Characterizing the role of Gds1p in the *Saccharomyces cerevisiae* environmental stress response

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ABSTRACT

The transcription factors Msn2p and Msn4p are major components of the *Saccharomyces cerevisiae* environmental stress response. Their transient activation/deactivation is dependent on a regulatory network centering on nucleocytoplasmic shuttling but also includes a range of other mechanisms. The acetyltransferase complex, NuA4 has been implicated in repression of Msn2p yet the mechanism is largely unknown. Gds1p is an uncharacterized yeast protein identified in a recent study as a physical interactor and acetylation target of NuA4. Gds1 protein level is dependant both on NuA4 and environmental stress and our analysis shows it to be involved in the nuclear exclusion of Msn2p in the absence of stress. Unstressed cells lacking *GDS1* exhibit increased nuclear accumulation Msn2p and an increase in transcription of the stress reporter gene, *HSP12*. My work supports a model in which Gds1 and NuA4 can work independently to inhibit the Msn2/4 dependant yeast stress response in the absence of stress.

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LIST OF ABRIVIATIONS

AcK	acetylated lysine
ATM	ataxia-telangiectasia mutated
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
cDNA	complementary deoxyribonucleic acid
CoA	coenzyme A
co-IP	co-immunoprecipitation
CREB	cAMP response element-binding protein
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
ESR	environmental stress response
EtOH	ethanol
FITC	fluorescein isothiocyanate
G6PDH	glucose-6-phosphate dehydrogenase
GFP	green fluorescent protein
GTP	guanosine-5'-triphosphate
H2A	histone h 2 A
H2B	histone h 2 B
H_2O_2	hydrogen peroxide
H3	histone h 3
H4	histone h 4
HA	human influenza hemagglutinin
HCl	hydrochloric acid
HPR	horseradish peroxidase
HSE	heat shock element
HU	hydroxyurea
IgG	immunoglobulin G
IP	immunoprecipitation
KAT	lysine acetyltransferase
KDAC	lysine deacetylase
LC-MS/MS	liquid chromatography–mass spectrometry
LiCl	lithium chloride
mChIP	modified chromatin Immunoprecipitation
MMS	methyl methanesulfonate
MYC	myelocytomatosis oncogne
NaAc	sodium acetate

Nad+	nicotinamide adenine dinucleotide
NaOH	sodium hydroxide
NES	nuclear export sequence
NLS	nuclear localization sequence
NuA4	nucleosome acetyltransferase histone H4
OD_{600}	Optical Density at 600nm
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PKA	protein kinase A
PP2A	Protein phosphatase 2A
PTM	post translational modification
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
RPM	rounds per minute
SAGA	Spt-Ada-Gcn5 acetyltransferase
SC	synthetic complete media
SDS	sodium monododecyl sulfate
STRE	stress response element
TAP	tandem affinity purification
TE	tris-EDTA
TOR	target of rapamycin
TS	temperature sensitive
WCE	whole cell extract
WT	wild type
YPD	yeast Extract Peptone Dextrose
β-Gal	β-galactosidase

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CHAPTER 1: INTRODUCTION

The work presented in this thesis examines the cellular function of the previously uncharacterized *Saccharomyces cerevisiae* protein Gds1p in the context of the Msn2p and Msn4p dependant environmental stress response. This introduction will review (i) the yeast environmental stress response, centering on Msn2p and Msn4p regulation; (ii) the lysine acetyltransferase complex, NuA4 and acetylation of non-histone targets; and (iii) Gds1p and its likely role in the stress response.

1.1 The Msn2p/Msn4p environmental stress response

1.1.1 Msn2p/Msn4p function and structure

To single celled organisms such as yeasts, stresses as a result of environmental fluctuations are a constant and major assault on cell viability. Stresses such as temperature, pH, osmolarity, or nutrient fluctuation lead to rapid denaturation of proteins and, if left unchecked, cell death. In yeast, one mechanism through which yeast cells adapt to external stress is a rapid and coordinated, transcriptional response largely relying on the transcription factors Msn2p and Msn4p (Causton et al., 2001; Gasch et al., 2000). The transcription of approximately 15% of all yeast genes are affected in response to any environmental stress with ~300 genes being upregulated and ~600 genes being downregulated. These affected genes are termed "the environmental stress response" or ESR and function primarily to inhibit transcription, translation, and cell growth and to protect against protein denaturation (Causton et al., 2001; Gasch et al., 2000). 180 of the genes whose expression is modified during the ESR are dependant entirely on Msn2p and Msn4p while most others are at least partially dependant on Msn2p and Msn4p (Gasch et al., 2000). Other major transcription

factors and pathways involved in stress survival include Hsf1p, Yap1p, and Skn7p of the HOG pathway but these factors respond to specific stresses such as heat shock or oxidative stress (reviewed in (Morano et al., 2012). Since the stress response involves the transcription and translation of hundreds of proteins, it is a major draw on energy and takes cells out of their ideal metabolic state (Causton et al., 2001). Thus, the transcriptional remodelling must be fine tuned to be proportional to the intensity of the applied stress and rapidly repressed once cells have either adapted or stress is alleviated (De Wever et al., 2005; Kaniak et al., 2004).

Msn2p and Msn4p are partially redundant transcription factors required for the transcriptional activation of hundreds of genes in response to environmental stress, but are not required for cellular viability in the absence of stress (Causton et al., 2001; MartinezPastor et al., 1996; Schmitt and McEntee, 1996). The DNA binding domains of Msn2p and Msn4p are comprised of two adjacent zinc finger DNA binding domains located near the C-terminus and preceded by a nuclear localization signal (NLS) (Gorner et al., 2002; MartinezPastor et al., 1996). In addition to the NLS, Msn2p also contains an N-terminal nuclear export sequence (NES) which is functionally redundant to the NLS (Gorner et al., 2002). Finally, the transcription activation domain of both proteins is located in the region between amino acids 1-303 and can act independently of the NLS as well as the zinc finger domains (Boy-Marcotte et al., 2006) **(Figure 1.1)**. As the C-terminal domains exhibit a high degree of homology, it was initially suspected that Msn2p and Msn4p share a redundant function. It was later shown that although Msn2p appears to be the dominant member of the pair, deletion mutant analysis shows that Msn4p is sufficient for cell viability in response to stress.

Msn2p

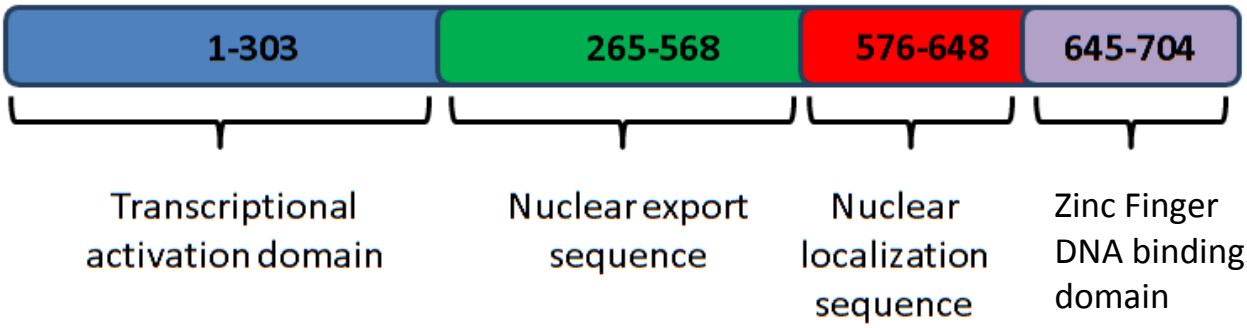

1-303
265-568
576-648
645-704
Transcriptional activation domain
Nuclear export sequence
Nuclear localization sequence
Zinc Finger DNA binding domain

Figure 1.1: Schematic of Msn2p environmental stress response transcription factor

Msn2p is composed of an N-terminal transcriptional activation domain followed by functionally redundant nuclear export and localization sequences whose phosphorylation state determines nucleocytoplasmic localization. The C-terminal domain is comprised of two repeated zinc finger DNA binding domains which through the coordination of a zinc ion promote the interaction between Msn2p and the large groove of the DNA double helix.

implying partially redundant function (Boy-Marcotte et al., 1998; Estruch and Carlson, 1993; Schmitt and McEntee, 1996).

Genes whose expression is controlled by Msn2p and Msn4p contain stress response element (STRE) sequences in their promoters that are recognized by the zinc finger domains of Msn2p and Msn4p (Estruch and Carlson, 1993; MartinezPastor et al., 1996; Wieser et al., 1991). STREs are characterized by the pentameric sequence CCCCT and the number of STREs located at a promoter influences transcriptional strength (Boy-Marcotte et al., 1999; Wieser et al., 1991). There is also often interplay between STREs and other promoter elements. For example the promoter of the heat shock gene *HSP82* contains two STREs and three heat shock elements (HSEs) controlled by the heat shock transcription factor Hsf1p (Boy-Marcotte et al., 1999). In contrast, the *HSP12* promoters contain only four STREs (Boy-Marcotte et al., 2006; Boy-Marcotte et al., 1999). Thus, under heat shock conditions, *HSP82* expression is driven by Msn2p, Msn4p, and Hsf1p and only requires the presence of one of the three transcription factors while *HSP12* transcription is driven only by Msn2p and/or Msn4p (Boy-Marcotte et al., 2006; Boy-Marcotte et al., 1999).

1.1.2 Msn2p/Msn4p subcellular localization

The primary mechanism by which Msn2p/Msn4p activity is regulated is sub-cellular localization. In the presence of stress Msn2p and Msn4p are localized to the nucleus were they are bound to STREs of stress related genes; however upon stress removal or adaptation to prolonged non lethal stress, the majority of these transcription factors rapidly relocalize to the cytoplasm (Gorner et al., 1998; Jacquet et al., 2003). To this end, multiple fine-tuned pathways function in parallel to influence Msn2p/Msn4p localization and are influenced by the type, intensity, and duration of the applied stress (Causton et al., 2001; Gasch et al.,

2000; Sadeh et al., 2011). Most crucial to the subcellular localization of Msn2p is the phosphorylation state of its NLS and NES. In particular the phosphorylation state of S288 of the NES and S620, S625, and S633 of the NLS play a large role in determining Msn2p localization (Boy-Marcotte et al., 2006; Gorner et al., 1998; Gorner et al., 2002). When exposed to stress, the NLS and NES of Msn2p is in a non-phosphorylated state. Once a stress is removed or adaptation occurs, the NLS and NES of Msn2p are phosphorylated resulting in Msn5p mediated nuclear export (Gorner et al., 1998; Gorner et al., 2002; Jacquet et al., 2003; Yoshida and Blobel, 2001). Msn5p is a karyopherin protein that when bound to Ran-GTP preferentially binds phosphorylated substrates and promotes their docking with the nuclear pore complex (Kaffman et al., 1998b; Yoshida and Blobel, 2001). Furthermore, Msn2p oscillates between the nucleus and cytoplasm with a frequency corresponding to the intensity or type of stress (Hao and O'Shea, 2012; Jacquet et al., 2003) potentially representing a mechanism for cellular adaptation or reduction of the stress response.

1.1.3 Protein kinase A and the target of rapamycin complex are the major regulators of Msn2p subcellular localization

Although multiple kinases are involved in the phosphorylation of the NES and NLS, protein kinase A (PKA) is the dominant kinase involved in Msn2p phosphorylation in the absence of stress. PKA activity is highly regulated through multiple signaling pathways. Briefly, in the absence of stress, the enzyme adenylase cyclase (Cyr1p) is abundant and active in cells leading to the production of cAMP. cAMP activates PKA by biding to the inhibitory PKA subunit Bcy1p and allowing its dissociation from the complex (Thevelein and de Winde, 1999). Active PKA is localized to the nucleus were it can phosphorylate Msn2p and Msn4p (Griffioen et al., 2000; Thevelein and de Winde, 1999). Upon environmental stress, two pathways of PKA inhibition exist. Under glucose depravation

conditions, the cell membrane bound G-protein coupled receptor Gpr1p is no longer bound to glucose inhibiting the downstream G-protein Gpa2p and leading to subsequent inhibition of adenylase cyclase (Kraakman et al., 1999; Wang et al., 2004; Xue et al., 1998). Alternatively, in response to environmental stresses leading to protein denaturation, the Hsp70 family chaperone protein, Ssa1p, shifts from being bound to and activating Cdc25p and instead binds denatured proteins (Geymonat et al., 1998; Wernerwashburne et al., 1987). As Cdc25p is a guanine exchange factor required for production of GTP and activation the Ras G-protein signaling members, its inactivation results in reduced adenylase cyclase and PKA activity (Broek et al., 1985; Broek et al., 1987; Geymonat et al., 1998). Thus, PKA can be inhibited through two alternate pathways upon stress, both of which inhibit cAMP production and lead to loss of Msn2p/Msn4p phosphorylation **(Figure 1.2)**. When core subunits of the PKA complex, such as the non-essential subunits *TPK2* and *TPK3,* are deleted constitutive nuclear localization and binding of STREs by Msn2p is observed indicating that PKA is required for nuclear export of Msn2p in the absence of stress (Gorner et al., 1998; Gorner et al., 2002). PKA also appears to have high and non specific affinity for Msn2p when active. When cells express the hyperactive isoform of the PKA subunit Ras2p ($Ras2^{Val19}$), Msn2p is hyperphosphorylated (Hao et al., 2013; Ma et al., 2012) Further, as PKA activity is dependent on cAMP, addition of excess cAMP in a strain lacking the ability to produce its own cAMP (*pde2Δ*) led to cytoplasmic localization of Msn2-GFP regardless of the presence or absence of stress (Boy-Marcotte et al., 2006). Similarly, in phosphomimic point mutants of four PKA consensus sites located in the NLS (serine to alanine mutations), Msn2-GFP is constitutively cytoplasmic (Hao et al., 2013). Conversely, when cells with responsive PKA mutant background are exposed to 1-NM-PP1, a specific inhibitor of PKA,

Msn2-GFP becomes localized to the nucleus regardless of the presence or absence of stress (Hao et al., 2013). Thus constitutively low PKA activity leads to nuclear localization of Msn2p, while constitutively high PKA activity leads to cytoplasmic Msn2p localization regardless of environmental stress applied. The essential function of PKA has even been linked to the inhibition of Msn2p and Msn4p as the lethal phenotype of the PKA mutant *tpk2-63*(Ts) (low PKA activity) at elevated temperature is partially rescued by knockout of either *MSN2* or *MSN4* and fully rescued in an *MSN2/MSN4* knockout (Smith et al., 1998b). These results suggest that the essential role of PKA is in the inhibition of Msn2p/Msn4p-dependent transcription of growth inhibiting genes such as the *YAK1* kinase (responsible for cell cycle arrest in the absence of glucose (Garrett et al., 1991)) in the absence of stress (Smith et al., 1998b). It is not known if PKA contributes to phosphorylation of cytoplasmic Msn2p in the absence of stress, but since Msn2p has been found in the nucleus in small quantities under stress free conditions (Mitchell et al., 2008), a plausible hypothesis is that in the absence of stress PKA phosphorylates any Msn2p that has been dephosphorylated and re-localized to the nucleus.

In addition to PKAs role in promoting nuclear export, the cytoplasmic retention of Msn2p under stress free environmental conditions is largely governed by target of rapamycin (TOR). Yeast possesses two forms of the TOR kinase complex,TOR1 and TOR2, which have a diverse array of functions including cell cycle, cellular metabolism, and stress survival (De Virgilio and Loewith, 2006; Loewith et al., 2002). The TOR signaling cascade is active when cells have access to a suitable carbon source and initiates a transcriptional program leading to growth and replication (Barbet et al., 1996; Thomas and Hall, 1997).

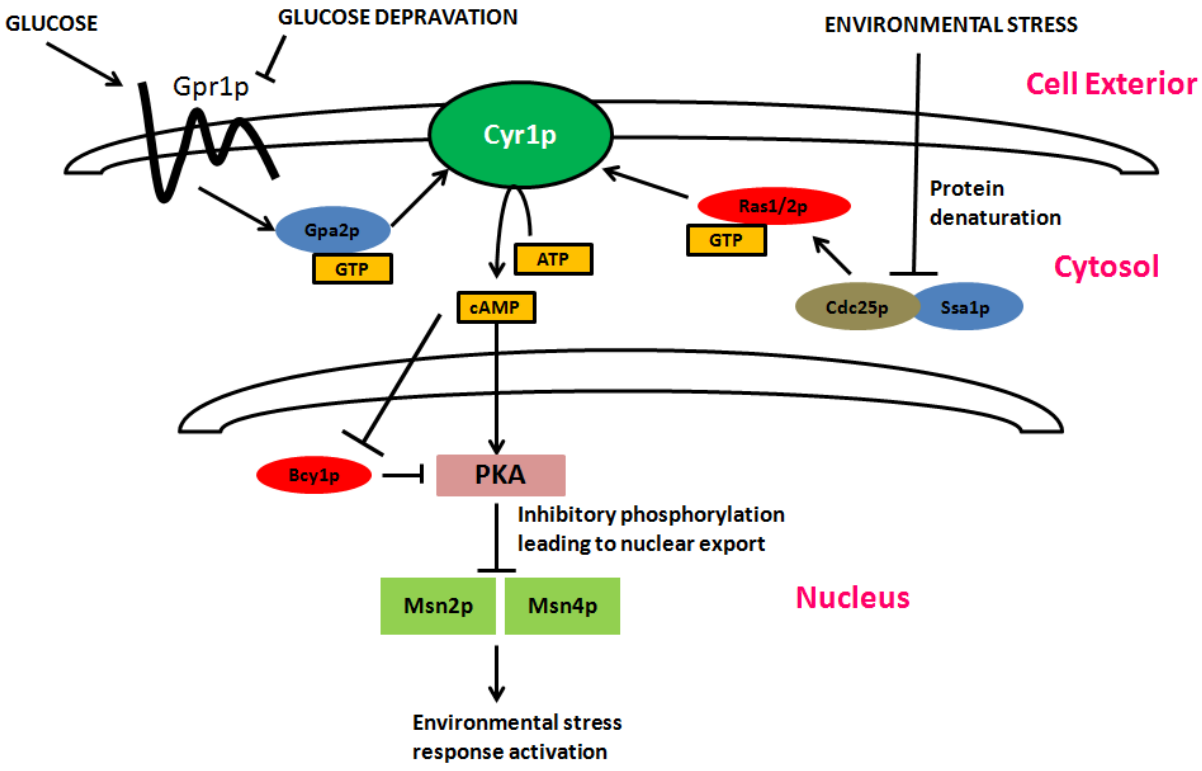

GLUCOSE
GLUCOSE DEPRAVATION
ENVIRONMENTAL STRESS
Gpr1p
Cell Exterior
Cyr1p
Protein
denaturation
Ras1/2p
GTP
Gpa2p
GTP
ATP
Cytosol
cAMP
Cdc25p
Ssa1p
Bcy1p
PKA
Inhibitory phosphorylation
leading to nuclear export
Nucleus
Msn2p
Msn4p
Environmental stress
response activation

Figure 1.2: PKA regulation of Msn2p and Msn4p in the presence and absence of Stress.

PKA represents the major mechanism of Msn2p nuclear export and its activity is modulates by cellular cAMP level. In the presence of glucose and absence of environmental stress, active Cyr1p produces cAMP leading to the activation of PKA and nuclear export of Msn2p and Msn4p. In the absence of glucose, the Cyr1p activator, Gpa2p is inhibited leading to decreased PKA activity and activation of the Msn2p/Msn4p ESR. In response to environmental stresses leading to protein denaturation, the Cyr1p activators, Ras1p and Ras2p are inhibited leading to low cAMP levels and inhibition of PKA. With the exception of Msn2p and Msn4p, all members of this pathway have human homologues.

It has been shown that upon treatment with the rapamycin, a specific small molecular inhibitor of the TOR complex, Msn2-GFP rapidly relocalize to the nucleus in the absence of stress. TOR promotes cytoplasmic localization of Msn2p, and likely Msn4p, through two poorly defined mechanisms. First, TOR promotes the interaction between Msn2p and the 14-3-3 cytoplasmic tethering proteins Bmh1p and Bmh2p aiding in the cytoplasmic retention of Msn2p (Beck and Hall, 1999; Wang et al., 2009). Conversely rapamycin also inhibits the ability of Bmh2p to bind both Msn2p and Msn4p (Beck and Hall, 1999). Further, TOR may also inhibit Msn2p export from the cytoplasm by inhibiting the formation of PP2A/Cdc55p, a complex that may be responsible for dephosphorylation of the Msn2p NLS and nuclear retention (Santhanam et al., 2004). Taken together these results suggest that under optimal nutrient conditions, active TOR complex promotes cytoplasmic Msn2p localization by promoting the interaction of Msn2p with 14-3-3 cytoplasmic tethering proteins as well as direct phosphorylation of Tap42p inhibiting the phosphatase activity of PP2A against Msn2p. In conditions of nutrient scarcity, TOR is inhibited leading to the formation of the active PP2A/Cdc55p complex, dephosphorylation of the Msn2p NLS and nuclear retention (DiComo and Arndt, 1996; Jiang and Broach, 1999; Santhanam et al., 2004) **(Figure 1.3)**. Controversy still surrounds both of these models as some groups have shown that 14-3-3 proteins are not involved in cytoplasmic retention of Msn2p (Gorner et al., 2002; Santhanam et al., 2004; Smith et al., 1998b), and *TAP42* deletion mutants do not show increased nuclear localization of Msn2p in stress free conditions (Beck and Hall, 1999). It is also unknown if PP2A/Cdc55 directly acts on Msn2p. Furthermore, *S. cerevisiae* TOR complex has not been shown to be inhibited in response to any stress other than nutrient limitations (although *S.*

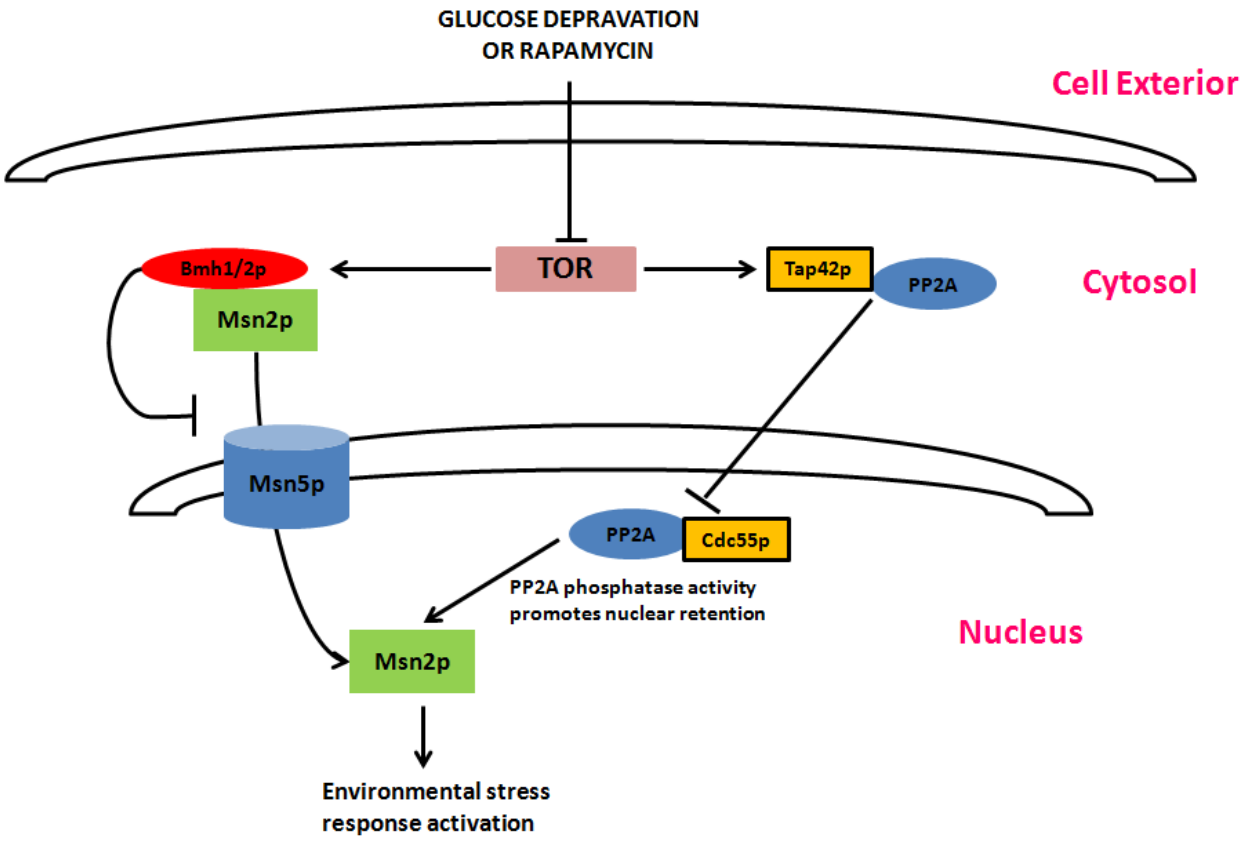

GLUCOSE DEPRAVATION
OR RAPAMYCIN
Cell Exterior
TOR
Bmh1/2p
Msn2p
Tap42p
PP2A
Cytosol
Msn5p
PP2A
Cdc55p
PP2A phosphatase activity
promotes nuclear retention
Nucleus
Msn2p
Environmental stress
response activation

Figure 1.3: Proposed model of TOR mediated cytoplasmic retention of Msn2p.

The TOR complex likely promotes cytoplasmic retention of Msn2p when glucose is present by promoting the interaction between Msn2p and the cytoplasmic tethering 14-3-3 proteins; Bmh1p and Bmh2p. Furthermore, threw promoting an interaction between Tap42p and PP2A, and preventing the Cdc55p/PP2A interaction, TOR may indirectly inhibiting nuclear retention of Msn2p. All members of these pathways with exceptions of Tap42p and Msn2p have human homologues.

pombe TOR complex does seem to be inhibited by a variety of stresses (Weisman and Choder, 2001)) suggesting other mechanisms of Msn2p and Msn4p cytoplasmic retention in the absence of stress are likely.

1.1.4 Additional regulators of Msn2p/Msn4p activity

Although the major regulators of Msn2p and Msn4p activity appear to be PKA and TOR, various other minor regulatory mechanisms exist to further fine-tune the Msn2p/Msn4p dependant ESR. The acetyltransferase complex NuA4 may play a complementary role to TOR while the kinases Gsk3h, Snf1p and the elongator component Srb10p are known to phosphorylate Msn2p and can promote either activation or repression of the Msn2p/Msn4p ESR.

Srb10p plays a similar role to PKA by phosphorylating the NLS of Msn2p already localized to STREs and promoting promoter eviction and nuclear export as well as proteasomal degradation of Msn2p (Chi et al., 2001; Lallet et al., 2004). In cells exposed to prolonged, non-fatal heat shock (38°C), Msn2p protein levels begin to decline in a proteasome and Srb10p dependant manner in as rapidly as 15 minutes leading to an eventual decrease in the protein level of the Msn2p/Msn4p controlled gene *HSP12* in cycloheximide treated cells (Lallet et al., 2004). Furthermore, the observed degradation is dependent on the zinc finger domain of Msn2p suggesting that the Srb10p containing elongator complex which is localized to the promoters of actively transcribed genes can phosphorylate promoter bound Msn2p leading to its degradation (Kuchin et al., 1995; Lallet et al., 2004). Supporting the notion that Srb10p only plays a minor role in Msn2p localization, *srb10-2* cells harboring a partially active Srb10p mutant, only display a minor increase (from 0% nuclear to between 15% and 30% nuclear) in nuclear Msn2p localization in the absence of stress (Chi et al.,

2001). Taken together, the role of Srb10p is likely in the inhibition of the Msn2p ESR after cellular adaptation to non lethal stress.

Snf1p, a cAMP dependant serine/threonine kinase and component of the SWI/SNF chromatin remodeling complex has been shown to phosphorylate Msn2p and promote nuclear exclusion during prolonged periods of stress, after initial Msn2p nuclear localization (Celenza and Carlson, 1984; De Wever et al., 2005; Hardie, 2007; Thompsonjaeger et al., 1991). Snf1p is a ubiquitously localized kinase whose activity towards nuclear localized Msn2p is specific to conditions of glucose depravation. Under abundant glucose conditions, the presence of glucose-6-phosphate contributes to the activation of the protein phosphatase type 1 complex, Glc7p/Reg1p, which in turn dephosphorylates and inhibits Snf1p (Ludin et al., 1998; Sanz et al., 2000). Evidence also exists that Glc7p/Reg1p contributes to direct dephosphorylation of Msn2p leading to prolonged nuclear localization in the absence of glucose (De Wever et al., 2005). Under conditions of glucose depravation, Snf1 auto-inhibition is relived (Jiang and Carlson, 1996) and association with the regulatory subunit Gal82p leads to nuclear localization (Vincent et al., 2001) and activation of genes required for glucose depravation survival and use of alternative carbon sources (Ronne, 1995). In response to prolonged periods of non lethal, low glucose availability (>160 minutes), Snf1p has been shown to phosphorylate S582, a PKA consensus site on the Msn2p NLS leading to gradual loss of Msn2p nuclear localization and transcription of ESR genes (De Wever et al., 2005; Mayordomo et al., 2002). The phosphorylation and cytoplasmic relocalization of Msn2p is dependent on the Snf1p/Gal82p interaction as well as loss of Glc7p/Reg1p activity (De Wever et al., 2005; Mayordomo et al., 2002). Thus Snf1p provides a mechanism for

reducing the intensity of the Msn2p/Msn4p stress response once cells have adapted to low glucose conditions.

In contrast to the inhibitory roles of PKA, Srb10p, and Snf1p, the four yeast homologues to mammalian Gsk3 (glycogen synthase kinase 3 homologues) promote Msn2p transcriptional activity through direct phosphorylation (Hirata et al., 2003). The yeast Gsk3p homologues: Mck1p, Rim11p, Mrk1p, and Ygk3 were initially characterized as being required for transition through mitosis and meiosis (Bowdish et al., 1994; Neigeborn and Mitchell, 1991; Shero and Hieter, 1991). Although no single delete displays ESR defects, deletion of all four Gsk3p homologues displays temperature sensitivity (Hirata et al., 2003). It was initially thought that the Gsk3p homologues were required for nuclear localization of Msn2p, but Msn2-GFP localization was normal in the quadruple mutant both in the absence of stress and directly after application of environmental stress such as heat shock, osmotic shock, and oxidative stress (Hirata et al., 2003). In the quadruple Gsk3p homologue mutant, under stress conditions, Msn2-GFP is localized to the nucleus but remains unbound to STREs and the transcription of ESR genes such as *PGM2* and *DDR2* are repressed. Further, when the Msn2p zinc finger is replaced with a LexA biding domain driving β-Gal production, deletion of the Gsk3p homologues does not affect β-Gal production (Hirata et al., 2003). Taken together, it can be inferred that the Gsk3p homologues are required for Msn2p binding to STREs in the presence of stress and not in either its nucleocytoplasmic shuttling or transcriptional activity. It is unknown if this in accomplished through direct phosphorylation of Msn2p or alternative mechanisms.

A potential secondary mechanism to TOR and PKA inhibition of Msn2p in the absence of stress is the direct inhibition of a small pool of nuclear Msn2p which is localized

to STREs but remains inactive. This effect may be mediated by the conserved yeast acetyltransferase complex NuA4 (described in section 1.2) as transcription of ESR related genes such as *HSP12* are up regulated in an Msn2p/Msn4p dependant manner under non stress conditions in a variety of NuA4 mutants (Mitchell et al., 2008; Sadeh et al., 2011). NuA4-dependent inhibition of Msn2p/Msn4p transcription, in the absence of stress, is still poorly understood. Histone H4 point mutants mimicking constitutive deacetylation (lysine to arginine mutations) mimic the phenotype observed in histone acetylation defective NuA4 mutant backgrounds, showing an increase in transcription of Msn2p/Msn4p related stress response genes under non-stress conditions (Dion et al., 2005; Lindstrom et al., 2006). This suggests that NuA4 based acetylation of histone tails changes promoter architecture and prevents binding of Msn2p/Msn4p. Contrary to this model, Msn4p can be detected bound to STREs in the absence of stress in both wild type and mutant NuA4 backgrounds (Mitchell et al., 2008). Another possibility is that NuA4-dependent acetylation of histones is changing local promoter environments and localization of other factors. Alternatively, but not mutually exclusive, NuA4 may have other targets that mediate some or all of the repression of Msn2/4 activity. Indeed, a direct physical interaction has been detected between NuA4 and Msn4p (Mitchell et al., 2008) and Msn4p is an in vitro target of NuA4 (Mitchell et al., 2013), but there is no evidence of Msn4 being acetylated in vivo. NuA4 could also be repressing Msn2/4 through other non-histone targets.

Thus, *Saccharomyces cerevisiae* employs a variety of complementary mechanisms to modulate and fine tune the Msn2p/Msn4p dependant environmental stress response **(see**

1.2 Non-histone lysine acetylation and NuA4

1.2.1 Acetylation as a post translational protein modification

Lysine acetyl transferences (KATs) catalyse the addition of acetyl moieties onto the epsilon amino groups of lysine's on target substrates. This reaction is achieved by transferring the acetyl group of the acetyl-CoA onto the lysine residue leaving behind Coenzyme A. The acetylation reaction is antagonized by lysine deacetylases (KDACs) either through the conversion of a water molecule to acetate or by the use of NAD+ leading to the production of 2`- and 3`-O-acetyl ADP-ribose **(Figure 1.5)**. As lysine is a positively charged amino acid, addition of an acetyl moiety neutralizes the charge and can have major effects on substrate structure and function.

The classical view of protein acetylation, focused on histone acetylation and transcriptional regulation. This work began 51 years ago with the observation that a large portion trypsin or hydrolytically digested histone fractions contained masked N-terminal lysine and that the masking effect was due to the presence of acetyl groups (Phillips, 1963). Core, conserved eukaryotic histones are composed of an octomer containing two of each of H3, H4, H2A, and H2B subunits (Luger et al., 1997). Wrapped around each histone are 146 base pairs of DNA forming the core structure of a chromosome; the nucleosome (Luger et al., 1997).

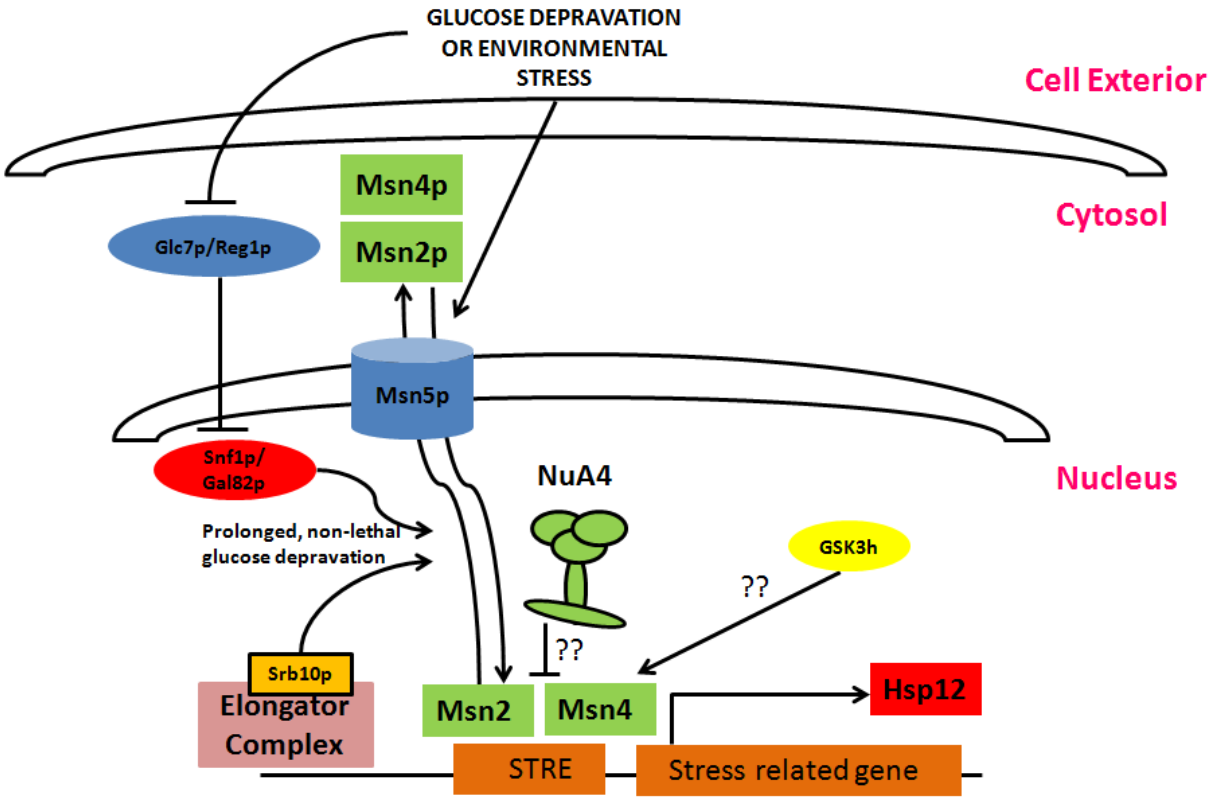

GLUCOSE DEPRAVATION
OR ENVIRONMENTAL
STRESS
Cell Exterior
Cytosol
Nucleus
Msn4p
Msn2p
Glc7p/Reg1p
Msn5p
Snf1p/
Gal82p
Prolonged, non-lethal
glucose depravation
NuA4
GSK3h
??
??
Srb10p
Elongator
Complex
Msn2
Msn4
Hsp12
STRE
Stress related gene

Figure 1.4: Minor pathways of Msn2p/Msn4p regulation.

In addition to the regulatory effects of PKA and TOR, Msn2p localization is futher fine tuned by a host of minor regulatory mechanisms. The kinases Snf1p and Srb10p contribute to nuclear export of Msn2p after cellular adaptation to stress. Srb10p also promotes proteasomal degradation of Msn2p. The lysine acetyltransferase complex, NuA4, inhibits Msn2p/Msn4p based transcription in the absence of stress threw unknown mechanisms. Either through direct phosphorylation of Msn2p or threw indirect mechanisms, the four yeast Gsk3 homologues (GSK3h) are required for localization of Msn2p to STREs during stress.

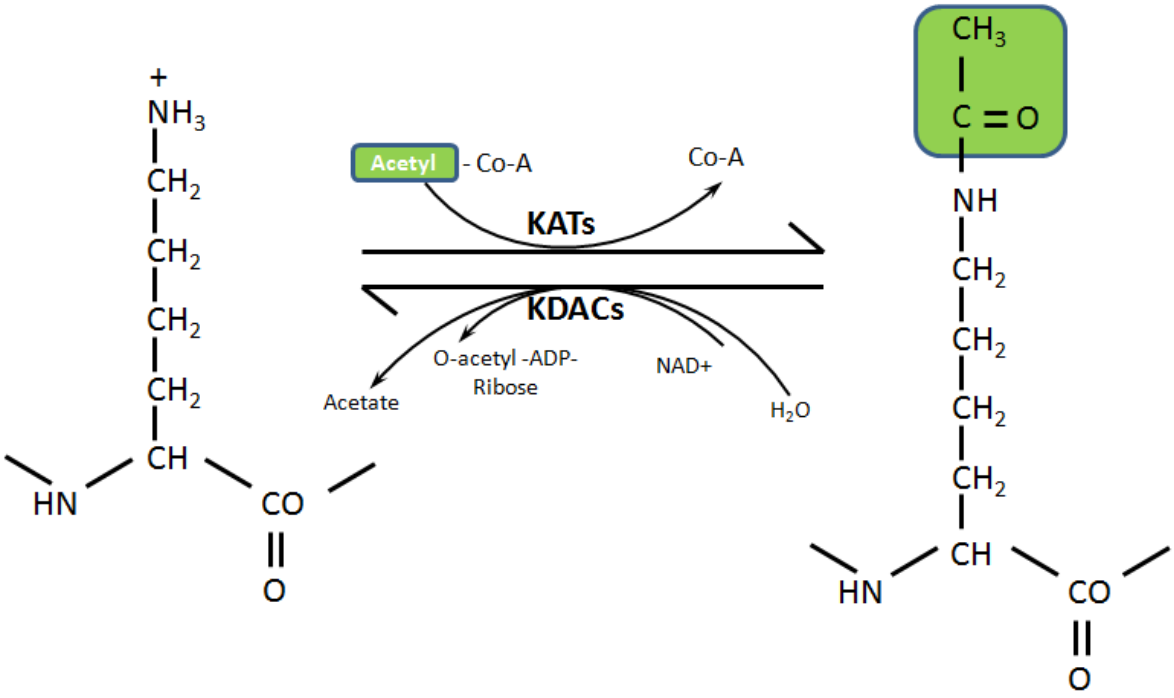

Acetyl
- Co-A
Co-A
KATs
KDACs
O-acetyl -ADP-
Ribose
NAD+
Acetate
H_2O

Figure 1.5: Reversible acetylation of lysine residues.

The transfer of acetyl groups from acetyl coenzyme A to the ε amino groups of lysine residues is catalysed by lysine acetyltransferases (KATs) and neutralizes the positive charge of lysine. The reverse reaction is catalysed by lysine deacetylase (KDACs) and requires either a water molecule or NAD+.

The winding of chromatin around histones allows for the tight and compact packaging of chromatin into chromosomes and restricts the access of DNA associated proteins such as DNA/RNA polymerases and transcription factors. Thus lack of histone tail acetylation is associated with chromosome compaction, transcriptional inhibition, and DNA silencing (Kuo and Allis, 1998; Roth et al., 2001; Shahbazian and Grunstein, 2007). Acetylation of lysine residues, in combination with other post translational modifications (PTMs) on histone tales loosens the histone-chromatin interaction and allows for protein access to chromatin (Minard et al., 2009). Thus acetylation of histone tails is associated with loosening of chromatin, transcriptional activation, DNA damage repair, and replication (Kuo and Allis, 1998; Roth et al., 2001; Shahbazian and Grunstein, 2007). Furthermore, many of these functions are related to acetylation of specific lysine residues of different histone subunits. Histone H4, the major histone target of NuA4, has been well studied and the functions of multiple lysine residues are known. For example, K16 acetylation regulates chromatin compression and transcriptional silencing (Ma et al., 1998; Shogren-Knaak et al., 2006); K5, 8, and 12 are involved in global transcriptional regulation and nucleosome deposition (de Nadal et al., 2004; Ma et al., 1998; Tyler et al., 1999), and all four sites are required for double stranded DNA break repair (Bird et al., 2002; Shahbazian and Grunstein, 2007; Shogren-Knaak et al., 2006).

1.2.2 Acetylation of non-histone protein targets

Over the last decade the field of non-histone acetylation has emerged revealing the widespread regulatory role that acetylation plays. Initially, low-throughput protein specific studies identified several non-histone KAT targets, but the method was inefficient as

acetylation was detected on a protein by protein basis. Recently, primarily through the use of high-throughput proteomic screens, thousands of non-histone acetylation sites have been found across multiple organisms and tissue types. Primarily, groups have used α-acetyl lysine antibodies coupled to magnetic beads to purify acetylated proteins from cell lysates followed by LC-MS/MS for identification. Acetylomes have been assembled from mouse (Kim et al., 2006) and human liver cell lines (Zhao et al., 2010), the HeLa cell line (Kim et al., 2006), fruit fly (Weinert et al., 2011), budding yeast (Henriksen et al., 2012a), salmonella (Wang et al., 2010), and *E. coli* (Zhang et al., 2009). More recently our group performed a similar screen in budding yeast were whole cell lysates were first enriched for NuA4 interaction partners through use of NuA4 coupled magnetic beads followed by LC-MS/MS (Mitchell et al., 2013). Common to all these screens are a large host of chromatin associated proteins involved in transcription, DNA replication, DNA damage repair, transcription factors, and histones. However, the majority of identified acetylated proteins in most of these studies though were non chromatin related and were involved in a wide range of processes including, but not limited to: various stress responses, trafficking, cell cycle, ribosomal function, cytoskeleton organization, kinase regulation, and metabolism.

The earliest non-histone acetylation target discovered was α-tubulin. Acetylation of α-tubulin in sperm flagella of a variety as well as both nuclear and cytoplasmic α-tubulin of organisms contributes to microtubule stability and assembly (Piperno and Fuller, 1985a; Piperno et al., 1987). Common to the above mentioned proteomic studies was the identification of almost all enzymes involved in glucose metabolism as well as many enzymes involved in other metabolic pathways. Acetylation of phosphoenolpyruvate carboxykinase (PEPCK), the enzyme catalysing the rate limiting step of glycolysis, promotes

its degradation through an ubiquitin mediated mechanism (Jiang et al., 2011; Zhao et al., 2010). Similarly, the M2 Isoform of Pyruvate kinase is acetylated in humans in response to high glucose leading to chaperone-mediated autophagy and degradation (Lv et al., 2011). Sphingosine kinase 1 (SK1) is involved in cell membrane formation and stability. Acetylation of SK1 blocks ubiquitination sites preventing degradation and leading to increased cell size and slow growth rate (Yu et al., 2012). In relation to cell cycle control, acetylation of cyclin dependant kinase 1 (Cdk1p) is essential for cell viability in yeast (Choudhary et al., 2009) and acetylation of cyclin dependant kinase 9 reduces its activity leading to decreased general activity of RNA polymerase II in human cells (Sabo et al., 2008). ATM kinase is required for the human DNA damage response and acetylation by the Tip60 acetyltransferase complex (mammalian homolog of NuA4) upregulates ATM activity in response to DNA damage (Sun et al., 2007). Acetylation can even create binding sites promoting protein/protein interaction through bromodomains. Acetylation of p53 promotes its association with the bromodomain of CBP and is required of p53 mediated activation of quiescence of apoptosis (Mujtaba et al., 2004). Finally, acetylation can influence protein subcellular localization. WRN DNA helicase is translocated from nucleolus to nucleoplasm in response to DNA damage (Blander et al., 2002). Thus non-histone acetylation can act as an enzymatic on/off switch, regulate protein/protein interactions, and influence cellular localization of proteins. Unfortunately, these are some of the very few known examples of phenotypes associated acetylation despite the knowledge that thousands of eukaryotic proteins are acetylated. Much work remains to be done in ascertaining the full extent of the role of non-histone acetylation.

The importance of further studying the role of non-histone acetylation lies in the uncharacterized involvement of human KATs and KDACs complexes in diverse pathologies. One of the main classes of current anti-cancer drugs are KDAC inhibitors which lead to hyperacetylation of histones and a slowdown in transcription and cell cycle progression in various cancer types (Arif et al., 2010; Bolden et al., 2006; Spange et al., 2009). In fact, Tip60 expression is significantly reduced in both lung and colon cancer cells lines potentially leading loss of p53 activation and inability to imitate apoptosis in response to the high stress state of a cancer cell (Lund et al., 2002). Further, mutants of the Tip60 KAT domain fail to initiate apoptosis in response to DNA damage stress (Ikura et al., 2000). Acetylation has also been implicated in various autoimmune diseases such as Alzheimer's (Cao and Sudhof, 2001; Dietz and Casaccia, 2010), Crohn's disease (Tsaprouni et al., 2011), and inflammatory lung disease (Ito et al., 2007).

1.2.3 The NuA4 lysine acetyltransferase Yeast nucleosome acetyltransferase histone H4 complex (NuA4) is a highly conserved 13 subunit complex **(Figure 1.6)** with close homologous in other yeasts, *C. elegans, D. melanogaster,* and humans (Ceol and Horvitz, 2004; Doyon and Cote, 2004; Kusch et al., 2004). Of particular interest, the human homologue of NuA4, Tip60 contains homologues to 12 of the NuA4 subunits as well as homologues to all subunits of the yeast SWR1 chromatin remodeling complex (Doyon and Cote, 2004). The histone acetyltransferase activity of yeast NuA4 was first identified *in vitro*. A nucleosome array containing the *HIV1* gene packaged *in vitro* with 11 histones and lacking any free DNA was used in transcriptional assay with and without different KATs.

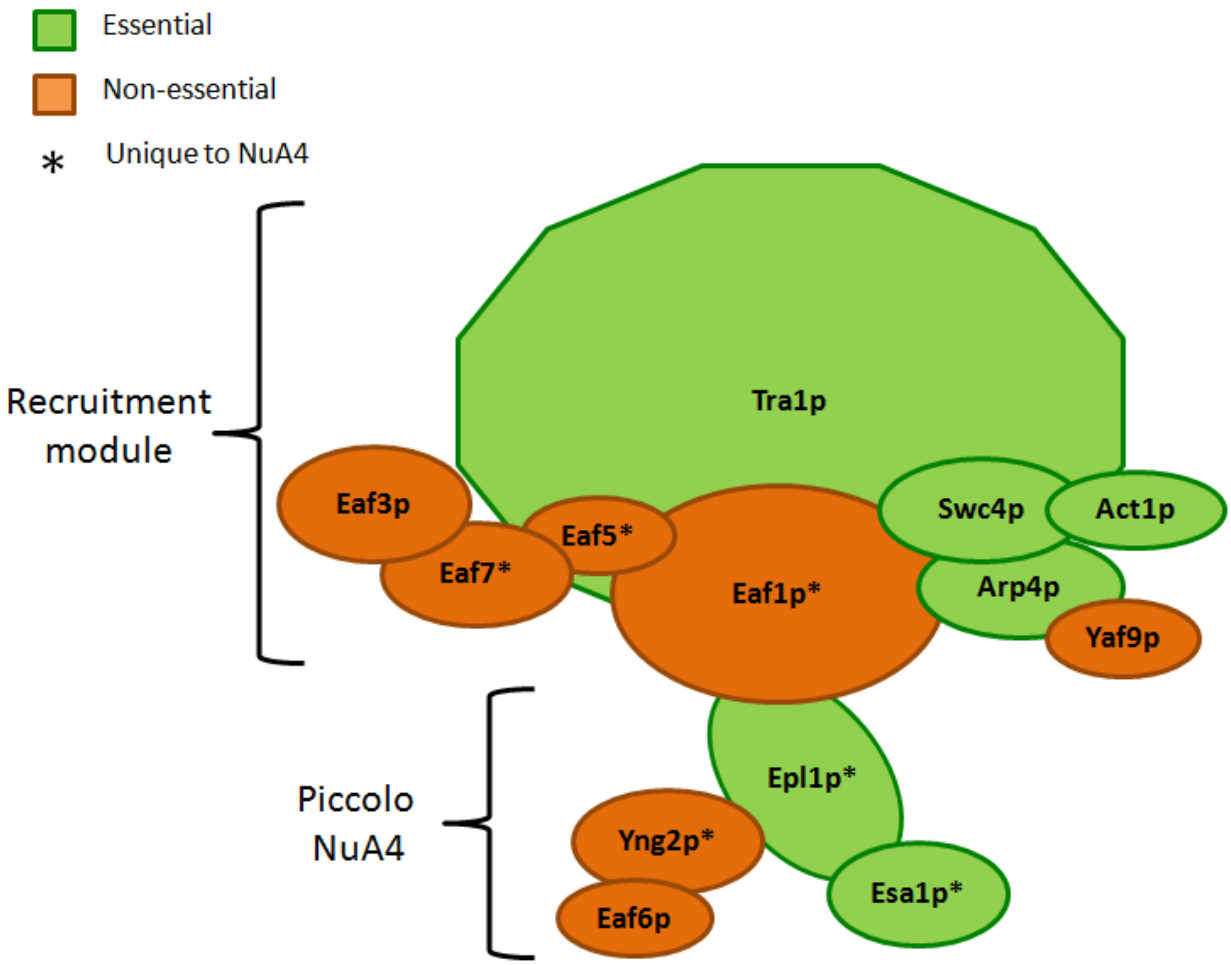

Essential
Non-essential
* Unique to NuA4
Recruitment module
Piccolo NuA4
Tra1p
Eaf3p
Eaf5*
Eaf7*
Eaf1p*
Swc4p
Act1p
Arp4p
Yaf9p
Epl1p*
Yng2p*
Eaf6p
Esa1p*

Figure 1.6: Model of the NuA4 Lysine Acetyltransferase Complex.

NuA4 is composed of 13 subunits, 6 of which are essential and 6 of which are unique to NuA4. The catalytic core of the complex (Piccolo NuA4) contains the catalytic subunit Esa1p . The recruitment module of the complex is hypothesised to target NuA4 to specific genomic loci or non histone proteins and is connected to Piccolo NuA4 by Eaf1p.

Green – essential
Orange – non essential
* Unique to NuA4

Upon the addition of HeLa cell nuclear extract to stimulate transcription, the nucleosome bound *HIV1* gene transcription was ~200 fold lower than a nucleosome free control. Addition of purified NuA4 relived the inhibition in a histone and acetyl-CoA dependant manner and NuA4 directly acetylated histone H4 (Steger et al., 1998).

NuA4 has been implicated in the regulation of a wide variety of cellular processes and although some of these functions are likely governed by H4 acetylation and transcriptional regulation, a growing body of evidence suggests that non-histone acetylation contributes to NuA4 regulatory activity (Bird et al., 2002; Choy et al., 2001; Mitchell et al., 2013; Mitchell et al., 2008). By direct acetylation of the gluconeogenesis enzyme Pck1p, and not through regulation of mRNA or protein levels, NuA4 activates the enzyme allowing for gluconeogenesis and use of non-fermentable carbon sources (Lin et al., 2009). NuA4 is also implicated in DNA damage repair as multiple NuA4 mutants display sensitivity to DNA damaging agents (Auger et al., 2008; Keogh et al., 2006). Autoacetylation of the Yng2p subunit of NuA4 during double stranded DNA brakes promotes complex stability and localization to the site of damage, while deacetylation leads to proteasomal degradation of Yng2p and dissociation of NuA4from DNA (Lin et al., 2008). Despite its role in histone acetylation, NuA4's major regulatory mechanism of action is likely through the acetylation of non-histone targets. The myriad of physical interactions between NuA4 and non-histone proteins combined with microarray data showing relatively minor transcriptional defects in fitness compromised and drug sensitive NuA4 mutants are evidence of NuA4s non chromatin related functions (Choy et al., 2001; Krogan et al., 2004; Lindstrom et al., 2006; Mitchell et al., 2008; Zhang et al., 2004).

The catalytic subunit of NuA4 is Esa1p and functions by formation of an Esa1-acetyl-CoA-histone ternary complex (Allard et al., 1999; Berndsen et al., 2007; Clarke et al., 1999; Yan et al., 2000). Recombinant Esa1p expressed and purified from bacteria carries histone acetyltransferase activity and yeast containing a temperature sensitive allele of *ESA1* coding for *esa1-ts,* is both lethal and loses H4 acetylation *in vivo* at the restrictive temperature (Clarke et al., 1999). These results show that Esa1p is the essential catalytic subunit of NuA4, and that NuA4 is a yeast KAT responsible for H4 acetylation. Further, NuA4 can also acetylate the H2A variant histone Htz1p leading to an inhibition in the spread of heterochromatin (Babiarz et al., 2006; Keogh et al., 2006). The remainder of the NuA4 complex consists of the non-essential subunits; Eaf1p, Eaf3p, Eaf5p, Eaf6p, Eaf7p, Yaf9p, and Yng2p, and the essential subunits; Act1p, Arp4p, Epl1p, Swc4p, and Tra1p (Doyon and Cote, 2004) **(Figure 1.6)**. Esa1p, Epl1p, Yng2p, and Eaf6p can also form a distinct complex termed piccolo NuA4 able to acetylate global H4 in an untargeted manner (Boudreault et al., 2003). This suggests that the remainder of the complex is involved in targeting NuA4 to specific genomic loci or non-histone targets. The majority of NuA4 subunits can also be found in other complexes involved in acetylation and chromatin remodeling (Doyon and Cote, 2004) and thus phenotypes related to their deletion may not be related to NuA4. The primary mutants used in this study, *esa1-ts, eaf1Δ,* and *eaf7Δ* are all specific to NuA4/Piccolo NuA4.

Eaf1p is the central scaffolding subunit of the NuA4 complex responsible for full complex integrity. Deletion of *EAF1* results in the dissociation of the Piccolo NuA4 subcomplex from the remaining subunits as demonstrated through Esa1-TAP IP of the NuA4 complex in a wild type versus *eaf1Δ* genetic background (Auger et al., 2008; Mitchell et al.,

2008). Supporting the model that Piccolo NuA4 is responsible for the essential global histone H4 acetylation function of NuA4 while the recruitment module targets NuA4 to specific genomic loci or non histone targets, *eaf1Δ* mutants display a myriad of cellular defects including sensitivity to heat, microtubule destabilization, DNA damaging agents (MMS and hydroxyurea), caffeine, and display a slow growth phenotype (Auger et al., 2008; Chang et al., 2002; Dudley et al., 2005; Mitchell et al., 2008; Sinha et al., 2008; White et al., 2009). Eaf7p on the other hand, is part of nonessential subcomplex of NuA4 comprised of Eaf7p, Eaf5p, and Eaf3p and a member of the recruitment module (Auger et al., 2008; Mitchell et al., 2008). Deletion of *EAF7* leads only to very mild fitness defects (Deutschbauer et al., 2005) and results in an increased ability for cells to withstand environmental stress such as heat shock due to an accumulation of stress related proteins (such as Hsp12p) in the absence of stress (Sadeh et al., 2011). These phenotypes suggest that Eaf7p is involved in the NuA4 dependant inhibition of the ESR under stress free conditions.

Our group recently developed a novel method of identification of NuA4 physical interactors and acetylation targets (mChIP-KAT-MS/MS) (Mitchell et al., 2013). As Gds1p was one of the top hits in this screen, we decided to further examine Gds1p function as a downstream validation of the technique. Furthermore, preliminary work **(figure 3.1)** suggested a role for Gds1p in the yeast environmental stress response, a process that NuA4 is known to be involved in (Mitchell et al., 2008; Sadeh et al., 2011).

1.3 Gds1p - an uncharacterized yeast protein

Gds1p is a previously uncharacterized protein with very little predicted structure or domains and no homologues outside of fungi. Using secondary structure predictive software

such as GOR IV, APSSP, CFSSP, and JPRED3 Gds1p is predicted to be composed mostly of random coils with few very short (1-2 turns) interspersed α-helixes. BLASTn alignments revealed only small stretches of homology with predicted, uncharacterized proteins found in other yeast species and BLASTp searches reveal no domain homology to any other peptide sequence and limited homology to Gds1p like sequences in other yeasts. Gds1p was initially suggested as being required for robust yeast growth on glycerol as the exclusive carbon source and was found to be a multicopy suppressor of the *nam9-1* mutant which displays defects in glycerol utilization (Konopinska et al., 1995). Several high-throughput functional genomic screens have also suggested a role for Gds1p in inhibiting the spread of heterochromatin, transcriptional regulation, and retrotransposon mobility (Nyswaner et al., 2008; Oki et al., 2004; Tronnersjo et al., 2007). Interestingly, Oki et al., also identified many subunits of NuA4 as potential barriers to the spread of heterochromatin and Nyswaner et al., identified Gds1p as a binding partner and potential regulator of the stress related zinc finger transcription factor Gis1p. Recently, our group discovered, by use of an *in vitro* NuA4 interaction/acetylation target screen that Gds1p is both a physical interactor and *in vitro* acetylation target of Esa1p (Mitchell et al., 2013). Further by mass spectroscopy, six NuA4-dependent in vitro acetylation sites were detected; K348, K351, K354, K365, K366, K408 and one in vivo acetylation on K87 was detected, however the KAT responsible for this site is unknown (Mitchell et al., 2013). Our group found, by use of a modified chromatin immunoprecipitation (mChIP) method examining over 100 nuclear proteins, that Gds1p only co-purified with Esa1-TAP and Msn4-TAP in log-phase cells under stress free conditions (Lambert et al., 2009a).

Section 1.4: Hypothesis and Objectives

Hypothesis: Based on preliminary work **(Figure 1A)**, I hypothesise that Gds1p is a novel regulator of the yeast environmental stress response through the regulation Msn2p and/or Msn4p transcriptional activity and that Gds1p is also involved in NuA4 dependant repression of the environmental stress response.

Objective 1: To provide a functional and phenotypic characterization of Gds1p

Objective 2: To elucidate the role of Gds1p and NuA4 in the regulation of the Msn2p/Msn4p dependant stress response.

CHAPTER 2: MATERIALS AND METHODS

2.1 Yeast strains

Yeast strains used in this study are listed in Table S1. All strains are derived from the S288c genetic background. Strains containing gene deletions or epitope tag integrations were generated by a PCR based homologous recombination methodology and confirmed by PCR analysis (Longtine et al., 1998).

2.2 Gds1 interactome mChIP

Gds1 and its associated protein network were isolated through mChIP (Lambert et al., 2009a) of endogenously TAP-tagged *GDS1*. Cells were harvested from a 500ml culture grown to mid-log phase in rich media (YPD) at 25°C by gentle centrifugation (3000rpm, 3min). Pellets were washed in 25ml water and frozen on liquid nitrogen in 1.5ml tubes. Pellets were lysed by adding an equal volume mChIP lysis buffer [100mM Hepes pH 8.0, 20mM magnesium acetate, 300mM sodium acetate, 10% glycerol (vol/vol), 10mM EGTA, 0.1mM EDTA, and protease inhibitor mixture prior to use (Sigma; P8215)] and glass beads (BioSpec Products, 11079105) followed vortexing for 5-7 one minute pulses with incubation on ice between pulses. Whole cell extract was subjected to sonication (3 x 20s power level 4 pulses with incubation on ice between pulses on Misonix Sonicator) prior the addition of NP-40 (1% vol/vol). Whole cell lysates were clarified by centrifugation (3000rpm, 10min, 4°C). NuPAGE Novex gradient gel SDS/PAGE (4–12%Bis•Tris Gel; Invitrogen, NP0321) was used to separate proteins and visualized by silver stain. Bands were excised for identification

by mass spectroscopy. Samples were processed by LC-MS/MS on an LTQ-Orbitrap XL mass spectrometer (Thermo-Electron) as previously described (Mitchell et al., 2013).

2.3 Serial spot dilution growth assays

Yeast strains were grown to mid-log phase under non-stress conditions (YPD 25°C). Cultures were diluted to an OD_{600} of 0.1 in a 96 well plate and three 1/10 serial dilutions were performed. 4.6µl of each dilution (OD_{600} 0.1, 0.01, 0.001, 0.0001) was spotted on the indicated media and allowed to grow at the indicated temperature for 2-3 days. Plates were photographed using epi-white imaging on a Molecular Imager ChemiDoc XRS System (BioRad). Dilution assay growth experiments were repeated in triplicate using different isolates of each strain. Chemicals / conditions used: heat stress (37°C), DNA damaging agents (0.08% MMS, Sigma M4016, 100mM HU, H8627, Sigma), microtubule stress (60µg/ml Benomyl, Sigma 381586), ethanol (5%), oxidative stress (0.4mM hydrogen peroxide), mild acetic stress (50mM acetic acid), osmotic stress (0.5M NaCl), metal ion stress (1.5mM $NiSO_4$, 227676, Sigma), or 8.0mM caffeine (Sigma C0750). For the alternate carbon source plates, 2% glycerol (Fisher, BP2291) or 2% xylose (Huixian City Hongtai Foodstuff Chemical Company, HT 100205) was utilized.

2.4 GFP-trap purification and TAP tag immunopurification

Cells were grown, harvested, and lysed as described in 2.1. For GFP IPs, 40µl of GFP-trap magnetic beads (Chromotek, Germany; Cat. #: GTM-20) were added to 15-20mg of whole cell lysate and incubated for 2 hours with end over end rotation at 4°C. For TAP IPs, 15mg of whole cell extract was incubated with 40µl magnetic Dynabeads (catalog no.

143-01; Dynal, Invitrogen) crossed-linked to rabbit immunoglobulin G (IgG) (catalog no. PP64; Chemicon) and incubated for two hours at 4°C with end over end rotation. Beads were collected and washed 3x with 1ml cold wash buffer (lysis buffer + 0.5% NP-40 [vol/vol]). Proteins were eluted off beads by addition of 20-40 µl loading dye (50 mM Tris, pH 6.8, 2% SDS, 0.1% bromophenol blue, 10% (vol/vol) glycerol) followed by heating at 65°C for 10 minutes. Loading dye was then transferred to new tubes and boiled at 95°C 10 minutes followed by addition of β-2-mercaptoethanol to 100 mM. Protein concentration was determined using Bradford reagent (Bio-Rad, USA; Cat. #: 500-0006).

2.5 Immunoblotting

150µg of proteins from whole cell extracts or The indicated percentage of protein eluted from beads of immunoprecipitation were separated by SDS-PAGE. Acetylation was detected using anti-acetyl lysine (Cell Signaling; Cat. #: 9681; dilution 1/500), GFP fusion protein was detected using α-GFP (Roche; Cat. #: 11814460001; dilution 1/3000), TAP fusion protein was detected using α-TAP (Thermo Scientific, CAB1001; dilution 1/5000), MYC fusion protein was detected using α-MYC (Roche Applied Science 11667149001; dilution 1/800), HA fusion proteins were detected by using α-HA (Covance, MMS-101P; dilution 1:1000), phosphorylated PKA substrates were detected by α-PKA phosphorylated substrate (α-sub, 1:5,000, Cell Signaling #9624), Msn2p was detected by α-Msn2 antibody (1:10,000, gift from Vivien Measday), phosphorylated Msn2p was detected by α-P-CREB (S133) antibody (1:1000, Cell Signaling), and anti-Pgk1 (1:10,000, Invitrogen). Unless otherwise indicated, G6PDH was used as a loading control and was detected with anti-G6PDH (Sigma, A9521 1/10,000). HPR coupled secondary antibodies used in this study

were: goat anti-rabbit IgG (Chemicon, catalog no. AP307P, 1:5000), and goat anti-mouse IgG (BioRad, catalog no. 170-6516, 1:5000). The fluorescent secondary antibody used was Alexa Flour 680 goat ani rabbit. Membranes were developed using Western Chemiluminescent HRP Substrate Detection System (Millipore, catalog no. WBKLS0500) and imaged using either chemiluminescence imaging on a Molecular Imager ChemiDoc XRS System (BioRad) or both chemiluminescence and flourecence imaging using a LI-COR Odyssey Fc (Li-COR Biosciences, USA).

2.6 Fluorescence microscopy of Gds1-GFP and Msn2-GFP

Strains containing endogenouly C-terminally GFP tagged *MSN2* or *GDS1* were grown to mid log phase (OD_{600} of 0.5-07) and imaged live in absence of fixation agents. 1ml of culture was gently spun down (3000rpm, 30seconds) and re-suspended in 50µl synthetic media. 2µl were immediately spotted onto a glass plate and imaged immediately using brightfield and FITC filters. Microscopy was performed using a Leica DMI 6000 florescent microscope (Leica Microsystems GmbH, Wetzler Germany), equipped with a Sutter DG4 light source (Sutter Instruments, California, USA), Ludl emission filter wheel with Chroma band pass emission filters (Ludl Electronic Products Ltd., NY, USA) and Hamamatsu Orca AG camera (Hamamatsu Photonics, Herrsching am Ammersee, Germany). Images were collected and analyzed using Velocity 4.3.2 Build 23 (Perkin Elmer). Nuclear localization of Msn2-GFP was scored by eye with any visible enrichment of GFP signal in the nucleus being scored as nuclear Msn2-GFP. ~300-600 cells were counted per strain from three independent replicates.

2.7 Quantification of Gds1-GFP protein level

A strain containing endogenously C-terminal GFP tagged Gds1p was grown in a single culture to an OD_{600} of 0.5 under non-stress conditions (YPD 25°C). The culture was split into 25ml fractions and cells were pelleted by gentle centrifugation (3000rpm 3 minutes). Pellets were re-suspended in a volume of 25ml YPD or a variety of stressors added to the YPD : 37°C, pH 4.1 (75µl 14.3N HCl), pH 9.0 (57.2µl 10N NaOH), 1.5M sorbitol (6.85g sorbitol), 8.0mM caffeine (0.04g caffeine, Sigma C0750), 7% EtOH (1.75ml 100% EtOH in 23.25ml YPD), 0.4mM H_2O_2 (1.4µl H_2O_2), 800ng/ml rapamycin (Sigma, R8781), 0.08% methyl methanesulfonate (MMS, Sigma M4016), and 60µg/ml Benomyl (Sigma 381586), and 150mM LiCl. Starvation was induced by washing a pellet twice with synthetic complete (SC) media lacking glucose and re-suspending in 25ml SC media lacking glucose. Cells were harvested from each condition at 30 minutes and 2 hours, washed in 50ml ice cold ddH_2O and flash frozen on dry-ice. Whole cell extracts were separated by SDS-PAGE and Western blotting was performed as describe above. α-GFP immunoblots were performed as described above and blots were exposed using a LI-COR system (Li-COR Biosciences, USA). Band intensity was analyzed using Image Studio V2.0 and standardized to unstressed Gds1-GFP protein level. Briefly, boxes of equal size were drawn around each Gds1-GFP protein band and standardized to corresponding loading control bands (Gds1-GFP band intensity / corresponding loading control band intensity). Total Relative band intensity corrected to loading control was then used as a measurement of relative Gds1-GFP abundance and compared to untreated Gds1-GFP protein level.

2.8 Quantitative real-time PCR

13ml of cells from 50ml of mid-log-phase culture (OD_{600} between 0.6 to 0.8) grown in YPD at 25°C were collected by centrifugation and washed twice with 5ml of cold water. Cells were re-suspended in 2ml of Tri-Reagent (Sigma-Aldrich, USA; Cat. #: T9424) and followed by the addition of 1ml of acid-washed glass beads (Fisher Scientific, USA; Cat. #: 35535). The cell pellet was then frozen in liquid nitrogen and store at -80C until ready for processing the samples. All the centrifugations were performed at 4°C. The samples were allowed to be thawed at room temperature (RT), vortexed at maximum speed for 5 minutes, followed by the addition of 500µl of chloroform and vortexed again for 15 seconds. The samples were incubated for 10 minutes at room temperature and then centrifuged at 3200g for 20 minutes. The aqueous layer was transferred equally between two RNAse-free eppendorf tubes. 1ml of isopropanol was added and incubated on ice for 10 minutes. The RNA was centrifuged at 12000g for 25 minutes. The pellet was washed with 500µl of ethanol 75% made with DEPC water. The pellet was again centrifuged at 7500g for 5 minutes, dried and resuspended in a final volume of 40µl of nuclease-free water (Ambion, USA; Cat. #: AM9937). 10ug of RNA (ND-1000 spectrophotometer, NanoDrop Technologies, USA) was treated with DNAse (RQ1 RNAse-free DNAse, Promega, USA; Cat. #: M6101) for 30 minutes at 37°C as per the manufacturer's instructions. The RNA was precipitated by adding 400µl of Nuclease-free water and 500µl of phenol/chloroform. The RNA was vortexed at maximum speed for 1 minute and centrifuged at 12000g for 5 minutes. The aqueous layer was transferred to a new RNAse-free eppendord, and 5µl of NaAc 3M and 1ml of Ethanol 100% was subsequently added. The precipitated RNA was incubated at -80°C overnight and centrifuged at 12000g for 30 minutes. The pellet was washed with

500µl of 70% ethanol made with Nuclease-free water, centrifuged again at 7500g for 5 minutes, dried and resuspended in 20µl Nuclease-free water. The RNA was normalized at 200 ng/µl with Nuclease-free water. RT-PCR was performed as per the manufacturer's instructions with 2.5µg of RNA in a final volume of 20µl (High Capacity cDNA RT Kit, Applied Biosystems, USA; Cat. #: 4368814). qPCR was performed with the cDNA as per the manufacturer's instructions in a final volume 10µl (SsoFast EvaGreen Supermix; Bio-Rad, USA; Cat. #: 172-5201). Primer used for the qPCR:

HSP12 forward 5'-GTCTGACGCAGGTAGAAAAGG-3; *HSP12* reverse 5'-CTTCTTGGTTGGGTCTTCTTC-3';

TDH3 forward 5'-CTGTCAAGTTGAA CAAGGAAACCAC-3'; *TDH3* reverse 5'-CAACGTGTTCAACCAAGTCGACAA-3'

2.9 Hsp12-GFP protein level analysis by flow cytometry

Strains containing endogenously C-terminally GFP tagged *HSP12* were grown to mid log (OD_{600} of 0.5-07) phase in YPD then diluted 1/1000 in ice cold citrate buffer (50mM sodium citrate pH 7.4 with citric acid). Samples were immediately analysed by flow cytometry (Beckman Coulter Cyan ADP 9 – Analyzer, USA). GFP was excited by a 488nm solid state laser and emission was collected through a 540/40nm FITC filter. A minimum of 10,000 cells were counted per sample using HyperCyt Software Interface CyAn softwere and median fluorescent intensity was extracted from histograms using Kaluza Flow Cytometry Analysis software.

2.10 Heat shock viability assay

Viability assays were performed as in (Sadeh et al., 2011) with modifications. Yeast strains were grown to an OD_{600} of 0.4 under non-stress conditions (YPD 25°C). Two 250µl aliquots of each culture was gently pelleted (3000rpm for 3 minutes) and re-suspended in YPD either pre-warmed to 25°C or 48°C. Cultures were incubated at the indicated temperature for 11 minutes and immediately diluted 1:900. 100µl of dilutions were plated on YPD in quadruplicate and incubated at 25°C for two days. Colonies were counted manually and strain viability was calculated as the survival ratio between mutant strain and WT. (# colonies treated mutant / # colonies untreated mutant) / (# colonies treated WT / # colonies untreated WT). Experiment is an average of three biological replicates.

2.11 Chromatin Immunoprecipitation-Multiplex PCR

TAP-mChIP was performed as described above but DNA was sheared by sonication to an average size of 500bp (3x 10sec setting 2 on the Misonix Sonicator 3000). Following immunoprecipitation, beads were re-suspended in 500µl TE (1M Tris-HCl pH 8.0) and 12.5 µl protein K (Invitrogen, 25530015) and incubated with end over end rotation at 37°C for 2 hours. Phenol-Chloroform extraction was performed to isolate DNA resulting in 50µl DNA samples dissolved in TE buffer. 1µl glycogen was added to samples followed by 1ml ice cold 100% ethanol and two hour incubation at -20°C. DNA was collected by centrifugation at 13000rpm for 15 minutes and pellets were washed with 1ml 70% ethanol followed by air-drying and re-suspension in 50ul TE buffer. Immunoprecipitated DNA was amplified using multiplex PCR with the following primer pairs: *HSP12* forward (5'-CGCAAGCATTAATACAACCC-3'), *HSP12* reverse (5`-

CGCAATTGAGGAAGTAGAAC-3) and no ORF control forward (5`-GGCTGTCAGAATATGGGGCCGTAGTA-3`)

reverse (5`-CACCCCGAAGCTGCTTTCACAATAC-3`).

PCR products were resolved on a 3% agarose gel and visualized by SYBR Safe (Invitrogen S33102).

2.12 Hsp12-GFP flow cytometry mini-screen

Two libraries expressing Hsp12-GFP combined with single or double gene deletions were constructed by robotic mating techniques using a Singer RoToR HDA (Singer Instruments, United Kingdom) as previously described (Tong and Boone, 2006; Winzeler et al., 1999). Briefly, quarry strains constructed expressed either Hsp12-GFP::URA (YKB 3286) or Hsp12-GFP::URA *gds1Δ::NAT* (YKB 3287) (the query strains) were robotically mated to deletion mutant strains (Tong et al., 2001) corresponding to putative Gds1p interactors. Gds1p putative interactors were manually picked from the deletion mutant array (DMA) and cultured in two 96 well plates. Strains were then robotically pinned from the liquid media onto square YPD plates containing G418 for selection (mini-DMA). The α-mating type query strains were plated as lawns on square YPD plates and allowed to grow for one day at 25°C followed by direct robotic pinning of the min-DMA onto the lawns for mating. Mating was allowed to occur for one day at 25°C and diploids were plated on appropriate selection (SC-URA + G418 or SC-URA +G418 + cloNAT (Werner Bioagents, 5.0000)). Colonies were then pinned on sporulation media (10g potassium acetate, 1g yeast extract, 0.5g glucose, 0.1g amino acid supplement powder [2g histidine, 10g leucine, 2g lysine, 2 g uracil] / liter of media) and allowed to sporulate 5 days at 22°C. To select for

mating type a haploid progeny, the sporulated strains were plated on SC- HIS –ARG –LYS + canavanine (50mg/L) for two days at 25°C. A second round of haploid MATa selection was performed as above. Finally, to select for strains carrying the appropriate genotype (either *HSP12-GFP::URA xxxΔ::KAN* or *HSP12-GFP::URA gds1Δ::NAT xxxΔ::KAN,* were gene *xxx* is a putative Gds1p interactor), colonies were pinned on SC –URA –HIS –LYS –ARG +MSG + canavanine (50mg/L) + G418 + cloNAT. Strains were allowed to grow two days at 25°C and the selection process was repeated three times. The two libraries (mini-arrays) produced consisted each of two 96 colony solid media plates and were grown on synthetic media lacking uracil and containing either G418 (Sigma, A1720) alone or G418 and clonNAT to select for the presence of Hsp12-GFP and the absence of the gene / genes of interest. The flow cytometry screen was performed once. Cells from the constructed mini-arrays were pinned into rich liquid media (YPD) in 96 well plates and grown to mid log phase at 25°C. Cultures were diluted ~1/1000 into ice cold citrate buffer (50mM sodium citrate pH 7.4 with citric acid) and immediately analysed by flow cytometry (Beckman Coulter Cyan ADP 9 – Analyzer, USA) as described above.

2.13 Construction of Gds1p point mutants

Point mutants were constructed using the QuikChange Lighting Site-Directed Mutagenesis kit (Agilent Technologies, 210518) as per manufacturer instructions. Primers were designed with a mismatch base pair at the lysine codon to be mutated flanked by ~10bp complementary DNA on each side. Mutagenesis PCR was conducted as per manufacturer instructions using 10ng of double stranded plasmid DNA containing WT *GDS1*. Mutated plasmid was transformed into 45µl XL10-Gold ultracompetent cells (Agilent Technologies ,

200314) with 2µl β-mercaptoethanol added. The *GDS1* point mutant was then PCR amplified from the plasmid along with a NAT resistance marker and co-transformed into a *gds1Δ* strain caring a G418 resistance marker as described (Longtine et al., 1998).

Primers:

K87 to R forward;
GAAGGACTTTGGTGCTCCCAAAAGAGACAATCCATTATTAGAA

reverse: TTCTAATAATGGATTGTCTCTTTTGGGAGCACCAAAGTCCTTC

K348, 342, 343 to R forward:
GCCAAGGAGAACCAGAACTACTAGACCTGGCAGACA

reverse: TGTCTGCCAGGTCTAGTAGTTCTGGTTCTCCTTGGC

K345, 351, 354 R forward:
AGAACTACTAAACCTGGCAGACAAACAAGATCCCAATCTTTATC

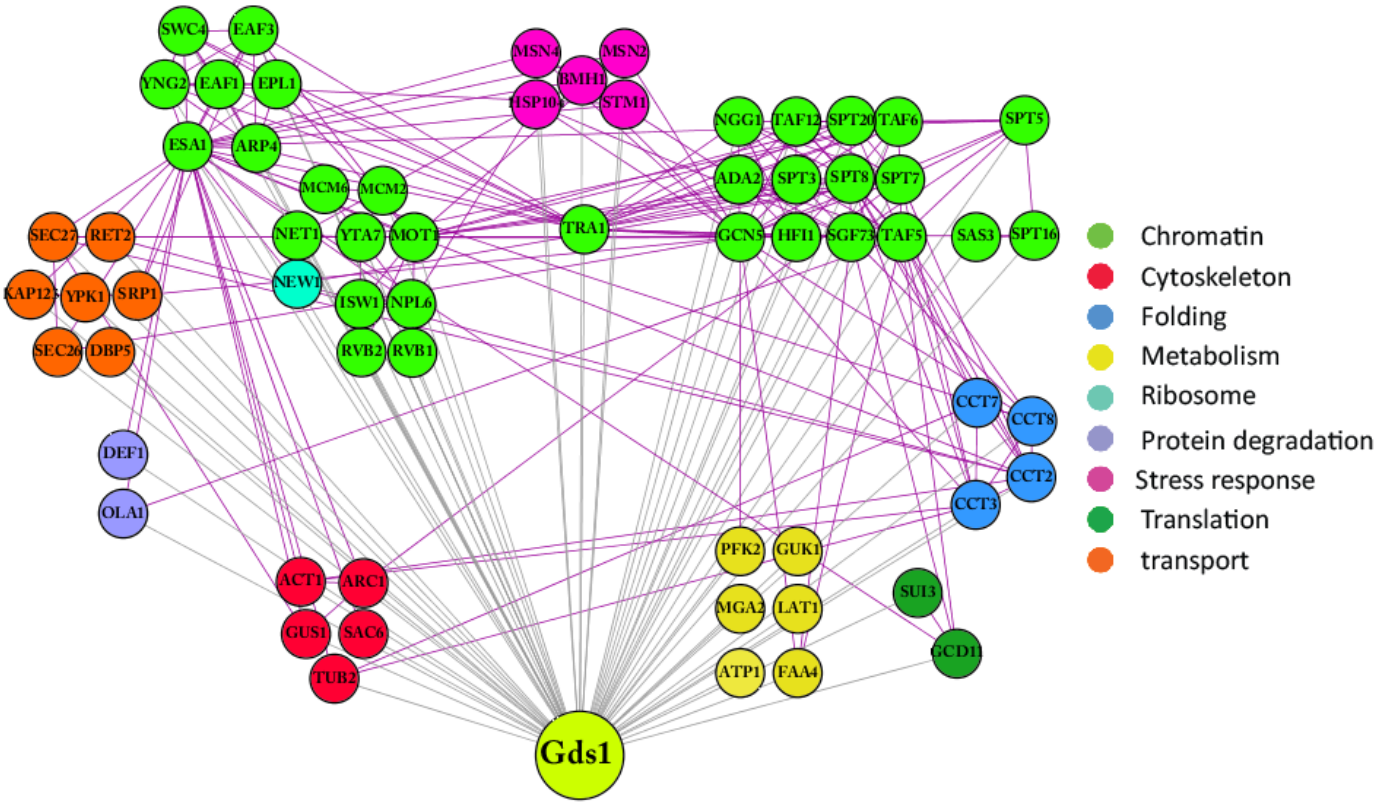
reverse: GATAAAGATTGGGATCTTGTTTGTCTGCCAGGTTTAGTAGTTCT

K408 to R forward: GCTGCACCAAGGCTTTCTAGGCTTTTGCCGAAAA

reverse: TTTTCGGCAAAAGCCTAGAAAGCCTTGGTGCAGC

CHAPTER 3: RESULTS

3.1 Gds1 physically interacts with NuA4 and the yeast stress response transcription factors Msn2p and Msn4p. To begin to uncover the role of Gds1p, a former PhD student, Leslie Mitchell, applied mChIP (Lambert et al., 2009b) to cells expressing Gds1-TAP and used mass spectrometry (MS) to identified interaction partners. mChIP is a variant of traditional TAP purifications that has been successfully applied to a wide range of bait proteins to increase the depth of protein interactions (Kennedy et al., 2011; Lambert et al., 2010; Mitchell et al., 2013) and when applied to Gds1-TAP identified interaction partners largely involved in chromatin-related processes (**Figure 1A and Table S1**). Confirming previously studies, Gds1-TAP co-purified 9 out of 13 subunits of NuA4 along with both Msn4p and Msn2p. In addition the Gds1p interactome was enriched for nuclear localized proteins (**Figure 1B**), including co-purification of 13 out of 21 subunits of the SAGA acetyltransferase complex, stress related transcription factors, a wide range of chromatin associated proteins and enzymes involved both in glycolysis and fatty acid metabolism (**Figure 1A,B and Table S1**). To determine whether Gds1p is required for the co-purification of NuA4 and Msn4p, I assessed the interaction in the presence and absence of Gds1p (**Figure 1C**). I found that the Msn4-TAP can immunopurify the NuA4 specific component Eaf7-MYC independently of the presence of Gds1p suggesting that Gds1p is not required for the interaction between NuA4 and Msn4p. The Gds1p interactome is evidence of a physical interaction between NuA4, Gds1p and Msn2p/Msn4p and suggests that Gds1p may play a role in transcription or other chromatin processes, including the Msn2p/Msn4p dependent environmental stress response.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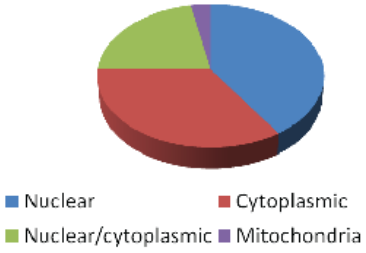
YNG2
EAF1
EPL1
ESA1
ARP4
MSN4
MSN2
BMH1
HSP104
STM1
NGG1
TAF12
SPT20
TAF6
SPT5
ADA2
SPT3
SPT8
SPT7
MCM6
MCM2
SEC27
RET2
NET1
YTA7
MOT1
TRA1
GCN5
HFI1
SGF73
TAF5
SAS3
SPT16
KAP123
YPK1
SRP1
NEW1
ISW1
NPL6
SEC26
DBP5
RVB2
RVB1
CCT7
CCT8
CCT2
CCT3
DEF1
OLA1
PFK2
GUK1
MGA2
LAT1
SUI3
GCD1
ATP1
FAA4
ACT1
ARC1
GUS1
SAC6
TUB2
Gds1
Chromatin
Cytoskeleton
Folding
Metabolism
Ribosome
Protein degradation
Stress response
Translation
transport

B


Nuclear
Cytoplasmic
Nuclear/cytoplasmic
Mitochondria

C

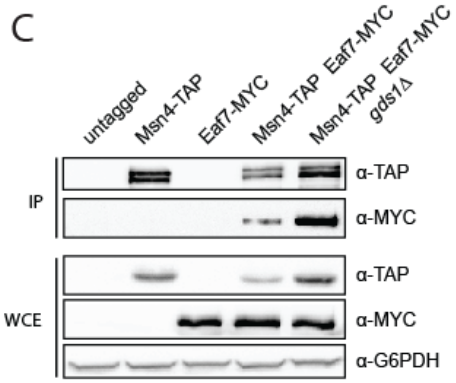

untagged
Msn4-TAP
Eaf7-MYC
Msn4-TAP Eaf7-MYC
Msn4-TAP Eaf7-MYC gds1Δ
IP
α-TAP
α-MYC
WCE
α-TAP
α-MYC
α-G6PDH

Figure 3.1: The Gds1 protein interaction network.

A, the Gds1 physical interactome is enriched for the NuA4 and SAGA acetyltransferase complexes as well as transcription factors involved in the stress response. C-terminal TAP tagged Gds1 (YKB 2163) and its associated proteins were purified by mChIP and identified by LC-MS/MS. Interacting proteins are grouped by cellular process (node color/label) and multiple members of the NuA4 and SAGA complex are circled. Gray lines indicate physical interactions identified in this study while purple lines represent known physical interactions listed in The Biogrid (http://thebiogrid.org/). **B**, Cellular localization of proteins in Gds1 network. Localization annotation is based on a global study (Huh et al., 2003). **C**, the physical interaction between NuA4 and Msn4p is not dependent on Gds1. C-terminally TAP tagged Msn4 (YKB 2959) was immunopurified in a strain background containing C-terminally MYC tagged Eaf7 (YKB 1062) in the presence (YKB1062) or absence (YKB 3018) of *GDS1*. Immunopreciptations (IPs) and whole cell extracts (WCEs) were analysed by Western blot and probed with anti-TAP (α-TAP), MYC (α-MYC), or glyceraldehyde-6-dehydrogenase (α-G6DPH) antibodies. The co-purification between the NuA4 subunit Eaf7p and Msn4p persists in a *gds1Δ* background.

A, Interactome experiment performed by L. Mitchell. Data analysis and processing performed by M. Cotrut.

3.2 Gds1p is not required for growth on alternate carbon sources or for the activation of the ESR. The Gds1-TAP interactome taken together with initial reports of Gds1p being required for survival on glycerol as a carbon source (Konopinska et al., 1995) led me to hypothesise that Gds1p is required for cell stress survival not only related to alternate carbon sources but also other Msn2p/Msn4p activating stresses. To test this hypothesis I tested the ability of *gds1Δ* cells to grow and survive under a wide variety of chemical and environmental stress conditions **(Figure 3.2)**. Surprisingly, I found that Gds1p is not required for cell growth on glycerol or the five carbon sugar xylose as the sole carbon source **(Figure 3.2)**, nor is it required for growth in the presence of heat stress (37°C), DNA damaging agents (0.08% MMS, 100mM HU), microtubule stress (60µg/ml Benomyl), ethanol (5%), oxidative stress (0.4mM hydrogen peroxide), mild acetic stress (50mM acetic acid), osmotic stress (0.5M NaCl), metal ion stress (1.5mM Ni+), or 8.0mM caffeine **(Figure 3.2)**. Thus Gds1p is not required to survive these stresses suggesting it is not involved in the initiation or maintenance of the ESR, nor is it required for growth on glycerol or xylose.

3.3 NuA4 regulates the acetylation state and protein level of Gds1. Our group previously determined that Gds1p is acetylated *in vivo* and is a substrate of NuA4 *in vitro* (Mitchell et al., 2013). Therefore, I next sought to determine if Gds1p acetylation state *in vivo* is dependent on NuA4. To determine whether Gds1 is acetylated *in vivo* we immunoprecipitated Gds1-GFP and probed with an anti-lysine acetyl antibody. As a control for cross-reactivity of the anti-lysine acetyl antibody to the GFP tag we also immunopurified GFP-Tub1. While mammalian tubulin is acetylated to stabilize microtubule structures (LeDizet and Piperno, 1987; Piperno and Fuller, 1985b) this

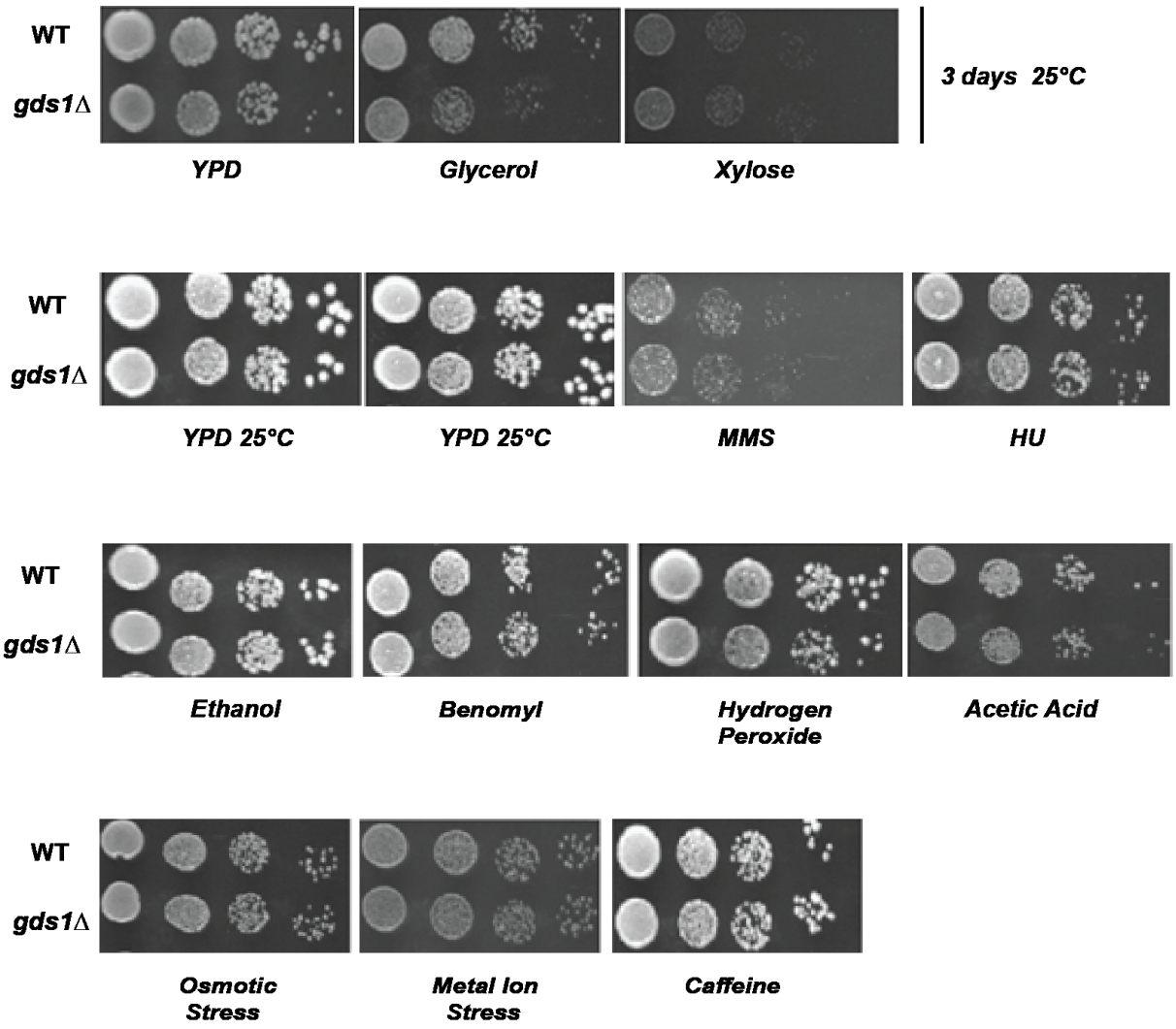

WT
gds1Δ
3 days 25°C
YPD
Glycerol
Xylose
WT
gds1Δ
YPD 25°C
YPD 25°C
MMS
HU
WT
gds1Δ
Ethanol
Benomyl
Hydrogen Peroxide
Acetic Acid
WT
gds1Δ
Osmotic Stress
Metal Ion Stress
Caffeine

Figure 3.2: Gds1p is not required for growth on alternate carbon sources or in the presence of various stresses.

Wild type (WT, YKB 779) and *gds1Δ* (YKB 2644) cells were grown to mid-log phase under non-stress conditions (rich media, 25°C). Cultures were diluted to an OD_{600} of 0.1 and 10-fold serial dilutions were spotted on plates containing the indicated carbon source or YPD in combination with heat stress (37°C), DNA damaging agents (0.08% MMS, 100mM HU), microtubule stress (60μg/ml Benomyl), ethanol (5%), oxidative stress (0.4mM hydrogen peroxide), mild acetic stress (50mM acetic acid), osmotic stress (0.5M NaCl), metal ion stress (1.5mM Ni+), or 8.0mM caffeine.

modified lysine is not conserved in *S. cerevisiae* and acetylation of Tub1p has not been reported (Henriksen et al., 2012b). Sylvain Huard and I determined that Gds1-GFP is acetylated *in vivo* as detected by Western analysis (**Figure 3.3A**), whereas Tub1-GFP displays a negligible signal. To test whether acetylation of Gds1p is dependent on NuA4, I introduced two acetylation deficient mutants, *esa1-L254P* and *eaf1Δ,* into a Gds1-GFP expressing strain and assessed Gds1-GFP acetylation status at 25^{o}C, a condition where both strains display reduced histone H4 acetylation (Clarke et al., 1999; Krogan et al., 2004). While the acetylation state of Gds1-GFP is reduced in *esa1-L254P* (**Figure 3.3B**), in *eaf1Δ* cells we could not assess Gds1-GFP acetylation status as its protein levels in the whole cell extract (WCE) is dramatically reduced. The reduced Gds1-GFP protein levels are not a reflection of transcriptional defects in *eaf1Δ* cells, as *GDS1* mRNA expression level is elevated in the absence of *EAF1* (**Figure 3.3C**). Sylvain Huard and I attempted to look at the acetylated state of Gds1-GFP at the non-permissive temperature in an *esa1-L254P* background, but found that the level of Gds1-GFP is dramatically reduced in the whole cell extract in both wild type and *esa1-L254P* background after four hours at 37^{o}C (data not shown, but further explored below). This work determined that the *in vivo* acetylation state of Gds1p is partially dependent on NuA4, and surprisingly determined that the protein levels of Gds1 are decreased in mutant backgrounds deficient in NuA4 activity.

3.4 Gds1-GFP is a nuclear protein that rapidly disappears upon stress. As Gds1 co-purifies with numerous nuclear localized proteins (**Figure 3.1B**) and protein levels decrease at elevated temperatures (**Figure 3.4A**), I next examined Gds1-GFP cellular localization at both 25^{o}C and after heat shock. I could not detect Gds1-GFP in fixed cells, however in live cells Gds1-GFP localized to the nucleus in the absence of stress (**Figure 3.4A**). Supporting

the disappearance of Gds1p upon heat shock (see below), Gds1-GFP was undetectable within 30 minutes at 37^{o}C (**Figure 3.4A**). To further asses Gds1-GFP protein levels in response to stress, cells expressing Gds1-GFP were grown under stress free conditions (25^{o}C YPD) prior to being exposed to a wide variety of environmental stress, include those that activate Msn2p/Msn4p and those that likely do not. Msn2p/Msn4p activating stresses tested were: heat stress, glucose depravation, oxidative stress, low pH, 7% ethanol treatment, osmotic stress through sorbitol, alkaline pH, rapamycin and benomyl (Beck and Hall, 1999; Causton et al., 2001; Gasch et al., 2000; Lucau-Danila et al., 2005; MartinezPastor et al., 1996; Sadeh et al., 2011). In fact, Msn2p is required for transcription of ~90% of genes that are induced regardless of environmental stress applied (Causton et al., 2001). Therefore, while MMS, caffeine and LiCl have not yet been implicated in the Msn2p/Msn4p dependant stress response, they likely elicit a stress response as *msn2Δ* mutants are sensitive to these stresses (Hillenmeyer et al., 2008). Gds1-GFP protein levels were then assessed by quantitative Western blot after 30 minutes and 2 hours of stress exposure (**Figure 3.4B**). Gds1 protein level depletion varied depending on the stress applied with the most robust and rapid depletion occurring upon heat stress at 37^{o}C, alkaline stress at pH 9.0, carbon starvation, and DNA damage by MMS. It is interesting to note that Gds1-GFP is depleted in 30 minutes when cells are exposed to pH 4.1 or 1.5M sorbitol media, but is restored to ~40% or 90% of wild type levels respectively after two hours. Of the two non-Msn2p/Msn4p implicated stresses tested; caffeine and LiCl, Gds1-GFP protein levels persist after 30 minutes but are markedly reduced after 2 hours. Thus, Gds1p is a nuclear protein and environmental stresses lead to depletion of Gds1p protein level with variable kinetics depending on the nature of the stress.

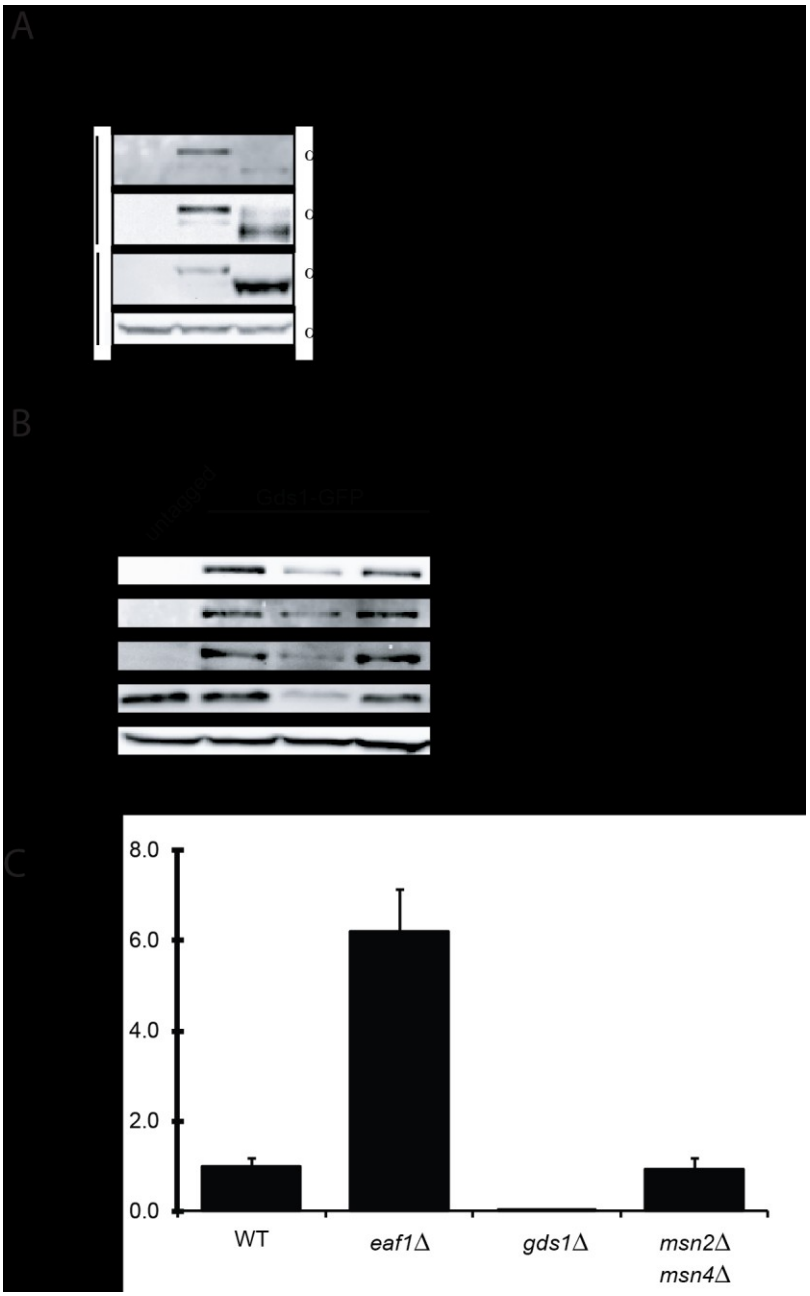

A
B
Gds1-GFP
C
8.0
6.0
4.0
2.0
0.0
WT
eaf1Δ
gds1Δ
msn2Δ
msn4Δ

Figure 3.3: NuA4 contributes to Gds1p acetylation *in vivo* and regulates its protein level.

A, Gds1p is acetylated *in vivo*. Gds1 was immunopurified from yeast cells through a C-terminal GFP tag (YKB2609) as compared to an untagged strain (YKB 779 WT) and an N-terminal Tub1 GFP fusion protein (YKB1189). Immunopurified products and whole cell extract were subjected to Western blot analysis with anti-acetyl lysine (α-AcK), GFP (α-GFP), or glyceraldehyde-6-dehydrogenase (α-G6DPH) antibodies. **B,** Gds1 *in vivo* acetylation status is partially dependent on NuA4. Gds1-GFP (YKB2609) was immunopurified from two acetylation deficient NuA4 mutant strains *eaf1Δ* (YKB2839) and *esa1-L254P* (YKB2628) as compared to an acetyltransferase competent strain (YKB2609) and an untagged control (YKB779, WT). Immunopurification and Western blotting were performed as in **A,** except here WCE samples were also blotted for histone H4 acetylation (α-AcK H4). **C,** GDS1 mRNA expression is increased in *eaf1Δ* cells. Yeast cells were harvested at mid-log phase from cultures grown at 25°C. Wildtype (YKB779, WT) cells were compared to deletion mutants of *EAF1* (YKB 42*)*, *GDS1* (YKB 2644), and the stress responsive transcription factors *MSN2* and *MSN4* (YKB 1093). mRNA was isolated and *GDS1* mRNA level was assessed by quantitative PCR and standardized to the Wildtype *GDS1* transcription level. Three biological replicates were performed with technical duplicates.

* **A, C.** performed by S. Huard with strains made by M.Cotrut. **B.** Western blotting performed by S.Huard, strains were made and grown by M.Cotrut and IP was performed by M.Cotrut.

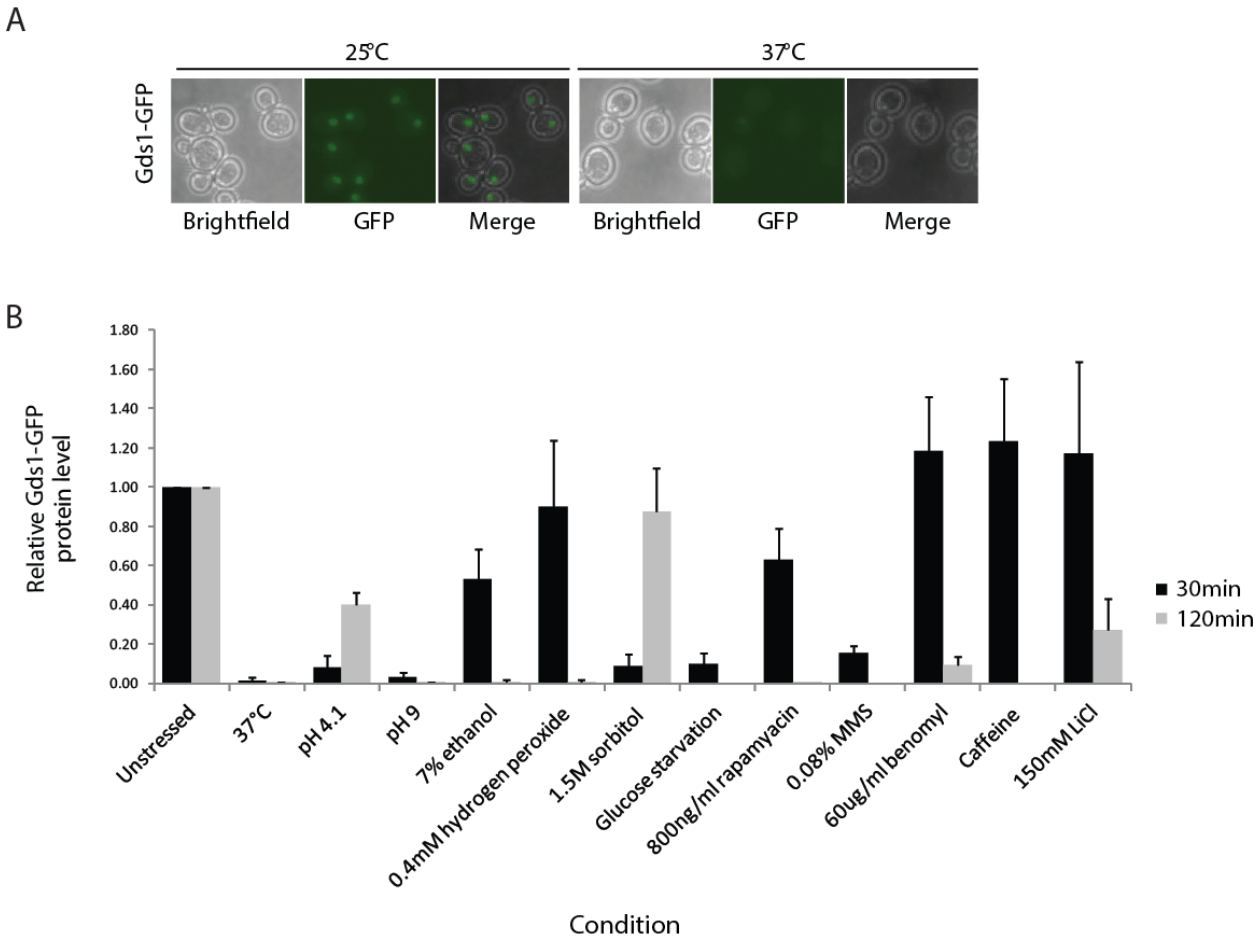
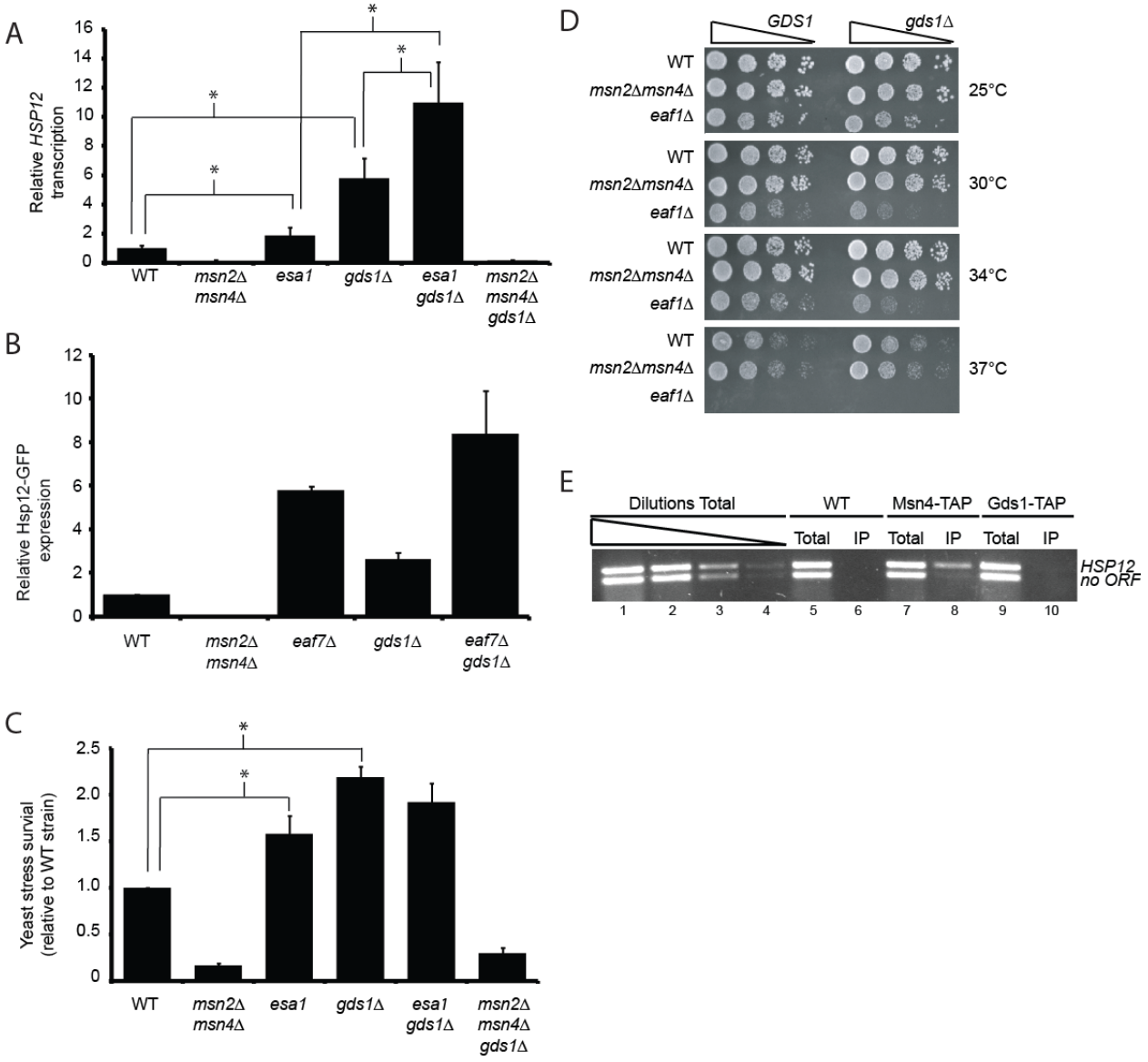

A
25°C
37°C
Gds1-GFP
Brightfield
GFP
Merge
Brightfield
GFP
Merge
B
Relative Gds1-GFP protein level
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
30min
120min
Unstressed
37°C
pH 4.1
pH 9
7% ethanol
0.4mM hydrogen peroxide
1.5M sorbitol
Glucose starvation
800ng/ml rapamycin
0.08% MMS
60ug/ml benomyl
Caffeine
150mM LiCl
Condition

Figure 3.4: Gds1-GFP is a nuclear protein that rapidly disappears upon stress.

A, Gds1-GFP is nuclear protein in the absence of stress and rapidly disappears after heat shock. Cells expressing endogenous C-terminally tagged Gds1 (YKB 2609) grown under optimal conditions (YPD, 25^{o}C) in the absence of stress were either exposed to a 30 minute heat at 37^{o}C shock or maintained at 25°C prior to imaging live. **B**, Gds1-GFP protein level is reduced in response to a variety of environmental stresses and at variable degrees. Strains Gds1-GFP (YKB 2609) was treated with a variety of environmental stresses as indicated for 30 minutes or 2 hours prior to lysis. Whole cell extracts were resolved by SDS-PAGE, subjected to Western blot analysis and probed with α-GFP and α-G6DPH antibodies. Results are displayed as Gds1-GFP protein level standardized to levels in unstressed cells and are averages of three experiments. Error bar represents one standard deviation.

3.5 Gds1p and NuA4 inhibit the transcription of the Msn2p/Msn4p target *HSP12* in the absence of stress. The reciprocal co-immunopurifications between Msn4p, NuA4 and Gds1p suggest that Gds1 may have a role in regulating the transcriptional stress response. Therefore we first asked if Gds1p impacts the transcription of the exclusive Msn2p/Msn4p target gene *HSP12* (MartinezPastor et al., 1996; Schmitt and McEntee, 1996) under non-stress conditions. We found that similar to *eaf1*Δ (Mitchell et al., 2008) and *esa1-L254P* cells, *HSP12* expression is derepressed under non-stress conditions in the absence of Gds1p and that this derepression is completely dependent on Msn2p and Msn4p (**Figure 3.5A**). A caveat to this result is that both *eaf1Δ* (Auger et al., 2008; Krogan et al., 2004) and *esa1-L254P* (Clarke et al., 1999) display growth defects, hence the Msn2p/Msn4p derepression in these NuA4 mutants could reflect a permanently stressed state. Therefore I also measured the impact of *gds1Δ* and *eaf7Δ,* a subunit of NuA4 that displays little or no impact on cell viability (Krogan et al., 2004), on Hsp12-GFP protein expression at 25°C. In agreement with previous studies (Sadeh et al., 2011), *eaf7Δ* displayed increased Hsp12-GFP signal as did *gds1Δ* (**Figure 3.5B***)*. As a secondary method to measure the role of Gds1p in Msn2p/Msn4p repression, I sought to determine if deletion of Gds1p confers protection to extreme heat shock. Msn2p/Msn4p activity is required to protect cells from extreme stress (MartinezPastor et al., 1996) and mutants that derepress Msn2p/Msn4p activity in the absence of stress, like that of NuA4 mutant *eaf7Δ*, display increased stress survival (Sadeh et al., 2011). Similarly, I discovered that after 11 min at 48°C, either deletion of *GDS1* or ablation of *esa1* acetyltransferase activity promotes cell survival (**Figure 3.5C**). Similar to *HSP12* transcription, the increased heat shock survival of *gds1Δ* cells is almost entirely mediated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Relative HSP12 transcription
16
14
12
10
8
6
4
2
0
*
*
*
*
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msn2Δ msn4Δ
esa1
gds1Δ
esa1 gds1Δ
msn2Δ msn4Δ gds1Δ
B
Relative Hsp12-GFP expression
12
10
8
6
4
2
0
WT
msn2Δ msn4Δ
eaf7Δ
gds1Δ
eaf7Δ gds1Δ
C
Yeast stress survial (relative to WT strain)
2.5
2.0
1.5
1.0
0.5
0
*
*
WT
msn2Δ msn4Δ
esa1
gds1Δ
esa1 gds1Δ
msn2Δ msn4Δ gds1Δ
D
GDS1
gds1Δ
WT
msn2Δmsn4Δ
eaf1Δ
25°C
30°C
34°C
37°C
E
Dilutions Total
WT
Msn4-TAP
Gds1-TAP
Total
IP
HSP12
no ORF
1
2
3
4
5
6
7
8
9
10

Figure 3.5: Gds1p regulates the yeast stress response through the stress responsive transcription factors Msn2p and Msn4p.

A, Gds1 negatively regulates expression of *HSP12*. mRNA from cells with the indicated gene mutations wild type (WT, YKB779), *msn2Δ msn4Δ* YKB 1093, *esa1* (*esa1-L254P*) (YKB860), *gds1Δ (*YKB 2644), *gds1Δ esa1* (*esa1-L254P*) (YKB 2943), *msn2Δ msn4Δ gds1Δ (*YKB 2841) were harvested following growth to mid-log phase at 25°C and analysed by RT-qPCR. **B,** *Hsp12-GFP* expression is increased in *GDS1* and NuA4 mutants. Flow cytometry was used to assess mean GFP fluorescence from the indicated strains: *HSP12-GFP* (YKB 3198, WT), *HSP12-GFP msn2Δ msn4Δ* (YKB 3278), *HSP12-GFP eaf7Δ* (YKB 3199), *HSP12-GFP gds1Δ* (YKB 3200), and *HSP12-GFP gds1Δ eaf7Δ* (YKB 3276). **C,** Gds1p and NuA4 mutants exhibit increased tolerance to heat stress. The same strains studied in **A,** were used in a viability assay to test cell survival following brief exposure (11 minutes) to extreme heat (48°C) as compared to cells maintained at 25°C. Results are an average of three experiments and are standardized to the survival rate of wild type cells (YKB 779) **D**, *GDS1* is not required for growth under temperature stress and displays a negative genetic interaction with *EAF1*. The indicated strains (WT 779, *gds1Δ* YKB 2644, *msn2Δ msn4Δ* YKB 1093, *msn2Δ msn4Δ gds1Δ* YKB 2841, *eaf1Δ YKB2839, eaf1Δ gds1Δ* YKB 3075) were grown to mid-log phase under non-stress conditions (rich media, 25°C). Cultures were diluted to an OD_{600} of 0.1 and 10-fold serial dilutions were plated on YPD at the indicated temperature and allowed to grow three days. The combined *eaf1Δ gds1Δ* shows a stronger synthetic sick interaction than the single mutants alone at temperatures above 30°C **E**, Gds1-TAP does not localize to the *HSP12* promoter. Modified ChIP (see Materials and Methods) was performed using untagged (YKB 779), Msn4-TAP (YKB1035), and Gds1-TAP (YKB 2163) strains. Total or immunoprecipitated (IP) DNA was subjected to multiplex PCR amplification using primers specific to the promoter of *HSP12* and an intergenic region on chromosome V (no ORF).

* Student T-test $P<0.05$

A. Strains made by M. Cotrut, qPCR performed by S. Huard, data analysis and organization performed by M. Cotrut and S. Huard.

E. Replicate shown preformed by A. Hamza. Two replicates also performed by M. Cotrut showed the same trends.

through Msn2p/Msn4p function. While, I found that *esa1-L254Pgds1Δ* cells and *eaf7Δgds1Δ* cells display additive derepression (the combined double mutant has higher HSP12 transcription than either single mutant) of *HSP12* transcription (**Figure 3.5A**) and Hsp12-GFP protein levels (**Figure 3.5B**) respectively, I did not detected additive ability for stress survival (**Figure 3.5C**). Potentially the differences may be attributed to the limitations in sensitivity of the heat stress survival assay as the two fold increase in stress survival seen in the single mutants already represents an ~90% survival rate. The observation that there are additive effects in both *HSP12* transcription and translation suggests that Gds1p and NuA4 act through two separate pathways to inhibit Msn2p/Msn4p. When both pathways are impeded (*gds1Δ,* NuA4 defective double mutant) the effect seen is greater than in single mutants were the complementary pathway can compensate for the loss of the other. To address whether Gds1p and NuA4 repress Msn2p/Msn4p activity through parallel or direct pathways, I examined synthetic genetic interactions between *EAF1* and *GDS1*. Supporting the hypothesis that NuA4 and *GDS1* work in parallel pathways, *eaf1Δ gds1Δ* cells display a conditional, moderate synthetic sick genetic interaction at temperatures over 30°C (**Figure 3.5D**). To address whether Gds1p inhibits Msn2p/Msn4p activity directly at promoter sites, we tested for Gds1-TAP localization at the promoter of *HSP12* using mChIP followed by PCR (Mitchell et al., 2011). Though we could detect Msn4-TAP on the promoter of *HSP12* as expected (Mitchell et al., 2011) we could not detect Gds1-TAP (**Figure, 3.5E**). Together, this suggests that Gds1p represses Msn2p/Msn4p activity under non-stress conditions and though Gds1p is a nuclear protein, its site of action may not be directly at STREs.

3.6 Nuclear exclusion of Msn2-GFP is dependent on Gds1p and NuA4. The major regulatory mechanism of Msn2p/Msn4p activity is localization; under non-stress conditions

the majority of Msn2p is localized to the cytoplasm and upon environmental stress it migrates to the nucleus (Gorner et al., 1998; Gorner et al., 2002; Jacquet et al., 2003). Therefore, I examined the localization of Msn2-GFP in both wild type and *gds1Δ* backgrounds at 25°C. While the majority of Msn2-GFP was localized to the cytoplasm in wild type cells as expected, in *gds1Δ* cells more than 70% of cells displayed Msn2-GFP enrichment in the nucleus (**Figure 3.6A, B**). Similar effects were seen for *eaf7Δ* cells, a deletion mutant subunit of NuA4 that though has little impact on cell viability displays derepression of *HSP12* transcription and improved heat stress survival (**Figure 3.5A and (Sadeh et al., 2011)**). My work suggests that both NuA4 and Gds1p are playing a role in excluding Msn2-GFP from the nucleus under non-stress conditions. While it is possible that NuA4 and Gds1p promote cytoplasmic retention of Msn2p in the absence of stress, the majority of both Gds1p (**Figure, 3.4A**) and NuA4 (Galarneau et al., 2000) are localized to the nucleus, suggests a role either in impeding Msn2p entry into the nucleus or aiding in its export.

3.7 Gds1p and NuA4 likely inhibit the Msn2p dependant stress response independently of PKA. In the absence of stress or once a stress is removed, cells rapidly inhibit Msn2p/Msn4p based transcription. This is largely mediated through PKA dependant phosphorylation of Msn2p, and presumably Msn4p, leading to nuclear export (Gorner et al., 1998; Gorner et al., 2002; Jacquet et al., 2003). As Gds1p and NuA4 also appear to act on Msn2p in the absence of stress, I asked if their deletion resulting in changes in PKA activity. Using an α-phosphorylated PKA substrate antibody, Lina Mah in the lab of Vivien Measday lab, probed whole cell extracts from *gds1Δ, eaf1Δ, gds1Δ eaf1Δ* cells and from cells expressing the overactive allele of the PKA component Ras2 ($Ras2^{VAL19}$- high PKA activity)

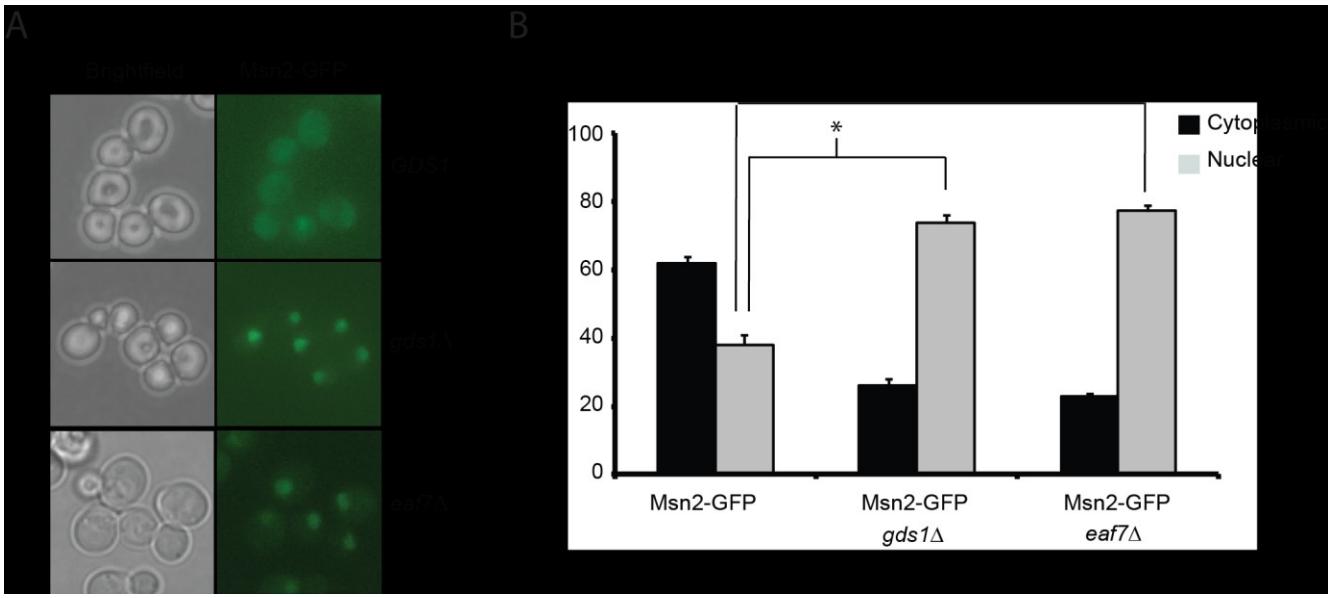
A
Brightfield
Msn2-GFP
GDS1
gds1Δ
eaf7Δ
B
100
80
60
40
20
0
*
Msn2-GFP
Msn2-GFP
gds1Δ
Msn2-GFP
eaf7Δ

Figure 3.6: Gds1p and NuA4 regulate Msn2p localization.

A, Cells expressing endogenous C-terminally GFP tagged Msn2p in the presence (YKB 2970) or absence (YKB 3085) of Gds1p or the non-essential NuA4 subunit, Eaf7p (YKB 3162), were examined by fluorescence microscopy. **B**, The localization of Msn2-GFP in each cell (nuclear or cytoplasmic) expressed as a percentage of total cells counted was determined from triplicate experimental repeats (300-600 cells per strain per repeat). Error bar represents one standard deviation. Cells displaying any discernible increase in nuclear GFP signal were counted as nuclear.

* Student T-test $P<0.05$

backgrounds in the absence of stress as a measure PKA activity (Chang et al., 2004; Deminoff et al., 2006; Ma et al., 2012). They found that neither the absence of *GDS1* or *EAF1* had an effect on the phosphorylation levels of PKA substrates (**Figure 3.7A**). Similarly under non-stress conditions, Msn2p phosphorylation levels were not impacted by deletion of *GDS1* or *EAF1* (**Figure 3.7B**). This suggests that neither deletion of *GDS1* or *EAF1* result in a decrease in overall PKA activity. Surprisingly, in the double mutant, *gds1Δ eaf1Δ*, there were modest increases in both PKA activity and Msn2p phosphorylation. As Msn2-GFP nuclear localization increases in both *gds1Δ* and *eaf7Δ* mutants (**Figure 3.4A, B**), the increased phosphorylation state of Msn2p and PKA substrates suggests cells are attempting to counter increased Msn2p nuclear influx by upregulating PKA phosphorylation of Msn2p. Another possibility is that Gds1p and/or NuA4 are necessary to mediate all or some of the PKA-dependent localization of Msn2p/Msn4p. Alternatively, Gds1p and/or NuA4 may work in parallel with PKA to exclude Msn2p from the nucleus in the absence of stress. To assess the latter two possibilities I looked at an Hsp12-GFP reporter gene by flow cytometry in the presence of underactive or overactive PKA (**Figure 3.7C, D**). In a strain constitutively expressing the PKA inhibitor *BCY1* (low PKA activity) nuclear localization of Msn2p/Msn4p increases resulting in an increase in Hsp12-GFP expression (Cameron et al., 1988; Gorner et al., 1998; Gorner et al., 2002; Ma et al., 2012). If PKA and Gds1p and/or NuA4 were working within the same pathway I would expect no additive increase in Hsp12-GFP expression in a strain combining *BCY1* overexpression and deletion of *GDS1* or *EAF7*. My results though may show additive affects between PKA downregulation and the *gds1Δ* and *eaf7Δ* mutants suggesting parallel pathways of promoting Msn2p nuclear exclusion (**Figure 3.7C**). To ascertain whether the Msn2p/Msn4p activating effects (Msn2p nuclear

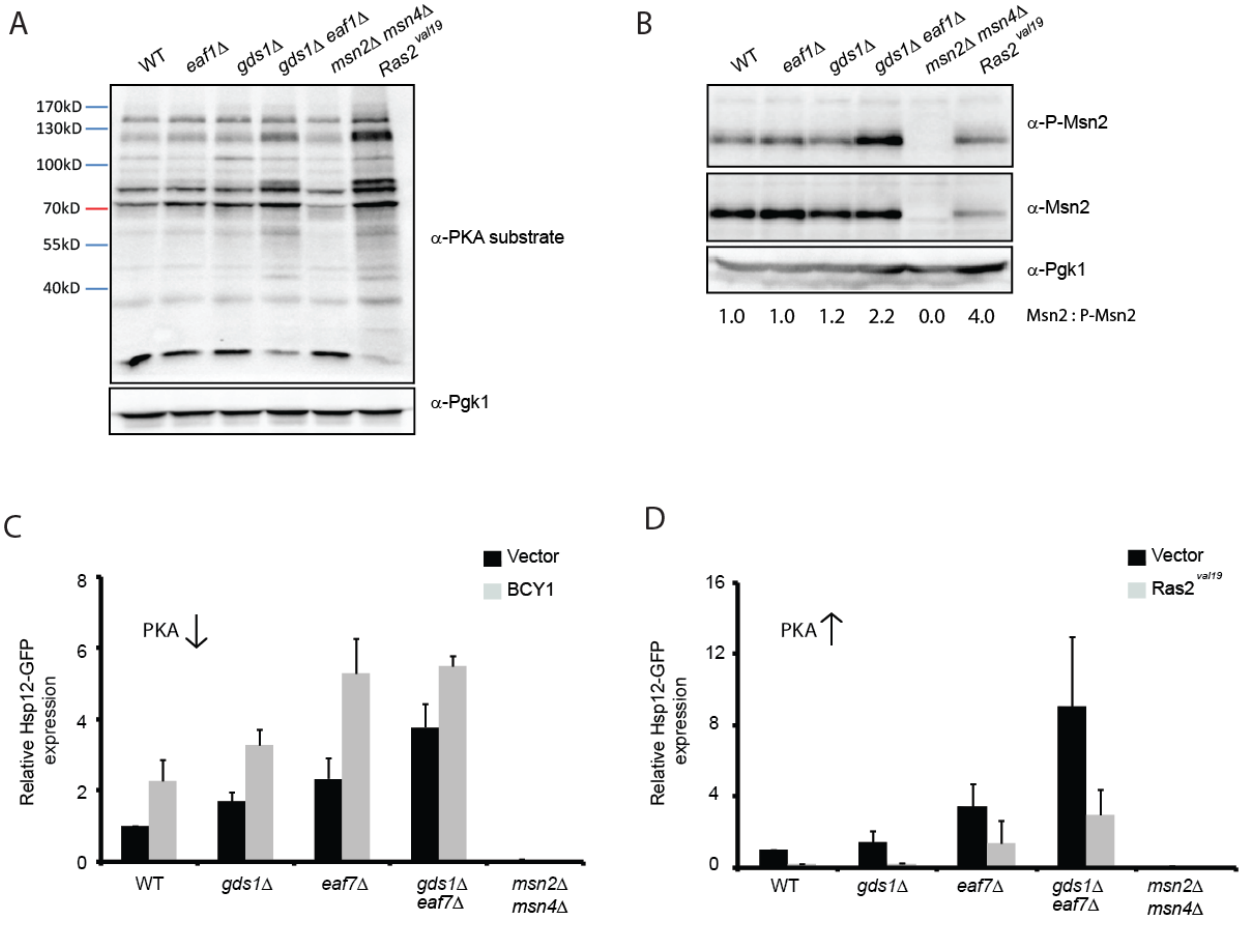
A
WT
eaf1Δ
gds1Δ
gds1Δ eaf1Δ
msn2Δ msn4Δ
Ras2val19
170kD
130kD
100kD
70kD
55kD
40kD
α-PKA substrate
α-Pgk1
B
WT
eaf1Δ
gds1Δ
gds1Δ eaf1Δ
msn2Δ msn4Δ
Ras2val19
α-P-Msn2
α-Msn2
α-Pgk1
1.0 1.0 1.2 2.2 0.0 4.0
Msn2 : P-Msn2
C
Vector
BCY1
PKA
Relative Hsp12-GFP expression
0
2
4
6
8
WT
gds1Δ
eaf7Δ
gds1Δ eaf7Δ
msn2Δ msn4Δ
D
Vector
Ras2val19
PKA
Relative Hsp12-GFP expression
0
4
8
12
16
WT
gds1Δ
eaf7Δ
gds1Δ eaf7Δ
msn2Δ msn4Δ

Figure 3.7: Gds1p and NuA4 regulate Msn2 independently of PKA.

A, Deletion of *GDS1* or *EAF1* does not impact global PKA activity. The indicated cells (wild type (WT, YKB 779), *eaf1Δ (*YKB 42)*, gds1Δ (*YKB 2644), *gds1Δeaf1Δ (*YKB 3075)*, msn2Δ msn4Δ* (YKB 1093)*,* and cells expressing $Ras2^{VAL19}$ [overactive allele of PKA component]) were grown to mid log phase, whole cell extracts resolved by SDS-PAGE and Western analysis was conducted using α-PKA substrate antibodies or α-Pgk1 antibodies as a loading control. Image is representative of three replicates. **B***,* Eaf1p and Gds1p do not affect Msn2p phosphorylation levels in unstressed cells. Whole cell extracts from the same strains described in **A**, were probed for Msn2 phosphorylation level using α-CREB antibodies, Msn2p protein level with α-Msn2 antibodies and Pgk1p protein levels as a loading control. Ratios are mutant:wildtype and standardized to the loading control **C**, PKA and Gds1p/Eaf7p work in parallel to suppress Msn2p/Msn4p activity. Relative Hsp12-GFP protein levels were determined by flow cytometry in the mutant strains: wild type (YKB 3198), *gds1Δ* (YKB 3200)*, eaf7Δ* (YKB 3199) *, gds1Δeaf7Δ* (YKB 3276)*,* and *msn2Δ msn4Δ* (YKB 3278), containing either a vector control (pRS416) or overexpressing the PKA inhibitor *BCY1*. **D***,* Hyperactive PKA can suppress *gds1Δ* and *eaf7Δ* induction of Hsp12-GFP. Relative Hsp12-GFP protein levels were determined by flow cytometry in the mutant strains described in (**C**) using either the vector control pRS326 or a vector expressing the hyperactive allele of the PKA component; $Ras2^{VAL19}$. For panel C and D, all experiments were performed with cells grown in the absence of environmental stresses and are averages of three biological repeats. Error bar represents standard deviation.

A, B performed by L. Ma in the Vivien Measday lab using strains made by M.Cotrut

enrichment) seen in *gds1Δ* and *eaf7Δ cells* are dominant to the repressive effects (Msn2p cytoplasmic enrichment) of PKA, I also assessed Hsp12-GFP expression in a strain background expressing the overactive allele of the PKA component Ras2 ($Ras2^{VAL19}$) (high PKA activity) (Ma et al., 2012; Ramachandran et al., 2011; Toda et al., 1985) (**Figure 3.7D**). In the $Ras2^{VAL19}$ background, there is almost complete abolition of Hsp12-GFP detection. I observed that the induction of HSP12-GFP displayed in *gds1Δ, eaf7Δ* and *gds1Δ eaf7Δ* mutant cells was decreased by the overactivation of PKA. Together these results suggest that Gds1p and NuA4 regulation of Msn2p/Msn4p localization and activity may be through a pathway independent of PKA. Further examination though is still required to definitively determine if Gds1p plays a role in PKA regulation.

3.6 Hsp12-GFP mini flow cytometry feasibility screen to identify potential mechanism by with Gds1p inhibition of Msn2p/Msn4p. In an attempt to discern the mechanism through which Gds1impacts Msn2/Msn4 activity, I designed a functional genomics screen based on the Hsp12-GFP flow cytometry screen that I determined was sensitive enough to detect additive expression defects of gds1Δ and eaf7Δ cells (**Figure 3.5B**). I hypothesized that proteins of the Gds1p interactome (Figure 3.1A) if they were working within the direct pathway with Gds1 to suppress Msn2p/Msn4p that when deleted would **a**) display increase Hsp12-GFP levels and b) this increase would not be additive in combination with *gds1Δ* **(Figure 3.8)**. Thus, I used robotics-based systematic genetic array methodology techniques (Tong et al., 2001), to create strains expressing Hsp12-GFP, as a Msn2p/Msn4p activity reporter, combined with non-essential deletion mutations of all known Gds1p physical interactors (manually identified from Biogrid as well as this study).

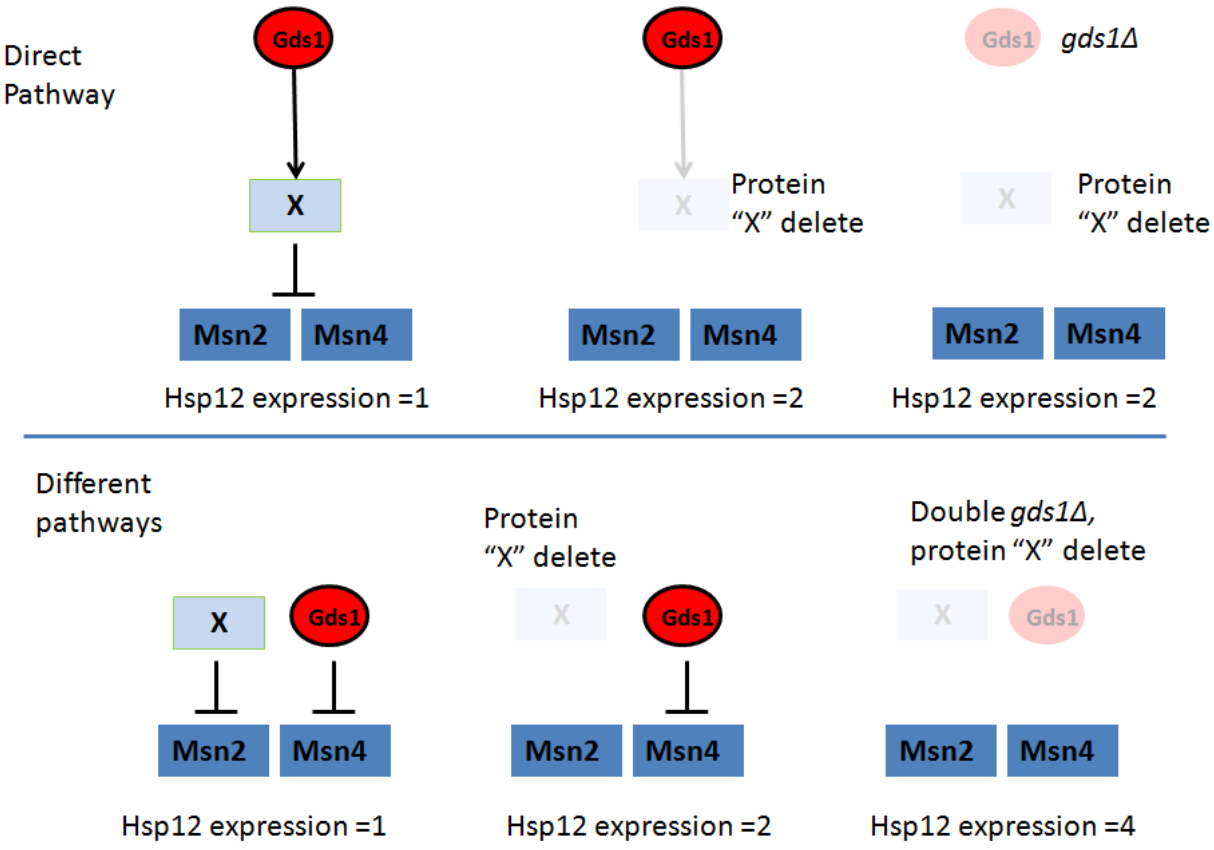
Direct
Pathway
Gds1
X
Msn2
Msn4
Hsp12 expression =1
Gds1
X
Protein
"X" delete
Msn2
Msn4
Hsp12 expression =2
Gds1
gds1Δ
X
Protein
"X" delete
Msn2
Msn4
Hsp12 expression =2
Different
pathways
X
Gds1
Msn2
Msn4
Hsp12 expression =1
Protein
"X" delete
X
Gds1
Msn2
Msn4
Hsp12 expression =2
Double gds1Δ,
protein "X" delete
X
Gds1
Msn2
Msn4
Hsp12 expression =4

Figure 3.8 Model for identification of proteins working within the same pathway as Gds1p.

A, Deletion of a protein "X" working within the same pathway as Gds1p results in an increase in Hsp12-GDP protein level. The further deletion of *GDS1* shows no additive increase in Hsp12-GFP level as the inhibitory pathway has already been eliminated. **B,** Deletion of a protein "X" working within a pathway parallel to Gds1p in combination with deletion of *GDS1* results in an additive increase in HSP12-GFP protein level as two parallel Msn2p/Msn4p inhibitory pathways have been abolished. Although this is a simplified model, it has been used on multiple occasions to assign proteins to specific pathways and even identify the pathways or proteins on which drugs work on (Costanzo et al., 2010; Hillenmeyer et al., 2008; Tong et al., 2001)

Any deletion mutant changing Hsp12-GFP protein level likely regulates or is involved in the Msn2p/Msn4p stress response. In parallel, I also created a second library expressing Hsp12-GFP in double mutant background *gds1Δ* with deletion mutants of Gds1p interactors (for example; Hsp12-GFP expression in a *gds1Δ eaf7Δ* strain). In total I screened 156 deletion mutants in both backgrounds (*HSP12-GFP*, *HSP12-GFP gds1Δ)* by flow cytometry to identify relative GFP abundance in each strain **(Table 3.1)**. Unfortunately, though I could detect Hsp12-GFP expression in the query stains prior to mating, and though post mating procedure I did detect an increase in HSP12-GFP levels in gds1Δ background (27 vs 37 mean florescent units, Table 3.1), only 44 strains expressed detectable GFP (mean fluorescence > 5 likely expressing GFP) listed in **Table 3.1**. Furthermore, two controls that should have resulted in higher Hsp12-GFP expression, *msn5Δ* and *tpk3Δ* (Gorner et al., 1998; Gorner et al., 2002; Sadeh et al., 2011), showed little or no Hsp12-GFP expression **(Table 3.1)**. Although some errors in strain production are common in large scale screens of this nature, the unexpected results of the controls combined with many strains having no Hsp12-GFP expression is an indication of widespread issues in the mating procedure. Thus, this screen was only performed once and the library will have to be re-constructed with more rigid selection procedures and controls at multiple steps of the robotic mating process. During the robotic mating process, I also identified seven negative genetic interactors of *GDS1* (combination of interactor deletion with *gds1Δ* displays slower growth phenotype than interactor deletion strain alone) and one positive genetic interactor (deletion of *GDS1* partially rescues interactor deletion slow growth phenotype) **(Table 3.2).**

Table 3.1: Mean Hsp12-GFP fluorescence in Gds1p interactor deletion screen

Interactor deletion	Strain background: Hsp12-GFP	Strain background: Hsp12-GFP *gds1Δ*
	Mean fluorescence minus background	
WT	27.19	36.56
ade3Δ	29.75	29.52
ade5Δ	8.29	-
apb140Δ	5.43	-
atp1Δ	17.92	-
bzz1Δ	10.95	-
fpr3Δ	5.06	9.67
gal80Δ	27.83	17.14
gsfΔ	22.62	-
hfi1Δ	22.69	-
hse1Δ	52.03	-
hsp82Δ	12.36	-
iki3Δ	33.17	10.79
imd4Δ	22.86	-
ioc2Δ	8.19	-
isw1Δ	6.91	-
msn5Δ	5.75	21.39
num1Δ	11.76	-
oac1Δ	6.17	-
pau13Δ	11.32	5.55
pfk1Δ	15.62	19.24
pho86Δ	26.68	-
rbg2Δ	39.7	-
rnr4Δ	25.86	13.2
snf2Δ	8.91	4.26
srs2Δ	11.36	-
ssd1Δ	-	9.22
vma5Δ	11.08	-
vma8Δ	21.53	-
vps1Δ	23.43	10.86
vtc3Δ	7.96	-
ypk1Δ	37.44	4.49

Table 3.2: Gds1p genetic interactors identified in during construction of flow cytometry screen library

Hsp12-GFP *gds1Δ*		
Interactor deletion	Gene description	Interaction +/-
ada5Δ	SAGA component required or complex integrity.	+
bfr1Δ	Component of mRNP complex involved in secretion and nuclear segregation.	-
eaf3Δ	NuA4 and Rpd3 subunit. May be involved in silencing.	-
hfi1Δ	SAGA component and transcriptional activator	-
npl3Δ	RNA binding protein and inhibitor of eIF4G. Protein abundance increases upon DNA replication stress. Shuttled between nucleus and cytoplasm.	-
sgf73Δ	SAGA component involved in transcriptional pre-activation. Shuttled to nucleus upon hypoxia.	-
srb10Δ	RNA pol II component and Msn2p inhibitor	-

Interactions: + (positive genetic interaction)

- (negative genetic interaction)

Hence this screen is a preliminary starting point for future exploration of Gds1p pathways and requires further work and more robust controls. Some possible insights gained from this preliminary screen are addressed in the discussion section.

CHAPTER 4: DISCUSSION

Nucleocytoplasmic shuttling is the primary mechanism of Msn2p regulation. In the absence of stress, Msn2p is cytoplasmic, while in response to stress, it is rapidly shuttled to the nucleus and becomes transcriptionally active (Gorner et al., 1998; Gorner et al., 2002; Hao et al., 2013; Jacquet et al., 2003). While PKA mediated phosphorylation of Msn2p has been identified as the major regulator of Msn2p localization (Gorner et al., 1998; Smith et al., 1998b), many other factors such as TOR (Beck and Hall, 1999; Mayordomo et al., 2002), Snf1p (De Wever et al., 2005; Mayordomo et al., 2002), Srb10p (Chi et al., 2001), and NuA4 (Lindstrom et al., 2006; Mitchell et al., 2008; Sadeh et al., 2011) have also been implicated in Msn2p shuttling or regulation. Gds1p was originally described as a protein required for budding yeast growth on glycerol as a carbon source (Konopinska et al., 1995) but little characterization has been performed since. In this study I assign novel cellular function to Gds1p as an inhibitor of the Msn2p/Msn4p dependant stress response, likely through promoting nuclear exclusion of Msn2p in the absence of stress. I show that Gds1p is a nuclear protein whose cellular abundance rapidly decreases in response to stress and is not required for growth on glycerol as a carbon source as initially described. Further, I show that the NuA4 lysine acetyltransferase complex plays a similar role in inhibiting the Msn2p/Msn4p dependant stress response, but likely acts in a parallel pathway with Gds1p. However, I cannot exclude the possibility of NuA4 also having a role in Gds1p regulation, likely though direct acetylation.

4.1 Gds1 localization and protein level regulation

Proteomic studies present here (**Figure 1A, B**) and elsewhere (Lambert et al., 2010; Mitchell et al., 2013; Mitchell et al., 2008) have determined that Esa1p (catalytic subunit of NuA4), Gds1p and Msn2p/Msn4p co-purify each other. In agreement with *in vitro* studies showing a direct interaction between NuA4 and Msn4p (Mitchell et al., 2008), Gds1p is not required to mediate this interaction (**Figure 1C**). The Gds1p interactome presented here (**Figure 1A**) suggests that Gds1p is a nuclear protein with roles in chromatin related processes as well as the environmental stress response. In agreement, I show that in the absence of stress Gds1-GFP is a nuclear protein **(Figure 3.4A)**, but when treated with a variety of stressors, Gds1-GFP protein level is often rapidly diminished **(Figure 3.4B)**. Interestingly, the rate of disappearance varies with the stress applied suggesting that Gds1p may be involved in the fine-tuning of the Msn2p/Msn4p stress response and that the full disappearance of Gds1p protein level is required for full activation of Msn2p/Msn4p. In certain conditions (acidic or hyper-osmotic environment as well as LiCl treatment), I detected a re-emergence of Gds1p protein level after prolonged stress (2 hours). This suggests that Gds1p may be involved in cellular adaption to prolonged, non-lethal stress with its re-emergence helping to downregulate Msn2p/Msn4p transcriptional activity. In agreement, studies have shown that Msn2p oscillates between the nucleus and cytoplasm with variable, but predictable frequency depending on the nature and intensity, and duration of an applied stress allowing for sustained, but diminished activation of ESR genes (Hao et al., 2013; Hao and O'Shea, 2012; Jacquet et al., 2003). It is then possible that the fluctuation in Gds1p protein level upon stress adaptation correlates directly with Msn2p oscillation and can mediate cellular adaptation to stress. For example, Msn2p rapidly and completely re-

localizes to the nucleus in response to osmotic shock, but soon after Msn2p is largely translocated back to the cytoplasm (Hao and O'Shea, 2012). Similarly, upon osmotic shock Gds1p protein level is rapidly diminished but restored to near stress free levels after two hours **(Figure 3.4B)**. In contrast, hydrogen peroxide induced oxidative stress leads to sustained Msn2p nuclear localization (Hao and O'Shea, 2012) and Gds1p protein levels are completely eliminated after two hours of peroxide treatment **(Figure 3.4B)**. As my work is only correlative, future studies will use real-time cell imaging to explore the localization and protein levels of Gds1p and Msn2p. These experiments can shed light on whether Gds1p can fine tune the intensity and duration of Msn2 activity in response to different stress types and intensities.

4.2 Gds1p and NuA4 inhibit Msn2p/Msn4p transcriptional activity

As Gds1p was initially described as a protein required for growth on glycerol as the sole carbon source (Konopinska et al., 1995), and displays physical interactions with Msn2p and Msn4p, I first hypothesised that Gds1p was required for activation of the Msn2p/Msn4p stress response. **Figure 3.2** though shows that *gds1Δ* cell viability remains normal in response to growth on alternate carbon sources or under stress. This is in agreement with the observation that Gds1p protein level is rapidly diminished in response to stress or carbon source depravation **(Figure 3.4B)**. Rather the disappearance of Gds1p upon stress suggested a role for Gds1p in the inhibition of the stress response. We show that deletion of *GDS1* or perturbation of the NuA4 complex both result in an increase in transcription and translation of the exclusively Msn2p/Msn4p controlled gene *HSP12* (Boy-Marcotte et al., 2006; Boy-Marcotte et al., 1998) **(Figure 3.5A, B)**. The increased survival of both *GDS1* and NuA4

mutants in response to sudden, extreme heat stress also suggests their involvement in repressing a global stress response that may extend beyond Msn2p/Msn4p **(Figure 3.5C)**. This study provided initial clues into the mechanism of Msn2p/Msn4p inhibition by Gds1p and suggests a novel mechanism of NuA4 involvement as both *gds1Δ* and *eaf7Δ* mutants display increased Msn2-GFP nuclear localization in the absence of stress **(Figure 3.6)**.

4.3 Do Gds1p and NuA4 work together to inhibit Msn2p/Msn4p?

The physical interaction between NuA4 and Gds1p and the ability of NuA4 acetylate Gds1p *in vitro,* suggests that NuA4 may be acting through Gds1p to inhibit Msn2p/Msn4p **(Figure 1A)** (Mitchell et al., 2013). Further, we show that Gds1p protein level is dependent on NuA4 **(Figure 3.3B)** independently of NuA4 transcriptional regulation **(Figure 3.3C)**. These results suggested that NuA4 mediated acetylation of Gds1p may be promoting protein stability and nuclear export of Msn2p in the absence of stress. As NuA4 has been shown to acetylate and mask ubiquitinatable lysine residues (Lin et al., 2008), one possibility is that Gds1p deacetylation followed by ubiquitination and proteasomal degradation may lead to rapid loss of Gds1p protein level upon stress. Contrary to this hypothesis, when I mutated seven of eight identified Gds1p acetylation sites from lysine to arginine (mimicking constitutive lack of acetylation), there was no effect on Gds1p protein level and acetylation state was inconclusive **(Supplemental Figure S1, A, B)**. An increase in stress survival was observed similar to a *gds1Δ* **(Supplemental Figure S1, C)**. The increased stress survival may be due to partial loss of acetylation but may also be due to loss protein function due to miss folding. Although this result suggests that NuA4 and acetylation play little or no role in Gds1p function, it is also possible that other key acetylation sites exist and have yet to be identified. Further, Gds1p may have unidentified SAGA specific acetylation sites as Gds1p

co-purified the majority of the SAGA complex **(Figure 1A)**. Regardless, the fact that GDS1 mRNA expression is not impacted in NuA4 mutants **(Figure 3.3C)**, suggests that NuA4 is regulating Gds1p protein levels though a yet to be discerned mechanism.

Despite the physical interaction and potential role of NuA4 regulating Gds1p protein levels, multiple lines of evidence suggest they are not acting in a simple direct pathway. We found additive effects in both *HSP12* transcription and expression in double *gds1Δ esa1-TS* and *gds1Δ eaf7Δ* mutants **(Figure 3.5A, B)** and a negative genetic interaction between *GDS1* and *EAF1* **(Figure 3.5D)**. These results suggest parallel pathways of NuA4 and Gds1p regulation of Msn2p/Msn4p. The negative genetic interaction may be caused by excess stress response protein expression resulting from perturbation of two independent repressor pathways. I also found that the physical interaction between NuA4 and Msn4p is not dependant on Gds1p and that unlike NuA4 (Mitchell et al., 2008), Gds1p is not localized to the STRE of the *HSP12* promoter **(Figure 3.1C, 3.5E)**. Taken together these results suggest largely parallel pathways of NuA4 and Gds1p regulation of Msn2p, but the possibility still remains that unidentified NuA4 mediated acetylation sites regulate Gds1p protein level. See **Figure 4.1** for a proposed model of Gds1p function.

4.4 Gds1p and NuA4 do not promote Msn2p nuclear exclusion through regulation of PKA

How are Gds1p and NuA4 promoting Msn2p nuclear exclusion? As PKA is the major promoter of Msn2p/Msn4p nuclear exclusion, I hypothesised that Gds1p and/or NuA4 may inhibit PKA activity in the absence of stress. PKA promotes the Msn5p mediated export of Msn2p and Msn4p by phosphorylation of their N-terminal NES

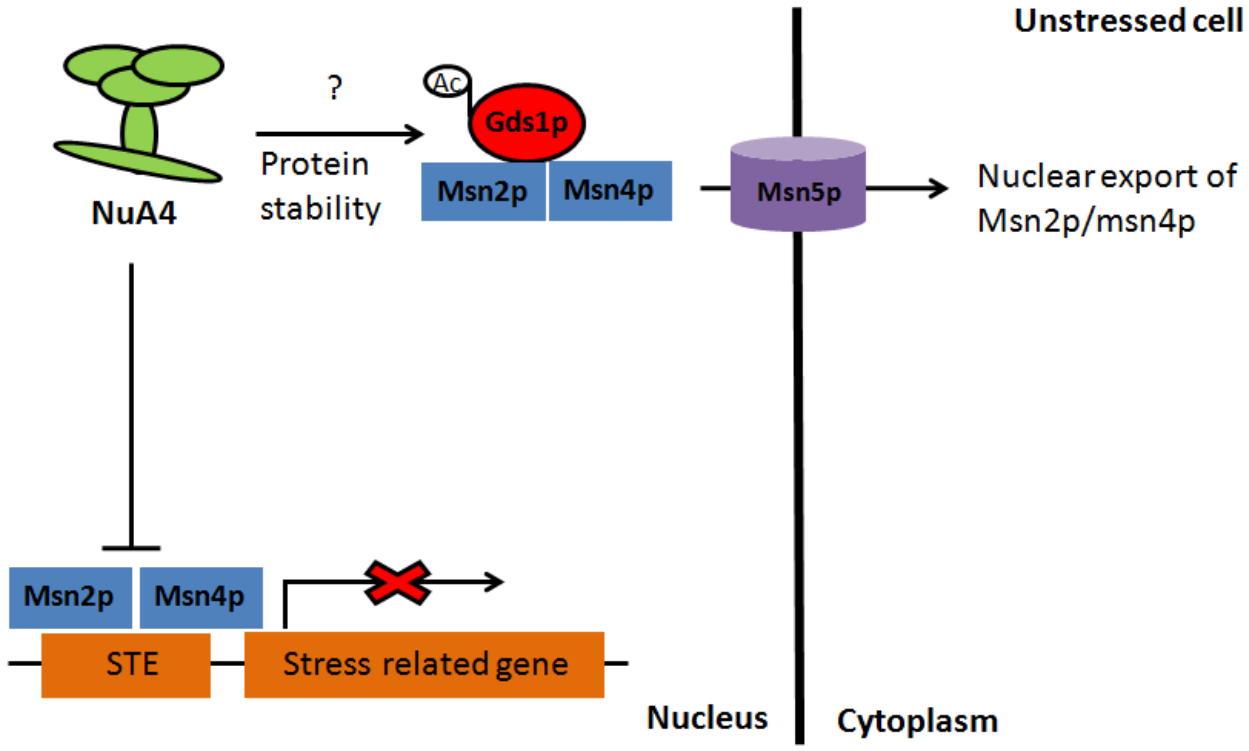
Unstressed cell
NuA4
?
Protein
stability
Ac
Gds1p
Msn2p
Msn4p
Msn5p
Nuclear export of
Msn2p/msn4p
Msn2p
Msn4p
STE
Stress related gene
Nucleus
Cytoplasm

Figure 4.1: possible model of Gds1p function in the absence of stress

In this model NuA4 directly inhibits Msn2p/Msn4p while promoting Gds1p stability through acetylation in the absence of stress. Gds1p physically interacts with Msn2p/Msn4p and leading to Msn5p mediated nuclear export.

(Gorner et al., 1998; Jacquet et al., 2003; Yoshida and Blobel, 2001). We show that neither deletion of *GDS1* nor NuA4 mutants impact the general activity of PKA, nor do they impact the phosphorylation levels of Msn2 in the absence of stress (**Figure 3.7 *A, B***). Further, by examining protein levels of Hsp12-GFP in strains harboring overactive (overexpression of $RAS2^{val19}$) or underactive (overexpression of *BCY1*) PKA, I show that NuA4 and Gds1p are regulating Msn2p/Msn4p activity through a pathway distinct from that of PKA (**Figure 3.7 C, D**). Indeed, in *gds1Δeaf1Δ* cells, which like cells deficient in both *GDS1* and subunits of NuA4 display increased *HSP12* expression (data set not shown), Msn2p phosphorylation is increased (**Figure 3.6 B**) suggesting the cell is attempting to export Msn2p to counterbalance its inappropriate nuclear localization. Though our work indicates that Gds1p and NuA4 are working in a pathway distinct from the primary pathway regulating Msn2p localization during non stress conditions, it does not exclude the possibility that this novel pathway is mediating the effects of TOR or Ssn3 (Balciunas and Ronne, 1995; Beck and Hall, 1999; Chi et al., 2001), also both implicated in Msn2p localization under non stress conditions. Regardless, as *gds1Δ* and *eaf1Δ* cells do not display decreased Msn2p phosphorylation (**Figure 3.7 B**), it suggests the mechanism maybe distinct from phosphorylation of the NLS of Msn2p. Further, as endogenous Msn2p protein levels do not substantially change in *eaf1Δ, gds1Δ* or *gds1Δeaf1Δ* cells (α-Msn2, **Figure 3.7B**) it is unlikely that Gds1p is regulating Msn2p protein levels as is the case with Ump1p (Erkina et al., 2008; Sadeh et al., 2011)

Intriguingly, our interactome **(Figure 3.1A)** detected a physical interaction between Gds1-TAP and Msn5p, the karyopherin responsible for Msn2p nuclear export (Durchschlag et al., 2004; Kaffman et al., 1998a). As Gds1 is exclusively nuclear, and shows physical

interactions with nuclear pore proteins, could Gds1p be contributing to trafficking Msn2p to nuclear pores? It is also possible that Gds1p affects other nuclear pore tethering or export regulating factors. Many karyopherin exportin proteins exist and may be regulated by Gds1p including Crm1 (Stade et al., 1997), Kap109 (Cook et al., 2005; Xiao et al., 1993), and Los1(Hellmuth et al., 1998). This hypothesis is unlikely as Gds1p has not yet been found to physically interact with any exportin other than Msn5p, and Msn2p nuclear export has only been tied to Msn5p (Durchschlag et al., 2004). Alternatively, Ntf2p and other importin family karyopherins are required for Ran-GTP nuclear import and may be affected by Gds1p (Gorlich et al., 1995; Ribbeck et al., 1998; Smith et al., 1998a). This hypothesis though is unlikely as it would affect global protein shuttling.

4.5 Hsp12-GFP mini-screen provides clues into Gds1p mechanism of action

To elucidate the pathway through which Gds1p inhibits Msn2p/Msn4p in the absence of stress, I created two mini libraries of strains expressing either Hsp12-GFP in strains deleted for individual Gds1p physical interactors with and without *gds1Δ*. The screen employed flow cytometry to detect Hsp12-GFP fluorescent signal as readout of Msn2p/Msn4p activity where deletions mutants in which GFP signal is increased over WT likely represent Gds1p physical interactors that also play a role in the inhibition of the ESR. If the combination of a Gds1p interactor deletion with the deletion of *GDS1* caused additive increase in GFP signal, the likely interpretation of this genetic interaction is that the two genes share a function but accomplish it by parallel pathways. If, on the other hand, the double deletion does not cause an additive increase in GFP signal over either single delete, a

possible interpretation is that the two genes work within the same pathway. Unfortunately, there were technical issues incorporating the *HSP12-GFP* gene into the mini-array and the screen only identified one gene fitting the model for working within the Gds1 pathway: *ADE3*. Deletion of *ADE3* leads to a slight, and probably negligible, increase in GFP signal compared to WT (29.7 vs 27.2) while the combined *ade3Δ gds1Δ* has no additive effect **(Table 3.1).** Ade3p is involved in single carbon alteration and amino acid synthesis (Song and Rabinowitz, 1993) suggesting a role for Gds1p in the amino acid starvation response. As the screen was only performed once and the difference in mean GFP fluorescence between WT and the single mutant was slight (27.2 and 29.7), further examination of this phenotype needs to be done before any conclusions can be made. A far more common occurrence in the screen were instances were a Gds1p interactor deletion led to a decrease in GFP signal and the double interactor/*GDS1* deletion did not recover or increase GFP signal. A possible interpretation of this scenario is that the Gds1p interactor is involved in the activation of the stress response, and Gds1p may be involved in its inhibition. *SNF2,* the catalytic subunit of the SWI/SNF chromatin remodeling complex, is involved in chromatin remodeling, silencing events, non-fermentable carbon source metabolism, and activation of the stress response (including binding to and activating transcription from Hsf1p and Msn2/Msn4 mediated promoters during heat shock) (Abrams et al., 1986; Erkina et al., 2008; Hirschhorn et al., 1992; Shivaswamy and Iyer, 2008). I found that deletion of *SNF2* led to decreased GFP signal and the additional deletion of *GDS1* did not alleviate the decrease **(Table 3.1)**. Thus, Gds1p may be involved in the regulation of the SWI/SNF or in its targeting to stress related promoters. *PAU13* is a gene of unknown function whose transcription increases upon ethanol shock (Alexandre et al., 2001; Viswanathan et al., 1994). The decrease in GFP signal in both

the *pau13Δ* and *pau13Δ gds1Δ* double mutant could shed light on both Gds1p and Pau13p mechanisms of action during ethanol exposure if further explored.

In conclusion, we have determined that NuA4 and Gds1 are work together to inhibit Msn2p/Msn4p activity under non-stress conditions. The identification of novel regulator Gds1p, emphasises that the cell has develop multiple redundant pathways ensure that Msn2p/Msn4p stress response pathway is exquisitely in tune at all times.

CONTRIBUTION OF COLABORATORS

Figure 1A performed by Leslie Mitchell (Baetz lab) with Data analysis and organization performed by Michael Cotrut

Figure 3.3A, C performed by Sylvain Huard (Baetz lab). Strains made by M.Cotrut

Figure 3.5A performed by Sylvain Huard. Strains made by M.Cotrut. E, performed in part by Akil Hamza (Baetz lab)

Figure 3.7A,B performed by Lina Mah (Measday lab). Strains made by M.Cotrut

REFRENCES

Abrams, E., Neigeborn, L., and Carlson, M. (1986). MOLECULAR ANALYSIS OF SNF2 AND SNF5, GENES REQUIRED FOR EXPRESSION OF GLUCOSE-REPRESSIBLE GENES IN SACCHAROMYCES-CEREVISIAE. Molecular and Cellular Biology *6*, 3643-3651.

Alexandre, H., Ansanay-Galeote, V., Dequin, S., and Blondin, B. (2001). Global gene expression during short-term ethanol stress in Saccharomyces cerevisiae. Febs Letters *498*, 98-103.

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

Arif, M., Senapati, P., Shandilya, J., and Kundu, T.K. (2010). Protein lysine acetylation in cellular function and its role in cancer manifestation. Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms *1799*, 702-716.

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

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

Balciunas, D., and Ronne, H. (1995). 3 SUBUNITS OF THE RNA-POLYMERASE-II MEDIATOR COMPLEX ARE INVOLVED IN GLUCOSE REPRESSION. Nucleic Acids Research *23*, 4421-4425.

Barbet, N.C., Schneider, U., Helliwell, S.B., Stansfield, I., Tuite, M.F., and Hall, M.N. (1996). TOR controls translation initiation and early G1 progression in yeast. Mol Biol Cell *7*, 25-42.

Beck, T., and Hall, M.N. (1999). The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. Nature *402*, 689-692.

Berndsen, C.E., Albaugh, B.N., Tan, S., and Denu, J.M. (2007). Catalytic mechanism of a MYST family histone acetyltransferase. Biochemistry *46*, 623-629.

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

Blander, G., Zalle, N., Daniely, Y., Taplick, J., Gray, M.D., and Oren, M. (2002). DNA damage-induced translocation of the Werner helicase is regulated by acetylation. Journal of Biological Chemistry *277*, 50934-50940.

Bolden, J.E., Peart, M.J., and Johnstone, R.W. (2006). Anticancer activities of histone deacetylase inhibitors. Nature Reviews Drug Discovery *5*, 769-784.

Boudreault, A.A., Cronier, D., Selleck, W., Lacoste, N., Utley, R.T., Allard, S.P., Savard, J., Lane, W.S., Tan, S., and Cote, J. (2003). Yeast enhancer of Polycomb defines global Esal-dependent acetylation of chromatin. Genes & Development *17*, 1415-1428.

Bowdish, K.S., Yuan, H.E., and Mitchell, A.P. (1994). ANALYSIS OF RIM11, A YEAST PROTEIN-KINASE THAT PHOSPHORYLATES THE MEIOTIC ACTIVATOR IME1. Molecular and Cellular Biology *14*, 7909-7919.

Boy-Marcotte, E., Garmendia, C., Garreau, H., Lallet, S., Mallet, L., and Jacquet, M. (2006). The transcriptional activation region of Msn2p, in Saccharomyces cerevisiae, is regulated by stress but is insensitive to the cAMP signalling pathway. Molecular Genetics and Genomics *275*, 277-287.

Boy-Marcotte, E., Lagniel, G., Perrot, M., Bussereau, F., Boudsocq, A., Jacquet, M., and Labarre, J. (1999). The heat shock response in yeast: differential regulations and contributions of the Msn2p/Msn4p and Hsf1p regulons. Mol Microbiol *33*, 274-283.

Boy-Marcotte, E., Perrot, M., Bussereau, F., Boucherie, H., and Jacquet, M. (1998). Msn2p and Msn4p control a large number of genes induced at the diauxic transition which are repressed by cyclic AMP in Saccharomyces cerevisiae. Journal of Bacteriology *180*, 1044-1052.

Broek, D., Samiy, N., Fasano, O., Fujiyama, A., Tamanoi, F., Northup, J., and Wigler, M. (1985). DIFFERENTIAL ACTIVATION OF YEAST ADENYLATE-CYCLASE BY WILD-TYPE AND MUTANT RAS PROTEINS. Cell *41*, 763-769.

Broek, D., Toda, T., Michaeli, T., Levin, L., Birchmeier, C., Zoller, M., Powers, S., and Wigler, M. (1987). THE SACCHAROMYCES-CEREVISIAE CDC25 GENE-PRODUCT REGULATES THE RAS/ADENYLATE CYCLASE PATHWAY. Cell *48*, 789-799.

Cameron, S., Levin, L., Zoller, M., and Wigler, M. (1988). CAMP-INDEPENDENT CONTROL OF SPORULATION, GLYCOGEN-METABOLISM, AND HEAT-SHOCK RESISTANCE IN S-CEREVISIAE. Cell *53*, 555-566.

Cao, X.W., and Sudhof, T.C. (2001). A transcriptively active complex of APP with Fe65 and histone acetyltransferase Tip60. Science *293*, 115-120.

Causton, H.C., Ren, B., Koh, S.S., Harbison, C.T., Kanin, E., Jennings, E.G., Lee, T.I., True, H.L., Lander, E.S., and Young, R.A. (2001). Remodeling of yeast genome expression in response to environmental changes. Mol Biol Cell *12*, 323-337.

Celenza, J.L., and Carlson, M. (1984). CLONING AND GENETIC-MAPPING OF SNF1, A GENE REQUIRED FOR EXPRESSION OF GLUCOSE-REPRESSIBLE GENES IN SACCHAROMYCES-CEREVISIAE. Molecular and Cellular Biology *4*, 49-53.

Ceol, C.J., and Horvitz, H.R. (2004). A new class of C-elegans synMuv genes implicates a Tip60/NuA4-like HAT complex as a negative regulator of ras signaling. Developmental Cell *6*, 563-576.

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

Chang, Y.W., Howard, S.C., and Herman, P.K. (2004). The Ras/IPKA signaling pathway directly targets the Srb9 protein, a component of the general RNA polymerase II transcription apparatus. Mol Cell *15*, 107-116.

Chi, Y., Huddleston, M.J., Zhang, X.L., Young, R.A., Annan, R.S., Carr, S.A., and Deshaies, R.J. (2001). Negative regulation of Gcn4 and Msn2 transcription factors by Srb10 cyclin-dependent kinase. Genes & Development *15*, 1078-1092.

Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., Olsen, J.V., and Mann, M. (2009). Lysine Acetylation Targets Protein Complexes and Co-Regulates Major Cellular Functions. Science *325*, 834-840.

Choy, J.S., Tobe, B.T.D., Huh, J.H., and Kron, S.J. (2001). Yng2p-dependent NuA4 histone H4 acetylation activity is required for mitotic and meiotic progression. Journal of Biological Chemistry *276*, 43653-43662.

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

Cook, A., Fernandez, E., Lindner, D., Ebert, J., Schlenstedt, G., and Conti, E. (2005). The structure of the nuclear export receptor Cse1 in its cytosolic state reveals a closed conformation incompatible with cargo binding. Mol Cell *18*, 355-367.

Costanzo, M., Baryshnikova, A., Bellay, J., Kim, Y., Spear, E.D., Sevier, C.S., Ding, H.M., Koh, J.L.Y., Toufighi, K., Mostafavi, S.*, et al.* (2010). The Genetic Landscape of a Cell. Science *327*, 425-431.

de Nadal, E., Zapater, M., Alepuz, P.M., Sumoy, L., Mas, G., and Posas, F. (2004). The MAPK Hog1 recruits Rpd3 histone deacetylase to activate osmoresponsive genes. Nature *427*, 370-374.

De Virgilio, C., and Loewith, R. (2006). The TOR signalling network from yeast to man. International Journal of Biochemistry & Cell Biology *38*, 1476-1481.

De Wever, V., Reiter, W., Ballarini, A., Ammerer, G., and Brocard, C. (2005). A dual role for PP1 in shaping the Msn2-dependent transcriptional response to glucose starvation. Embo Journal *24*, 4115-4123.

Deminoff, S.J., Howard, S.C., Hester, A., Warner, S., and Herman, P.K. (2006). Using substrate-binding variants of the cAMP-dependent protein kinase to identify novel targets and a kinase domain important for substrate interactions in Saccharomyces cerevisiae. Genetics *173*, 1909-1917.

Deutschbauer, A.M., Jaramillo, D.F., Proctor, M., Kumm, J., Hillenmeyer, M.E., Davis, R.W., Nislow, C., and Giaever, G. (2005). Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast. Genetics *169*, 1915-1925.

DiComo, C.J., and Arndt, K.T. (1996). Nutrients, via the Tor proteins, stimulate the association of Tap42 with type 2A phosphatases. Genes & Development *10*, 1904-1916.

Dietz, K.C., and Casaccia, P. (2010). HDAC inhibitors and neurodegeneration: At the edge between protection and damage. Pharmacological Research *62*, 11-17.

Dion, M.F., Altschuler, S.J., Wu, L.F., and Rando, O.J. (2005). Genomic characterization reveals a simple histone H4 acetylation code. Proceedings of the National Academy of Sciences of the United States of America *102*, 5501-5506.

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

Dudley, A.M., Janse, D.M., Tanay, A., Shamir, R., and Church, G.M. (2005). A global view of pleiotropy and phenotypically derived gene function in yeast. Molecular Systems Biology *1*.

Durchschlag, E., Reiter, W., Ammerer, G., and Schuller, C. (2004). Nuclear localization destabilizes the stress-regulated transcription factor Msn2. The Journal of biological chemistry *279*, 55425-55432.

Erkina, T.Y., Tschetter, P.A., and Erkine, A.M. (2008). Different requirements of the SWI/SNF complex for robust nucleosome displacement at promoters of heat shock factor and Msn2- and Msn4-regulated heat shock genes. Molecular and Cellular Biology *28*, 1207-1217.

Estruch, F., and Carlson, M. (1993). 2 HOMOLOGOUS ZINC-FINGER GENES IDENTIFIED BY MULTICOPY SUPPRESSION IN A SNF1 PROTEIN-KINASE MUTANT OF SACCHAROMYCES-CEREVISIAE. Molecular and Cellular Biology *13*, 3872-3881.

Galarneau, L., Nourani, A., Boudreault, A.A., Zhang, Y., Heliot, L., Allard, S., Savard, J., Lane, W.S., Stillman, D.J., and Cote, J. (2000). Multiple links between the NuA4

histone acetyltransferase complex and epigenetic control of transcription. Mol Cell *5*, 927-937.

Garrett, S., Menold, M.M., and Broach, J.R. (1991). THE SACCHAROMYCES-CEREVISIAE YAK1-GENE ENCODES A PROTEIN-KINASE THAT IS INDUCED BY ARREST EARLY IN THE CELL-CYCLE. Molecular and Cellular Biology *11*, 4045-4052.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., and Brown, P.O. (2000). Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell *11*, 4241-4257.

Geymonat, M., Wang, L.L., Garreau, H., and Jacquet, M. (1998). Ssa1p chaperone interacts with the guanine nucleotide exchange factor of ras Cdc25p and controls the cAMP pathway in Saccharomyces cerevisiae. Mol Microbiol *30*, 855-864.

Gorlich, D., Kostka, S., Kraft, R., Dingwall, C., Laskey, R.A., Hartmann, E., and Prehn, S. (1995). 2 DIFFERENT SUBUNITS OF IMPORTIN COOPERATE TO RECOGNIZE NUCLEAR-LOCALIZATION SIGNALS AND BIND THEM TO THE NUCLEAR-ENVELOPE. Current Biology *5*, 383-392.

Gorner, W., Durchschlag, E., Martinez-Pastor, M.T., Estruch, F., Ammerer, G., Hamilton, B., Ruis, H., and Schuller, C. (1998). Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. Genes & Development *12*, 586-597.

Gorner, W., Durchschlag, E., Wolf, J., Brown, E.L., Ammerer, G., Ruis, H., and Schuller, C. (2002). Acute glucose starvation activates the nuclear localization signal of a stress-specific yeast transcription factor. Embo Journal *21*, 135-144.

Griffioen, G., Anghileri, P., Imre, E., Baroni, M.D., and Ruis, H. (2000). Nutritional control of nucleocytoplasmic localization of cAMP-dependent protein kinase catalytic and regulatory subunits in Saccharomyces cerevisiae. Journal of Biological Chemistry *275*, 1449-1456.

Hao, N., Budnik, B.A., Gunawardena, J., and O'Shea, E.K. (2013). Tunable Signal Processing Through Modular Control of Transcription Factor Translocation. Science *339*, 460-464.

Hao, N., and O'Shea, E.K. (2012). Signal-dependent dynamics of transcription factor translocation controls gene expression. Nat Struct Mol Biol *19*, 31-U47.

Hardie, D.G. (2007). AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat Rev Mol Cell Biol *8*, 774-785.

Hellmuth, K., Lau, D.M., Bischoff, F.R., Kunzler, M., Hurt, E., and Simos, G. (1998). Yeast Los1p has properties of an exportin-like nucleocytoplasmic transport factor for tRNA. Molecular and Cellular Biology *18*, 6374-6386.

Henriksen, P., Wagner, S.A., Weinert, B.T., Sharma, S., Bacinskaja, G., Rehman, M., Juffer, A.H., Walther, T.C., Lisby, M., and Choudhary, C. (2012a). Proteome-wide Analysis of Lysine Acetylation Suggests its Broad Regulatory Scope in Saccharomyces cerevisiae. Mol Cell Proteomics *11*, 1510-1522.

Henriksen, P., Wagner, S.A., Weinert, B.T., Sharma, S., Bacinskaja, G., Rehman, M., Juffer, A.H., Walther, T.C., Lisby, M., and Choudhary, C. (2012b). Proteome-wide analysis of lysine acetylation suggests its broad regulatory scope in Saccharomyces cerevisiae. Molecular & cellular proteomics : MCP *11*, 1510-1522.

Hillenmeyer, M.E., Fung, E., Wildenhain, J., Pierce, S.E., Hoon, S., Lee, W., Proctor, M., St Onge, R.P., Tyers, M., Koller, D.*, et al.* (2008). The chemical genomic portrait of yeast: Uncovering a phenotype for all genes. Science *320*, 362-365.

Hirata, Y., Andoh, T., Asahara, T., and Kikuchi, A. (2003). Yeast glycogen synthase kinase-3 activates Msn2p-dependent transcription of stress responsive genes. Mol Biol Cell *14*, 302-312.

Hirschhorn, J.N., Brown, S.A., Clark, C.D., and Winston, F. (1992). EVIDENCE THAT SNF2/SWI2 AND SNF5 ACTIVATE TRANSCRIPTION IN YEAST BY ALTERING CHROMATIN STRUCTURE. Genes & Development *6*, 2288-2298.

Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., and O'Shea, E.K. (2003). Global analysis of protein localization in budding yeast. Nature *425*, 686-691.

Ikura, T., Ogryzko, V.V., Grigoriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. (2000). Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. Cell *102*, 463-473.

Ito, K., Charron, C.E., and Adcock, I.M. (2007). Impact of protein acetylation in inflammatory lung diseases. Pharmacology & Therapeutics *116*, 249-265.

Jacquet, M., Renault, G., Lallet, S., De Mey, J., and Goldbeter, A. (2003). Oscillatory nucleocytoplasmic shuttling of the general stress response transcriptional activators Msn2 and Msn4 in Saccharomyces cerevisiae. Journal of Cell Biology *161*, 497-505.

Jiang, R., and Carlson, M. (1996). Glucose regulates protein interactions within the yeast SNF1 protein kinase complex. Genes & Development *10*, 3105-3115.

Jiang, W., Wang, S., Xiao, M., Lin, Y., Zhou, L., Lei, Q., Xiong, Y., Guan, K.-L., and Zhao, S. (2011). Acetylation Regulates Gluconeogenesis by Promoting PEPCK1 Degradation via Recruiting the UBR5 Ubiquitin Ligase. Mol Cell *43*, 33-44.

Jiang, Y., and Broach, J.R. (1999). Tor proteins and protein phosphatase 2A reciprocally regulate Tap42 in controlling cell growth in yeast. Embo Journal *18*, 2782-2792.

Kaffman, A., Rank, N.M., O'Neill, E.M., Huang, L.S., and O'Shea, E.K. (1998a). The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus. Nature *396*, 482-486.

Kaffman, A., Rank, N.M., O'Neill, E.M., Huang, L.S., and O'Shea, E.K. (1998b). The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus. Nature *396*, 482-486.

Kaniak, A., Xue, Z.X., Macool, D., Kim, J.H., and Johnston, M. (2004). Regulatory network connecting two glucose signal transduction pathways in Saccharomyces cerevisiae. Eukaryotic Cell *3*, 221-231.

Kennedy, M.A., Kabbani, N., Lambert, J.P., Swayne, L.A., Ahmed, F., Figeys, D., Bennett, S.A.L., Bryan, J., and Baetz, K. (2011). Srf1 Is a Novel Regulator of Phospholipase D Activity and Is Essential to Buffer the Toxic Effects of C16:0 Platelet Activating Factor. PLoS Genet *7*.

Keogh, M.C., Mennella, T.A., Sawa, C., Berthelet, S., Krogan, N.J., Wolek, A., Podolny, V., Carpenter, L.R., Greenblatt, J.F., Baetz, K.*, et al.* (2006). The Saccharomyces cerevisiae histone H2A variant Htz1 is acetylated by NuA4. Genes & Development *20*, 660-665.

Kim, S.C., Sprung, R., Chen, Y., Xu, Y.D., Ball, H., Pei, J.M., Cheng, T.L., Kho, Y., Xiao, H., Xiao, L.*, et al.* (2006). Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mol Cell *23*, 607-618.

Konopinska, A., Szczesniak, B., and Boguta, M. (1995). Nucleotide sequence of the GDS1 gene of Saccharomyces cerevisiae. Yeast *11*, 1513-1518.

Kraakman, L., Lemaire, K., Ma, P.S., Teunissen, A., Donaton, M.C.V., Van Dijck, P., Winderickx, J., de Winde, J.H., and Thevelein, J.M. (1999). A Saccharomyces cerevisiae G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. Mol Microbiol *32*, 1002-1012.

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

Kuchin, S., Yeghiayan, P., and Carlson, M. (1995). CYCLIN-DEPENDENT PROTEIN-KINASE AND CYCLIN HOMOLOGS SSN3 AND SSN8 CONTRIBUTE TO TRANSCRIPTIONAL CONTROL IN YEAST. Proceedings of the National Academy of Sciences of the United States of America *92*, 4006-4010.

Kuo, M.H., and Allis, C.D. (1998). Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays *20*, 615-626.

Kusch, T., Florens, L., MacDonald, W.H., Swanson, S.K., Glaser, R.L., Yates, J.R., Abmayr, S.M., Washburn, M.P., and Workman, J.L. (2004). Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. Science *306*, 2084-2087.

Lallet, S., Garreau, H., Poisier, C., Boy-Marcotte, E., and Jacquet, M. (2004). Heat shock-induced degradation of Msn2p, a Saccharomyces cerevisiae transcription factor, occurs in the nucleus. Molecular Genetics and Genomics *272*, 353-362.

Lambert, J.-P., Mitchell, L., Rudner, A., Baetz, K., and Figeys, D. (2009a). A novel proteomics approach for the discovery of chromatin-associated protein networks. Molecular & cellular proteomics : MCP *8*, 870-882.

Lambert, J.P., Fillingham, J., Siahbazi, M., Greenblatt, J., Baetz, K., and Figeys, D. (2010). Defining the budding yeast chromatin-associated interactome. Molecular Systems Biology *6*.

Lambert, J.P., Mitchell, L., Rudner, A., Baetz, K., and Figeys, D. (2009b). A novel proteomics approach for the discovery of chromatin-associated protein networks. Molecular & cellular proteomics : MCP *8*, 870-882.

LeDizet, M., and Piperno, G. (1987). Identification of an acetylation site of Chlamydomonas alpha-tubulin. Proceedings of the National Academy of Sciences of the United States of America *84*, 5720-5724.

Lin, Y.Y., Lu, J.Y., Zhang, J.M., Walter, W., Dang, W.W., Wan, J., Tao, S.C., Qian, J., Zhao, Y.M., Boeke, J.D.*, et al.* (2009). Protein Acetylation Microarray Reveals that NuA4 Controls Key Metabolic Target Regulating Gluconeogenesis. Cell *136*, 1073-1084.

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

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

Loewith, R., Jacinto, E., Wullschleger, S., Lorberg, A., Crespo, J.L., Bonenfant, D., Oppliger, W., Jenoe, P., and Hall, M.N. (2002). Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol Cell *10*, 457-468.

Longtine, M.S., McKenzie, A., Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast *14*, 953-961.

Lucau-Danila, A., Lelandais, G., Kozovska, Z., Tanty, V., Delaveau, T., Devaux, F., and Jacq, C. (2005). Early expression of yeast genes affected by chemical stress. Molecular and cellular biology *25*, 1860-1868.

Ludin, K., Jiang, R., and Carlson, M. (1998). Glucose-regulated interaction of a regulatory subunit of protein phosphatase 1 with the Snf1 protein kinase in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America *95*, 6245-6250.

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

Lund, A.H., Turner, G., Trubetskoy, A., Verhoeven, E., Wientjens, E., Hulsman, D., Russell, R., DePinho, R.A., Lenz, J., and van Lohuizen, M. (2002). Genome-wide retroviral insertional tagging of genes involved in cancer in Cdkn2a-deficient mice. Nature Genetics *32*, 160-165.

Lv, L., Li, D., Zhao, D., Lin, R., Chu, Y., Zhang, H., Zha, Z., Liu, Y., Li, Z., Xu, Y.*, et al.* (2011). Acetylation Targets the M2 Isoform of Pyruvate Kinase for Degradation through Chaperone-Mediated Autophagy and Promotes Tumor Growth. Mol Cell *42*, 719-730.

Ma, L.N., Ho, K., Piggott, N., Luo, Z.L., and Measday, V. (2012). Interactions Between the Kinetochore Complex and the Protein Kinase A Pathway in Saccharomyces cerevisiae. G3-Genes Genomes Genet *2*, 831-841.

Ma, X.J., Wu, J.S., Altheim, B.A., Schultz, M.C., and Grunstein, M. (1998). Deposition-related sites K5/K12 in histone H4 are not required for nucleosome deposition in yeast. Proceedings of the National Academy of Sciences of the United States of America *95*, 6693-6698.

MartinezPastor, M.T., Marchler, G., Schuller, C., MarchlerBauer, A., Ruis, H., and Estruch, F. (1996). The Saccharomyces cerevisiae zinc finger proteins Msn2p and Msn4p are required for transcriptional induction through the stress-response element (STRE). Embo Journal *15*, 2227-2235.

Mayordomo, I., Estruch, F., and Sanz, P. (2002). Convergence of the target of rapamycin and the Snf1 protein kinase pathways in the regulation of the subcellular localization of Msn2, a transcriptional activator of STRE (stress response element)-regulated genes. Journal of Biological Chemistry *277*, 35650-35656.

Minard, M.E., Jain, A.K., and Barton, M.C. (2009). Analysis of Epigenetic Alterations to Chromatin During Development. Genesis *47*, 559-572.

Mitchell, L., Huard, S., Cotrut, M., Pourhanifeh-Lemeri, R., Steunou, A.L., Hamza, A., Lambert, J.P., Zhou, H., Ning, Z.B., Basu, A.*, et al.* (2013). mChIP-KAT-MS, a method to map protein interactions and acetylation sites for lysine

acetyltransferases. Proceedings of the National Academy of Sciences of the United States of America *110*, E1641-E1650.

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

Mitchell, L., Lau, A., Lambert, J.P., Zhou, H., Fong, Y., Couture, J.F., Figeys, D., and Baetz, K. (2011). Regulation of Septin Dynamics by the Saccharomyces cerevisiae Lysine Acetyltransferase NuA4. Plos One *6*.

Morano, K.A., Grant, C.M., and Moye-Rowley, W.S. (2012). The Response to Heat Shock and Oxidative Stress in Saccharomyces cerevisiae. Genetics *190*, 1157-1195.

Mujtaba, S., He, Y., Zeng, L., Yan, S., Plotnikova, O., Sachchidanand, Sanchez, R., Zeleznik-Le, N.J., Ronai, Z., and Zhou, M.M. (2004). Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation. Mol Cell *13*, 251-263.

Neigeborn, L., and Mitchell, A.P. (1991). THE YEAST MCK1 GENE ENCODES A PROTEIN-KINASE HOMOLOG THAT ACTIVATES EARLY MEIOTIC GENE-EXPRESSION. Genes & Development *5*, 533-548.

Nyswaner, K.M., Checkley, M.A., Yi, M., Stephenst, R.M., and Garfinkel, D.J. (2008). Chromatin-associated genes protect the yeast genome from Ty1 insertional mutagenesis. Genetics *178*, 197-214.

Oki, M., Valenzuela, L., Chiba, T., Ito, T., and Kamakaka, R.T. (2004). Barrier proteins remodel and modify chromatin to restrict silenced domains. Molecular and Cellular Biology *24*, 1956-1967.

Phillips, D.M. (1963). PRESENCE OF ACETYL GROUPS IN HISTONES. Biochemical Journal *87*, 258-&.

Piperno, G., and Fuller, M.T. (1985a). MONOCLONAL-ANTIBODIES SPECIFIC FOR AN ACETYLATED FORM OF ALPHA-TUBULIN RECOGNIZE THE ANTIGEN IN CILIA AND FLAGELLA FROM A VARIETY OF ORGANISMS. Journal of Cell Biology *101*, 2085-2094.

Piperno, G., and Fuller, M.T. (1985b). Monoclonal antibodies specific for an acetylated form of alpha-tubulin recognize the antigen in cilia and flagella from a variety of organisms. The Journal of cell biology *101*, 2085-2094.

Piperno, G., Ledizet, M., and Chang, X.J. (1987). MICROTUBULES CONTAINING ACETYLATED ALPHA-TUBULIN IN MAMMALIAN-CELLS IN CULTURE. Journal of Cell Biology *104*, 289-302.

Ramachandran, V., Shah, K.H., and Herman, P.K. (2011). The cAMP-Dependent Protein Kinase Signaling Pathway Is a Key Regulator of P Body Foci Formation. Mol Cell *43*, 973-981.

Ribbeck, K., Lipowsky, G., Kent, H.M., Stewart, M., and Gorlich, D. (1998). NTF2 mediates nuclear import of Ran. Embo Journal *17*, 6587-6598.

Ronne, H. (1995). GLUCOSE REPRESSION IN FUNGI. Trends in Genetics *11*, 12-17.

Roth, S.Y., Denu, J.M., and Allis, C.D. (2001). Histone acetyltransferases. Annu Rev Biochem *70*, 81-120.

Sabo, A., Lusic, M., Cereseto, A., and Giacca, M. (2008). Acetylation of conserved lysines in the catalytic core of cyclin-dependent kinase 9 inhibits kinase activity and regulates transcription. Molecular and Cellular Biology *28*, 2201-2212.

Sadeh, A., Movshovich, N., Volokh, M., Gheber, L., and Aharoni, A. (2011). Fine-tuning of the Msn2/4-mediated yeast stress responses as revealed by systematic deletion of Msn2/4 partners. Mol Biol Cell *22*, 3127-3138.

Santhanam, A., Hartley, A., Duvel, K., Broach, J.R., and Garrett, S. (2004). PP2A phosphatase activity is required for stress and Tor kinase regulation of yeast stress response factor Msn2p. Eukaryotic Cell *3*, 1261-1271.

Sanz, P., Alms, G.R., Haystead, T.A.J., and Carlson, M. (2000). Regulatory interactions between the Reg1-Glc7 protein phosphatase and the Snf1 protein kinase. Molecular and Cellular Biology *20*, 1321-1328.

Schmitt, A.P., and McEntee, K. (1996). Msn2p, a zinc finger DNA-binding protein, is the transcriptional activator of the multistress response in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America *93*, 5777-5782.

Shahbazian, M.D., and Grunstein, M. (2007). Functions of site-specific histone acerylation and deacetylation. In Annu Rev Biochem, pp. 75-100.

Shero, J.H., and Hieter, P. (1991). A SUPPRESSOR OF A CENTROMERE DNA MUTATION ENCODES A PUTATIVE PROTEIN-KINASE (MCK1). Genes & Development *5*, 549-560.

Shivaswamy, S., and Iyer, V.R. (2008). Stress-dependent dynamics of global chromatin remodeling in yeast: Dual role for SWI/SNF in the heat shock stress response. Molecular and Cellular Biology *28*, 2221-2234.

Shogren-Knaak, M., Ishii, H., Sun, J.M., Pazin, M.J., Davie, J.R., and Peterson, C.L. (2006). Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science *311*, 844-847.

Sikorski, R.S., and Hieter, P. (1989). A SYSTEM OF SHUTTLE VECTORS AND YEAST HOST STRAINS DESIGNED FOR EFFICIENT MANIPULATION OF DNA IN SACCHAROMYCES-CEREVISIAE. Genetics *122*, 19-27.

Sinha, H., David, L., Pascon, R.C., Clauder-Munster, S., Krishnakumar, S., Nguyen, M., Shi, G., Dean, J., Davis, R.W., Oefner, P.J.*, et al.* (2008). Sequential Elimination of Major-Effect Contributors Identifies Additional Quantitative Trait Loci Conditioning High-Temperature Growth in Yeast. Genetics *180*, 1661-1670.

Smith, A., Brownawell, A., and Macara, I.G. (1998a). Nuclear import of Ran is mediated by the transport factor NTF2. Current Biology *8*, 1403-1406.

Smith, A., Ward, M.P., and Garrett, S. (1998b). Yeast PKA represses Msn2p/Msn4p-dependent gene expression to regulate growth, stress response and glycogen accumulation. Embo Journal *17*, 3556-3564.

Song, J.M., and Rabinowitz, J.C. (1993). FUNCTION OF YEAST CYTOPLASMIC-C(1)-TETRAHYDROFOLATE SYNTHASE. Proceedings of the National Academy of Sciences of the United States of America *90*, 2636-2640.

Spange, S., Wagner, T., Heinzel, T., and Kramer, O.H. (2009). Acetylation of non-histone proteins modulates cellular signalling at multiple levels. International Journal of Biochemistry & Cell Biology *41*, 185-198.

Stade, K., Ford, C.S., Guthrie, C., and Weis, K. (1997). Exportin 1 (Crm1p) is an essential nuclear export factor. Cell *90*, 1041-1050.

Steger, D.J., Eberharter, A., John, S., Grant, P.A., and Workman, J.L. (1998). Purified histone acetyltransferase complexes stimulate HIV-1 transcription from preassembled nucleosomal arrays. Proceedings of the National Academy of Sciences of the United States of America *95*, 12924-12929.

Sun, Y., Xu, Y., Roy, K., and Price, B.D. (2007). DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. Molecular and Cellular Biology *27*, 8502-8509.

Thevelein, J.M., and de Winde, J.H. (1999). Novel sensing mechanisms and targets for the cAMP-protein kinase A pathway in the yeast Saccharomyces cerevisiae. Mol Microbiol *33*, 904-918.

Thomas, G., and Hall, M.N. (1997). TOR signalling and control of cell growth. Current Opinion in Cell Biology *9*, 782-787.

Thompsonjaeger, S., Francois, J., Gaughran, J.P., and Tatchell, K. (1991). DELETION OF SNF1 AFFECTS THE NUTRIENT RESPONSE OF YEAST AND RESEMBLES MUTATIONS WHICH ACTIVATE THE ADENYLATE-CYCLASE PATHWAY. Genetics *129*, 697-706.

Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K., and Wigler, M. (1985). IN YEAST, RAS PROTEINS ARE CONTROLLING ELEMENTS OF ADENYLATE-CYCLASE. Cell *40*, 27-36.

Tong, A.H., and Boone, C. (2006). Synthetic genetic array analysis in Saccharomyces cerevisiae. Methods in molecular biology (Clifton, NJ) *313*, 171-192.

Tong, A.H.Y., Evangelista, M., Parsons, A.B., Xu, H., Bader, G.D., Page, N., Robinson, M., Raghibizadeh, S., Hogue, C.W.V., Bussey, H.*, et al.* (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science *294*, 2364-2368.

Tronnersjo, S., Hanefalk, C., Balciunas, D., Hu, G.Z., Nordberg, N., Muren, E., and Ronne, H. (2007). The jmjN and jmjC domains of the yeast zinc finger protein Gis1 interact with 19 proteins involved in transcription, sumoylation and DNA repair. Molecular Genetics and Genomics *277*, 57-70.

Tsaprouni, L.G., Ito, K., Powell, J.J., Adcock, I.M., and Punchard, N. (2011). Differential patterns of histone acetylation in inflammatory bowel diseases. Journal of Inflammation-London *8*.

Tyler, J.K., Adams, C.R., Chen, S.R., Kobayashi, R., Kamakaka, R.T., and Kadonaga, J.T. (1999). The RCAF complex mediates chromatin assembly during DNA replication and repair. Nature *402*, 555-560.

Vincent, O., Townley, R., Kuchin, S., and Carlson, M. (2001). Subcellular localization of the Snf1 kinase is regulated by specific beta subunits and a novel glucose signaling mechanism. Genes & Development *15*, 1104-1114.

Viswanathan, M., Muthukumar, G., Cong, Y.S., and Lenard, J. (1994). SERIPAUPERINS OF SACCHAROMYCES-CEREVISIAE - A NEW MULTIGENE FAMILY ENCODING SERINE-POOR RELATIVES OF SERINE-RICH PROTEINS. Gene *148*, 149-153.

Wang, C., Skinner, C., Easlon, E., and Lin, S.-J. (2009). Deleting the 14-3-3 Protein Bmh1 Extends Life Span in Saccharomyces cerevisiae by Increasing Stress Response. Genetics *183*, 1373-1384.

Wang, Q.J., Zhang, Y.K., Yang, C., Xiong, H., Lin, Y., Yao, J., Li, H., Xie, L., Zhao, W., Yao, Y.F.*, et al.* (2010). Acetylation of Metabolic Enzymes Coordinates Carbon Source Utilization and Metabolic Flux. Science *327*, 1004-1007.

Wang, Y., Pierce, M., Schneper, L., Guldal, C.G., Zhang, X.Y., Tavazoie, S., and Broach, J.R. (2004). Ras and Gpa2 mediate one branch of a redundant glucose signaling pathway in yeast. Plos Biology *2*, 610-622.

Weinert, B.T., Wagner, S.A., Horn, H., Henriksen, P., Liu, W.S.R., Olsen, J.V., Jensen, L.J., and Choudhary, C. (2011). Proteome-Wide Mapping of the Drosophila Acetylome Demonstrates a High Degree of Conservation of Lysine Acetylation. Science Signaling *4*.

Weisman, R., and Choder, M. (2001). The fission yeast TOR homolog, tor1(+), is required for the response to starvation and other stresses via a conserved serine. Journal of Biological Chemistry *276*, 7027-7032.

Wernerwashburne, M., Stone, D.E., and Craig, E.A. (1987). COMPLEX INTERACTIONS AMONG MEMBERS OF AN ESSENTIAL SUBFAMILY OF HSP70 GENES IN SACCHAROMYCES-CEREVISIAE. Molecular and Cellular Biology *7*, 2568-2577.

White, M.A., Riles, L., and Cohen, B.A. (2009). A Systematic Screen for Transcriptional Regulators of the Yeast Cell Cycle. Genetics *181*, 435-446.

Wieser, R., Adam, G., Wagner, A., Schuller, C., Marchler, G., Ruis, H., Krawiec, Z., and Bilinski, T. (1991). HEAT-SHOCK FACTOR-INDEPENDENT HEAT CONTROL OF TRANSCRIPTION OF THE CTT1 GENE ENCODING THE CYTOSOLIC CATALASE-T OF SACCHAROMYCES-CEREVISIAE. Journal of Biological Chemistry *266*, 12406-12411.

Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H.*, et al.* (1999). Functional characterization of the S-cerevisiae genome by gene deletion and parallel analysis. Science *285*, 901-906.

Xiao, Z.X., McGrew, J.T., Schroeder, A.J., and Fitzgeraldhayes, M. (1993). CSE1 AND CSE2, 2 NEW GENES REQUIRED FOR ACCURATE MITOTIC CHROMOSOME SEGREGATION IN SACCHAROMYCES-CEREVISIAE. Molecular and Cellular Biology *13*, 4691-4702.

Xue, Y., Batlle, M., and Hirsch, J.P. (1998). GPR1 encodes a putative G protein-coupled receptor that associates with the Gpa2p G(alpha) subunit and functions in a Ras-independent pathway. Embo Journal *17*, 1996-2007.

Yan, Y., Barlev, N.A., Haley, R.H., Berger, S.L., and Marmorstein, R. (2000). Crystal structure of yeast Esa1 suggests a unified mechanism for catalysis and substrate binding by histone acetyltransferases. Mol Cell *6*, 1195-1205.

Yoshida, K., and Blobel, G. (2001). The karyopherin Kap142p/Msn5p mediates nuclear import and nuclear export of different cargo proteins. The Journal of cell biology *152*, 729-740.

Yu, H., Shao, Y., Gao, L., Zhang, L., Guo, K., Wu, C., Hu, X., and Duan, H. (2012). Acetylation of sphingosine kinase 1 regulates cell growth and cell-cycle progression. Biochemical and Biophysical Research Communications *417*, 1242-1247.

Zhang, H.Y., Richardson, D.O., Roberts, D.N., Utley, R., Erdjument-Bromage, H., Tempst, P., Cote, J., and Cairns, B.R. (2004). The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation,

and Htz1 replacement near telomeres. Molecular and Cellular Biology *24*, 9424-9436.

Zhang, J.M., Sprung, R., Pei, J.M., Tan, X.H., Kim, S., Zhu, H., Liu, C.F., Grishin, N.V., and Zhao, Y.M. (2009). Lysine Acetylation Is a Highly Abundant and Evolutionarily Conserved Modification in Escherichia Coli. Mol Cell Proteomics *8*, 215-225.

Zhao, S.M., Xu, W., Jiang, W.Q., Yu, W., Lin, Y., Zhang, T.F., Yao, J., Zhou, L., Zeng, Y.X., Li, H.*, et al.* (2010). Regulation of Cellular Metabolism by Protein Lysine Acetylation. Science *327*, 1000-1004.

Appendix A

Table S1- Strain list

Strain	**Auxotrophies**	**Reference or Source**
YKB779	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63*	(Sikorski and Hieter, 1989)
YKB 2163	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 GDS1-TAP::HIS3*	This study
YKB 440	*MATa trpΔ ura3-1 leu2-3,112 his3-11,15 ade2-1 can1-100 ESA1-TAP::TRP*	TAP collection
YKB 2703	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 GDS1-HA::KAN*	This study
YKB 2705	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 GDS1-HA::KAN ESA1-TAP::TRP*	This study
YKB 1035	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 MSN4-TAP*	(Mitchell et al., 2008)
YKB 2959	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 GDS1-HA::KAN MSN4-TAP*	This study
YKB 2609	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 GDS1-GFP::KAN*	This study
YKB 1233	*MATα his3Δ1 leu2Δ lys2Δ ura3Δ GFP-TUB1::URA3*	This study
YKB 2839	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 GDS1-GFP::KAN eaf1Δ::KAN*	This study
YKB 2628	*MATα ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 esa1ΔHIS3 GDS1-GFP::KAN esa1-L245P::URA3*	This study
YKB 42	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 eaf1Δ::KAN*	(Mitchell et al., 2008)
YKB 2644	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 gds1Δ::KAN*	This study
YKB 1093	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 met15Δ0 msn2Δ::TRP msn4Δ::KAN*	(Mitchell et al., 2008)
YKB 860	*MATa his3-Δ200 leu2-3,112 trp-1Δ1 ura3-52 ade2-101 esa1ΔHIS3 esa1-L245P::URA3*	(Clarke et al., 1999)
YKB 2943	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 gds1ΔKAN esa1ΔHIS3 esa1-L245P::URA3*	This study
YKB 2841	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 met15Δ0 msn2Δ::TRP msn4::KAN gds1Δ::KAN*	This study
YKB 3075	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 gds1Δ::KAN eaf1Δ::NAT*	This study

YKB 2961	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63MSN4-TAP::TRP*	This study
YKB 2970	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 MSN2-GFP::HIS*	This study
YKB 3085	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 MSN2-GFP::HIS gds1Δ::KAN*	This study
YKB 3162	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 MSN2-GFP::HIS eaf7Δ::KAN*	This study
YKB 3198	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 HSP12-GFP::HIS*	This study
YKB 3199	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 HSP12-GFP::HIS eaf7Δ::KAN*	This study
YKB 3200	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 HSP12-GFP::HIS gds1Δ::KAN*	This study
YKB 3276	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 HSP12-GFP::HIS gds1Δ::KAN eaf7Δ::TRP*	This study
YKB 3278	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 HSP12-GFP::HIS msn2Δ::TRP msn4::KAN gds1Δ::KAN*	This study
YKB 3279	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 GDS1 K 87, 342, 343, 345, 348, 351, 354, 408R –HA:: NAT*	This study
YKB 3280	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 GDS1 K87R –HA:: NAT*	This study
YKB 1690	*MATa ade2-101 his3-Δ200 lys2-801 leu2-Δ1 ura3-52 trp1-Δ63 EPL1-HA::KAN*	Mitchell et al., 2013

Table S2 – Gds1-TAP physical interactome

	Gene Name	Mascot Score	# Unique Peptide	# Peptide Hits	% Coverage
YJL111W	CCT7	342	2	6	4%
YGL207W	SPT16	412	6	7	7%
YDR190C	RVB1	235	3	5	7%
YPL235W	RVB2	221	2	4	5%
YBR245C	ISW1	465	3	8	4%
YBL023C	MCM2	251	4	5	5%
YGL201C	MCM6	419	3	8	4%
YMR037C	MSN2	87	2	2	3%
YKL062W	MSN4	512	3	10	6%
YBL052C	SAS3	230	2	4	3%
YFL039C	ACT1	2312	7	42	19%
YJL081C	ARP4	2410	10	40	25%
YDR359C	EAF1	9463	29	164	33%
YPR023C	EAF3	911	7	16	21%
YFL024C	EPL1	5573	31	102	38%
YOR244W	ESA1	2694	15	49	31%
YHR090C	YNG2	1887	5	31	17%
YHR099W	TRA1	23719	90	414	28%
YGR002C	SWC4	2806	14	48	27%
YJL076W	NET1	147	2	3	2%
YMR091C	NPL6	214	2	4	5%
YBR081C	SPT7	2282	13	39	11%
YLR055C	SPT8	1192	7	19	14%
YGL066W	SGF73	2229	6	36	14%
YPL254W	HFI1	1375	4	21	11%
YDR392W	SPT3	384	4	7	16%
YOL148C	SPT20	554	3	11	6%
YGR252W	GCN5	433	5	8	14%
YDR448W	ADA2	474	4	9	11%
YDR176W	NGG1	634	3	13	4%
YDR145W	TAF12	1983	12	39	28%
YBR198C	TAF5	1887	10	33	16%
YGL112C	TAF6	1050	6	18	15%
YML010W	SPT5	350	3	6	3%
YGR270W	YTA7	914	6	16	7%
YPL082C	MOT1	152	3	3	2%
YDR129C	SAC6	625	4	11	8%
YFL037W	TUB2	254	2	5	5%
YKL054C	DEF1	832	4	14	8%
YBR025C	OLA1	724	5	13	14%
YJL008C	CCT8	352	4	7	8%
YIL142W	CCT2	174	2	3	4%
YJL014W	CCT3	97	2	2	4%
YLL026W	HSP104	653	8	13	10%
YBL099W	ATP1	164	2	3	4%

YER177W	BMH1	312	2	5	9%
YMR246W	FAA4	143	2	3	3%
YOR355W	GDS1	12283	17	203	40%
YIR033W	MGA2	643	3	12	4%
YPL226W	NEW1	787	4	15	4%
YLR150W	STM1	238	4	5	16%
YNL071W	LAT1	282	4	6	9%
YMR205C	PFK2	293	3	6	6%
YDR454C	GUK1	0	2	8	6%
YPL237W	SUI3	193	2	4	7%
YKL126W	YPK1	155	2	3	4%
YER025W	GCD11	361	4	7	9%
YGL105W	ARC1	168	2	3	6%
YGL245W	GUS1	1423	10	25	15%
YDR238C	SEC26	266	2	5	2%
YGL137W	SEC27	322	2	5	3%
YFR051C	RET2	246	2	5	3%
YER110C	KAP123	1218	7	19	8%
YNL189W	SRP1	248	2	4	4%
YOR046C	DBP5	164	3	3	9%

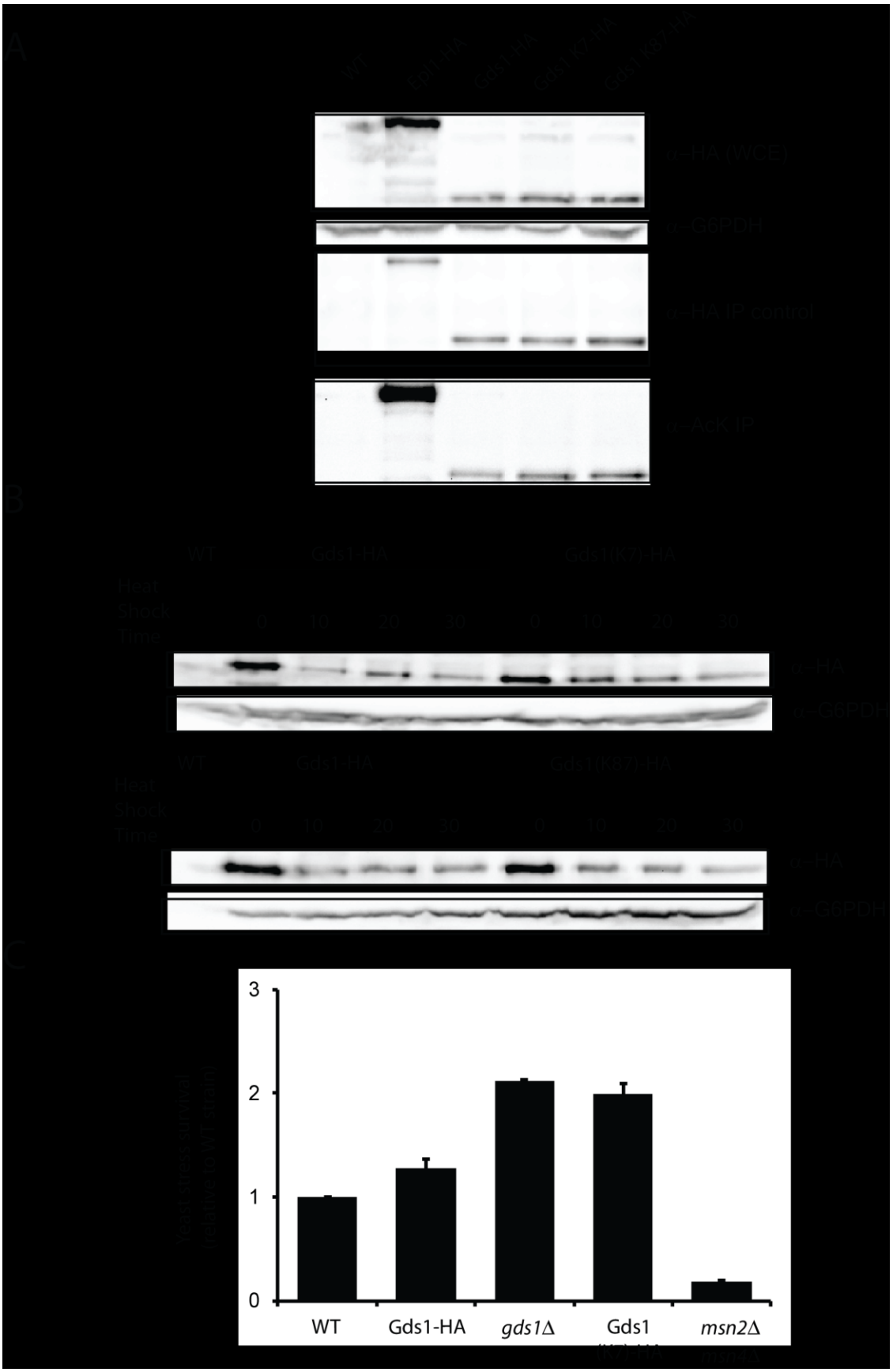
A
WT
Gds1-HA
Gds1(K87)-HA
α-HA (WCE)
α-G6PDH
α-HA IP control
α-AGK IP
B
WT
Gds1-HA
Gds1(K87)-HA
Heat Shock Time
0
10
20
30
α-HA
α-G6PDH
C
3
2
1
0
WT
Gds1-HA
gds1Δ
Gds1
msn2Δ

Figure S1: Gds1p acetylation site point mutants are degraded upon heat shock but confer increased stress survival

A. Gds1p acetylation site point mutants may retain acetylation. Gds1-HA (YKB 2703), Gds1K7-HA(YKB 3279)*, Gds1K87-HA (YKB 3280)** and Epl1-HA (YKB 1690) were immunopurified from yeast cells through α-AcK coupled magnetic beads or α-HA coupled magnetic beads compared to an untagged strain (YKB 779 WT). Immunopurified products and whole cell extract were subjected to Western blot analysis with anti HA (α-HA) or glyceraldehyde-6-dehydrogenase (α-G6DPH) antibodies as indicated. Results are inconclusive as the uncoupled beads control (not shown) immunopurified Gds1-HA as well as the point mutants. n=1 **B.** Gds1-HA and Gds1-HA point mutant protein level is reduced in response heat shock. Strains Gds1-HA (YKB 2703), Gds1K7-HA(YKB 3279), and Gds1K87-HA (YKB 3280) were heat shocked at 37°C for the indicated time period prior to lysis. Whole cell extracts were resolved by SDS-PAGE, subjected to Western blot analysis and probed with α-HA and α-G6DPH antibodies. n=1 **C.** Gds1-HA point mutants exhibit increased tolerance to heat stress similar to *gds1Δ* cells. Strain Gds1-HA (YKB 2703), *gds1Δ* (YKB 2644), Gds1K7-HA(YKB 3278), and *msn2Δ msn4Δ* (YKB 1093) were used in a viability assay to test cell survival following brief exposure (11 minutes) to extreme heat (48°C) as compared to cells maintained at 25°C. Results are an average of two experiments and are standardized to the survival rate of wild type cells (YKB 779).

*Gds1K7-HA refers to a seven lysine to arginine site point Gds1p point mutant – K87, 342, 343, 345, 351, 348, 354R

**Gds1K87-HA refers to a single lysine to arginine site point Gds1p point mutant – K87R