HISTONE H2A-S122 IS REQUIRED FOR NUCLEAR AND MITOCHONDRIAL GENOME STABILITY

By

Shannon G. Uffenbeck

RECOMMENDED:

Dr. Miki Ii

Dr. Kristin O'Brien

Dr. Thomas Kuhn, Advisory Committee Co-Chair

Dr. Jocelyn Krebs, Advisory Committee Chair

Dr. William Simpson
Chair, Department of Chemistry and Biochemistry

APPROVED:

Dr. Paul Layer
Dean, College of Natural Science and Mathematics

Dr. John Eichelberger
Dean of the Graduate School

8/20/2013

Date

HISTONE H2A-S122 IS REQUIRED FOR NUCLEAR AND MITOCHONDRIAL GENOME STABILITY

A

DISSERTATION

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSPHY

By

Shannon R. Uffenbeck, M.S.

Fairbanks, Alaska

August 2013

Abstract

Organization and maintenance of the mitochondrial and nuclear genomes are vastly different, yet I have shown that a single serine in the H2A C-terminal tail (H2A-S122) is critical for stability of both genomes in the budding yeast, *Saccharomyces cerevisiae*. Phosphorylation of H2A-S122 has previously been implicated in the spindle assembly checkpoint (SAC), however I show that by mutating the serine to an alanine (H2A-S122A), the resulting aneuploidy occurs at a much higher rate than is observed by deleting its immediate downstream kinase *BUB1*. Furthermore, the H2A-S122A mutant displays an increased susceptibility to DNA damaging agents that is not observed in *bubΔ1* deletion cells. Our studies also implicate H2A-S122 as critical to the maintenance of the mitochondrial genome, as upon introduction of the H2A-S122A mutation, cells rapidly lose their mitochondrial genomes.

Table of Contents

Page

List of Figures

Page

List of Tables

Page

INTRODUCTION

DNA holds the blueprint of a cell's existence. The simple sequences of deoxyribonucleotides found within the nucleus and mitochondria provide a mechanism for transferring hereditary information that determines the health, function, and productivity of a given cell. Maintaining that sequence with a high degree of fidelity is critical, as increased rates of mutation can have devastating effects for the cell and are associated with a number of diseases including cancer. Due to the importance of genetic stability, it is not surprising that the primary mechanisms maintaining both the mitochondrial and nuclear genomes are highly conserved. Thus the work discussed in this thesis using the budding yeast *Saccharomyces cerevisiae* is highly relevant in terms of human genome stability and health. As is explained in detail below, the mitochondrial and nuclear genomes use very different strategies for maintaining their respective genomes; however, I have found that one amino acid residue on the C-terminal tail of histone H2A (serine-122) is critical for the maintenance of both genomes. This important residue is clearly involved in multiple cellular pathways that are required for nuclear and mitochondrial genome stability.

I.1 Nuclear Genome Environment

The fundamental genome organization of nuclear DNA is highly conserved between all eukaryotic organisms, with chromatin condensation being the primary mechanism in which DNA is stored, organized and maintained within the nucleus. Chromatin, the native compacted form of DNA, is organized into varying levels of condensation, and is the mechanism that allows large amounts of genetic material to fit into the relatively small nucleus. The basic level of compaction is the wrapping of DNA around a histone octamer comprised of two copies each of the core histones H2A, H2B, H3 and H4 (for review see: Wolffe, 1999). These DNA-histone complexes, called nucleosomes, are linked together by a variable length of linker DNA that can be associated with linker histones. Each core histone has an amino-terminal tail, and histone H2A has a significant carboxy-terminal tail as well. Histone tail regions are not critical for nucleosome integrity (Ausio et al., 1989; Whitlock and Simpson, 1977); instead they allow for more flexible interactions between DNA and other

nucleosomes. Histone tails play a significant role in further compaction of chromatin through interactions with other nucleosomes (for review see: Zheng and Hayes, 2003).

The level of compaction varies between the most tightly packed form, referred to as heterochromatin, and the less and more variably compacted form, euchromatin. DNA confined within heterochromatin is generally inaccessible to DNA binding proteins which functions to both protect the DNA as well as regulate its accessibility, while euchromatin-associated DNA is more easily accessed and is stereotypically associated with active transcription even though plenty of euchromatic DNA is not being actively transcribed at any given time (for review see: Fedorova and Zink, 2008). The various levels of compaction not only function in packaging the DNA, but also serve as a mode of regulation as the DNA packaged into nucleosomes is generally inaccessible to the basal transcriptional machinery. To overcome the naturally repressive state of chromatin structure, the cell uses chromatin modifying complexes to manipulate DNA-histone interactions. Chromatin modifying complexes, or factors that recruit such complexes, are often recruited to elements within the promoter sequences that are more readily accessible within condensed chromatin, due to nucleosome positioning or maintenance of nucleosome-free regions, or by opportunistic binding to transiently exposed sites, thus creating a dynamic means of control in which the regulation of gene expression depends on the accessibility of promoter sequences within the greater context of chromatin structure (for review see: Wolffe, 1999).

Chromatin modifying complexes use a variety of mechanisms to alter chromatin structure, allowing the cell a number of ways in which it can regulate access to its condensed DNA. Chromatin remodeling enzymes utilize ATP to mechanically alter chromatin structure by moving, removing or exchanging either the entire nucleosome itself or particular histones within the nucleosome (Hargreaves and Crabtree, 2011). Histone modifying enzymes alter chromatin structure directly by creating localized changes of electronegativity that alter the level of condensation or indirectly by creating novel binding sites for DNA binding proteins that alter chromatin structure (for review see: Berger, 2007). Frequently, it is a histone modification event that triggers the recruitment of a chromatin remodeling enzyme to a particular locus, thus underscoring the fact that these two mechanisms are often interdependent.

Histone modifying complexes alter the state of chromatin through covalent modifications. This involves the addition or removal of many different moieties, such as acetyl, phosphate, or methyl groups (Fischle et al., 2003; Iizuka and Smith, 2003). Depending on the type of modification, localized changes in electrical charge can occur. This can alter the DNA-histone binding affinity, thus increasing or decreasing the stability of the single nucleosome or alter inter-nucleosome interactions, resulting in changes in chromatin condensation levels. Histone modifications can also result in the generation of novel binding surfaces for other factors, such as chromatin remodelers, repressors, or transcription factors (for review see: Spencer and Davie, 1999).

The large number of possible histone modification patterns allows for highly specific and complex signaling mechanisms. The reversibility of these marks further enhances the regulatory powers held by the post-translational modification of histones. For example, the acetylation via histone acetyltransferases (HATs) of histone tails is associated with active transcription (Brownell et al., 1996; Struhl, 1998) while deacetylation through histone deacetylase (HDAC) complexes normally results in gene repression (Kadosh and Struhl, 1998; Laherty et al., 1997; Nagy et al., 1997). For this particular example, the acetylation of lysine residues affects level of condensation of the chromatin by neutralizing the positive charge of the lysine and reducing the affinity between the histone tail and the phosphate backbone of DNA. Additionally, lysine acetylation can also create a novel binding site that is recognized by proteins containing bromodomains (acetyl-lysine recognition domains).

While histone tail acetylation appears to function primarily within the context of transcriptional activation or repression, phosphorylation of histone tails is associated with a much wider variety of cellular processes. Due to the importance of kinase cascades in a number of cell signaling pathways, it is not surprising that phosphorylation of histone tails is critical for both cell-cycle and DNA damage signaling in addition to roles in gene activation and chromosome condensation (for review see: Berger, 2010). In the context of this thesis, phosphorylation is of particular interest as H2A-S122 is phosphorylated *in vivo*, and it is presumed that its phosphorylation status is the key to its importance in maintaining both mitochondrial and nuclear genome stability ((Moore et al., 2007; Wyatt et al., 2003).

I.2 Mitochondrial Genome Environment

The mitochondrial genome is much smaller than the nuclear genome, thus it does not require the level of condensation found within the nucleus. Still, mtDNA exists within the context of mitochondrial chromatin, the nucleoid. Nucleoids do not contain canonical histone proteins; however the high-mobility group-like protein Abf2 packages mtDNA. HMG-like proteins have a weaker affinity for DNA than histones, thus in yeast the level of mtDNA compaction (190 nm structure) is approximately seven-fold less than that seen in the nuclear mitotic chromosome (Brewer et al., 2003). Much like histones within the nuclear genome, Abf2 both protects and mediates access to mtDNA. Cells lacking Abf2 (*abf2Δ*) are more sensitive to nuclease digestion (Newman et al., 1996) and oxidative stress (O'Rourke et al., 2002). Furthermore, *abf2Δ* cells rapidly lose mtDNA unless mitochondrial function is required for growth (Chen and Butow, 2005; Kucej and Butow, 2007). In addition to protecting mtDNA, Abf2-dependent condensation offers a mechanism for regulating access to mtDNA. Respiring cells (which demand increased oxidative phosphorylation) display decreased amounts of Abf2 in relation to mtDNA levels resulting in a lessening of mtDNA condensation (Kucej et al., 2008). The decrease in condensation would allow increased levels of mtDNA transcription and repair during respiration.

In addition to Abf2, there are approximately 25 additional proteins that associate with the nucleoid (Kucej and Butow, 2007). Not surprisingly, some of these proteins function in replication, transcription, repair, recombination or packaging of mtDNA. There are also a number of heat shock proteins involved in typical chaperone functions that are required for protein import and quality control. However, some of these proteins may have nucleoid specific functions as well. For instance, besides its well established role in mitochondrial protein import, Hsp60 is also required for nucleoid division through its speculative role in linking mtDNA *ori* sequences with the inner mitochondrial membrane (Kaufman et al., 2003). Of the 25 nucleoid-associated proteins, approximately 15 are metabolic enzymes that affect mitochondrial genome stability, thus providing a mechanism for coupling metabolism needs to mitochondrial genome regulation (Chen and Butow, 2005; Kucej and Butow, 2007).

In *S. cerevisiae*, one cell contains one to ten mitochondria that can fuse to form a dynamic filamentous mega-mitochondrial structure that alters dramatically depending on the energy

requirements of the cell. The number of nucleoids per cell also varies, but has an approximate upper limit of about 40 with each nucleoid containing one to ten copies of the mitochondrial genome (Kucej and Butow, 2007; Williamson, 2002). Unlike mammalian mitochondrial genomes which are found in circular form, the vast majority of mtDNA in yeast is found in a linear conformation and is heterologous in length (Maleszka et al., 1991). Interestingly, the mtDNA structure appears to be somewhat fluid as mother cell mitochondrial genomes are comprised of concatamers while the corresponding buds contain a much higher percentage of circular monomers (Ling and Shibata, 2002). Although the mechanism and significance of this finding is yet unknown, it is easy speculate that the difference in bud mtDNA structure is related to mechanisms that contribute to the asymmetric distribution of aging determinants in yeast cell division. In addition to other aging associated factors (oxidized proteins, rDNA circles, and catalase activation; (Erjavec et al., 2008; Shcheprova et al., 2008), evidence suggests that mitochondria with reduced membrane potential are sequestered in the mother cell during cytokinesis (Lai et al., 2002). These mechanisms would account for the ability of yeast to reset their biological clock to almost zero in daughter cells by keeping damaged proteins and other age determinants in the mother cell (for review see: Laun et al., 2007).

In *S. cerevisiae*, approximately equal numbers of mitochondria are segregated to opposite poles of the yeast cell, the bud tip and the mother cell tip, during cell division. Mitochondria move bidirectionally along actin filaments that span between the bud tip and mother cell tip (Fehrenbacher et al., 2004). Nucleoid-associated mitochondrial outer-membrane proteins Mmm1, Mdm10, Mdm12 form a complex, referred to as the mitochore, that directly links the nucleoid to actin cables with the help of Puf3 (Boldogh et al., 1998; Boldogh et al., 2003; Garcia-Rodriguez et al., 2007). Peraza-Reyes et al. propose a model in which mitochondria flow along actin cables away from the bud and only those mitochondria generating enough energy (thus are highly functioning) are able to overcome the actin cable flow and migrate against the actin cable flow towards the bud (Peraza-Reyes et al., 2010). Anchorage of mitochondria to the appropriate pole requires Ypt11 and Mmr1 at the bud pole and Dnm1 and Num1 at the mother tip pole which is critical for proper mitochondrial segregation (Boldogh et al., 2004; Cerveny et al., 2007; Peraza-Reyes et al., 2010). There is also evidence that the

tethering of the mitochondria in the bud is coordinated with the tethering of the endoplasmic reticulum (Swayne et al., 2011).

The mitochore physically links the nucleoid to the cytoskeleton; thus it is not surprising that mitochore proteins are critical for maintaining mitochondrial morphology and inheritance (Boldogh et al., 1998; Boldogh et al., 2003). It is also likely that the mitochore has a direct role in mitochondrial genome maintenance as cells carrying a temperature-sensitive mutant of the mitochore protein, Mmm1 (*mmm1-1*), display a decrease in mitochondrial DNA staining after only one hour of growth at the non-permissive temperature (Hobbs et al., 2001). Mitochore mutant cells also display increased inhibition of contractile-ring closure during cytokinesis, implicating the importance of the mitochore in cell cycle regulation (Garcia-Rodriguez et al., 2009).

I.3 Nuclear and Mitochondrial Communication

According to the *Saccharomyces* genome database (www.yeastgenome.org), there are approximately 1004 proteins that have been isolated from highly purified mitochondrial extracts, however only eight are encoded by the mitochondrial genome in *S. cerevisiae*. The vast majority of mitochondrial proteins are encoded within the nucleus, synthesized in the cytosol and transported into mitochondria. Thus communication between the two organelles is critical for maintaining the proper function of the mitochondria in its many roles, including energy production, nutrient production, phospholipid synthesis, calcium signaling, apoptosis, and iron-sulfur biogenesis. Communication between the two organelles has been traditionally referred to as anterograde signaling, where nuclear-perceived conditions are signaled to the mitochondria affecting mitochondrial gene expression, or retrograde signaling, where mitochondrial conditions are signaled to the nucleus resulting in changes in nuclear gene expression (Yazgan and Krebs, 2012). However, as our understanding has increased, it has become clear that communication between mitochondria and the nucleus is not unidirectional; therefore it is more appropriate to refer to the mitochondria-nuclear crosstalk in terms of the information conveyed. Woo et al. characterizes three established types of mitochondrial-nuclear crosstalk: hypoxic signaling (facilitates cellular adaptation to hypoxia via mitochondrial and nuclear transcriptional changes), retrograde regulation (alters nuclear

transcription when respiration is compromised) and intergenomic signaling (alters nuclear transcription in the absence of a mitochondrial genome; (Woo et al., 2009). Recently, Rodley et al. have proposed a novel mode of communication that is based on the transport of reverse transcribed mitochondrial RNA transcripts into the nucleus where they act directly with nuclear DNA to alter transcription (Rodley et al., 2012). Furthermore, multiple mechanisms have been identified that indicates mitochondrial-nuclear crosstalk within the context of cell cycle regulation. In human cells, mtDNA replication is active throughout the cell cycle, however replication and transcription are predominantly coordinated with the cell cycle(Chatre and Ricchetti, 2013). In yeast, mitochondrial status also plays a role in modulating the cell cycle through two independent mitochondrial cell cycle checkpoints that will be described in greater detail below. Currently, we have abundant evidence suggesting that communication pathways must exist; however, our understanding of the mechanisms behind such communication is minimal. As our mitochondrial research techniques improve, we will begin to gain a much better understanding of the many ways in which mitochondrial and nuclear coordination takes place.

I.4 Modification of Histone H2A

H2AX is the predominant H2A variant in *S. cerevisiae*. Like H2AX variants found in most eukaryotes, it has a highly conserved SQEY domain found on the C-terminal tail that is required for DNA damage signaling (Table I1). The globular domain of histone proteins is highly conserved; however the flexible N- and C-terminal tails are much more variable. To date three residues of the H2A C-terminal tail have been shown to undergo phosphorylation *in vivo*. Serine 122, threonine 126, and serine 129 have been shown to be important for proper cellular function in *S. cerevisiae* (Downs et al., 2000; Moore et al., 2007; Wyatt et al., 2003). Phospho-specific antibodies for all three of the residues demonstrate that the residues are phosphorylated in response to DNA damage *in vivo,* however the level of phosphorylation in response to the different DNA damaging agents varies between the different residues (Moore et al., 2007). Mutational analysis using strains in which the threonine or serine has been mutated to an alanine, thus losing its potential for phosphorylation, suggests that there may be some functional redundancy between the residues (Moore et al., 2007; Wyatt et al., 2003).

Using two-dimensional electrophoresis to ascertain H2A phosphorylation levels, Wyatt et al. show that even under non-DNA damaging conditions, total H2A phosphorylation remains constant when one or two of the three residues are mutated to an alanine, while deletion of the entire C-terminal tail obliterates H2A phosphorylation; further indicating a redundancy within the three residues (Wyatt et al., 2003). While H2A-T126 is clearly important for telomeric silencing and certain DNA repair responses in *S. cerevisiae* (Moore et al., 2007; Wyatt et al., 2003), this residue is not conserved in more complex eukaryotes, therefore I will focus on the well-conserved H2A-S122 and H2A-S129 residues.

The SQE(Y/L) domain of H2AX is highly conserved throughout eukaryotic organisms (Downs et al., 2000; Rogakou et al., 1998). Serine 129 in yeast (serine 139 in humans) is found within the context of the SQE(Y/L) motif and plays a major role in DNA damage responses (reviewed in (Lukas et al., 2011; Morrison and Shen, 2005). H2AX proteins in which the SQE(L/Y) serine has been a phosphorylated in response to a DNA break are referred to as γH2AX. Phosphorylation resulting in γH2AX in yeast is carried out by the kinases Mec1 and Tel1 (ATR and ATM in mammals) and results in localized chromatin relaxation through recruitment of the acetyltransferase complex NuA4 and its subsequent acetylation of histone H4 (Downs et al., 2004; Downs et al., 2000). NuA4 and two ATP-dependent chromatin remodeling enzymes, INO80 and SWR1, that are also recruited to double strand breaks, all contain a common subunit, Arp4, that has been shown to directly bind γH2AX (Downs et al., 2004). The importance of γH2AX dependent chromatin remodeler recruitment is not entirely clear as studies have shown that H2AX is required for the retention of damage response factors but is dispensable for the initial signaling of damage (Celeste et al., 2003). γH2AX is also involved in the recruitment of Rad9 to the DNA break where the Mec1 kinase activates Rad9 resulting in triggering the G1/S phase DNA damage checkpoint (Javaheri et al., 2006). Following repair, γH2AX is evicted by Swr1 and is then dephosphorylated by the HTP-C phosphatase complex. Dephophorylation of γH2AX is required for the cell cycle to continue following DNA repair (Keogh et al., 2006; van Attikum et al., 2007).

The role that H2A-S122 plays in DNA repair is much less understood. H2A-S122 is phosphorylated in response to DNA damage induced by menadione, phleomycin, and MMS;

however, levels of phospho-S122 appear to decrease from constitutive levels at 6 hours following UV irradiation (Moore et al., 2007). The change of phosphorylation status does not necessarily correlate with phenotype severity at these timepoints. For instance, cells in which H2A-S122 has been mutated to an alanine (H2A-S122A), thus losing the ability to be phosphorylated, are severely affected by both menadione and MMS treatment. However, levels of H2A-S122 phosphorylation are much higher in menadione treated cells while MMS treated cells demonstrate a more subtle increase in H2A-S122 phosphorylation. It is important to note that this data was obtained from a single time point 6 hours after the various DNA damaging treatments. Unpublished data in our lab has shown that the phosphorylation profiles for the different mutant strains vary dramatically across a time course following damage, and in fact a temporal sequence of phosphorylation of T126, S129, then S122 occurs during persistent MMS exposure (Humpal and Krebs, unpublished).

The relationship between H2A-S122 and H2A-S129 within the context of DNA repair is unclear. Data from the monitoring of H2A phosphorylation (via two dimensional electrophoresis) suggests that a decreased capacity for phosphorylation of the H2A-S122A mutant is compensated for by increased phosphorylation of T126 and/or S129 (Wyatt et al., 2003). However, phenotypic data also suggests that H2A-S122 and H2A-S129 may function independently as the H2A-S122A+ H2A-S129A double mutant displays much stronger menadione, bleomycin, and UV phenotypes than either individual mutant alone (Moore et al., 2007).

In addition to the role for H2A-S122 in DNA damage responses, it has been shown that H2A-S122 is also critical for genomic stability through its role in the spindle assembly checkpoint (SAC). In the fission yeast *Schizosaccharomyces pombe* as well as in HeLa cells, the Bub1 kinase directly phosphorylates H2A-S122 and is critical for the spindle assembly checkpoint (Kawashima et al., 2010; Yamagishi et al., 2010). While it is clear that the H2A-S122 residue is a substrate for the Bub1 kinase within the context of the spindle assembly checkpoint, it is questionable whether the Bub1 kinase acts on H2A-S122 in response to DNA damage. Kawashima et al. demonstrated that both H2A-S122A and *bub1* deletion (*bub1Δ*) strains are sensitive to the DNA topoisomerase inhibitor camptothecin (Kawashima et al., 2010); however given the high rates of aneuploidy due to decreased SAC function, it is

difficult to ascertain whether the camptothecin phenotypes are due to the mutations themselves or are downstream effects of aneuploidy, as the authors did not confirm the ploidy state of their strains in this experiment. The role of H2A-S122 within the SAC will be discussed more in depth below.

I.5 DNA Damage

Both mitochondrial and nuclear genomes are targeted by endogenous and exogenous sources of DNA damage; however, the types of damage most associated with each genome as well as the mode of repair are genome specific. Due to the nuclear genome's larger size (i.e. more gene diversity) and highly condensed state, it is not surprising that the DNA damage recognition and repair mechanisms utilized by the nuclear genome are more diverse than those observed in the mitochondrial genome. Meanwhile, the small mitochondrial genome does not encode for any replication or repair genes; therefore all such proteins must be encoded within the nuclear genome and transported into the mitochondria. While this is highly efficient for the cell, it makes the study of mitochondria-specific replication and repair much more difficult as excluding nuclear contamination while obtaining sufficient mitochondrial material for *in vitro* assays is not trivial. This has led to such a discrepancy in the amount of nuclear and mitochondrial replication and repair research done that one review has termed the more neglected mitochondrial genome the Cinderella of the cell (Holt, 2009). Thus it is not entirely clear whether some of the known nuclear mechanisms are truly absent from the mitochondrial genome or simply haven't been documented in mitochondria (for review see: Kazak et al., 2012).

In the context of the nucleus, there are five general repair mechanisms utilized by cells: homologous recombination (HR), non-homologous end-joining (NHEJ), nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MR) (for reviews see: Ataian and Krebs, 2006; Kanaar et al., 1998). The three excision repair pathways (NER, BER, and MR) recognize and correct for distorted, damaged, or incorrect nucleotides. Following damage recognition, the lesion is excised and then replaced by DNA polymerase using the complementary DNA strand as template. When both strands of the double helix are severed cells utilize either of the two double strand break repair pathways (HR and NHEJ)

depending on whether homologous sequence is available. As the names infer, HR utilizes homology to replace any lost genetic material while NHEJ directly ligates two ends regardless of the sequence. Because of the homology requirement, HR is predominately used during S phase (following DNA replication) and G2 of the cell cycle.

Of course, all of these repair mechanisms occur within the context of chromatin, thus histone-modifying enzymes and ATP-dependent chromatin remodeling enzymes are critical for all nuclear repair mechanisms. The role of ATP-dependent chromatin remodeling enzymes is to physically manipulate the chromatin structure so that the lesion and surrounding areas are accessible to the repair machinery. Histone modifying enzymes on the other hand mediate signaling that is required for damage recognition, repair factor recruitment, cell cycle coordination, and repair resolution (for reviews see: Ataian and Krebs, 2006; Chambers and Downs, 2007; Huertas et al., 2009; Humpal et al., 2009).

While there is overlap between nuclear and mitochondrial genome repair strategies, mitochondrial genome repair has clearly evolved to suit its particular genomic environment. There are three factors that most likely contribute to the differences between mitochondrial and nuclear repair mechanisms. First, the mitochondrial genome is not packaged into chromatin and thus alleviates the need for chromatin remodeling and/or modifying enzymes. Second, due to its close proximity to the oxidative phosphorylation machinery the mitochondrial genome is especially vulnerable to reactive oxygen species generated through electron transport. Third, there are multiple mitochondrial genomes in one cell, resulting in individual genomes being somewhat more expendable.

Base excision repair is the primary mechanism for correcting oxidized bases (Croteau and Bohr, 1997) thus it is not surprising that a functioning BER pathway is required for mitochondrial genome stability (Rosenquist et al., 1997; Vongsamphanh et al., 2001; You et al., 1999). BER consists of recognizing and removing the damaged base and recruiting DNA polymerase to fill in the gap. There are two branches of nuclear BER depending on whether a single nucleotide is cleaved, single nucleotide BER (SN-BER) or a larger section of bases are removed, long patch BER (LP-BER) and evidence suggests that both branches are utilized within mitochondria as well (Akbari et al., 2008; Liu et al., 2008; Szczesny et al., 2008). BER is the best understood mitochondrial repair mechanism with isoforms for a

number of key nuclear BER components having been shown to localize to mitochondria (Rosenquist et al., 1997; Vongsamphanh et al., 2001; You et al., 1999). Most of the research to date on mitochondrial BER has been done using mutational genetics. These approaches have provided a list of players involved, but have left researchers with only a superficial understanding of the mechanistic details. Even so, it has been increasingly clear that nuclear repair proteins may have different functions within mitochondrial repair. One example of this is *MSH1*, one of six MutS homologs in *S. cerevisiae*. MutS homologs are involved in mismatch repair, but *MSH1* is the only MutS homolog that has been isolated from mitochondria (Chi and Kolodner, 1994; Reenan and Kolodner, 1992a, b). It has long been known that *MSH1* is essential for mitochondrial function; however the context of its function has been a mystery as no other mismatch repair proteins have been isolated from mitochondrial extracts. Only relatively recently has mutational analysis provided evidence that both branches of the mitochondrial BER pathway have evolved independently to incorporate the function of Msh1, with SN-BER using Msh1 for damage recognition while LP-BER requires the ATPase activity of Msh1 for repair completion (Pogorzala et al., 2009). Mookerjee et al. also suggest that the extent of mitochondrial genetic disruption in *Δmsh1* mutants cannot be accounted for by the point mutation rate of the mutants. They hypothesize that Msh1 also plays a role in maintaining larger-scale mitochondrial DNA stability that is independent of excision repair (Mookerjee et al., 2005; Pogorzala et al., 2009). Clearly, Msh1 function has diverged from the role that it displays within the nucleus.

Data suggestive of mitochondrial-specific homologous recombination and non-homologous end-joining pathways has slowly begun to accumulate (Chi and Kolodner, 1994; Coffey et al., 1999; Kajander et al., 2001; Kraytsberg et al., 2004; Lakshmipathy and Campbell, 1999; Lockshon et al., 1995; Thyagarajan et al., 1996). Mitochondrial homologous recombination within the context of double strand break repair and stalled or collapsed replication forks has long been speculated, but early attempts to use comparative mitochondrial DNA analysis in humans to demonstrate mitochondrial recombination has been contradictory (Elson et al., 2001; Innan and Nordborg, 2002; Jorde and Bamshad, 2000; Piganeau and Eyre-Walker, 2004; White and Gemmell, 2009; Wiuf, 2001). More recently however, yeast biochemical and genetic assays indicate that there are at least two recombination proteins, Mhr1 (a

proposed ATP-independent recombinase) and Mgm101 (a Rad52-related protein), that have been found to localize to the mitochondria and are critical for mitochondrial genome stability (Ling and Shibata, 2002; Masuda et al., 2010; Mbantenkhu et al., 2011). In addition, a mitochondrial Holliday junction resolving endonuclease, Cce1, which functions through a recombination process that has yet to be fully characterized, is critical for proper mtDNA segregation. Cells lacking *CCE1* (*cce1Δ*) exhibit improper mtDNA aggregation through linked Holiday junctions (Lockshon et al., 1995). Furthermore, the nuclear HR proteins Rad51, Rad51C, and Xrcc3 have been found to localize in human mitochondria and their deletion causes a decrease in mitochondrial DNA copy number (Sage et al., 2010). Meanwhile, recent genetic assays by Kalifa et al. in yeast provide strong evidence for multiple HR and NHEJ pathways in mitochondria. Their data indicates that both the MRX and Ku complexes play a direct role in the repair of mitochondrial double strand breaks, however their roles are partially redundant (Kalifa et al., 2012).

One interesting conundrum facing mitochondrial repair is the high level of homologous sequence within mitochondrial genomes. High homology and multiple mitochondrial genomes within nucleoids would increase the likelihood for HR repair over NHEJ in double strand break repair, however the high homology would also increase the likelihood for inadvertent deletions. Interestingly, approximately 85% of mtDNA deletions found in humans are flanked by short directly repeated sequences which could very well be due to deletions from improper HR. In support of this possibility is data from Kalifa et al. suggesting that in yeast HR gives rise to mtDNA deletions at a much higher rate than in nuclear HR repair (Kalifa et al., 2012). It is easy to speculate that HR may be the large-scale repair pathway hypothesized by Mookerjee et al. as large deletions caused by improper HR would cause more genetic instability than single point mutations (Mookerjee et al., 2005).

I.6 Cell Cycle Checkpoints

Cell cycle checkpoints are surveillance pathways that ensure proper cell progression by monitoring specific processes at key intervals during the cell cycle. There are key checkpoints that occur at specific points in the cell cycle such as the spindle assembly checkpoint (SAC) which monitors spindle formation during mitotic metaphase, or general

checkpoints such as DNA repair checkpoints that can be triggered throughout the cell cycle. The fundamental role of a checkpoint is to detect a cellular problem (via sensor molecules), communicate that problem (via signal transducers), halt cell cycle progression (via effector molecules) and finally restart the cell cycle upon resolution or adaptation. There are a relatively large number of very specific sensing molecules that feed into a small number of effector kinases allowing many types of triggers to initiate a general checkpoint kinase cascade.

I.6.a DNA Damage and Replication Checkpoints

In *S. cerevisiae*, DNA damage checkpoints initiate a delay at the G1/S and G2/M transitions as well as during S-phase (Paulovich et al., 1997; Siede et al., 1993; Weinert and Hartwell, 1988). In addition, another S-phase checkpoint is triggered by replication stress (Santocanale and Diffley, 1998). In *S. cerevisiae*, two highly conserved phosphoinositol-3-kinase-related kinase (PIKK) family members Mec1 and Tel1 function as both damage sensors and/or signal transducers depending on the type of damage encountered. Other damage sensors can also activate Mec1 and Tel1, increasing the number of triggers that influence the PIKK-associated kinase cascade (for review see: Putnam et al., 2009). One important target of both PIKK family members is H2A-S129 as the phosphorylated residue (γ-H2AX) serves to recruit repair proteins to the site of damage as well as mark the beginning of the central kinase cascade crucial for checkpoint activation (Downs et al., 2000; Shroff et al., 2004). Effector molecules for the kinase cascade are Chk1 and Rad53 that target a wide variety of proteins that suppress cell cycle progression, initiate repair, and alter the transcriptome (Branzei and Foiani, 2006; Chen and Sanchez, 2004). A basic overview of this process is summarized in Figure I1. In response to damage or replication failure, Mec1 and Ddc2 are recruited to RPA-coated single stranded DNA (Ball et al., 2005; Rouse and Jackson, 2002; Zou and Elledge, 2003). Other cofactors recruited by the single-strand to double-strand DNA interface activate Mec1. Once activated, Mec1 phosphorylates H2A-S129 (Downs et al., 2004; Downs et al., 2000). Depending on whether the checkpoint is being activated in response to DNA damage or due to a stalled replication fork checkpoint mediator Rad9 or Mrc1, respectively, is recruited by γ-H2AX (Hammet et al., 2007; Javaheri et al., 2006;

Naylor et al., 2009). The recruitment of Rad9 to the proximity of Mec1 allows Mec1 phosphorylation of Rad9 which in turn recruits Rad53 (Emili, 1998; Schwartz et al., 2002; Sun et al., 1998; Vialard et al., 1998). Mec1 then phosphorylates Rad53 resulting in its activation (Sweeney et al., 2005). Once activated, Rad53 drifts away from the site of damage where it acts upon a whole host of factors depending on when in the cell cycle it is activated (for review see: Finn et al., 2012).

Recent evidence suggests that in addition to its roles in recruiting DSB repair proteins and initiating the DNA damage checkpoint, γ-H2AX is also a trigger for the SAC checkpoint (described in detail below). A DSB greater than 19kb away from the centromere results in H2A-S129 phosphorylation near the centromere which results in a Mad2-dependent cell-cycle arrest (Dotiwala et al., 2010). This collaborates findings by others that suggest that there is a good deal of cross-talk and synergistic interactions between the DNA damage and spindle assembly checkpoints (Clemenson and Marsolier-Kergoat, 2006; Garber and Rine, 2002; Kim and Burke, 2008).

I.6.b SAC checkpoint

Prior to the beginning of anaphase, the spindle assembly checkpoint (SAC) monitors chromosome alignment at the spindle equator. Chromosome segregation requires proper microtubule attachment to each chromosome centromere (CEN) which is mediated by large multi-protein complexes called kinetochores. The SAC is triggered by two situations: improper tension between sister chromatids and/or insufficient binding between kinetochores and microtubules. The number of microtubules that connect to a given kinetochore varies by species; in *S. cerevisiae*, each chromosomal kinetochore attaches to a single microtubule (Winey et al., 1995). Centromeres in *S. cerevisiae* are referred to as point centromeres as they are localized to a relatively small region consisting of approximately 125bp of DNA (Fitzgerald-Hayes et al., 1982). Centromeres are also comprised of centromeric nucleosomes that contain a centromere-specific histone H3 variant, referred to as Cse4 in budding yeast. In *S. cerevisiae* it has long been thought that one Cse4 containing nucleosome aligns perfectly with the centromeric-specific DNA sequence, however recent evidence suggests that there could be up to three Cse4 nucleosomes per kinetochore (Coffman et al., 2011; Cole

et al., 2011; Furuyama and Biggins, 2007; Henikoff and Henikoff, 2012; Lawrimore et al., 2011); this remains unresolved.

In addition to Cse4 nucleosomes, the kinetochore is comprised of at least 60 proteins that form approximately 9 discrete subcomplexes that function to monitor and mediate chromosome movement through conformational changes (Cheeseman and Desai, 2008; McAinsh et al., 2003; Santaguida and Musacchio, 2009). Figure I2 provides a schematic showing how a few of the better-understood kinetochore complexes assemble in relation to the centromere and the microtubule. The CBF3 complex binds directly with two binding domains that reside within the centromeric-specific DNA sequence (Cole et al., 2011). CBF3 components have also been shown to localize with the plus-ends of growing and shrinking microtubules following spindle disassembly which is presumed to assist in the reassociation of microtubules to kinetochores during the next cell division (Bouck and Bloom, 2005; Tanaka et al., 2005). The COMA complex is analogous to the CCAN complex in vertebrates, which has been shown to recruit the vertebrate Cse4 homolog, CENP-A, to the centromere and anchor kinetochores to CENP-A nucleosomes (Gascoigne and Cheeseman, 2011; Liu et al., 2003; Matson et al., 2012; Wan et al., 2009). The KMN complex is essential for both microtubule/centromere binding as well as SAC signaling. The KMN complex binds directly with the microtubule and that interaction is stabilized by the circular oligomerization of Dam1. Dam1 is required for kinetochore stability as it adds the necessary stability required for the level of tension caused by chromosomal movement (Tien et al., 2010).

Along the entire length of the chromosomes, sister chromatids are held together by the protein complex cohesin. Both cohesin and another complex called condensin are enriched in pericentric chromatin and together they function in compaction of pericentric chromatin as well as spindle length regulation (D'Ambrosio et al., 2008; Eckert et al., 2007; Ng et al., 2009; Stephens et al., 2011). During meiosis I, cohesin is degraded along the chromosome except at the pericentromere where it is protected by shugoshin (Sgo1) (Katis et al., 2004; Kerrebrock et al., 1992; Kitajima et al., 2004; Marston et al., 2004). Sgo1 is important for both sensing tension between sister chromatids as well as sister chromatid biorientation along the meiosis II spindle (Kiburz et al., 2008; Klein et al., 1999).

When mitosis is unperturbed, the anaphase-promoting complex (APC) and its co-activator Cdc20 trigger anaphase and chromosome segregation (Schwab et al., 1997; Visintin et al., 1997; Yu, 2007). APC, an E3 ubiqitin ligase, catalyzes the ubiquitination of Pds1 (securin in humans) resulting in the destruction of Pds1. The absence of Pds1 liberates Esp1 (separase in humans) allowing Esp1 to separate the sister chromatids by cleaving the cohesin complex.

Insufficient tension between sister chromatids at the kinetochore or lack of chromosome to microtubule binding activates the spindle assembly checkpoint which ultimately blocks the cell cycle by inhibiting APC and Cdc20 (for review see: Luo and Yu, 2012; Murray, 2011). Two critical complexes involved in the sensing of inappropriate kinetochore connections and SAC activation are the mitotic checkpoint complex, MCC, and the chromosomal passenger complex, CPC (also referred to as the Aurora kinase complex). In *S. cerevisiae* the MCC is composed of Cdc20, Mad2, Mad3, and Bub3 and functions to inhibit the APC. Mad1 and Mad2 form an initial complex at unattached kinetochores that binds a cytosolic Mad2, resulting in conformational changes of the cytosolic Mad2 allowing it to bind to Cdc20 (Kulukian et al., 2009; Shah et al., 2004). Experiments using a Mad2-Mad3 fusion protein indicate that Mad3 is also required for stable binding of Mad2 and Cdc20. Mad2-Cdc20 binding has been found to be sufficient for inhibition of APC activity (Lau and Murray, 2012).

The chromosomal passenger complex contains four members: Ipl1 (human: Aurora B), Sli15 (human: INCENP), Bir1 (human: Suvivin) and Nbl1 (human: Borealin). The genes encoding the four CPC members are all essential in *S. cerevisiae* as they all are required for the proper localization and/or function of the CPC. Ipl1 is the catalytic subunit while Sli15, Bir1, and Nbl1 function to regulate the complex's activity. An in vitro study demonstrates that a complex of Bir1 and Sli15 can directly link microtubules and centromeres thus it has been proposed that the CPC may regulate Ipl1 via tension-dependent changes in the complex (Sandall et al., 2006). In response to inadequate tension between sister chromatids, Ipl1 phosphorylates the kinetochore stabilizing protein Dam1allowing for the Ipl1 mediated release of aberrant kinetochore-microtubule attachments (Tien et al., 2010). This process is required for the proper biorientation of sister chromatids.

Along with Ipl1, there are two other highly conserved protein kinases, Mps1 and Bub1 that regulate SAC activity. In the absence of a microtubule-kinetochore attachment, phosphorylation of Spc105 by Mps1 recruits Bub1 to the kinetochore while proper microtubule attachment results in the phosphatase PP1 interaction with Spc105 that leads to Spc105 dephosphorylation and the release of Bub1 (London et al., 2012). In the presence of an improper microtubule attachment, Bub1 binds with Bub3, which targets and complexes with Mad1 (Musacchio and Salmon, 2007). In addition to the role Bub1 plays in the recruitment of the MCC complex, work in both human cells and fission yeast *S. pombe* has shown that H2A-S122 is a phosphorylation target of Bub1 and that phospho-H2A-S122 is required for the recruitment of shugoshin (Sgo1 in *S. cerevisiae*) to the pericentromere (Fernius and Hardwick, 2007; Kawashima et al., 2010; Yamagishi et al., 2010). As stated above, the CPC complex is also involved in the biorientation of sister chromatids; however, genetic evidence suggests that Bub1 and Sgo1 function independently of the CPC complex and that both pathways are critical for proper biorientation (Storchova et al., 2011). The mechanism for how Bub1 and Sgo1 modulate chromatin structure in terms of biorientation is yet unclear, however Haase et al. propose that the chromatin structure acts as a spring that can be mechanically amplified around the metaphase spindle and that the cell is able to fine tune the spring in response to mitotic spindle damage through the post-translational modification of H2A (Haase et al., 2012).

At the onset of anaphase, sister chromatids are separated as part of the normal cell cycle. The loss of tension caused by the cleavage of the cohesin complex in metaphase would instigate the SAC; however, in anaphase the loss of tension is detected by SAC surveillance yet the SAC is not triggered. There are three known mechanisms preventing the activation of the SAC in anaphase. First, the Cdc14 phosphatase inactivates the checkpoint through its dephosphorylation of Sli15 (Mirchenko and Uhlmann, 2010). Second, the beginning of anaphase initiates the movement of the aurora complex from the centromere to the spindle mid-zone, presumably preventing the aurora complex from reengaging the SAC (Vazquez-Novelle and Petronczki, 2010). And third, the phosphorylation of Bub1 at T566 results in Bub1 degradation in anaphase. Bub1 degradation is required for the adaptation of the spindle checkpoint to prolonged mitotic arrest (Goto et al., 2011).

I.6.c Mitochondrial Checkpoints

In *S. cerevisiae*, there are two known pathways for integrating mitochondrial status into cell cycle regulation. The first is a checkpoint that inhibits cytokinesis due to mitochondrial inheritance defects (Garcia-Rodriguez et al., 2009). Deletion of mitochore proteins or mutations that inhibit mitochondrial inheritance produce multi-budded cells as a result of defects in cytokinesis. Those defects are suppressed by hyperactivation of the mitotic exit network (MEN) which is the signaling pathway associated with spindle pole body that drives mitotic exit.

The second mitochondrial inheritance checkpoint is triggered by a complete loss of mitochondrial DNA. In the absence of a mitochondrial genome, cells are halted prior to S-phase (Crider et al., 2012). The G1-to-S-phase inhibition requires Rad53, presumably linking genome status to the cell cycle via the DNA damage checkpoint. It is interesting that this checkpoint is only triggered after the mitochondrial genome has been lost, leaving its purpose a bit of a mystery. It could be that the checkpoint halts the cell cycle prior to S-phase in the hopes of finding a mate that could supply an intact mitochondrial genome. Alternatively, this checkpoint may have evolved as a competitive advantage: cells containing mitochondrial genomes would further outcompete cells lacking mitochondrial genomes due to their delayed cell cycle. This would not give an advantage to individual cells per se; however, it would give an advantage to a given related population.

Previous work in our lab has shown that H2A-S122 is critical for a number of stress responses as converting this residue to an alanine, thus losing its ability to be phosphorylated, results in increased sensitivity to a broad range of DNA damaging agents, osmotic stress, heat stress, and ethanol exposure. H2A-S122 has previously been shown to be involved in the spindle assembly checkpoint in fission yeast and humans thus I used whole genome microarray data to verify increased rates of aneuploidy as an indication of a dysfunctional SAC in *S. cerevisiae* as well. During the course of our investigations of this residue, I also found that it is required for mitochondrial genome stability. While the loss of the mitochondrial loss does exacerbate some stress response phenotypes it is clear that the role H2A-S122 plays in some DNA damage responses is independent of both mitochondrial status as well as Bub1 mediated phosphorylation. The work described in this thesis

characterizes the importance of H2A-S122 in both nuclear and mitochondrial genome stability as described in the following Specific Aims.

I.7 Specific Aims

I.7.a Specific Aim 1: *To characterize H2A-S122-dependent mitochondrial genome loss.* Previous work in our lab indicates that the introduction of the H2A-S122A mutation results in a loss of mitochondrial function. PCR data indicates that the mitochondrial function loss is due to a loss of mitochondrial DNA. The loss of mitochondrial genome foci observed in the H2A-S122A mutant could be due to mitochondrial rearrangement or it could be due to a total loss of the mitochondrial genome. I used fluorescent microscopy to observe mitochondrial genome status. Additionally, I characterized the kinetics of mitochondrial genome loss using mitochondrial function as a readout for mitochondrial genome status.

I.7.b Specific Aim 2: *To characterize role(s) of H2A-S122 in cell-cycle regulation and to determine whether these roles are dependent on the kinase Bub1*. There is mounting evidence that phosphorylation of H2A-S122 is critical to the activation of the Spindle Assembly Checkpoint (SAC) when tension between sister chromatids is insufficient. Aneuploidy is a direct consequence of SAC defects, thus I used aneuploidy as a readout of SAC function in the H2A-S122A mutant. Of course, the role H2A-S122 (phosphorylation) likely plays in the tension sensing arm of the SAC does not preclude it from having other roles within the SAC or other checkpoints. In fact, the DNA repair kinases Mec1 and Tel1 have increased rates of aneuploidy in the absence of DNA damage, yet have a functioning SAC. For this experiment I used flow cytometry to observe cell cycle defects of two double mutant strains: one of which combines the H2A-S122A mutation with a *Δbub1* mutation, the other combines the H2A-S122A strain with the *bub1ΔK* mutation that lacks the *BUB1* kinase domain. I compared cell cycle characteristics of the double mutant strains to the individual single mutants to determine whether H2A-S122 functions in cell cycle regulation, and whether any role(s) are dependent on Bub1 kinase activity.

I.7.c Specific Aim 3: *To determine whether H2A-S122A DNA damage sensitivities are a consequence of mitochondrial genome loss*. The H2A-S122A mutant is sensitive to a variety

of DNA damaging agents, however the role of H2A-S122 in DNA damage has yet to be elucidated. Previous work has shown that mitochondrial dysfunction leads to certain types of genomic instability (Veatch et al., 2009), suggesting the possibility that H2A-S122A DNA damage phenotypes are simply downstream effects of mitochondrial genome instability. To determine what role, if any, mitochondrial genome loss has on the H2A-S122A DNA damage phenotypes, I used phenotypic plating assays to compare DNA damage sensitivities among a large number of individual H2A-S122A mutants that had varying degrees of mitochondrial genome loss.

I.8 Project Relevance

The impact of this research to a variety of health fields is significant, as both mitochondrial and nuclear genome instability have been implicated in a number of diseases. Both cell cycle and DNA repair defects within the nuclear genome lead to severe genomic instability, which can eventually result in cancer and death. Aneuploidy has a well-established presence in cancers, although there is still much debate as to whether aneuploidy is a cause of cancer, a downstream effect of cellular transformation, or both (Duesberg et al., 1998; Zimonjic et al., 2001). New research also suggests a role for aneuploidy in a number of age-related pathologies, including both normal and accelerated aging (Ricke and van Deursen, 2013). Meanwhile mitochondrial genome instability has been implicated in a number of diseases, including but not limited to: neurological disorders, muscular diseases, endrometriosis, heart disease, cancer, as well as the general aging processes (Baruffini et al., 2007; Fogg et al., 2011; Govatati et al., 2012; Olgun and Akman, 2007). This research may provide better understanding of the mechanisms involved maintaining both genomes.

Table I.1: H2A-S122 is highly conserved throughout eukaryotes. Sequence alignments of the C-terminal end of H2A show a high level of conservation in the globular domain (highlighted in purple), while the C-terminal tail (unhighlighted) is highly variable except for the conserved SQEY domain (red font) found in H2AX variants and the S/T-122 (highlighted in aqua) which is conserved in both the canonical H2A and H2AX.

S. cerevisiae	H2Aα	93	DELNKLLGNVTIAQGGVLPNIHQNLLPKKSAKATKASQEL
S. pombe	H2Aα	93	EELNKLLGHVTIAQGGVVPNINAHLLPKTSGRTGKPSQEL
H. sapiens	H2AX	92	EELNKLLGGVTIAQGGVLPNIQAVLLPKKTSATVGPKAPSGGKKATQASQEY
M. musculus	H2Ax	92	EELNKLLGGVTIAQGGVLPNIQAVLLPKKSSATVGPKAPAVGKKASQASQEY
X. laevis	H2Ax	92	EELNKLLGGVTIAQGGVLPNIQAVLLPKKSSGGVSTSGKKSSQQSQEY
D. melanogaster	H2A	92	EELNKLLSGVTIAQGGVLPNIQAVLLPKKTEKKA------
H. sapiens	H2A	92	EELNKLLGRVTIAQGGVLPNIQAVLLPKKTESHHKAKGK-
M. musculus	H2Atype1	92	EELNKLLGRVTIAQGGVLPNIQAVLLPKKTESHHKAKGK-
V. carteri	H2A	91	EELGKLLGDVTIASGGVLPNIHAVLLPKKSKGGKGEEAA-
G. gallus	H2A	92	EELNKLLGKVTIAQGGVLPNIQAVLLPKKTDS-HKAKAK-
O. mykiss	H2A	92	EELNKLLGGVTIAQGGVLPNIQAVLLPKKTEKAVKAK---
C. elegans	H2A	93	EELNKLLAGVTIAQGGVLPNIQAVLLPKKTGGDKE-----
T.thermophile	H2A	96	EELNKLMANTTIADGGVLPNINPMLLPSKTKKSTEPEH--
X. laevis	H2Atype1	92	EELNKLLGGVTIAQGGVLPNIQSVLLPKKTESAKSAKSK-
A. thaliana	H2A	93	EELSKLLGDVTIANGGVMPNIHNLLLPKKT--GASKPSAEDD

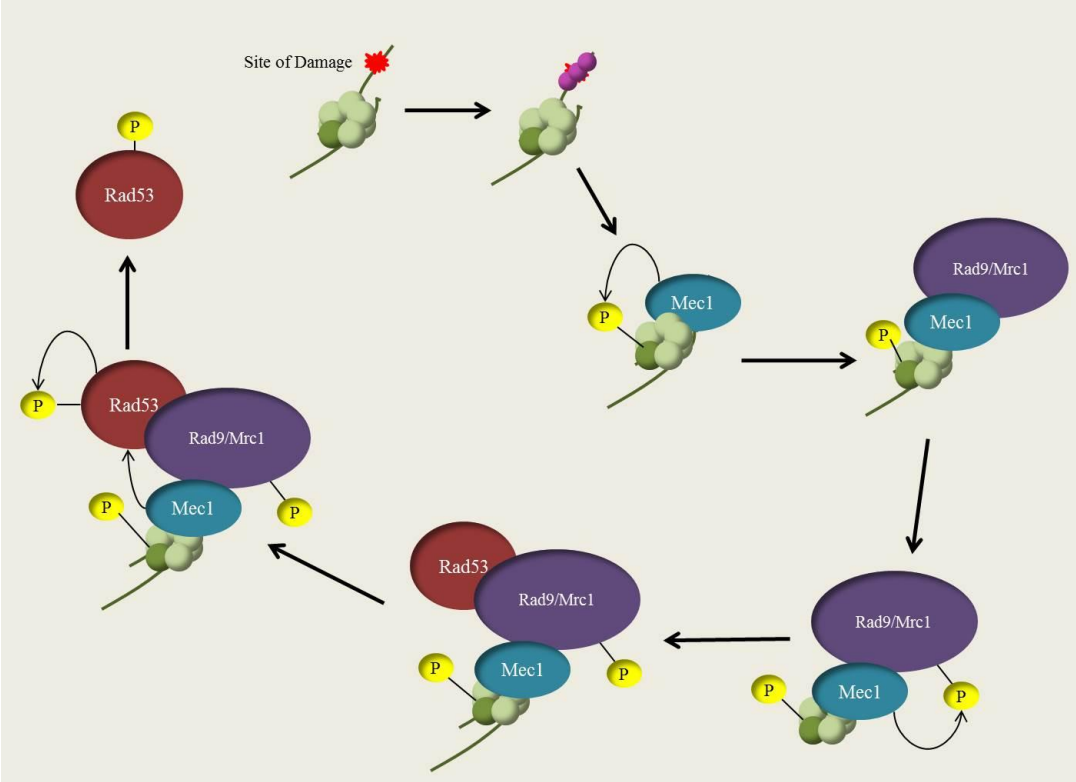

Figure I.1: Diagram of the DNA damage checkpoint. Mec1 is recruited to RPA (purple circles) coated single stranded DNA where it phosphorylates H2A-S129 (H2A is designated by dark green circles). This results in the recruitment of Rad9 or Mrc1 depending on whether the single stranded DNA is caused by DNA repair or a stalled replication fork. Mec1 then phosphorylates Rad9/Mrc1 which results in Rad53 recruitment. Activation of Rad53 is achieved through a combination of Mec1 phosphorylation in addition to self-phosphorylation. Activated Rad53 is then free to drift away from the site of damage.

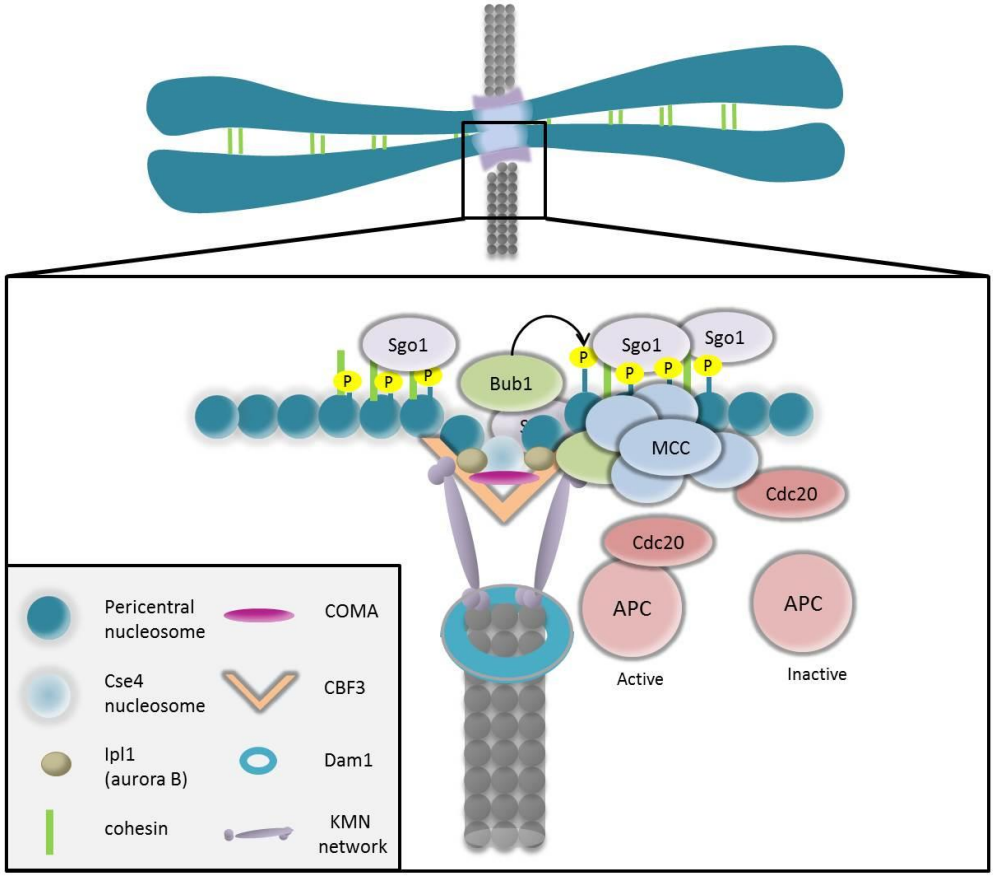

Figure I.2: H2A-S122 Phosphorylation is critical for SAC function. The large kinetochore complex physically links the microtubule and centromere. The kinetochore associated proteins are generally categorized into those that bind the microtubule (Dam1), those that bind the centromere (COMA and CBF3) and those that link the microtubule (KMN). The MCC, APC, and the Ipl1 containing CPC complex monitor for inappropriate kinetochore connections and/or insufficient tension at the kinetochore. Although the mechanisms are not entirely clear, Bub1 dependent phosphorylation of H2A-S122 is required for the recruitment of Sgo1 to the kinetochore and SAC activation.

CHAPTER 1:

H2A-S122 is Required for Nuclear and Mitochondrial Genome Stability in *Saccharomyces cerevisiae*[1]

1.1 ABSTRACT

Organization and maintenance of the mitochondrial and nuclear genomes are vastly different, yet we have shown that a single serine in the H2A C-terminal tail (H2A-S122) is critical for stability of both genomes in the budding yeast, *Saccharomyces cerevisiae*. H2A-S122 has previously been implicated in the spindle assembly checkpoint (SAC), however we show that by mutating the serine to an alanine (H2A-S122A) the resulting aneuploidy occurs at a much higher rate than is observed by deleting its immediate downstream kinase *BUB1*. Furthermore, the H2A-S122A mutant displays an increased susceptibility to DNA damaging agents than is not observed in *bub1Δ* deletion cells. Our studies also implicate H2A-S122 as critical to the maintenance of the mitochondrial genome as upon introduction of the H2A-S122A mutation, cells rapidly lose their mitochondrial genome.

1.2 INTRODUCTION

Post-translational modification of specific histone tails plays a role in the regulation of a number of cellular processes and is critical for maintaining genomic stability. In *Saccharomyces cerevisiae*, the C-terminal tail of histone H2AX holds two serine residues (S122 and S129) that are phosphorylated in response to DNA damage and are critical for genomic stability (Downs et al., 2000; Harvey et al., 2005; Moore et al., 2007). Phosphorylation of H2A-S129 by the kinase Mec1, analogous to the phosphorylation of H2AX-S139 by ATM in humans (γ-H2AX), results in the recruitment of chromatin remodeling enzymes and other repair proteins that either assist in the repair process itself or alter cell cycle progression through DNA damage checkpoint (Downs et al., 2004; Morrison et al., 2004; van Attikum et al., 2004).

The role of H2A-S122 in genomic stability is much less understood. Phosphorylation of the equivalent residue in *Drosophila melanogaster* (H2A-T119) is regulated both temporally and spatially by mitotic kinases (Aihara et al., 2004; Brittle et al.,

[1] Uffenbeck S.R., and Krebs J.E. in preparation for submission to *G3: Genes, Genomes, Genetics*

2007). H2A-T119 is phosphorylated throughout chromatin during interphase but becomes localized only at centromeres between prometaphase and anaphase (Brittle et al., 2007). Work in *Schizosaccharomyces pombe* has demonstrated that centromeric phosphorylation of H2A-S122 is mediated by the spindle assembly checkpoint (SAC) kinase Bub1, and localization of shugoshin proteins to the kinetochore is dependent on that phosphorylation (Kawashima et al., 2010; Yamagishi et al., 2010). The SAC is crucial for the proper segregation of chromosomes as it delays anaphase initiation until it has detected both a microtubule-to-kinetochore connection as well as sufficient tension between sister chromatids (Biggins and Murray, 2001; Rieder et al., 1994) (for review see: Clarke and Bachant, 2008).

S. cerevisiae cells in which the H2A-S122 residue has been replaced with an alanine (H2A-S122A) are highly susceptible to a wide range of DNA damaging agents (Harvey et al., 2005; Moore et al., 2007). Our lab has previously demonstrated an increase in H2A-S122 phosphorylation following DNA damage in *S. cerevisiae* (Moore et al., 2007) while work done in HeLa cells has shown that irradiation-induced phosphorylation of H2A-T121 is Bub1 dependent (Yang et al., 2012). This work is consistent with the hypothesis that H2A-T119 phosphorylation detected in *Drosophila* during interphase is in response to DNA damage.

In this study, we report that H2A-S122 is critical for both nuclear and mitochondrial genome stability. We show that H2A-S122A mutant cells have a much higher rate of aneuploidy than *BUB1* deletion cells and that the DNA damage phenotypes observed in H2A-S122A mutant cells are independent of Bub1. Furthermore, we show that the introduction of the H2A-S122A mutation results in a rapid loss of the mitochondrial genome. Our findings demonstrate that H2A-S122 is required for both mitochondrial and nuclear genome stability.

1.3 MATERIALS AND METHODS

1.3.a Strains and Plasmids

Saccharomyces cerevisiae strains and plasmids utilized in this study are listed in Supplemental Table 1. The wildtype and H2A-S122A strains used were created by transforming either FY406 or JKY98 with JKP18 or JKP22 respectively. Successful transformation was verified by selection on HIS dropout media followed by selection against

pSAB6 using 5-FOA. Numbering of amino acids in H2A follows the convention of counting the initial methionine as position 1.

Wildtype, H2A-S122A, and H2A-S122A *bub1Δ* clones used for the mitochondrial function, DNA damage assays, and CGH assays were recreated via plasmid shuffle for each individual assay. The *bub1Δ* strain was made and immediately stored as a glycerol stock, and a fresh culture was obtained from the original -80°C stock for all experiments. For all microscopy and flow cytometry analysis, wildtype and H2A-S122A cultures were grown from -80°C stocks of cells that had been obtained immediately following 5-FOA selection.

1.3.b Mitochondrial Function Assays

Twelve clones (colonies) from each strain were randomly selected from 5-FOA and grown in liquid YPD overnight such that they would be at log phase the next day. The log phase liquid culture was then diluted and an equal amount of liquid culture was plated onto YPD (glucose) and YPG (glycerol) plates for determining the glycerol/glucose ratio for colony passage one. After 72 hours of growth, one colony from the YPD plate was randomly chosen for the next colony passage. The clones were taken through six colony passaging events.

1.3.c Microscopy

Indicated strains were transformed with the pVT100U-mtGFP plasmid constitutively expressing a mitochondria-targeted GFP protein (Westermann, 2000). Cells were cultured in SD –Ura media (to maintain the plasmids) until mid-log phase when they were collected and fixed using ethanol. Following fixation, they were incubated with .1µg/ml of DAPI for 5 minutes. Cells were then washed and resuspended in H_2O. Three-microliters of mid-log phase cultures were placed on poly-Lysine coated microscope slides and covered with a coverslip. Bright field and fluorescent microscopy was done using a Leica microscope equipped with a 100X oil-immersion objective and operated by the FW-4000 program. All images were taken with a mono-color CCD camera. The fluorescent images were processed by the deconvolution algorithm of the program to minimize the effects of optical distortion.

1.3.d DNA Damage Assays

Logarithmically growing cells were diluted to an OD_{600} of 0.2 and 5µl of ten-fold serial dilutions were plated onto YPD agar, YPG agar, or YPD agar containing 0.02% MMS,

3μg/mL phleomycin, or 200μM hydroxyurea. Pictures were taken at approximately 72 hours except for YPG plates which were photographed at approximately 144 hours.

1.3.e Comparative Genome Hybridization

Twelve clones from each strain were passaged six times as above and stored at -80°C. Cells taken from freezer stocks were plated on YPD and an isolated colony chosen at random from each clones was used to inoculate a 10mL liquid culture and allowed to grow overnight. DNA was isolated from the overnight culture. Purified DNA was sent to Roche Nimblegen (Reykjavik, Iceland) (Westermann and Neupert, 2000) where the samples were hybridized and analyzed. Hybridizations were performed on the CGH 385k array platform with a 45-75bp probe size and a 12-bp median spacing (catalog no. B2436001).

1.3.f Flow Cytometry

Cells grown to early log phase were treated with 10mg/ml of α-factor incubated at 30°C for two hours. Cells were then washed and resuspended in either YPD or YPD plus treatment. Collected cells were immediately fixed using 70% EtOH overnight. Cells were then washed with 1X PBS and treated with RNase (2mg/mL in 50mM Tris pH 8.0) for 4 hours at 37°C. Cells were then pelleted and treated with pepsin (5mg/ml in H_2O with 4.5μl of concentrated HCl per mL of H_2O) for 20 minutes at 37°C. Following pepsin treatment cells were pelleted and resuspended in 50mM Tris pH 7.5 and sonicated. Cells were then stained with 0.1 mM SYTOX Green. Cells were analyzed using a Beckman Coulter flow cytometer.

1.4 RESULTS

1.4.a H2A-S122 is required for nuclear genome stability

Work in *S. pombe* has shown that H2A-S122 is a phosphorylation target for Bub1 and the phosphorylation of S122 is required for activation of the spindle assembly checkpoint (SAC) (Kawashima et al., 2010). Cells lacking a functional SAC continue through mitosis regardless of whether chromosomes have properly attached to the spindle and are thus much more likely to have aneuploid chromosomes. We wished to determine whether mutation of H2A-S122 results in aneuploidy in *S. cerevisiae,* consistent with a SAC defect. We used Comparative Genome Hybridization (CGH) to detect aneuploidy events in *S. cerevisiae*.

To compare aneuploidy frequencies between H2A-S122A clones and isogenic *bub1Δ* clones, 12 clones of each strain were passaged 7 times and assayed for aneuploidy using CGH. The aneuploidy frequency was compared to prior work that characterized the aneuploidy rate of diploid *bub1Δ* cells (Table 1; (McCulley and Petes, 2010). Surprisingly, after six colony passages following the introduction of the mutations, only one *bub1Δ* clone displayed an aneuploid chromosome, while all but one H2A-S122A clones had at least one instance of aneuploidy, with the majority of clones containing multiple aneuploid chromosomes (Supplemental Table 1). Clearly the H2A-S122A mutant cells begin to accumulate aneuploid chromosomes much more rapidly than *bub1Δ* cells following introduction of the mutation (Table 1 and Sup Table 2). Furthermore, although diploid cells can sustain a greater number of survivable aneuploidy events than haploid cells, the haploid H2A-S122A clones have a higher aneuploid/euploid ratio than diploid *bub1Δ* clones even when the *bub1Δ* diploid cells had started with more aneuploidy (diploid data from (McCulley and Petes, 2010)). This data demonstrates an increased rate of aneuploidy in H2A-S122A mutant cells compared to *bub1Δ* cells, which adds to the accumulating evidence for involvement of the H2A-S122 residue in the spindle assembly checkpoint in *S. cerevisiae*.

1.4.b H2A-S122 is critical for mitochondrial function

S. cerevisiae is unique in its ability to function without a mitochondrial genome. Yeast colonies comprised of cells lacking functional mitochondria are usually smaller than their functional counterparts and are thus referred to as "petites." There are a number of nuclear- and mitochondria-encoded genes that are required to maintain mitochondrial genome stability, thus it is not surprising that as much as 10% of a wildtype cell population may be phenotypically petite (Contamine and Picard, 2000). Mitochondrial genome status is described as rho^{+} (cells contain a functional mitochondrial genome), rho^{-} (cells contain a mitochondrial genome that lacks function), or rho^{0} (cells completely lack a mitochondrial genome). For the purposes of this paper we use the designation $rho^{\blacktriangleright}$ to indicate cells that are in the early stages of progressive mitochondrial genome loss, described further below.

Colonies comprised of cells with the H2A-S122A mutation display a normal variance in size, although the average colony size is somewhat smaller than wildtype in the same background. Thus we were surprised to discover that these cells lack mitochondria-encoded gene transcripts when we performed a transcriptional microarray (data not shown). We

therefore sought to determine whether H2A-S122A cells were competent for respiration, an indicator of mitochondrial function.

We assayed mitochondrial function by plating the same number of cells onto glucose (a fermentable carbon source) and glycerol (a non-fermentable carbon source which requires an intact respiratory chain for utilization). The ratio of the number of colonies that grow on glycerol to the number of colonies growing on glucose provides a direct readout of mitochondrial function. We measured mitochondrial function over time in cells in which wildtype H2A or H2A carrying the S122A mutation was shuffled into either wildtype or *bub1Δ* cells. At each colony passage, one colony was chosen at random from the cells growing on glucose and then assayed for mitochondrial function as described in Materials and Methods. Clones carrying the S122A mutation rapidly lose mitochondrial function such that within six colony passages mitochondrial function is almost obliterated, with most clones showing zero growth on glycerol (Figure 1A). Wildtype cells exhibit some loss in mitochondrial function following the first passage as well which is likely an effect of the plasmid shuffle and 5-FOA selection. However; the rate of mitochondrial function loss in wildtype cells is slight over the remainder of the experiment. The kinetics of mitochondrial function loss is also strikingly different for wildtype and H2A-S122A cells. Wildtype clones that lose mitochondrial function exhibit a swift decline; going from normal function to no function within one passage, indicating a spontaneous event that results in loss of respiratory capability, consistent with typical petite formation. The kinetics of mitochondrial function loss in the H2A-S122A mutant clones are markedly different. While some clones lose mitochondrial function abruptly, most clones display a gradual decrease in function over the six passages (Figure 1B). Given time, all the H2A-S122A clones eventually demonstrate a complete loss of respiratory capacity.

Since H2A-S122 is a phosphorylation target for Bub1, we also looked at mitochondrial function in *bub1Δ* clones in addition to double mutant clones carrying both the H2A-S122A mutation and *BUB1*deletion (*bub1Δ*H2A-S122A). The *bub1Δ* clones consistently maintain a higher level of mitochondrial function than wildtype cells, while mitochondrial function loss in the double mutant is initially more severe than what is observed in the H2A-S122A mutation alone. However, the rate of loss over the course of the

experiment is less than in H2A-S122A cells (Figure 1A). These data indicate that the role for H2A-S122 in maintaining mitochondrial function does not require Bub1.

1.4.c Mitochondrial function loss in the H2A-S122A mutant is due to loss of the mitochondrial genome

Preliminary whole genome expression analysis of the H2A-S122A mutant revealed a complete lack of mitochondrial-encoded transcripts (data not shown). We used both quantitative polymerase chain reaction (qPCR) and fluorescence microscopy to examine mitochondrial genome status. Both methods indicate that the H2A-S122A mutation leads to the complete loss of the mitochondrial genome (PCR data not shown and Figure 2). DAPI-stained mitochondrial DNA foci are easily visualized in wildtype, *bub1Δ,* and H2A-S122A cells that recently acquired the H2A-S122A mutation (H2A-S122A rho^{+}). However, we are unable to detect mitochondrial DNA in H2A-S122A clones that have had the mutation over many generations (H2A-S122A rho^{0}) (Figure 2).

We also visualized mitochondrial structure using Green Fluorescent Protein (GFP) tagged with a mitochondrial matrix localization sequence (Westermann and Neupert, 2000). Wildtype cells transformed with the mt-GFP plasmid display normal filamentous mitochondria, while cells carrying the H2A-S122A mutation, even those newly acquired, display a slightly more punctate mitochondrial morphology (Figure 2).

1.4.d H2A-S122A cells do not exhibit increased mitochondrial point mutation rates or misexpression of nuclear-encoded genes involved in mitochondrial stability

There are a number of nuclear encoded genes that are required for mitochondrial function and genome stability (Contamine and Picard, 2000). We used whole genome expression analysis to look for altered transcription of nuclear encoded genes in log-phase cells immediately following the introduction of the H2A-S122A mutation. Of 1004 nuclear-encoded genes associated with mitochondrial localization, only one transcript, the T subunit of the mitochondrial glycine decarboxylase complex (*GCV1*), showed a greater than 2-fold change in gene expression and only 53 H2A-S122A transcripts had a 1.4-fold or greater increase or decrease from wildtype (Supplemental Table 3). This surprisingly low level of altered transcription of known mitochondrial effectors suggests that the mitochondrial instability in H2A-S122 mutant cells is not due to transcriptional defects.

One potential cause for mitochondrial genome instability is increased rates of mtDNA point mutagenesis, for example due to increased reactive oxygen species (ROS) resulting from inefficient respiration. A direct method of detecting point mutation rates in mitochondrial DNA is by assaying for erythromycin resistance (Chi and Kolodner, 1994). Erythromycin resistance is acquired through specific point mutations of either of the two mitochondrial *rib2* or *rib3* loci (Cui and Mason, 1989). We therefore assayed for erythromycin resistance and compared the H2A-S122A mutant to both wildtype cells and cells carrying a deletion for the helicase *Δpif1*, which is involved in the repair and recombination of mitochondrial DNA and is known to exhibit a high frequency of erythromycin resistance (Table 2; (O'Rourke et al., 2002). The frequency of erythromycin resistance in H2A-S122A mutant cells does not differ from the frequency observed in wildtype cells, while the *Δpif1* mutant cells display a 63-fold increase in frequency compared to wildtype cells. Mitochondrial genome loss exhibited by H2A-S122A mutant cells is thus not due to an increased point mutation rate in the mitochondrial DNA.

1.4.e H2A-S122 functions in DNA damage responses

Our lab and others have shown that the H2A-S122A mutant is sensitive to a wide variety of DNA damaging agents (Harvey et al., 2005; Moore et al., 2007; Wyatt et al., 2003). It is unclear whether the H2A-S122A DNA damage phenotypes are direct effects of the mutation or are downstream effects of aneuploidy, mitochondrial dysfunction, or both. We used semi-quantitative phenotypic analysis to determine DNA damage sensitivity in cells that had recently acquired the H2A-S122A mutation (H2A-S122A rho$^{\blacktriangleright}$) in order to address these questions.

It is clear that aneuploid chromosomes affect DNA repair pathways. However, aneuploidy can either increase or decrease the severity of the damage phenotype depending on which chromosomes are aneuploid. The most likely explanation for the different outcomes of aneuploidy are protein dosage effects due to stoichiometric alterations in protein levels (Sheltzer et al., 2011). By using cells immediately following their acquisition of the H2A-S122A mutation, we can reduce the influence of aneuploid chromosomes. However, since aneuploidy events occur rapidly in H2A-S122A cells, we also elected to test a large number of freshly acquired H2A-S122A clones so that variations in the phenotype due to specific aneuploidy events are only occasionally represented in the collection of clones.

Thus, the vast majority of clones present phenotypes similar to the representative clones shown in Figure 3.

Mitochondrial dysfunction has been shown to increase nuclear genome instability due to defective iron-sulfur cluster biogenesis (Veatch et al., 2009). If the H2A-S122A DNA damage phenotypes are, in fact due to decreased mitochondrial function, we would expect wildtype cells lacking mitochondrial function to display similar DNA damage phenotypes to that of the H2A-S122A mutant cells. Mitochondrial genomes are highly susceptible to treatment with ethidium bromide (Goldring et al., 1970); therefore we treated both wildtype and H2A-S122A cells with ethidium bromide and monitored the resulting loss of mitochondrial function by the lack of growth on glycerol. Our results indicate that mitochondrial dysfunction does not affect MMS or hydroxyurea phenotypes in wildtype cells (wildtype vs. wildtype EtBr) suggesting that MMS and hydroxyurea phenotypes are not significantly affected by mitochondrial dysfunction (Figure 3). Treatment with ethidium bromide does, however, exacerbate MMS phenotypes in H2A-S122A rho$^{\blacktriangleright}$ cells, most likely due to the increased opportunity for aneuploidy or other nuclear genome perturbations. The protocol for disrupting mitochondrial genome stability with ethidium bromide calls for at least three additional culture or colony passages following the introduction of the H2A-S122A mutation, resulting in an increased opportunity for aneuploidy development.

Both wildtype and H2A-S122A rho$^{\blacktriangleright}$ cells display an equivalent increase in sensitivity to the double strand break inducing agent phleomycin following treatment with ethidium bromide. This suggests that the H2A-S122A phleomycin sensitivity is, at least in part, due to decreased mitochondrial function (Figure 3). Due to the rapid decrease in mitochondrial function of the H2A-S122A rho$^{\blacktriangleright}$ cells, it is impossible to completely rule out the possibility of mitochondrial dysfunction being the primary cause of the phleomycin phenotype. However, the strong growth of the H2A-S122A rho$^{\blacktriangleright}$ cells on glycerol suggests that at least some of the displayed phleomycin phenotype is independent of mitochondrial function.

In human HeLa cells, Bub1 siRNA knock-down results in a reduced DNA damage response as well hyper-radiosensitivity. Furthermore, irradiation induces Bub1-dependent phosphorylation of the human H2A-S122 equivalent (H2A-T121). (Yang et al., 2012). Thus we were surprised that *bub1Δ* cells were no more sensitive to DNA damaging agents than

wildtype cells. Combining the *BUB1* deletion with the H2A-S122A mutation (*bub1Δ*H2A-S122A rho$^{▶}$) did not significantly exacerbate or suppress any of the DNA damage phenotypes. Although the double mutant does display an increased sensitivity to phleomycin compared to the H2A-S122A mutation alone, the decreased growth on glycerol indicates that the increased sensitivity is most likely due to decreased mitochondrial function (Figure 3). Together these results suggest that the sensitivity exhibited by the H2A-S122A mutant cells to DNA damage is independent of Bub1.

1.4.f The H2A-S122A mutant does not display DNA damage checkpoint dysfunction

One potential role for H2A-S122 in DNA damage responses is through DNA damage-induced cell cycle checkpoints. Downs et al. have previously shown that the H2A-S122A mutant strain lacks any obvious cell cycle defects under normal conditions (Downs et al., 2000); however, checkpoint function was not explicitly explored. To investigate the potential role of H2A-S122 in DNA damage checkpoints, H2A-S122A mutant cell cultures were synchronized using alpha-factor and exposed to hydroxyurea, menadione, and MMS, and then analyzed using flow cytometry. Following alpha-factor release, untreated cells begin to move through the cell cycle for all strains analyzed (Figure 4). Both H2A-S122A and the *bub1Δ* mutant cells exposed to DNA damaging agents fail to progress into S-phase, indicating that both mutant strains are capable of activating the DNA damage checkpoint. Furthermore, the H2A-S122A mutants as well as the *bub1Δ* cells were beginning to progress through the cell cycle 60 minutes after removal of the DNA damaging agents in a manner similar to that observed in wildtype cells. Our results indicate that the DNA damage phenotypes exhibited by H2A-S122A mutant cells are not due to altered DNA checkpoint dynamics.

While exploring the potential role for H2A-S122 in DNA damage checkpoints we also attempted to investigate the potential role for H2A-S122 in the mitochondrial inheritance checkpoint. Recent work by Crider et al. demonstrates that mitochondrial genome loss triggers a Rad53-dependent G1 to S phase checkpoint in *S. cerevisiae* (Crider et al., 2012). Remarkably, this mitochondrial DNA inheritance checkpoint is only triggered upon the loss of DNA, rather than the loss of particular mitochondria-encoded genes or loss of mitochondrial function. Because our data did not show any obvious transcriptional defects in

the H2A-S122A mutant that would account for the loss of mitochondrial genome stability, we thought it possible that H2A-S122 may have a role in this checkpoint.

Wildtype, H2A-S122A, and *bub1Δ* cells were treated with ethidium bromide and mitochondrial DNA loss was verified using fluorescent microscopy as was done by Crider et al. Their data indicated that approximately 50% of cells lacking mitochondrial DNA fail to progress into S phase from G1 and those that do progress do so at a slower pace. However, we are unable to reproduce the inheritance checkpoint in wildtype cells and are thus unable to determine whether H2A-S122 functions within this checkpoint (Figure 5). Crider et al. observed the mitochondrial inheritance checkpoint in multiple strain backgrounds including S288C, which is isogenic to the strains used in our work. Therefore we speculated that the checkpoint could be affected by histone dosage effects, as H2A and H2B are being expressed from a single-copy plasmid in our experimental strains, compared to the usual two chromosomal loci. We attempted to trigger the mitochondrial inheritance checkpoint in another S288C wildtype (JKY19); however we were again unable to reproduce the checkpoint observed by Crider et al. (Figure 5). As we followed their protocol exactly as described, we are unable to determine why we cannot reproduce the checkpoint in our laboratory. Since we were unable to observe the mitochondrial checkpoint in wildtype strains, we are unable to determine whether H2A-S122 functions within the checkpoint.

1.5 DISCUSSION

1.5a H2A-S122 and nuclear genome stability

Our data indicate that cells containing the H2A-S122A mutation have a much higher frequency of aneuploidy than isogenic *bub1Δ* cells. Using CGH, McCulley and Petes have previously characterized the aneuploidy frequency for diploid clones lacking *BUB1* (*bub1Δ*) (McCulley and Petes, 2010). However, there are two fundamental differences between these studies that should be noted. The first is that the strains used by McCulley and Petes are diploid, while our strains are haploid. There is a lack of published data directly comparing aneuploidy frequency between diploid and haploid cells; however it is clear that ploidy does alter the mechanisms involved in surviving aneuploidy (Jung et al., 2011). The second major difference is that we introduced the H2A-S122A mutation and *BUB1* deletion immediately prior to the first colony passage (CP) as described in the Materials and Methods. Therefore

CP 0 clones are less likely to have aneuploid chromosomes. In contrast, all 20 of the *bub1Δ* CP 0 clones used by McCulley and Petes already had multiple aneuploid chromosomes as the strains used in their experiment were previously passaged prior to the experiment (Table 1). Given the differences in ploidy and starting aneuploidy frequency, it is particularly striking that the haploid H2A-S122A clones in this study exhibit higher aneuploidy rates than the diploid *bub1Δ* clones in McCulley and Pete's study.

There are two possible explanations for the higher aneuploidy rate in H2A-S122A cells. One is that there is partial redundancy in Bub1 function within the context of the SAC. The simplest explanation of this would be kinase promiscuity for H2A-S122. Given the lack of DNA damage phenotype or mitochondrial genome loss in *bub1Δ* cells, and the fact that H2A-S122 is phosphorylated in response to some DNA damage (Moore et al., 2007), H2A-S122 is likely a substrate for a kinase(s) other than Bub1. Consistent with this hypothesis is the observed temporal and spatial regulation of H2A-T119 phosphorylation in *Drosophila* (Brittle et al., 2007). The H2A-S119 phosphorylation state is regulated by at least four different mitotic kinases (Aurora B, Cdc2, Cyclin B, and NHK-1), though it remains unclear whether any of these kinases directly target H2A-T119.

A second explanation is that the H2A-S122A mutation results in increased chromosome instability (CIN) via a pathway independent of the SAC. While this work does not directly address the frequency of non-SAC-dependent mechanisms of CIN, the increased sensitivity to DNA damaging agents that result in stalled replication forks of the H2A-S122A mutant suggest that higher levels of other chromosomal aberrations may be expected in this mutant. Our CGH analysis did not reveal significant segmental aneuploidies (gain or loss of large chromosome fragments rather than whole chromosomes), but the possibilities of translocations or increased smaller-scale mutations remain for future investigation.

A number of aneuploidy studies in yeast have demonstrated that certain chromosomes are more prone to aneuploidy depending on the genetic background of the cells (McCulley and Petes, 2010; Sheltzer et al., 2011; Spector and Fogel, 1992; Vernon et al., 2008; Zhu et al., 2012). Ploidy clearly influences whether particular chromosomal losses or gains can be tolerated (Zhu et al., 2012), thus it is important to note that the parental strain in which our wildtype, H2A-S122A, and *bub1Δ* strains were derived appears to have a duplication of chromosome III. The ramifications of a chromosome III duplication are unknown; however

no obvious phenotypes were observed in wildtype cells carrying the duplicated chromosome compared to a euploid wildtype of the same background, such as BY4741.

1.5.b H2A-S122 in mitochondrial genome stability

H2A-S122 is clearly critical for mitochondrial genome stability; however the mechanism behind its role remains elusive. Given the role H2A-S122 phosphorylation plays within the cell cycle during the activation of the spindle assembly checkpoint, it is not unreasonable to predict that it may also function to relay mitochondrial genome status to the cell cycle. To date, the mitochondrial inheritance checkpoint is the only known instance of mitochondrial genome status directly triggering a cell cycle checkpoint in *S. cerevisiae*; and while it has long been suspected that such a mitochondrial genome to cell cycle communication might exist, the mitochondrial inheritance checkpoint is somewhat surprising in that it results in a cell cycle delay only *after* the mitochondrial genome has been lost. This may lead to a selective advantage for rho^{+} cells as those with intact mitochondrial genomes will outcompete those cells that have lost their mitochondrial genome. An alternative (but not mutually exclusive) model is that the checkpoint pauses the cell cycle at the same point in which mating occurs, so that the cell has the opportunity to mate with a rho^{+} cell and thus restore its mitochondrial function. However, for a common lab strain that exists in a homogenic culture this checkpoint would be too little too late; we could certainly test this model in a mating assay in a mixed culture or using a mating type switching-competent strain. Clearly the mitochondrial genome instability exhibited by the H2A-S122A mutant is independent of the proposed mitochondrial inheritance checkpoint; however, further examination is needed to rule out a role for H2A-S122 in the checkpoint. Furthermore, as can be seen in both Figure 4A and 4B, the H2A-S122A rho$^{\blacktriangleright}$ cells often show a subtle accumulation of cells in S-phase that is not observed in wildtype or H2A-S122A rho^{0} cells. This may support a proposed role for H2A-S122 in cell cycle regulation during mitochondrial genome instability. Unfortunately, we are not yet able to rule out the possibility of this phenotype being a result of specific aneuploidy events.

The role, if any, which increased aneuploidy frequency in the H2A-S122A mutant has on its loss of mitochondrial genome stability, was not directly addressed in this study. All 12 clones analyzed for aneuploidy through CGH had lost mitochondrial function as ascertained by growth on glycerol, however only one clone had a normal karyotype (Supplemental Table

2); therefore we were unable to draw statistically significant conclusions from this data. Nonetheless, the rapid decline in mitochondrial function in H2A-S122A mutants (Figure 1) suggests that the mitochondrial genome loss is primarily independent of aneuploidy.

Our data clearly indicate that the role H2A-S122 plays in mitochondrial genome stability does not require its one known kinase Bub1, as *bub1Δ* cells do not display mitochondrial genome loss over the duration of our experiments (Figure 1). It is important to note that this does not rule out a role for Bub1 in the maintenance of mitochondrial function as it could share functional homology with another protein, enabling mitochondrial function even in the absence of Bub1. Interestingly, *bub1Δ* cells in our strain background have a low to nonexistent frequency of "petite" colonies, which represent the classic appearance of rho^- cells (data not shown). This, in conjunction with the observed increase in mitochondrial function in the *bub1Δ* cells (Figure 1), has led us to hypothesize that mitochondrial genome loss is synthetically lethal in *bub1Δ* cells, with the few petite colonies that exist arising due to suppressor mutations. This synthetic lethality appears to be isolated to our background as *bub1Δ* cells in the BY4741 background have normal petite frequencies; although given the high potential for aneuploidy it is difficult to know whether the phenotype is restricted to our background. More investigation is needed to determine what role, if any, Bub1 plays in the maintenance of mitochondrial function.

Communication between the mitochondria and nucleus is critical for proper cellular function. In addition to passing on transcriptional directives for more than 1000 nuclear-encoded mitochondria-localized proteins, mitochondrial genome status, organization and replication is coordinated with cell division. This work suggests that in addition to its role in the nuclear genome within the spindle assembly checkpoint, H2A-S122 also plays a critical role in the nucleus to mitochondrial communication network.

1.6 Acknowledgements

We would like to thank Oya Yazgan and Jason Bell for their assistance with microscopy work. Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103395. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

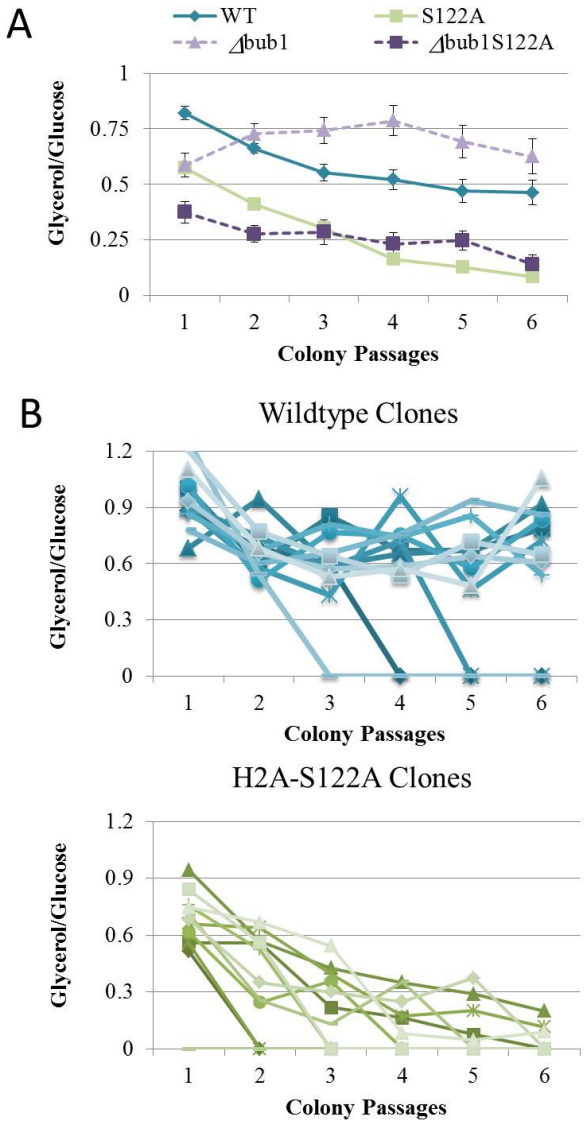

Figure 1: H2A-S122 is critical for mitochondrial function. (A) Plasmids containing either wildtype H2A-S122 or H2A-S122A were shuffled into either wildtype or *bub1Δ* cells. At each passage one colony was chosen at random from a glucose plate and analyzed for its mitochondrial function as described in Materials and Methods. At least 36 clones of each strain were passaged and analyzed for mitochondrial function. (B and C) Twelve individual clones were passaged and analyzed for mitochondrial function in Wildtype (B) and H2A-S122A (C) cells over six passages.

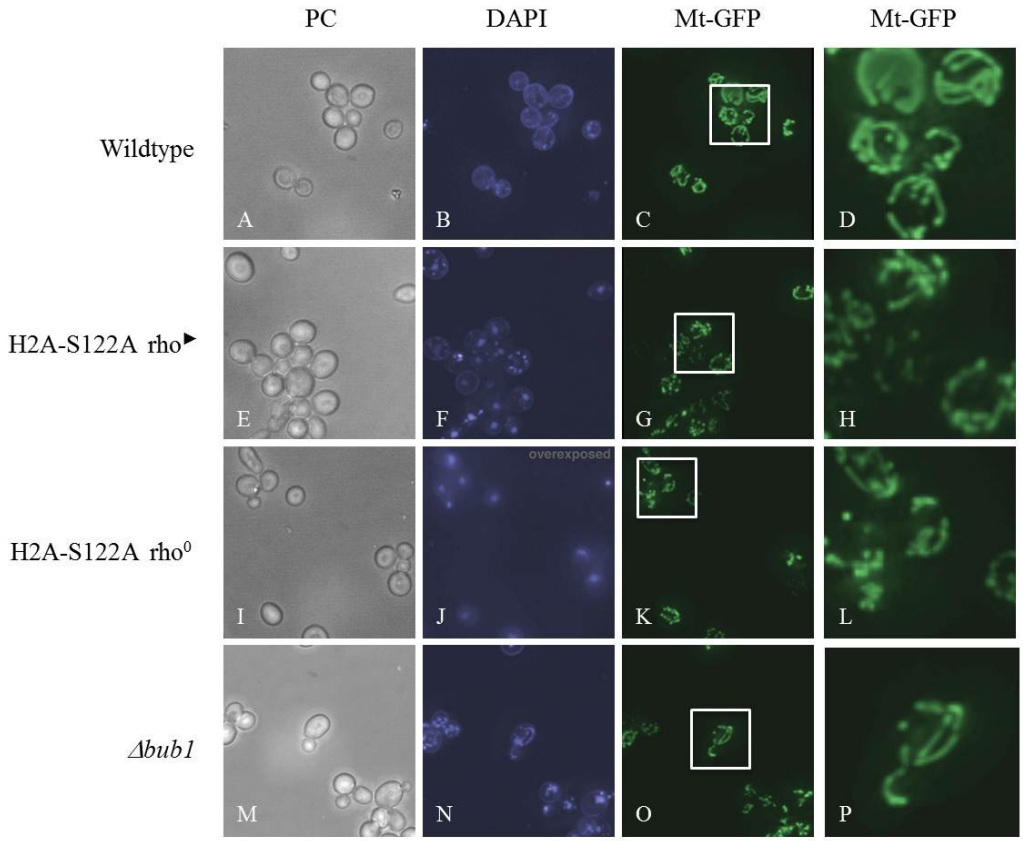

Figure 2: H2A-S122 is critical for maintaining the mitochondrial genome. Wildtype, H2A-S122A rho$^{\blacktriangleright}$, H2A-S122A rho^{0}, and *Δbub1* cells carrying the mt-GFP plasmid were stained with DAPI and visualized using fluorescent microscopy. Cells were visualized using phase contrast, while DAPI staining was visualized using xxx filter and the mitochondria matrix localized GFP was visualized using the xxx filter. Panels D, H, L, and P are enlargements of the outlined sections of panels C, G, K, and O respectively. Panel J showing DAPI stained S122 rho^{0} cells is overexposed to visualize the lack of mitochondrial DNA staining.

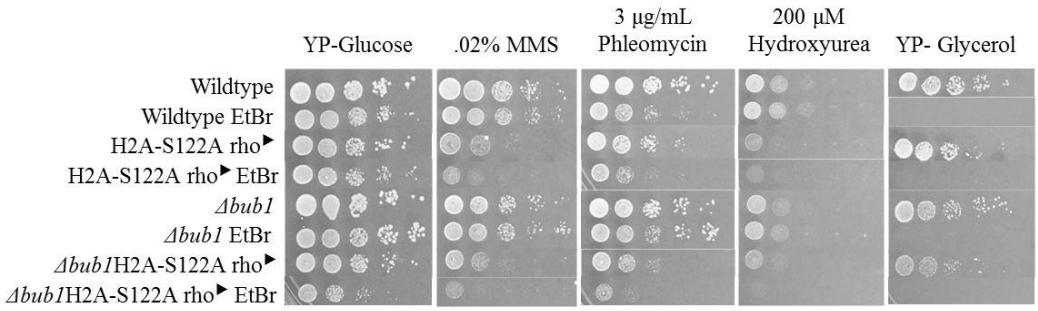

Figure 3: H2A-S122A DNA damage phenotypes are independent of mitochondrial function and Bub1 activity. DNA damage sensitivity is measured by plating ten-fold serial dilutions of cultured cells onto media containing 0.02% MMS, 3µg/mL phleomycin, or 200µM hydroxyurea. Ethidium bromide (EtBr) treatment was used to eliminate mitochondrial function, verified by a lack of growth on YP-glycerol.

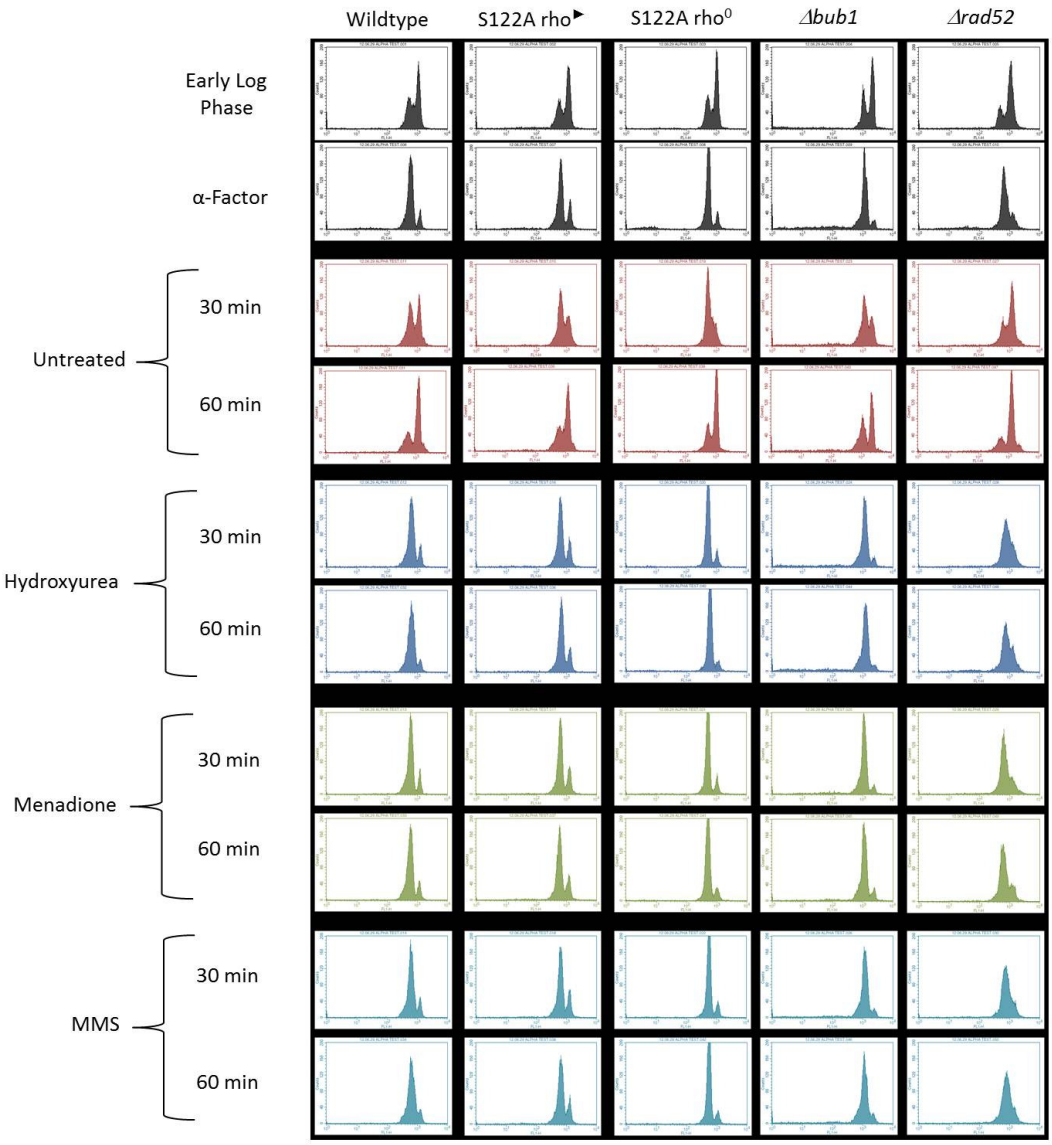

Figure 4: The H2A-S122A mutant displays an intact DNA damage cell cycle response following treatment with DNA damaging agents. For each strain log phase cells were treated with α-factor, inhibiting the majority of cells in G1. Upon release from α-factor, cells were grown in YPD media alone or media containing 200μM hydroxyurea, 2mM menadione, and .02% MMS for 60 minutes. Samples were collected at 30 minute intervals and analyzed using flow cytometry.

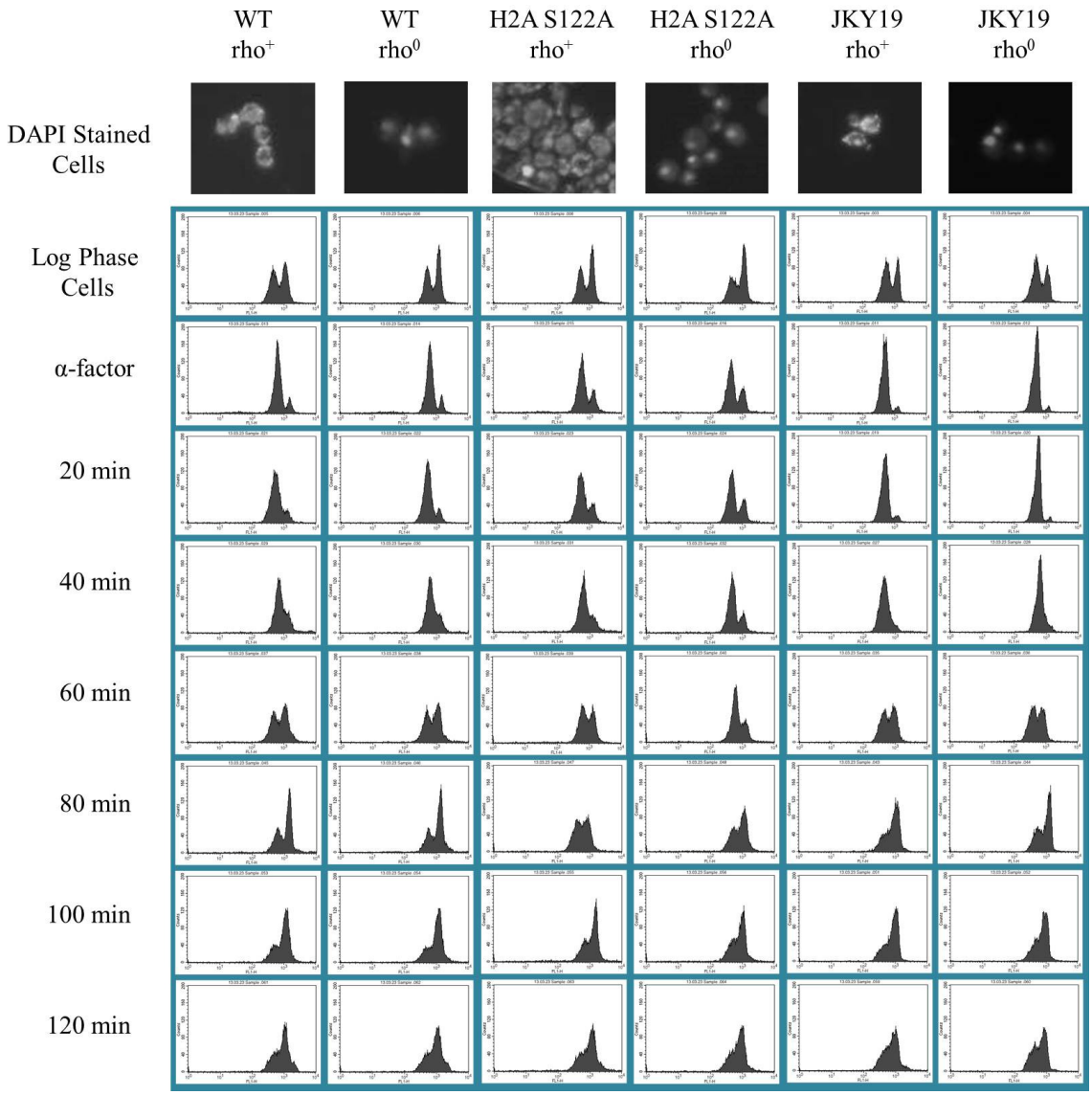

Figure 5: The mitochondrial inheritance checkpoint is not triggered in any of the strains analyzed. Cells were treated with EtBr to induce loss of the mitochondrial genome. (A) Mitochondrial genome loss was verified using flourescent microscopy. (B) For each strain log phase cells were treated with α-factor, inhibiting the majority of cells in G1. Cells were collected and analyzed using flow cytometry prior to treatment with α-factor (log phase cells) and then at 20-minute increments following the release from α-factor.

TABLE 1: **Aneuploidy Frequency.** The number and distribution of aneuploid chromosomes in *bub1Δ* and H2A-S122A strains are ascertained by comparative genome hybridization microarrays. The unparenthesied numbers refer to aneuploidy events that increased the number of chromosomes, while the numbers in parenthesis indicate aneuploidy events that resulted in a decrease in chromosome number. The number of colony passages prior to analysis is indicated by (CP #). No aneuploidy events were detected in wildtype cells in the course of the experiment.

	Diploid Cells		Haploid Cells‡£	
Chromosome number	*bub1Δ* (CP 0)*	*bub1Δ* (CP 5)*	H2A- S122A (CP 7)	*bub1Δ* (CP 7)
I	2 (1)	1	4	0
II	8	5	8	0
III	5 (1)	4	0 (2)	(1)
IV	0	0	0	0
V	3	1	1	0
VI	0	2	0	0
VII	0	0	0	0
VIII	18	8	3	0
IX	0 (2)	0 (1)	0	0
X	8	5	0	0
XI	0 (2)	0 (1)	1	0
XII	0	0	0	0
XIII	0	1	0	0
XIV	2 (1)	2	0	0
XV	0	0	0	0
XVI	7	6	4	0
Total number of aneuploids	60	37	23	1
Total number of chromosomes analyzed †	640	384	193	193
Aneuploid/euploid chromosomes	60/580 = .103	37/347 = .106	23/169=.136	1/191 = .005

*Data obtained from the supplemental data supplied by (McCulley and Petes, 2010)

†Total number of chromosomes calculated by multiplying the number of independent strains examined by either 32 (diploid cells) or 16 (haploid cells)

‡ Both the H2A-S122A mutation and *BUB1* deletion were introduced immediately prior to the first subculture, therefore the likelihood of either strain having aneuploid chromosomes at SC 0 is very low.

£ The parental strain in which these strains were derived has an incidental duplication of Chromosome III, thus allowing a decrease in Chromosome III copy number.

TABLE 2: Frequency of erythromycin resistance in H2A-S122A mutants

Genotype	Mutation Frequency[†]	Fold Effect[‡]
Wildtype	3.06×10^{-12}	1.00
H2A-S122A rho^{+}	2.90×10^{-12}	1.01
Δpif1	2.97×10^{-10}	63.61

[†] The number of erythromycin resistant colonies divided by the total number of colonies plated (based on 30-35 clones for each genotype)

[‡] Calculated relative to wildtype

SUPPLEMENTAL TABLE 1: Strains and Plasmids Used

Strain[¥]	Genotype	Plasmid
FY406*	*MAT**a** (hta1-htb1)Δ::LEU2 (hta2-htb2) Δ::TRP1 leu2 Δ1ura3-52 lys2 Δ1 lys2-128δ his3 Δ200 trp1Δ63*	pSAB6 *(HTA1-HTB1, URA3)*
JKY29†	Same as FY406	pJKP (*HTA1-HTB1, URA3*)
JKY38†	Same as FY406	pJKP18 (*HTA1-HTB1*, *HIS3*)
JKY33†	Same as FY406	pJKP22 (*hta1-S122A-HTB1*, *HIS3*)
JKY198	*MAT**a** (hta1-htb1)Δ::LEU2 (hta2-htb2) Δ::TRP1 (BUB1)::?? leu2 Δ1ura3-52 lys2 Δ1 lys2-128δ his3 Δ200 trp1Δ63*	pSAB6*(HTA1-HTB1, URA3)*
JKY19‡	*MAT**a** ura3-52 leu2Δ1 his3-Δ200 trp1-Δ1 ade2-101 lys2-801*	
JKY184	Same as JKY198	pJKP18 (*HTA1-HTB1*, *HIS3*)
JKY185	Same as JKY198	pJKP22 (*hta1-S122A-HTB1*, *HIS3*)

¥ All strains originate from the S288C background
* Hirschhorn et al (1995) (Hirschhorn et al., 1995)
† Moore et al. (2007) (Moore et al., 2007)
‡ (CY25) supplied by Craig Peterson

SUPPLEMENTAL TABLE 2: Aneuploidy distribution for wildtype, H2A-S122A, and *bub1Δ*clones

Strain	Addition of Chromosome	Loss of Chromosome	Growth on Glycerol (SC 7)†
Wildtype – 1	-	-	-
Wildtype – 2	-	-	X
Wildtype – 3	-	-	X
Wildtype – 4	-	-	-
Wildtype – 5	-	-	-
Wildtype – 6	-	-	-
Wildtype – 7	-	-	-
Wildtype – 8	-	-	-
Wildtype – 9	-	-	-
Wildtype – 10	-	-	X
Wildtype – 11	-	-	-
Wildtype – 12	-	-	-
H2A-S122A – 1	-	-	-
H2A-S122A – 2	16	-	-
H2A-S122A – 3	1,2,8,11	-	-
H2A-S122A – 4	2	-	-
H2A-S122A – 5	2	3	-
H2A-S122A – 6	1,16	-	-
H2A-S122A – 7	2,8	-	-
H2A-S122A – 8	2,5	-	-
H2A-S122A – 9	1,2	3	-
H2A-S122A – 10	2	-	-
H2A-S122A – 11	8	-	-
H2A-S122A –12	1,2	-	-
bub1Δ – 1	-	-	X
bub1Δ – 2	-	-	-
bub1Δ – 3		3	-
bub1Δ – 4	-	-	X
bub1Δ – 5	-	-	X
bub1Δ – 6	-	-	X
bub1Δ – 7	-	-	-
bub1Δ – 8	-	-	X
bub1Δ – 9	-	-	-
bub1Δ – 10	-	-	-
bub1Δ – 11	-	-	X
bub1Δ – 12	-	-	X

† X represents the presence of growth on glycerol for cells used in the CGH analysis as an indicator of mitochondrial function

1.7 References

Aihara, H., Nakagawa, T., Yasui, K., Ohta, T., Hirose, S., Dhomae, N., Takio, K., Kaneko, M., Takeshima, Y., Muramatsu, M., Ito, T., 2004. Nucleosomal histone kinase-1 phosphorylates H2A Thr 119 during mitosis in the early Drosophila embryo. Genes & development 18, 877-888.

Biggins, S., Murray, A.W., 2001. The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. Genes & development 15, 3118-3129.

Brittle, A.L., Nanba, Y., Ito, T., Ohkura, H., 2007. Concerted action of Aurora B, Polo and NHK-1 kinases in centromere-specific histone 2A phosphorylation. Experimental cell research 313, 2780-2785.

Chi, N.W., Kolodner, R.D., 1994. Purification and characterization of MSH1, a yeast mitochondrial protein that binds to DNA mismatches. The Journal of biological chemistry 269, 29984-29992.

Clarke, D.J., Bachant, J., 2008. Kinetochore structure and spindle assembly checkpoint signaling in the budding yeast, Saccharomyces cerevisiae. Frontiers in bioscience : a journal and virtual library 13, 6787-6819.

Contamine, V., Picard, M., 2000. Maintenance and integrity of the mitochondrial genome: a plethora of nuclear genes in the budding yeast. Microbiology and molecular biology reviews : MMBR 64, 281-315.

Crider, D.G., Garcia-Rodriguez, L.J., Srivastava, P., Peraza-Reyes, L., Upadhyaya, K., Boldogh, I.R., Pon, L.A., 2012. Rad53 is essential for a mitochondrial DNA inheritance checkpoint regulating G1 to S progression. The Journal of cell biology 198, 793-798.

Cui, Z., Mason, T.L., 1989. A single nucleotide substitution at the rib2 locus of the yeast mitochondrial gene for 21S rRNA confers resistance to erythromycin and cold-sensitive ribosome assembly. Current genetics 16, 273-279.

Downs, J.A., Allard, S., Jobin-Robitaille, O., Javaheri, A., Auger, A., Bouchard, N., Kron, S.J., Jackson, S.P., Cote, J., 2004. Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. Molecular cell 16, 979-990.

Downs, J.A., Lowndes, N.F., Jackson, S.P., 2000. A role for Saccharomyces cerevisiae histone H2A in DNA repair. Nature 408, 1001-1004.

Goldring, E.S., Grossman, L.I., Krupnick, D., Cryer, D.R., Marmur, J., 1970. The petite mutation in yeast. Loss of mitochondrial deoxyribonucleic acid during induction of petites with ethidium bromide. Journal of molecular biology 52, 323-335.

Harvey, A.C., Jackson, S.P., Downs, J.A., 2005. Saccharomyces cerevisiae histone H2A Ser122 facilitates DNA repair. Genetics 170, 543-553.

Hirschhorn, J.N., Bortvin, A.L., Ricupero-Hovasse, S.L., Winston, F., 1995. A new class of histone H2A mutations in Saccharomyces cerevisiae causes specific transcriptional defects in vivo. Molecular and cellular biology 15, 1999-2009.

Jung, P.P., Fritsch, E.S., Blugeon, C., Souciet, J.L., Potier, S., Lemoine, S., Schacherer, J., de Montigny, J., 2011. Ploidy influences cellular responses to gross chromosomal rearrangements in Saccharomyces cerevisiae. BMC genomics 12, 331.

Kawashima, S.A., Yamagishi, Y., Honda, T., Ishiguro, K., Watanabe, Y., 2010. Phosphorylation of H2A by Bub1 prevents chromosomal instability through localizing shugoshin. Science 327, 172-177.

McCulley, J.L., Petes, T.D., 2010. Chromosome rearrangements and aneuploidy in yeast strains lacking both Tel1p and Mec1p reflect deficiencies in two different mechanisms. Proceedings of the National Academy of Sciences of the United States of America 107, 11465-11470.

Moore, J.D., Yazgan, O., Ataian, Y., Krebs, J.E., 2007. Diverse roles for histone H2A modifications in DNA damage response pathways in yeast. Genetics 176, 15-25.

Morrison, A.J., Highland, J., Krogan, N.J., Arbel-Eden, A., Greenblatt, J.F., Haber, J.E., Shen, X., 2004. INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. Cell 119, 767-775.

O'Rourke, T.W., Doudican, N.A., Mackereth, M.D., Doetsch, P.W., Shadel, G.S., 2002. Mitochondrial dysfunction due to oxidative mitochondrial DNA damage is reduced through cooperative actions of diverse proteins. Molecular and cellular biology 22, 4086-4093.

Rieder, C.L., Schultz, A., Cole, R., Sluder, G., 1994. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. The Journal of cell biology 127, 1301-1310.

Sheltzer, J.M., Blank, H.M., Pfau, S.J., Tange, Y., George, B.M., Humpton, T.J., Brito, I.L., Hiraoka, Y., Niwa, O., Amon, A., 2011. Aneuploidy drives genomic instability in yeast. Science 333, 1026-1030.

Spector, L.M., Fogel, S., 1992. Mitotic hyperploidy for chromosomes VIII and III in Saccharomyces cerevisiae. Current genetics 21, 309-318.

van Attikum, H., Fritsch, O., Hohn, B., Gasser, S.M., 2004. Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. Cell 119, 777-788.

Veatch, J.R., McMurray, M.A., Nelson, Z.W., Gottschling, D.E., 2009. Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. Cell 137, 1247-1258.

Vernon, M., Lobachev, K., Petes, T.D., 2008. High rates of "unselected" aneuploidy and chromosome rearrangements in tel1 mec1 haploid yeast strains. Genetics 179, 237-247.

Westermann, B., Neupert, W., 2000. Mitochondria-targeted green fluorescent proteins: convenient tools for the study of organelle biogenesis in Saccharomyces cerevisiae. Yeast 16, 1421-1427.

Wyatt, H.R., Liaw, H., Green, G.R., Lustig, A.J., 2003. Multiple roles for Saccharomyces cerevisiae histone H2A in telomere position effect, Spt phenotypes and double-strand-break repair. Genetics 164, 47-64.

Yamagishi, Y., Honda, T., Tanno, Y., Watanabe, Y., 2010. Two histone marks establish the inner centromere and chromosome bi-orientation. Science 330, 239-243.

Yang, C., Wang, H., Xu, Y., Brinkman, K.L., Ishiyama, H., Wong, S.T., Xu, B., 2012. The kinetochore protein Bub1 participates in the DNA damage response. DNA repair 11, 185-191.

Zhu, J., Pavelka, N., Bradford, W.D., Rancati, G., Li, R., 2012. Karyotypic determinants of chromosome instability in aneuploid budding yeast. PLoS genetics 8, e1002719.

GENERAL CONCLUSIONS

The wide range of phenotypes observed in the H2A-S122A mutant strain illustrates the importance of this residue in cellular function. It is sensitive to a wide variety of stressors including temperature, osmotic, and mutagenic stress in addition to its clear role in the spindle assembly checkpoint and its importance in mitochondrial genome stability. The reversibility of phosphorylation makes this posttranslational modification especially useful as a signaling mark and it is likely that phosphorylation of H2A-S122 is critical for cellular function through stress signaling. Our lab have previously shown that the phosphorylation status of H2A-S122 changes in response to DNA damage (Moore et al., 2007); however the antibody used for those experiments has been exhausted and despite many attempts at reproducing this antibody we have been unable to obtain a sufficiently specific phospho-H2A-S122 antibody. Unfortunately this has prevented us from directly associating H2A-S122 phosphorylation status with the phenotypes exhibited by the H2A-S122A mutant or investigating potential kinases involved in H2A-S122 phosphorylation.

The work done by Kawashima et al. (2010) in both the fission yeast *S. pombe* and humans demonstrates that H2A-S122 is a direct target of Bub1, and our work adds indirect evidence that this is true in *S. cerevisiae* as well. Aneuploidy is a direct consequence of a dysfunctional spindle assembly checkpoint, thus I directly compared aneuploidy frequency between H2A-S122A mutants and *bub1Δ* cells. H2A-S122A mutant cells exhibit a remarkably high rate of aneuploidy which may indicate that it has additional roles in maintaining nuclear genome stability.

There are vast differences between the nuclear and mitochondrial genomes; however I show that H2A-S122 is critical for the stability of both genomes. As mentioned above, this is most likely dependent on post-translational modification of S122. Further research is needed to determine the mechanism(s) in which H2A-S122 is involved in mitochondrial genome stability. The potential for H2A-S122 to link mitochondrial genome status to the cell cycle is intriguing. The checkpoint kinase Rad53 (discussed in Figure I2) is critical for the DNA damage checkpoints as well as the mitochondrial inheritance checkpoint (Crider et al., 2012; Kim and Weinert, 1997; Sweeney et al., 2005). It also mediates mtDNA copy number by altering dNTP levels through the RNR pathway (Taylor et al., 2005).

Furthermore, Rad53 recruitment is downstream of H2A-S129 phosphorylation, thus we know that Rad53 is in the vicinity of H2A-S122.

More research is also needed to determine whether the mitochondrial genome instability observed in the H2A-S122 mutant is at least partially due to the aneuploidy state of the cells. Dosage effects from aneuploid chromosome are not well understood; however it is clear that these gross changes to the transcriptome can significantly alter gene expression, chromosome structure and arrangement which results in altered cellular function (Huettel et al., 2008). The universality of mitochondrial genome loss in H2A-S122A cells, coupled with the variety of aneuploidy events, indicate that it is not a specific aneuploid chromosome leading to mitochondrial genome destabilization. However, the perturbations in chromosome segregation could certainly interact with a pathway involved in mitochondrial genome segregation or maintenance.

In conclusion, the data presented here demonstrate the centrality of H2A-S122 in both mitochondrial and nuclear genome stability, opening new research directions in understanding the communication and coordination between the nuclear and mitochondrial genomes.

REFERENCES

Aihara, H., Nakagawa, T., Yasui, K., Ohta, T., Hirose, S., Dhomae, N., Takio, K., Kaneko, M., Takeshima, Y., Muramatsu, M., Ito, T., 2004. Nucleosomal histone kinase-1 phosphorylates H2A Thr 119 during mitosis in the early Drosophila embryo. Genes & development 18, 877-888.

Akbari, M., Visnes, T., Krokan, H.E., Otterlei, M., 2008. Mitochondrial base excision repair of uracil and AP sites takes place by single-nucleotide insertion and long-patch DNA synthesis. DNA repair 7, 605-616.

Ataian, Y., Krebs, J.E., 2006. Five repair pathways in one context: chromatin modification during DNA repair. Biochemistry and cell biology = Biochimie et biologie cellulaire 84, 490-504.

Ausio, J., Dong, F., van Holde, K.E., 1989. Use of selectively trypsinized nucleosome core particles to analyze the role of the histone "tails" in the stabilization of the nucleosome. Journal of molecular biology 206, 451-463.

Ball, H.L., Myers, J.S., Cortez, D., 2005. ATRIP binding to replication protein A-single-stranded DNA promotes ATR-ATRIP localization but is dispensable for Chk1 phosphorylation. Molecular biology of the cell 16, 2372-2381.

Baruffini, E., Ferrero, I., Foury, F., 2007. Mitochondrial DNA defects in Saccharomyces cerevisiae caused by functional interactions between DNA polymerase gamma mutations associated with disease in human. Biochimica et biophysica acta 1772, 1225-1235.

Berger, S.L., 2007. The complex language of chromatin regulation during transcription. Nature 447, 407-412.

Berger, S.L., 2010. Cell signaling and transcriptional regulation via histone phosphorylation. Cold Spring Harbor symposia on quantitative biology 75, 23-26.

Biggins, S., Murray, A.W., 2001. The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. Genes & development 15, 3118-3129.

Boldogh, I., Vojtov, N., Karmon, S., Pon, L.A., 1998. Interaction between mitochondria and the actin cytoskeleton in budding yeast requires two integral mitochondrial outer membrane proteins, Mmm1p and Mdm10p. The Journal of cell biology 141, 1371-1381.

Boldogh, I.R., Nowakowski, D.W., Yang, H.C., Chung, H., Karmon, S., Royes, P., Pon, L.A., 2003. A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. Molecular biology of the cell 14, 4618-4627.

Boldogh, I.R., Ramcharan, S.L., Yang, H.C., Pon, L.A., 2004. A type V myosin (Myo2p) and a Rab-like G-protein (Ypt11p) are required for retention of newly inherited mitochondria in yeast cells during cell division. Molecular biology of the cell 15, 3994-4002.

Bouck, D.C., Bloom, K.S., 2005. The kinetochore protein Ndc10p is required for spindle stability and cytokinesis in yeast. Proceedings of the National Academy of Sciences of the United States of America 102, 5408-5413.

Branzei, D., Foiani, M., 2006. The Rad53 signal transduction pathway: Replication fork stabilization, DNA repair, and adaptation. Experimental cell research 312, 2654-2659.

Brewer, L.R., Friddle, R., Noy, A., Baldwin, E., Martin, S.S., Corzett, M., Balhorn, R., Baskin, R.J., 2003. Packaging of single DNA molecules by the yeast mitochondrial protein Abf2p. Biophysical journal 85, 2519-2524.

Brittle, A.L., Nanba, Y., Ito, T., Ohkura, H., 2007. Concerted action of Aurora B, Polo and NHK-1 kinases in centromere-specific histone 2A phosphorylation. Experimental cell research 313, 2780-2785.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., Allis, C.D., 1996. Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell 84, 843-851.

Celeste, A., Fernandez-Capetillo, O., Kruhlak, M.J., Pilch, D.R., Staudt, D.W., Lee, A., Bonner, R.F., Bonner, W.M., Nussenzweig, A., 2003. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nature cell biology 5, 675-679.

Cerveny, K.L., Studer, S.L., Jensen, R.E., Sesaki, H., 2007. Yeast mitochondrial division and distribution require the cortical num1 protein. Developmental cell 12, 363-375.

Chambers, A.L., Downs, J.A., 2007. The contribution of the budding yeast histone H2A C-terminal tail to DNA-damage responses. Biochemical Society transactions 35, 1519-1524.

Chatre, L., Ricchetti, M., 2013. Prevalent coordination of mitochondrial DNA transcription and initiation of replication with the cell cycle. Nucleic acids research 41, 3068-3078.

Cheeseman, I.M., Desai, A., 2008. Molecular architecture of the kinetochore-microtubule interface. Nature reviews. Molecular cell biology 9, 33-46.

Chen, X.J., Butow, R.A., 2005. The organization and inheritance of the mitochondrial genome. Nature reviews. Genetics 6, 815-825.

Chen, Y., Sanchez, Y., 2004. Chk1 in the DNA damage response: conserved roles from yeasts to mammals. DNA repair 3, 1025-1032.

Chi, N.W., Kolodner, R.D., 1994. Purification and characterization of MSH1, a yeast mitochondrial protein that binds to DNA mismatches. The Journal of biological chemistry 269, 29984-29992.

Clarke, D.J., Bachant, J., 2008. Kinetochore structure and spindle assembly checkpoint signaling in the budding yeast, Saccharomyces cerevisiae. Frontiers in bioscience : a journal and virtual library 13, 6787-6819.

Clemenson, C., Marsolier-Kergoat, M.C., 2006. The spindle assembly checkpoint regulates the phosphorylation state of a subset of DNA checkpoint proteins in Saccharomyces cerevisiae. Molecular and cellular biology 26, 9149-9161.

Coffey, G., Lakshmipathy, U., Campbell, C., 1999. Mammalian mitochondrial extracts possess DNA end-binding activity. Nucleic acids research 27, 3348-3354.

Coffman, V.C., Wu, P., Parthun, M.R., Wu, J.Q., 2011. CENP-A exceeds microtubule attachment sites in centromere clusters of both budding and fission yeast. The Journal of cell biology 195, 563-572.

Cole, H.A., Howard, B.H., Clark, D.J., 2011. The centromeric nucleosome of budding yeast is perfectly positioned and covers the entire centromere. Proceedings of the National Academy of Sciences of the United States of America 108, 12687-12692.

Contamine, V., Picard, M., 2000. Maintenance and integrity of the mitochondrial genome: a plethora of nuclear genes in the budding yeast. Microbiology and molecular biology reviews : MMBR 64, 281-315.

Crider, D.G., Garcia-Rodriguez, L.J., Srivastava, P., Peraza-Reyes, L., Upadhyaya, K., Boldogh, I.R., Pon, L.A., 2012. Rad53 is essential for a mitochondrial DNA inheritance checkpoint regulating G1 to S progression. The Journal of cell biology 198, 793-798.

Croteau, D.L., Bohr, V.A., 1997. Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells. The Journal of biological chemistry 272, 25409-25412.

Cui, Z., Mason, T.L., 1989. A single nucleotide substitution at the rib2 locus of the yeast mitochondrial gene for 21S rRNA confers resistance to erythromycin and cold-sensitive ribosome assembly. Current genetics 16, 273-279.

D'Ambrosio, C., Schmidt, C.K., Katou, Y., Kelly, G., Itoh, T., Shirahige, K., Uhlmann, F., 2008. Identification of cis-acting sites for condensin loading onto budding yeast chromosomes. Genes & development 22, 2215-2227.

Dotiwala, F., Harrison, J.C., Jain, S., Sugawara, N., Haber, J.E., 2010. Mad2 prolongs DNA damage checkpoint arrest caused by a double-strand break via a centromere-dependent mechanism. Current biology : CB 20, 328-332.

Downs, J.A., Allard, S., Jobin-Robitaille, O., Javaheri, A., Auger, A., Bouchard, N., Kron, S.J., Jackson, S.P., Cote, J., 2004. Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. Molecular cell 16, 979-990.

Downs, J.A., Lowndes, N.F., Jackson, S.P., 2000. A role for Saccharomyces cerevisiae histone H2A in DNA repair. Nature 408, 1001-1004.

Duesberg, P., Rausch, C., Rasnick, D., Hehlmann, R., 1998. Genetic instability of cancer cells is proportional to their degree of aneuploidy. Proceedings of the National Academy of Sciences of the United States of America 95, 13692-13697.

Eckert, C.A., Gravdahl, D.J., Megee, P.C., 2007. The enhancement of pericentromeric cohesin association by conserved kinetochore components promotes high-fidelity chromosome segregation and is sensitive to microtubule-based tension. Genes & development 21, 278-291.

Elson, J.L., Andrews, R.M., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M., Howell, N., 2001. Analysis of European mtDNAs for recombination. American journal of human genetics 68, 145-153.

Emili, A., 1998. MEC1-dependent phosphorylation of Rad9p in response to DNA damage. Molecular cell 2, 183-189.

Erjavec, N., Cvijovic, M., Klipp, E., Nystrom, T., 2008. Selective benefits of damage partitioning in unicellular systems and its effects on aging. Proceedings of the National Academy of Sciences of the United States of America 105, 18764-18769.

Fedorova, E., Zink, D., 2008. Nuclear architecture and gene regulation. Biochimica et biophysica acta 1783, 2174-2184.

Fehrenbacher, K.L., Yang, H.C., Gay, A.C., Huckaba, T.M., Pon, L.A., 2004. Live cell imaging of mitochondrial movement along actin cables in budding yeast. Current biology : CB 14, 1996-2004.

Fernius, J., Hardwick, K.G., 2007. Bub1 kinase targets Sgo1 to ensure efficient chromosome biorientation in budding yeast mitosis. PLoS genetics 3, e213.

Finn, K., Lowndes, N.F., Grenon, M., 2012. Eukaryotic DNA damage checkpoint activation in response to double-strand breaks. Cellular and molecular life sciences : CMLS 69, 1447-1473.

Fischle, W., Wang, Y., Allis, C.D., 2003. Histone and chromatin cross-talk. Curr Opin Cell Biol 15, 172-183.

Fitzgerald-Hayes, M., Clarke, L., Carbon, J., 1982. Nucleotide sequence comparisons and functional analysis of yeast centromere DNAs. Cell 29, 235-244.

Fogg, V.C., Lanning, N.J., Mackeigan, J.P., 2011. Mitochondria in cancer: at the crossroads of life and death. Chinese journal of cancer 30, 526-539.

Furuyama, S., Biggins, S., 2007. Centromere identity is specified by a single centromeric nucleosome in budding yeast. Proceedings of the National Academy of Sciences of the United States of America 104, 14706-14711.

Garber, P.M., Rine, J., 2002. Overlapping roles of the spindle assembly and DNA damage checkpoints in the cell-cycle response to altered chromosomes in Saccharomyces cerevisiae. Genetics 161, 521-534.

Garcia-Rodriguez, L.J., Crider, D.G., Gay, A.C., Salanueva, I.J., Boldogh, I.R., Pon, L.A., 2009. Mitochondrial inheritance is required for MEN-regulated cytokinesis in budding yeast. Current biology : CB 19, 1730-1735.

Garcia-Rodriguez, L.J., Gay, A.C., Pon, L.A., 2007. Puf3p, a Pumilio family RNA binding protein, localizes to mitochondria and regulates mitochondrial biogenesis and motility in budding yeast. The Journal of cell biology 176, 197-207.

Gascoigne, K.E., Cheeseman, I.M., 2011. Kinetochore assembly: if you build it, they will come. Current opinion in cell biology 23, 102-108.

Goldring, E.S., Grossman, L.I., Krupnick, D., Cryer, D.R., Marmur, J., 1970. The petite mutation in yeast. Loss of mitochondrial deoxyribonucleic acid during induction of petites with ethidium bromide. Journal of molecular biology 52, 323-335.

Goto, G.H., Mishra, A., Abdulle, R., Slaughter, C.A., Kitagawa, K., 2011. Bub1-mediated adaptation of the spindle checkpoint. PLoS genetics 7, e1001282.

Govatati, S., Tipirisetti, N.R., Perugu, S., Kodati, V.L., Deenadayal, M., Satti, V., Bhanoori, M., Shivaji, S., 2012. Mitochondrial genome variations in advanced stage endometriosis: a study in South Indian population. PloS one 7, e40668.

Haase, J., Stephens, A., Verdaasdonk, J., Yeh, E., Bloom, K., 2012. Bub1 kinase and Sgo1 modulate pericentric chromatin in response to altered microtubule dynamics. Current biology : CB 22, 471-481.

Hammet, A., Magill, C., Heierhorst, J., Jackson, S.P., 2007. Rad9 BRCT domain interaction with phosphorylated H2AX regulates the G1 checkpoint in budding yeast. EMBO reports 8, 851-857.

Hargreaves, D.C., Crabtree, G.R., 2011. ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. Cell research 21, 396-420.

Harvey, A.C., Jackson, S.P., Downs, J.A., 2005. Saccharomyces cerevisiae histone H2A Ser122 facilitates DNA repair. Genetics 170, 543-553.

Henikoff, S., Henikoff, J.G., 2012. "Point" centromeres of Saccharomyces harbor single centromere-specific nucleosomes. Genetics 190, 1575-1577.

Hirschhorn, J.N., Bortvin, A.L., Ricupero-Hovasse, S.L., Winston, F., 1995. A new class of histone H2A mutations in Saccharomyces cerevisiae causes specific transcriptional defects in vivo. Molecular and cellular biology 15, 1999-2009.

Hobbs, A.E., Srinivasan, M., McCaffery, J.M., Jensen, R.E., 2001. Mmm1p, a mitochondrial outer membrane protein, is connected to mitochondrial DNA (mtDNA) nucleoids and required for mtDNA stability. The Journal of cell biology 152, 401-410.

Holt, I.J., 2009. Mitochondrial DNA replication and repair: all a flap. Trends in biochemical sciences 34, 358-365.

Huertas, D., Sendra, R., Munoz, P., 2009. Chromatin dynamics coupled to DNA repair. Epigenetics : official journal of the DNA Methylation Society 4, 31-42.

Huettel, B., Kreil, D.P., Matzke, M., Matzke, A.J., 2008. Effects of aneuploidy on genome structure, expression, and interphase organization in Arabidopsis thaliana. PLoS genetics 4, e1000226.

Humpal, S.E., Robinson, D.A., Krebs, J.E., 2009. Marks to stop the clock: histone modifications and checkpoint regulation in the DNA damage response. Biochemistry and cell biology = Biochimie et biologie cellulaire 87, 243-253.

Iizuka, M., Smith, M.M., 2003. Functional consequences of histone modifications. Curr Opin Genet Dev 13, 154-160.

Innan, H., Nordborg, M., 2002. Recombination or mutational hot spots in human mtDNA? Molecular biology and evolution 19, 1122-1127.

Javaheri, A., Wysocki, R., Jobin-Robitaille, O., Altaf, M., Cote, J., Kron, S.J., 2006. Yeast G1 DNA damage checkpoint regulation by H2A phosphorylation is independent of chromatin remodeling. Proceedings of the National Academy of Sciences of the United States of America 103, 13771-13776.

Jorde, L.B., Bamshad, M., 2000. Questioning evidence for recombination in human mitochondrial DNA. Science 288, 1931.

Jung, P.P., Fritsch, E.S., Blugeon, C., Souciet, J.L., Potier, S., Lemoine, S., Schacherer, J., de Montigny, J., 2011. Ploidy influences cellular responses to gross chromosomal rearrangements in Saccharomyces cerevisiae. BMC genomics 12, 331.

Kadosh, D., Struhl, K., 1998. Targeted recruitment of the Sin3-Rpd3 histone deacetylase complex generates a highly localized domain of repressed chromatin in vivo. Mol Cell Biol 18, 5121-5127.

Kajander, O.A., Karhunen, P.J., Holt, I.J., Jacobs, H.T., 2001. Prominent mitochondrial DNA recombination intermediates in human heart muscle. EMBO reports 2, 1007-1012.

Kalifa, L., Quintana, D.F., Schiraldi, L.K., Phadnis, N., Coles, G.L., Sia, R.A., Sia, E.A., 2012. Mitochondrial genome maintenance: roles for nuclear nonhomologous end-joining proteins in Saccharomyces cerevisiae. Genetics 190, 951-964.

Kanaar, R., Hoeijmakers, J.H., van Gent, D.C., 1998. Molecular mechanisms of DNA double strand break repair. Trends in cell biology 8, 483-489.

Katis, V.L., Galova, M., Rabitsch, K.P., Gregan, J., Nasmyth, K., 2004. Maintenance of cohesin at centromeres after meiosis I in budding yeast requires a kinetochore-associated protein related to MEI-S332. Current biology : CB 14, 560-572.

Kaufman, B.A., Kolesar, J.E., Perlman, P.S., Butow, R.A., 2003. A function for the mitochondrial chaperonin Hsp60 in the structure and transmission of mitochondrial DNA nucleoids in Saccharomyces cerevisiae. The Journal of cell biology 163, 457-461.

Kawashima, S.A., Yamagishi, Y., Honda, T., Ishiguro, K., Watanabe, Y., 2010. Phosphorylation of H2A by Bub1 prevents chromosomal instability through localizing shugoshin. Science 327, 172-177.

Kazak, L., Reyes, A., Holt, I.J., 2012. Minimizing the damage: repair pathways keep mitochondrial DNA intact. Nature reviews. Molecular cell biology 13, 659-671.

Keogh, M.C., Kim, J.A., Downey, M., Fillingham, J., Chowdhury, D., Harrison, J.C., Onishi, M., Datta, N., Galicia, S., Emili, A., Lieberman, J., Shen, X., Buratowski, S., Haber, J.E., Durocher, D., Greenblatt, J.F., Krogan, N.J., 2006. A phosphatase complex that dephosphorylates gammaH2AX regulates DNA damage checkpoint recovery. Nature 439, 497-501.

Kerrebrock, A.W., Miyazaki, W.Y., Birnby, D., Orr-Weaver, T.L., 1992. The Drosophila mei-S332 gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation. Genetics 130, 827-841.

Kiburz, B.M., Amon, A., Marston, A.L., 2008. Shugoshin promotes sister kinetochore biorientation in Saccharomyces cerevisiae. Molecular biology of the cell 19, 1199-1209.

Kim, E.M., Burke, D.J., 2008. DNA damage activates the SAC in an ATM/ATR-dependent manner, independently of the kinetochore. PLoS genetics 4, e1000015.

Kim, S., Weinert, T.A., 1997. Characterization of the checkpoint gene RAD53/MEC2 in Saccharomyces cerevisiae. Yeast 13, 735-745.

Kitajima, T.S., Kawashima, S.A., Watanabe, Y., 2004. The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature 427, 510-517.

Klein, F., Mahr, P., Galova, M., Buonomo, S.B., Michaelis, C., Nairz, K., Nasmyth, K., 1999. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 98, 91-103.

Kraytsberg, Y., Schwartz, M., Brown, T.A., Ebralidse, K., Kunz, W.S., Clayton, D.A., Vissing, J., Khrapko, K., 2004. Recombination of human mitochondrial DNA. Science 304, 981.

Kucej, M., Butow, R.A., 2007. Evolutionary tinkering with mitochondrial nucleoids. Trends in cell biology 17, 586-592.

Kucej, M., Kucejova, B., Subramanian, R., Chen, X.J., Butow, R.A., 2008. Mitochondrial nucleoids undergo remodeling in response to metabolic cues. Journal of cell science 121, 1861-1868.

Kulukian, A., Han, J.S., Cleveland, D.W., 2009. Unattached kinetochores catalyze production of an anaphase inhibitor that requires a Mad2 template to prime Cdc20 for BubR1 binding. Developmental cell 16, 105-117.

Laherty, C.D., Yang, W.-M., Sun, J.-M., Davie, J.R., Seto, E., Eisenman, R.N., 1997. Histone deacetylases associated with the mSin3 corepressor mediate Mad transcriptional repression. Cell 89, 349-356.

Lai, C.Y., Jaruga, E., Borghouts, C., Jazwinski, S.M., 2002. A mutation in the ATP2 gene abrogates the age asymmetry between mother and daughter cells of the yeast Saccharomyces cerevisiae. Genetics 162, 73-87.

Lakshmipathy, U., Campbell, C., 1999. Double strand break rejoining by mammalian mitochondrial extracts. Nucleic acids research 27, 1198-1204.

Lau, D.T., Murray, A.W., 2012. Mad2 and Mad3 cooperate to arrest budding yeast in mitosis. Current biology : CB 22, 180-190.

Laun, P., Bruschi, C.V., Dickinson, J.R., Rinnerthaler, M., Heeren, G., Schwimbersky, R., Rid, R., Breitenbach, M., 2007. Yeast mother cell-specific ageing, genetic (in)stability, and the somatic mutation theory of ageing. Nucleic acids research 35, 7514-7526.

Lawrimore, J., Bloom, K.S., Salmon, E.D., 2011. Point centromeres contain more than a single centromere-specific Cse4 (CENP-A) nucleosome. The Journal of cell biology 195, 573-582.

Ling, F., Shibata, T., 2002. Recombination-dependent mtDNA partitioning: in vivo role of Mhr1p to promote pairing of homologous DNA. The EMBO journal 21, 4730-4740.

Liu, P., Qian, L., Sung, J.S., de Souza-Pinto, N.C., Zheng, L., Bogenhagen, D.F., Bohr, V.A., Wilson, D.M., 3rd, Shen, B., Demple, B., 2008. Removal of oxidative DNA damage via

FEN1-dependent long-patch base excision repair in human cell mitochondria. Molecular and cellular biology 28, 4975-4987.

Liu, S.T., Hittle, J.C., Jablonski, S.A., Campbell, M.S., Yoda, K., Yen, T.J., 2003. Human CENP-I specifies localization of CENP-F, MAD1 and MAD2 to kinetochores and is essential for mitosis. Nature cell biology 5, 341-345.

Lockshon, D., Zweifel, S.G., Freeman-Cook, L.L., Lorimer, H.E., Brewer, B.J., Fangman, W.L., 1995. A role for recombination junctions in the segregation of mitochondrial DNA in yeast. Cell 81, 947-955.

London, N., Ceto, S., Ranish, J.A., Biggins, S., 2012. Phosphoregulation of Spc105 by Mps1 and PP1 regulates Bub1 localization to kinetochores. Current biology : CB 22, 900-906.

Lukas, J., Lukas, C., Bartek, J., 2011. More than just a focus: The chromatin response to DNA damage and its role in genome integrity maintenance. Nature cell biology 13, 1161-1169.

Luo, X., Yu, H., 2012. Mitosis: short-circuiting spindle checkpoint signaling. Current biology : CB 22, R128-130.

Maleszka, R., Skelly, P.J., Clark-Walker, G.D., 1991. Rolling circle replication of DNA in yeast mitochondria. The EMBO journal 10, 3923-3929.

Marston, A.L., Tham, W.H., Shah, H., Amon, A., 2004. A genome-wide screen identifies genes required for centromeric cohesion. Science 303, 1367-1370.

Masuda, T., Ling, F., Shibata, T., Mikawa, T., 2010. Analysis of DNA-binding sites on Mhr1, a yeast mitochondrial ATP-independent homologous pairing protein. The FEBS journal 277, 1440-1452.

Matson, D.R., Demirel, P.B., Stukenberg, P.T., Burke, D.J., 2012. A conserved role for COMA/CENP-H/I/N kinetochore proteins in the spindle checkpoint. Genes & development 26, 542-547.

Mbantenkhu, M., Wang, X., Nardozzi, J.D., Wilkens, S., Hoffman, E., Patel, A., Cosgrove, M.S., Chen, X.J., 2011. Mgm101 is a Rad52-related protein required for mitochondrial DNA recombination. The Journal of biological chemistry 286, 42360-42370.

McAinsh, A.D., Tytell, J.D., Sorger, P.K., 2003. Structure, function, and regulation of budding yeast kinetochores. Annual review of cell and developmental biology 19, 519-539.

McCulley, J.L., Petes, T.D., 2010. Chromosome rearrangements and aneuploidy in yeast strains lacking both Tel1p and Mec1p reflect deficiencies in two different mechanisms. Proceedings of the National Academy of Sciences of the United States of America 107, 11465-11470.

Mirchenko, L., Uhlmann, F., 2010. Sli15(INCENP) dephosphorylation prevents mitotic checkpoint reengagement due to loss of tension at anaphase onset. Current biology : CB 20, 1396-1401.

Mookerjee, S.A., Lyon, H.D., Sia, E.A., 2005. Analysis of the functional domains of the mismatch repair homologue Msh1p and its role in mitochondrial genome maintenance. Current genetics 47, 84-99.

Moore, J.D., Yazgan, O., Ataian, Y., Krebs, J.E., 2007. Diverse roles for histone H2A modifications in DNA damage response pathways in yeast. Genetics 176, 15-25.

Morrison, A.J., Highland, J., Krogan, N.J., Arbel-Eden, A., Greenblatt, J.F., Haber, J.E., Shen, X., 2004. INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. Cell 119, 767-775.

Morrison, A.J., Shen, X., 2005. DNA repair in the context of chromatin. Cell cycle 4, 568-571.

Murray, A.W., 2011. A brief history of error. Nature cell biology 13, 1178-1182.

Musacchio, A., Salmon, E.D., 2007. The spindle-assembly checkpoint in space and time. Nature reviews. Molecular cell biology 8, 379-393.

Nagy, L., Kao, H.-Y., Chakrvarti, D., Lin, R.J., Hassig, C.A., Ayer, D.E., Schreiber, S.L., Evans, R.M., 1997. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell 89, 373-380.

Naylor, M.L., Li, J.M., Osborn, A.J., Elledge, S.J., 2009. Mrc1 phosphorylation in response to DNA replication stress is required for Mec1 accumulation at the stalled fork. Proceedings of the National Academy of Sciences of the United States of America 106, 12765-12770.

Newman, S.M., Zelenaya-Troitskaya, O., Perlman, P.S., Butow, R.A., 1996. Analysis of mitochondrial DNA nucleoids in wild-type and a mutant strain of Saccharomyces cerevisiae that lacks the mitochondrial HMG box protein Abf2p. Nucleic acids research 24, 386-393.

Ng, T.M., Waples, W.G., Lavoie, B.D., Biggins, S., 2009. Pericentromeric sister chromatid cohesion promotes kinetochore biorientation. Molecular biology of the cell 20, 3818-3827.

O'Rourke, T.W., Doudican, N.A., Mackereth, M.D., Doetsch, P.W., Shadel, G.S., 2002. Mitochondrial dysfunction due to oxidative mitochondrial DNA damage is reduced through cooperative actions of diverse proteins. Molecular and cellular biology 22, 4086-4093.

Olgun, A., Akman, S., 2007. Mitochondrial DNA-deficient models and aging. Annals of the New York Academy of Sciences 1100, 241-245.

Paulovich, A.G., Margulies, R.U., Garvik, B.M., Hartwell, L.H., 1997. RAD9, RAD17, and RAD24 are required for S phase regulation in Saccharomyces cerevisiae in response to DNA damage. Genetics 145, 45-62.

Peraza-Reyes, L., Crider, D.G., Pon, L.A., 2010. Mitochondrial manoeuvres: latest insights and hypotheses on mitochondrial partitioning during mitosis in Saccharomyces cerevisiae. BioEssays : news and reviews in molecular, cellular and developmental biology 32, 1040-1049.

Piganeau, G., Eyre-Walker, A., 2004. A reanalysis of the indirect evidence for recombination in human mitochondrial DNA. Heredity 92, 282-288.

Pogorzala, L., Mookerjee, S., Sia, E.A., 2009. Evidence that msh1p plays multiple roles in mitochondrial base excision repair. Genetics 182, 699-709.

Putnam, C.D., Jaehnig, E.J., Kolodner, R.D., 2009. Perspectives on the DNA damage and replication checkpoint responses in Saccharomyces cerevisiae. DNA repair 8, 974-982.

Reenan, R.A., Kolodner, R.D., 1992a. Characterization of insertion mutations in the Saccharomyces cerevisiae MSH1 and MSH2 genes: evidence for separate mitochondrial and nuclear functions. Genetics 132, 975-985.

Reenan, R.A., Kolodner, R.D., 1992b. Isolation and characterization of two Saccharomyces cerevisiae genes encoding homologs of the bacterial HexA and MutS mismatch repair proteins. Genetics 132, 963-973.

Ricke, R.M., van Deursen, J.M., 2013. Aneuploidy in health, disease, and aging. The Journal of cell biology 201, 11-21.

Rieder, C.L., Schultz, A., Cole, R., Sluder, G., 1994. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. The Journal of cell biology 127, 1301-1310.

Rodley, C.D., Grand, R.S., Gehlen, L.R., Greyling, G., Jones, M.B., O'Sullivan, J.M., 2012. Mitochondrial-nuclear DNA interactions contribute to the regulation of nuclear transcript levels as part of the inter-organelle communication system. PloS one 7, e30943.

Rogakou, E.P., Pilch, D.R., Orr, A.H., Ivanova, V.S., Bonner, W.M., 1998. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. The Journal of biological chemistry 273, 5858-5868.

Rosenquist, T.A., Zharkov, D.O., Grollman, A.P., 1997. Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. Proceedings of the National Academy of Sciences of the United States of America 94, 7429-7434.

Rouse, J., Jackson, S.P., 2002. Interfaces between the detection, signaling, and repair of DNA damage. Science 297, 547-551.

Ruis, H., Schuller, C., 1995. Stress signaling in yeast. BioEssays : news and reviews in molecular, cellular and developmental biology 17, 959-965.

Sage, J.M., Gildemeister, O.S., Knight, K.L., 2010. Discovery of a novel function for human Rad51: maintenance of the mitochondrial genome. The Journal of biological chemistry 285, 18984-18990.

Sandall, S., Severin, F., McLeod, I.X., Yates, J.R., 3rd, Oegema, K., Hyman, A., Desai, A., 2006. A Bir1-Sli15 complex connects centromeres to microtubules and is required to sense kinetochore tension. Cell 127, 1179-1191.

Santaguida, S., Musacchio, A., 2009. The life and miracles of kinetochores. The EMBO journal 28, 2511-2531.

Santocanale, C., Diffley, J.F., 1998. A Mec1- and Rad53-dependent checkpoint controls late-firing origins of DNA replication. Nature 395, 615-618.

Schwab, M., Lutum, A.S., Seufert, W., 1997. Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. Cell 90, 683-693.

Schwartz, M.F., Duong, J.K., Sun, Z., Morrow, J.S., Pradhan, D., Stern, D.F., 2002. Rad9 phosphorylation sites couple Rad53 to the Saccharomyces cerevisiae DNA damage checkpoint. Molecular cell 9, 1055-1065.

Shah, J.V., Botvinick, E., Bonday, Z., Furnari, F., Berns, M., Cleveland, D.W., 2004. Dynamics of centromere and kinetochore proteins; implications for checkpoint signaling and silencing. Current biology : CB 14, 942-952.

Shcheprova, Z., Baldi, S., Frei, S.B., Gonnet, G., Barral, Y., 2008. A mechanism for asymmetric segregation of age during yeast budding. Nature 454, 728-734.

Sheltzer, J.M., Blank, H.M., Pfau, S.J., Tange, Y., George, B.M., Humpton, T.J., Brito, I.L., Hiraoka, Y., Niwa, O., Amon, A., 2011. Aneuploidy drives genomic instability in yeast. Science 333, 1026-1030.

Shroff, R., Arbel-Eden, A., Pilch, D., Ira, G., Bonner, W.M., Petrini, J.H., Haber, J.E., Lichten, M., 2004. Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Current biology : CB 14, 1703-1711.

Siede, W., Friedberg, A.S., Friedberg, E.C., 1993. RAD9-dependent G1 arrest defines a second checkpoint for damaged DNA in the cell cycle of Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America 90, 7985-7989.

Spector, L.M., Fogel, S., 1992. Mitotic hyperploidy for chromosomes VIII and III in Saccharomyces cerevisiae. Current genetics 21, 309-318.

Spencer, V.A., Davie, J.R., 1999. Role of covalent modifications of histones in regulating gene expression. Gene 240, 1-12.

Stephens, A.D., Haase, J., Vicci, L., Taylor, R.M., 2nd, Bloom, K., 2011. Cohesin, condensin, and the intramolecular centromere loop together generate the mitotic chromatin spring. The Journal of cell biology 193, 1167-1180.

Storchova, Z., Becker, J.S., Talarek, N., Kogelsberger, S., Pellman, D., 2011. Bub1, Sgo1, and Mps1 mediate a distinct pathway for chromosome biorientation in budding yeast. Molecular biology of the cell 22, 1473-1485.

Struhl, K., 1998. Histone acetylation and transcriptional regulatory mechanisms. Genes Dev 12, 599-606.

Sun, Z., Hsiao, J., Fay, D.S., Stern, D.F., 1998. Rad53 FHA domain associated with phosphorylated Rad9 in the DNA damage checkpoint. Science 281, 272-274.

Swayne, T.C., Zhou, C., Boldogh, I.R., Charalel, J.K., McFaline-Figueroa, J.R., Thoms, S., Yang, C., Leung, G., McInnes, J., Erdmann, R., Pon, L.A., 2011. Role for cER and Mmr1p in anchorage of mitochondria at sites of polarized surface growth in budding yeast. Current biology : CB 21, 1994-1999.

Sweeney, F.D., Yang, F., Chi, A., Shabanowitz, J., Hunt, D.F., Durocher, D., 2005. Saccharomyces cerevisiae Rad9 acts as a Mec1 adaptor to allow Rad53 activation. Current biology : CB 15, 1364-1375.

Szczesny, B., Tann, A.W., Longley, M.J., Copeland, W.C., Mitra, S., 2008. Long patch base excision repair in mammalian mitochondrial genomes. The Journal of biological chemistry 283, 26349-26356.

Tanaka, K., Mukae, N., Dewar, H., van Breugel, M., James, E.K., Prescott, A.R., Antony, C., Tanaka, T.U., 2005. Molecular mechanisms of kinetochore capture by spindle microtubules. Nature 434, 987-994.

Taylor, S.D., Zhang, H., Eaton, J.S., Rodeheffer, M.S., Lebedeva, M.A., O'Rourke T, W., Siede, W., Shadel, G.S., 2005. The conserved Mec1/Rad53 nuclear checkpoint pathway regulates mitochondrial DNA copy number in Saccharomyces cerevisiae. Molecular biology of the cell 16, 3010-3018.

Thyagarajan, B., Padua, R.A., Campbell, C., 1996. Mammalian mitochondria possess homologous DNA recombination activity. The Journal of biological chemistry 271, 27536-27543.

Tien, J.F., Umbreit, N.T., Gestaut, D.R., Franck, A.D., Cooper, J., Wordeman, L., Gonen, T., Asbury, C.L., Davis, T.N., 2010. Cooperation of the Dam1 and Ndc80 kinetochore complexes enhances microtubule coupling and is regulated by aurora B. The Journal of cell biology 189, 713-723.

Uffenbeck, S.R., Krebs, J.E., 2006. The role of chromatin structure in regulating stress-induced transcription in Saccharomyces cerevisiae. Biochemistry and cell biology = Biochimie et biologie cellulaire 84, 477-489.

van Attikum, H., Fritsch, O., Gasser, S.M., 2007. Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. The EMBO journal 26, 4113-4125.

van Attikum, H., Fritsch, O., Hohn, B., Gasser, S.M., 2004. Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. Cell 119, 777-788.

Vazquez-Novelle, M.D., Petronczki, M., 2010. Relocation of the chromosomal passenger complex prevents mitotic checkpoint engagement at anaphase. Current biology : CB 20, 1402-1407.

Veatch, J.R., McMurray, M.A., Nelson, Z.W., Gottschling, D.E., 2009. Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. Cell 137, 1247-1258.

Vernon, M., Lobachev, K., Petes, T.D., 2008. High rates of "unselected" aneuploidy and chromosome rearrangements in tel1 mec1 haploid yeast strains. Genetics 179, 237-247.

Vialard, J.E., Gilbert, C.S., Green, C.M., Lowndes, N.F., 1998. The budding yeast Rad9 checkpoint protein is subjected to Mec1/Tel1-dependent hyperphosphorylation and interacts with Rad53 after DNA damage. The EMBO journal 17, 5679-5688.

Visintin, R., Prinz, S., Amon, A., 1997. CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. Science 278, 460-463.

Vongsamphanh, R., Fortier, P.K., Ramotar, D., 2001. Pir1p mediates translocation of the yeast Apn1p endonuclease into the mitochondria to maintain genomic stability. Molecular and cellular biology 21, 1647-1655.

Wan, X., O'Quinn, R.P., Pierce, H.L., Joglekar, A.P., Gall, W.E., DeLuca, J.G., Carroll, C.W., Liu, S.T., Yen, T.J., McEwen, B.F., Stukenberg, P.T., Desai, A., Salmon, E.D., 2009. Protein architecture of the human kinetochore microtubule attachment site. Cell 137, 672-684.

Weinert, T.A., Hartwell, L.H., 1988. The RAD9 gene controls the cell cycle response to DNA damage in Saccharomyces cerevisiae. Science 241, 317-322.

Westermann, B., Neupert, W., 2000. Mitochondria-targeted green fluorescent proteins: convenient tools for the study of organelle biogenesis in Saccharomyces cerevisiae. Yeast 16, 1421-1427.

White, D.J., Gemmell, N.J., 2009. Can indirect tests detect a known recombination event in human mtDNA? Molecular biology and evolution 26, 1435-1439.

Whitlock, J.P., Jr., Simpson, R.T., 1977. Localization of the sites along nucleosome DNA which interact with NH2-terminal histone regions. The Journal of biological chemistry 252, 6516-6520.

Williamson, D., 2002. The curious history of yeast mitochondrial DNA. Nature reviews. Genetics 3, 475-481.

Winey, M., Mamay, C.L., O'Toole, E.T., Mastronarde, D.N., Giddings, T.H., Jr., McDonald, K.L., McIntosh, J.R., 1995. Three-dimensional ultrastructural analysis of the Saccharomyces cerevisiae mitotic spindle. The Journal of cell biology 129, 1601-1615.

Wiuf, C., 2001. Recombination in human mitochondrial DNA? Genetics 159, 749-756.

Wolffe, A., 1999. Chromatin Structure and Function, 3rd ed. Academic Press, San Diego.

Woo, D.K., Phang, T.L., Trawick, J.D., Poyton, R.O., 2009. Multiple pathways of mitochondrial-nuclear communication in yeast: intergenomic signaling involves ABF1 and affects a different set of genes than retrograde regulation. Biochimica et biophysica acta 1789, 135-145.

Wyatt, H.R., Liaw, H., Green, G.R., Lustig, A.J., 2003. Multiple roles for Saccharomyces cerevisiae histone H2A in telomere position effect, Spt phenotypes and double-strand-break repair. Genetics 164, 47-64.

Yamagishi, Y., Honda, T., Tanno, Y., Watanabe, Y., 2010. Two histone marks establish the inner centromere and chromosome bi-orientation. Science 330, 239-243.

Yang, C., Wang, H., Xu, Y., Brinkman, K.L., Ishiyama, H., Wong, S.T., Xu, B., 2012. The kinetochore protein Bub1 participates in the DNA damage response. DNA repair 11, 185-191.

Yazgan, O., Krebs, J.E., 2012. Mitochondrial and nuclear genomic integrity after oxidative damage in Saccharomyces cerevisiae. Frontiers in bioscience : a journal and virtual library 17, 1079-1093.

Yoshikawa, K., Furusawa, C., Hirasawa, T., Shimizu, H., 2008. Genome-wide analysis of the effects of location and number of stress response elements on gene expression in Saccharomyces cerevisiae. J Biosci Bioeng 106, 507-510.

You, H.J., Swanson, R.L., Harrington, C., Corbett, A.H., Jinks-Robertson, S., Senturker, S., Wallace, S.S., Boiteux, S., Dizdaroglu, M., Doetsch, P.W., 1999. Saccharomyces cerevisiae Ntg1p and Ntg2p: broad specificity N-glycosylases for the repair of oxidative DNA damage in the nucleus and mitochondria. Biochemistry 38, 11298-11306.

Yu, H., 2007. Cdc20: a WD40 activator for a cell cycle degradation machine. Molecular cell 27, 3-16.

Zheng, C., Hayes, J.J., 2003. Structures and interactions of the core histone tail domains. Biopolymers 68, 539-546.

Zhu, J., Pavelka, N., Bradford, W.D., Rancati, G., Li, R., 2012. Karyotypic determinants of chromosome instability in aneuploid budding yeast. PLoS genetics 8, e1002719.

Zimonjic, D., Brooks, M.W., Popescu, N., Weinberg, R.A., Hahn, W.C., 2001. Derivation of human tumor cells in vitro without widespread genomic instability. Cancer research 61, 8838-8844.

Zou, L., Elledge, S.J., 2003. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300, 1542-1548.

APPENDIX

Specific Aim: *To determine whether mitochondrial genomic instability in H2A-S122A mutants is due to transcriptional defects in nuclear-encoded genes***.** To address this aim I used whole genome microarray technology to measure transcriptional changes upon introduction of the H2A-S122A mutation.

Experimental Details and Results:

For this experiment, I created twelve H2A-S122A clones by introducing the H2A-S122A mutation on a plasmid as their sole source of histone H2A. I also created twelve new wildtype clones in the same manner. Every three days, these newly created strains were passaged onto new YPD plates. At the time of passage, I collected samples for RNA isolation and tested each strain for mitochondrial function by plating the same number of cells on plates containing either glycerol or glucose as the sole carbon source, and then using the ratio of colonies formed on media containing glycerol to those on glucose (glycerol/glucose ratio) to determine respiratory competence (i.e. mitochondrial function). This experiment was done in duplicate and the YPG/YPD ratio for the two independent experiments can be seen in Figure A1.

Once I completed six passages, I collected and pooled RNA for ten wildtype and ten H2A-S122A strains corresponding to three stages of respiratory function: before cells begin losing their ability to respire (high respiratory function), during respiration loss (low respiratory function), and post mitochondrial function loss (no respiratory function): when the wildtype glycerol/glucose ratio is greater than 85%, between 55 and 53%, and at 0% respectively and greater than 60%, between 40 and 38%, and at 0% respectively in the H2A-S122A mutant. The described method of passaging does not give rise to high glycerol/glucose ratios in the S122A mutant as the strain presumably begins the process of mitochondrial function loss early in colony development, so that by the time the colony is grown enough for sample collection and passage, the glycerol/glucose ratio is already quite low with an average below 60%.

To capture the H2A-S122A mutant cells prior to mitochondrial loss, I devised another collection method where the cells were grown in liquid media following the introduction of either wildtype-H2A or the S122A-H2A plasmids. While growing the cells in liquid media

allows us to capture earlier stages of mitochondrial loss, it is clear that this is a poor approach for observing mitochondrial loss over several passages, for in liquid culture those cells that maintain mitochondria unsurprisingly out-compete those cells that have lower glycerol/glucose ratios (Figure A2a). Fortunately, the first passage allows for the collection of RNA from respiratory-competent H2A-S122A mutants prior to mitochondria loss (log phase of passage 1) as well as from cells displaying clear mitochondrial dysfunction (stationary phase of passage 1). Thus I collected RNA from three wildtype H2A and three S122A-H2A clones for both the log and stationary phase of passage 1 (the glycerol/glucose ratio for these samples are shown in Figure A2b).

A total of 18 RNA samples (6 samples representing 60 pooled samples grown and passaged on plates and 12 individual samples grown and passaged in liquid media) were sent for gene expression analysis (synthesis of cDNA from the RNA and hybridization to microarrays to analyze RNA levels for approximately 6000 *Saccharomyces cerevisiae* genes).

There are a large number of nuclear encoded genes that influence mitochondrial replication, inheritance and genomic stability; however none of those genes exhibit an increase or decrease greater than two-fold in the high respiratory function group (data not shown). In fact, the transcriptomes of the S122A and WT cells with high respiratory function are quite similar in both the pooled colony and liquid culture samples (data not shown). This leads us to believe that the mitochondrial genome instability demonstrated by the S122A mutant is not likely to be due to specific transcriptional defects, though we have not formally ruled out that small effects on the expression of one or more genes might contribute to this phenotype.

While it does not appear that introduction of the S122A mutation *immediately* results in transcriptional perturbations, our data indicate that mutant cells undergo global transcriptional changes once the S122A cells demonstrate low respiratory function. Figure A3 shows that the number of genes being over-expressed compared to wildtype cells of similar respiratory capabilities is much higher in the low respiratory cells than in either the high-function or non-function groups. This suggests that as the S122A cells lose their ability to respire, the transcriptional response differs from that of wildtype cells. Yet, as mitochondrial function diminishes to nothing, the S122A cells once again have

transcriptomes similar to that of wildtype (Figure A3). Upon further analysis of the genes being over-expressed in the low respiratory function group, we find that most of these genes are regulated in response to stress.

Transcriptional responses to stress are highly regulated and include the up-regulation of specific stress protectant proteins, the up-regulation of general stress protectant proteins as well as the down-regulation of non-critical genes such as housekeeping genes. This tight regulation occurs through the use of transcriptional elements that are specific for particular transcription factors, allowing for a number of genes to be activated simulaniously by the same factor. Furthermore, a number of stress response genes are under the control of more than one stress response element. Chromatin architecture, (ie. post-translational modifications of pertinenant histone tails and the number and location of stress response elements) allows for the implemenation and fine tuning at each individual gene promotor (for review see: Uffenbeck and Krebs, 2006). STress Response Elements (STREs) are found in a number or stress response gene promoters and are regulated by the transcription factors Msn2 and Msn4 in response to a large variety of stresses; while the transcription factors Hsf1 and Yap1 regulate genes expressed in response to heat shock and oxidative stress respectiviely (for review see: Ruis and Schuller, 1995).

As stated above, I observe increased transcription levels for stress response genes in the low respiratory function S122A cells. Using a list of all STRE-containing genes and their location provided by Yoshikawa et al., I was able to calculate the prevalence of STRE containing genes in our dataset (Yoshikawa et al., 2008). In fact, of the 399 genes with a two-fold increase in transcript levels, 81% have an STRE in the promoter region, and of the 68 with a four-fold increase, 87% have an upstream STRE (Figure A4b). Up-regulation of STRE-containing genes is clearly beginning to occur in cells with high respiratory function, however by the time cells have lost all respiratory function their transcriptional stress responses have passed (Figure A4a and A4c). One explanation for the observed stress response is that as mitochondrial dysfunction increases, levels of reactive oxygen species are significantly increased due to inefficient oxidative phosphorylation. Once mitochondrial function has stopped all together, ROS caused by poor oxidative phosphorylation would diminish. Although the pattern of stress response corresponds with the expected increase and subsequent decrease in ROS, we would expect that the over-expressed genes would show

greater enrichment for genes regulated by Yap1. While the prevalence of Yap1 regulated genes is higher in the two-fold over-expressed genes compared to all genes on the microarray, there is actually a greater increase in enrichment for genes regulated by Hsf1 (Figure A4d-e) demonstrates that the increased enrichment of Hsf1 is not due to overlapping regulatory functions with Msn2 at the same genes. Therefore, it is clear that these cells are experiencing some degree of stress; however the cause of stress is unclear.

To analyze further transcriptional patterns of the low respiratory function S122A cells, gene ontology lists provided by the *Saccharomyces* Genome Database were used to categorize genes based on their function. The transcript levels (i.e. $\log_2$(S122A/WT)) for all genes in the associated category were then averaged. Interestingly, genes associated with the heat shock response were the most elevated group observed, while oxidative stress associated genes showed a less remarkable increase (Figure A5) which is consistent with the number of over-expressed genes regulated by the corresponding transcription factor (Figure A4d-e). Also showing greater increases in average transcript levels are those genes involved in autophagy and mitochondria-specific autophagy, known as mitophagy (Figure A5). Interestingly, known genes involved in mitochondrial maintenance and inheritance were only slightly elevated.

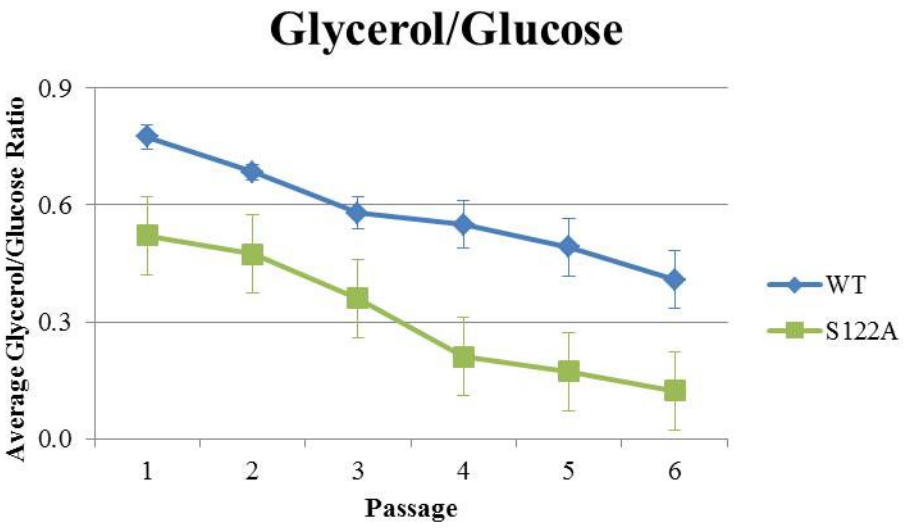

Figure A1: H2A-S122A leads to decreased mitochondrial function. Two batches of cells were transformed with either the wildtype plasmid (diamond) or the H2A-S122A mutant (square). Twelve large colonies from each transformation were selected and their lineage was followed over six passages. The strains were passaged every third day when each lineage was propagated from a randomly chosen colony and analyzed for mitochondrial function using glycerol/glucose ratio.

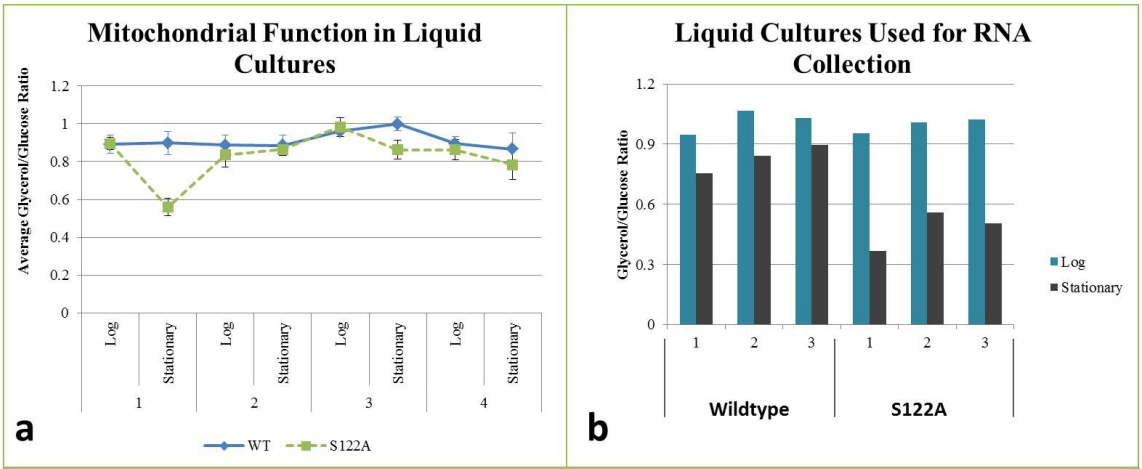

Figure A2: H2A-S122A leads to decreased mitochondrial function. **(a)** A batch of cells was transformed with either the wildtype plasmid (diamond) or the H2A-S122A mutant (square). Twelve large colonies from each transformation were selected and their lineage was followed over four complete passages with the cells being maintained only in liquid media. For each passage, cells were collected (for RNA extraction) and glycerol/glucose ratios were obtained during log phase (OD_{600} = 0.4 - 0.8) and then at or near stationary phase (after 24 hours) where they were also repassaged. **(b)** Three wildtype and mutant strains were chosen and RNA was collected for both the log and stationary phase of passage 1. The glycerol/glucose ratio for those samples are shown.

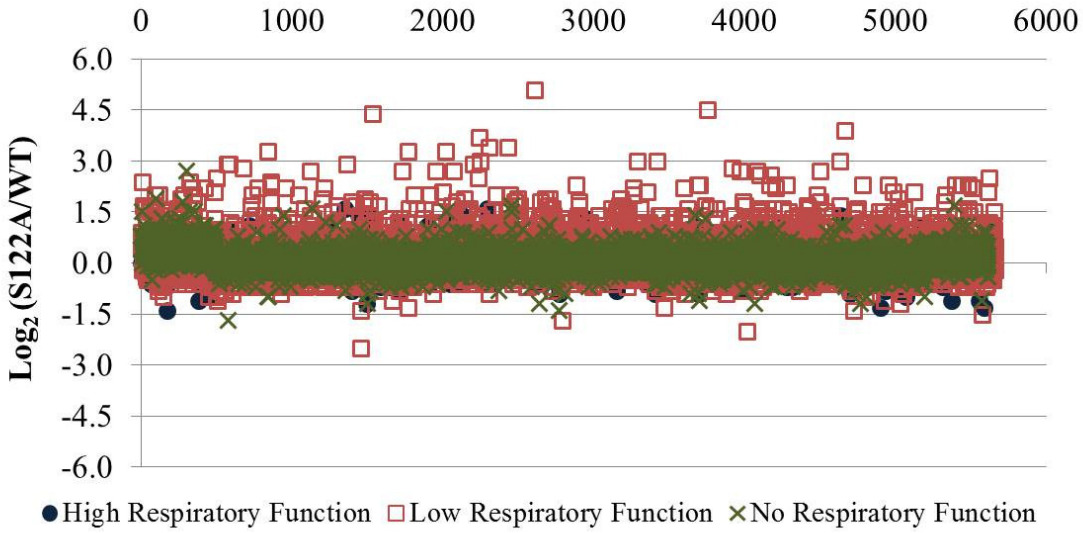

Figure A3: Loss of mitochondrial DNA does not appear to be caused by gross transcriptional defects. The log2 ratio of the H2A-S122A to wildtype transcript levels for each individual gene is plotted according to its position in the genome, ie. the first gene of chromosome one plotted to the farthest left.

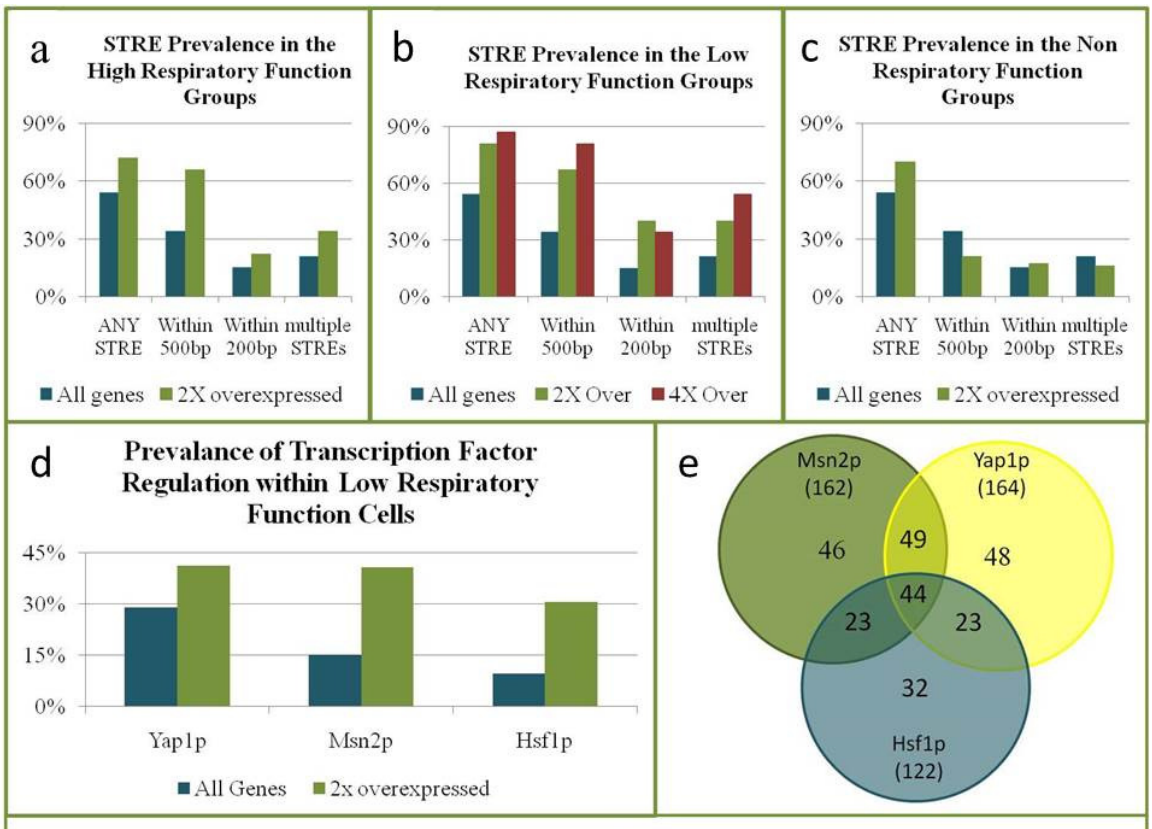

Figure A4: Stress response genes are up-regulated in H2A-S122A mutant cells during mitochondrial function loss. The percentage of stress response element (STRE) containing genes was found for all genes within the microarray vs. genes that were either two-fold or four-fold increased compared to wildtype for each respiratory function category (a-c). The percentage of genes known to be regulated b either Yap1p, Msn2p, or Hsf1p were also compared for all genes in the microarray and those with a 2-fold increase in the low respiratory function grouped (d). The Venn diagram demonstrates that the transcription factors disturbed for the 399 genes that exhibit a two-fold or greater in crease in transcript levels in the low functional group (e).

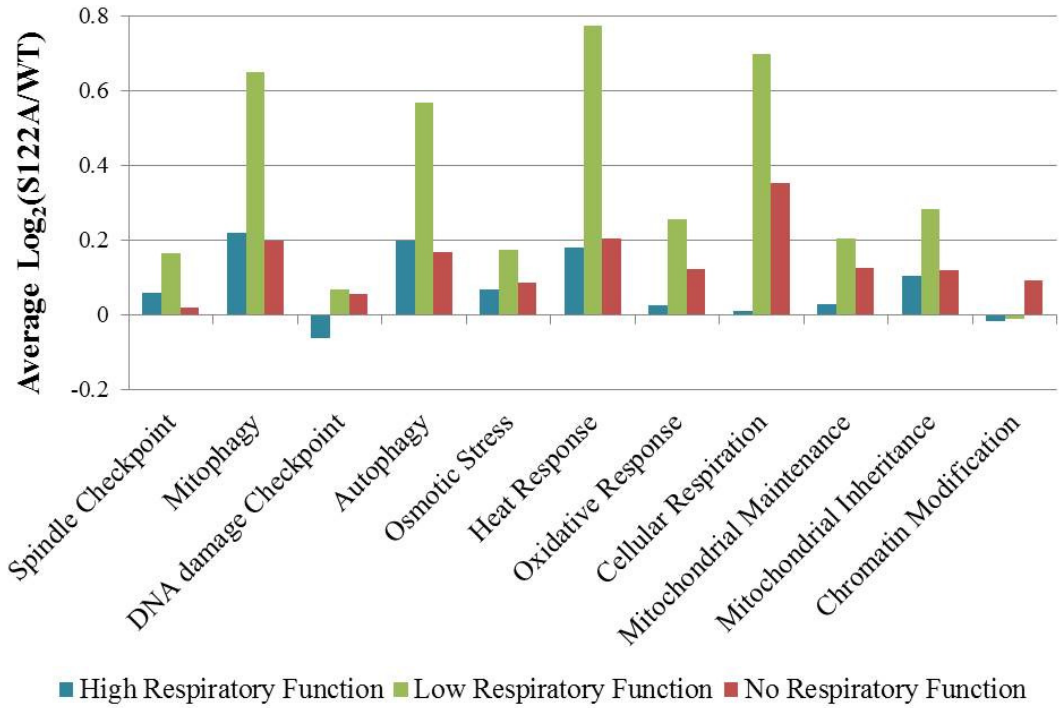

Figure A5: Genes associated with mitophagy, autophagy, heat shock, and respiration display increased transcription in low respiratory functioning H2A-S122A mutants. Gene onotology provided by the Saccharomyces database (www.yeastgenome.org) was used to determine genes belonging to each functional group. The log ratios for all genes for each functional group was then averaged.

References

Ruis, H., Schuller, C., 1995. Stress signaling in yeast. BioEssays : news and reviews in molecular, cellular and developmental biology 17, 959-965.

Uffenbeck, S.R., Krebs, J.E., 2006. The role of chromatin structure in regulating stress-induced transcription in Saccharomyces cerevisiae. Biochemistry and cell biology = Biochimie et biologie cellulaire 84, 477-489.

Yoshikawa, K., Furusawa, C., Hirasawa, T., Shimizu, H., 2008. Genome-wide analysis of the effects of location and number of stress response elements on gene expression in Saccharomyces cerevisiae. J Biosci Bioeng 106, 507-510.