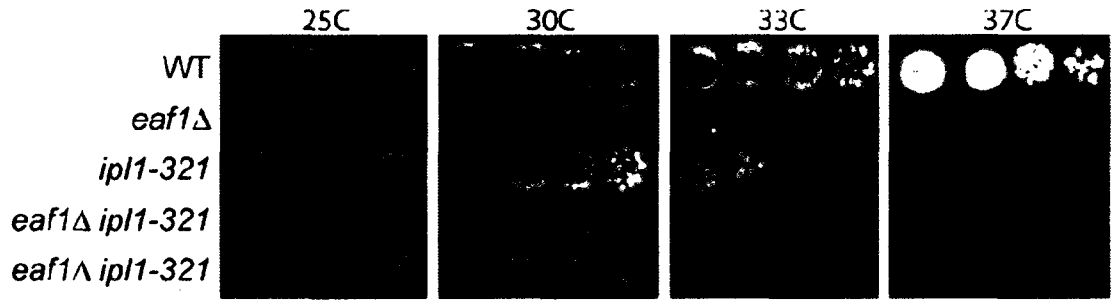

Figure 1. Eaf1 exhibits mild genetic interactions with the *ipl1-321* mutant. Yeast strains with the indicated genotypes (WT, YKB779; *eaf1ΔkanMX*, YKB44; *ipl1-321*, YKB1351; *ipl1-321 eaf1Δ::kanMX*, YKB1358) were serially diluted 10-fold, replica spotted onto YPD plates and grown at the indicated temperatures.

Table 1. Genetic interactions of *eaf1Δ* and Glc7/Ipl1 mutants.

Mutant genotype	**Phenotype**
ipl1-315 eaf1Δ	Viable
ipl1-321 eaf1Δ	Sickness
glc7-10 eaf1Δ	Suppressed
pGAL-*GLC7 eaf1Δ*	Sickness

ANDREA LAU

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University of Ottawa, Ottawa, ON — 2007 - 2009
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Bachelor of Science (Honours) in Biochemistry with Distinction

PUBLICATIONS AND CONFERENCES

Progress in Systems Biology, Ottawa, ON — 2009
Poster Presentation

- Synthetic dosage lethal (SDL) analysis of Yeast NuA4. **Andrea Lau**, Leslie Mitchell, Jean-Philippe Lambert, Ying Fong, Daniel Figeys, and Kristin Baetz. Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, Ontario, Canada.

International Conference on Yeast Genetics and Molecular Biology, Toronto, ON — 2008
Poster Presentation

- Synthetic dosage lethal (SDL) analysis of NuA4. **Andrea Y.T. Lau**, Ying Fong, Leslie Mitchell, Kristin Baetz. Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, Ontario, Canada.

Queen's University Department of Biochemistry, Kingston, ON — 2008
Published Article

- Rothnie, A., Conseil, G., **Lau, A.Y.T.**, Deeley, R.G., and Cole, S.P.C. (2008). Mechanistic differences between GSH transport by multidrug resistance protein 1 (MRP1/ABCC1) and GSH modulation of MRP1-mediated Transport. Molecular Pharmacology 74: 1630-1640.

AWARDS AND ACHIEVEMENTS

NSERC Alexander Graham Bell Canadian Graduate Scholarship — 2007 - 2009

- $17500 per annum, 2 year term

University of Ottawa SAD Award — 2007 - 2008

- $3000, strategic areas of development award

University of Ottawa Excellence Scholarship — 2007 - 2009

- Cost of tuition, awarded to students that have received external scholarships

NSERC Undergraduate Student Research Award — 2005

- Awarded to students conducting research for a summer at any university

Queen's University Dean's Honour List — 2003 - 2007

- Recognizes students that achieve a cumulative average over 80%

Queen's University Entrance Honours with Merit — 2003

- Recognizes students with admissions average over 90%