A Role for the Histone Variant HTZ1 in Meiotic Chromosome Segregation

by

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ABSTRACT

In Eukaryotic cells, the packaging of genomic DNA into chromatin has important consequences for all DNA-dependent transactions. Chromatin structure is highly regulated by a variety of complex processes that are not well understood. These include nucleosome remodeling and post-translational modification of histone proteins (Dunn & Kingston, 2007; Kouzarides, 2007; Workman, 2006). An additional mechanism for chromatin regulation is the replacement of conventional histones with specific non-allelic variants. H2AZ, a highly conserved variant of histone H2A, is of particular interest because it is essential for viability in multicellular organisms and it has been implicated in many distinct and even contradictory functions. Despite extensive evidence implicating H2AZ in maintenance of genome stability, centromere structure and function, and chromosome segregation, a role for H2AZ in meiosis has not been investigated. The budding yeast *Saccaromyces cerevisiae*, a classical model for cell division studies, constitutes a highly amenable system in which to approach this question. In this study, deletion of the *S. cerevisiae* H2AZ homologue, Htz1, resulted in classical meiotic defect phenotypes such as reduced sporulation efficiency, impaired spore viability, and displayed a reduced ability to progress through meiosis. Htz1 deletion strains also showed an increase in chromosome nondisjunction during both meiosis I and II and premature sister chromatid separation during meiosis I. These results suggest a novel role for H2AZ in regulating meiotic chromosome segregation and possibly in centromeric protection and kinetochocore co-orientation and further illustrate how defects in H2AZ function may contribute to human diseases such as cancer.

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ABBREVIATIONS

ATP	-	Adenosine TriPhosphate
bp	-	Base pairs *(DNA sequence length)*
BUB1	-	Budding Uninhibited by Benzimidazoles 1
CEN	-	Centromere
CenH3	-	Centromere-specific variant of histone H3
CPC	-	Chromosome Passenger Complex
DAPI	-	4',6-diamidino-2-phenylindole
H2AZ	-	H2A histone family, member Z
H3K9	-	Lysine 9 of histone 3
HTZ1	-	Histone Two A Z 1 *(Budding yeast H2AZ homolog)*
IPL1	-	Increase in PLoidy 1 *(Budding yeast Aurora B kinase homolog)*
GFP	-	Green Fluorescent Protein
kb	-	Kilobases *(DNA sequence length)*
PHT1	-	Pombe Histone Two A Z 1 *(Fission yeast H2AZ homolog)*
SAC	-	Spindle Assembly Checkpoint
SPO11	-	SPOrulation 11
SGO	-	ShuGOshin
Tet	-	Tetracycline
TetO	-	Tet operon
TetR	-	Tet repressor
TetR::GFP	-	Tet repressor/GFP fusion protein

NOTE: Standard S. cerevisiae gene notation is followed throughout the text:

- Fully capitalized names denote genes or DNA loci (e.g. HTZ1, SPO11);
- Lower case with a capitalized initial denotes protein (e.g. Htz1, Spo11);
- Italicized names indicate gene deletions (e.g. *htz1*, *spo11*).

INTRODUCTION

Chromatin structure and organization

In eukaryotic nuclei, the basic subunit of chromatin is the nucleosome, comprising ~146 base pairs of DNA wound around an octameric protein core that is composed of two copies each of histones H2A, H2B, H3, and H4. Nucleosomes are interspersed with histone H1-bound linker DNA and can be further compacted to form progressively higher order chromatin structures. Chromatin structure is non-randomly organized within the nucleus into specialized structures such as euchromatin and heterochromatin, regions of less and more densely packaged chromatin, respectively (Schneider & Grosschedl 2007; Polo & Almouzni, 2006; Fraser & Bickmore, 2007). Rather than serving a purely structural role, the regulation of chromatin structure can profoundly influence DNA-dependent processes by modulating the accessibility of the underlying genomic sequence. This sequence-independent 'epigenetic' regulation is therefore critical for DNA replication and repair, gene expression, and genome integrity.

Chromatin structure and organization can be modulated by a number of different mechanisms that together specify an epigenetic code for genome regulation (Goldberg *et al*., 2007; Bernstein *et al*., 2007). For example, ATP-dependent remodeling complexes, such as SWI/SNF superfamily members, can alter chromatin organization and nucleosomal DNA accessibility (Varga-Weisz & Becker, 2006; Choudhary & Varga-Weisz, 2007). In addition, histones are subject to a variety of covalent post-translational modifications, including methylation, acetylation, phosphorylation, ubiquitination, ADP-

ribosylation and SUMOylation. Such modifications can directly impact chromatin structure or act to recruit other regulatory factors and distinct patterns of histone modifications can be found to correlate with gene activity and mark regions of euchromatin vs. heterochromatin (Kouzarides, 2007; Cosgrove *et al*., 2004). Another important mechanism for chromatin regulation is the replacement of conventional histones within the nucleosome with non-allelic variants (Kamakaka & Biggins, 2005, Bernstein & Hake, 2006; Eirín-López & Ausió, 2007). While conventional histones are transcribed from multi-copy gene loci at the onset of S phase, replacement histones are encoded by evolutionarily conserved single genes and are transcribed in a cell cycle independent manner. Histone variants can vary widely in their amino acid sequence as compared to the major histones and their incorporation can affect nucleosome structure and stability. Interestingly, variants are generally localized to discrete genomic loci, unlike canonical histones which are randomly distributed throughout the genome (Polo & Almouzni, 2006; Krogan *et al*., 2003; Mizuguchi *et al*., 2004). Therefore, histone variants likely also contribute to the organization of structurally and functionally distinct chromatin domains. Collectively, these epigenetic processes function together with genetic factors and form the basis of a highly intricate network for genome regulation and function.

The histone variant H2AZ

H2AZ, a variant of H2A, is highly conserved from yeast to man (Iouzalen *et al*., 1996). It is more highly conserved than the canonical H2A, suggesting a functionally important role for this variant in all eukaryotes (Jackson & Gorovsky, 2000). Accordingly, H2AZ

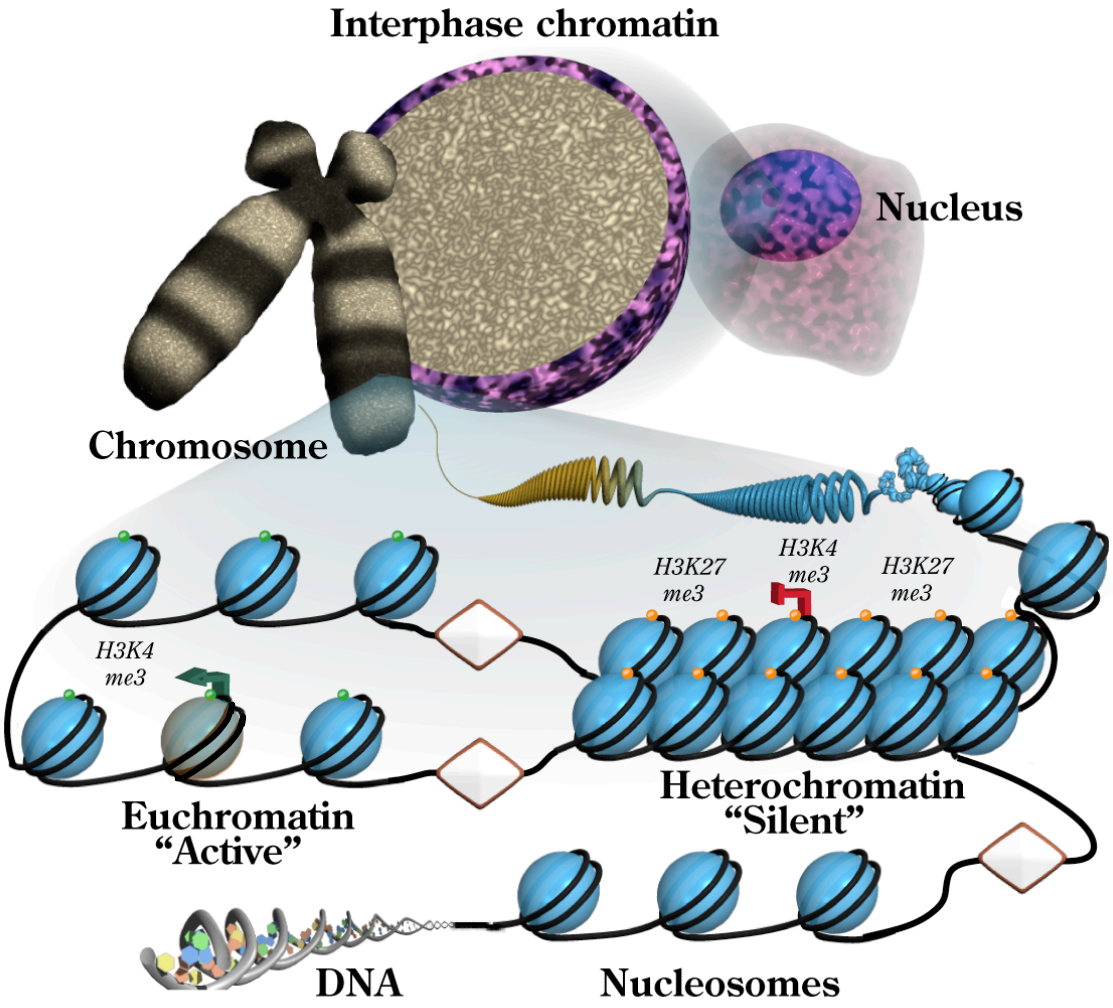


Figure 1: Epigenetics overview
The basic subunit of chromatin consists of nucleosomes *(blue spheres)*, comprising ~146 base pairs of DNA wound about an octameric protein core. Nucleosomes associate to form progressively higher order chromatin structures, compacting the genome into a suitable volume and organizing the nucleus into specialized structures such as euchromatin and heterochromatin. Factors that contribute to and help regulate this compaction and structural organization include ATP-dependent nucleosome remodeling, deposition of post-translational modifications *(represented by green and orange spheres)* onto histones, and targeted replacement of conventional histones with specialized variants *(shaded)*. Collectively, these epigenetic processes function together with genetic factors and form the basis of a highly intricate network for genome regulation and function.

is essential for viability in *Tetrahymena thermophila*, *Drosophila melanogaster*, *Xenopus laevis*, and mouse (Liu *et al*., 1996; van Daal & Elgin, 1992; Ridgway *et al*., 2004; Faast *et al.,* 2001). H2AZ has been implicated in a wide variety of distinct and even contradictory functions. For example, H2AZ has been connected to processes such as DNA replication, genome stability, cell cycle progression (Dhillon *et al*., 2006) and in both gene activation and repression (Allis *et al*., 1980; Santisteban *et al*., 2000, LaRochelle & Gaudreau, 2003; Swaminathan et al., 2005; Sarcinella *et al*., 2007), as well as in heterochromatin formation and in preventing the spreading heterochromatin domains (Swaminathan *et al*., 2005; Babiarz *et al*., 2006; Rangasamy *et al*., 2004; Meneghini *et al*., 2003). The extent to which these various functions are organism-, cell-type-, or context-specific remains unclear (Zlatanova & Thakar, 2008; Guillemette & Gaudreau, 2006;).

Epigenetic control of cell division and chromosome segregation

Faithful chromosome segregation during cell division is critical for genome integrity and stability, as segregation errors are irreversible and result in aneuploidy (Marston & Amon, 2004; Uhlmann, 2003; Ekwall, 2007). After DNA replication, sister chromatids are held together by cohesin complexes. Upon subsequent cell division, whether conservative (Mitosis) or reductional (Meiosis), spindle microtubules attach to chromosomes through kinetochore complexes assembled at the centromere. During mitosis and meiosis II, sister kinetochores attach to a different spindle pole, which results in proper chromatid segregation upon loss of cohesion. In contrast, meiosis proceeds by a stepwise loss of cohesion along the chromosome. meiosis I segregation of homologous

chromosomes requires loss of cohesion at the chromosome arms. Retention of cohesion at centromeres along with sister kinetochore co-orientation (attachment to the same spindle pole) ensures that sister chromatids co-segregate to the same daughter cell so that they can segregate properly during subsequent meiosis II (**Figure 2**; reviewed in Marston & Amon, 2004; Revenkova & Jessberger, 2005; Uhlmann, 2003). Accordingly, cell division is very tightly regulated, and several redundant and mutually reinforcing mechanisms are in place to prevent aberrant segregation events (Reviewed in Marston & Amon, 2004; Ruchaud *et al*., 2007; Musacchio & Salmon, 2007).

Centromeres are specialized chromatin domains that constitute the sites of kinetochore assembly and microtubule attachment (Reviewed in Ekwall, 2007; Black & Bassett, 2008; Vagnarelli *et al*., 2008). Centromeric DNA sequences, while believed to favor centromere assembly and stabilization, are not substantially conserved between species and are not sufficient for *de novo* centromere formation (du Sart *et al*., 1997). Rather, centromeres are primarily defined by the presence of the histone variant CenH3 in place of conventional H3. CenH3 is highly conserved and essential in all eukaryotes, and is sufficient to help establish and maintain centromeric domain identity (Kamakaka & Biggins, 2005; Ekwall, 2007). The distribution of CenH3-containing nucleosomes throughout the centromeric region (Blower *et al*., 2002; Sullivan & Karpen, 2004) nucleates assembly of the kinetochore complex (Reviewed in Cheeseman & Desai, 2008; Westermann *et al*., 2007). Different kinetochore components and subcomplexes are responsible for microtubule attachment, quality control checkpoints, and even help to stabilize the centromere by reinforcing CenH3 incorporation (Okada *et al*., 2006). Thus,

the major determinants of centromere identity and function are primarily epigenetic in nature (Gieni *et al*., 2008; Morris & Moazed, 2007).

Centromere formation in fission yeast (*Schizossacharomyces pombe*) and vertebrates also depends on flanking pericentric heterochromatin. This region is characterized by histone modifications such as tri-methylated lysine 9 of histone 3 (H3K9me3), an epigenetic mark that serves to recruit heterochromatin protein 1 (HP1), and its formation has been shown to require HP1 and the RNA interference machinery (Grewal & Jia., 2007; Verdel & Moazed, 2005). Preventing pericentric heterochromatin formation by interfering with H3K9 methylation, HP1 function, or RNAi impairs CenH3 deposition and kinetochore assembly, resulting in defects in chromosome cohesion and segregation and increased chromosome loss (Folko *et al*., 2008; Grewal & Jia, 2007; Durand-Dubief & Ekwall 2008; Pidoux & Allshire, 2005; Kanellopoulou *et al*., 2005). Thus, the establishment and maintenance of pericentric heterochromatin in *S. pombe* and metazoans by epigenetic factors constitutes another example of the importance of chromatin modulation in regulating the process of cell division.

Centromeres and kinetochores are the platforms for microtubule attachment and for the maintenance of cohesion, critical cell division processes in which Shugoshin (Sgo) proteins play a regulatory role (Kawashima *et al*., 2007; Lee *et al*., 2008; Watanabe, 2005 and references therein). Sgo proteins are structurally and functionally conserved from yeast to mammals; while budding yeast and *Drosophila melanogaster* have a single copy of Sgo (Sgo1 in *S. cerevisiae*), fission yeast, plants and mammals have two family

members which divide the two functions. Sgo proteins help ensure proper spindle attachment by recruiting Aurora B kinase to improper kinetochore-microtubule attachments (Kawashima *et al.,* 2007; Vanoosthuyse *et al*., 2007; Indjeian *et al*., 2005, 2007). Aurora B (*Ipl1* in *S. cerevisiae*) constitutes the enzymatic component of the Chromosome Passenger Complex (CPC), a master regulator of cell division (Reviewed in Vader & Lens, 2008; Ruchaud *et al*., 2007). Upon its recruitment, Aurora B causes improper connections to sever, triggering the Spindle Assembly Checkpoint (SAC) to arrest cell division progression until proper attachments are formed (Pinsky *et al*., 2006). The contribution of Shugoshin varies between species: *S. pombe* Sgo2 is essential for sensing improper connections whereas *S. cerevisiae* Sgo1 seems to provide only a minor contribution (Kiburz *et al*., 2008; Monje-Casas *et al*., 2007). In contrast, the role of Sgo in protecting centromeric cohesion is critical and highly conserved. Aurora B and the CPC are required for proper Sgo localization, by regulating the kinetochore and SAC component Bub1 (Fernius & Hardwick, 2007) and possibly also through a direct interaction between Sgo the CPC component INCENP (Resnick *et al*., 2006). Aurora B also contributes to stabilizing the association of protein phosphatase 2A, with whom Sgo1 interacts to maintain centromeric cohesion (Yu & Koshland, 2007; Tang *et al*., 2006). Thus, while Sgo has a critical role in promoting maintenance of cohesion, its function is dependent on interactions with Aurora B.

While their function depends on kinetochore components and on chromatin structure, more direct interactions of Sgo and Aurora B with pericentric heterochromatin have been reported. In *S. pombe* and mammals, HP1 has recently been shown to recruit Sgo directly

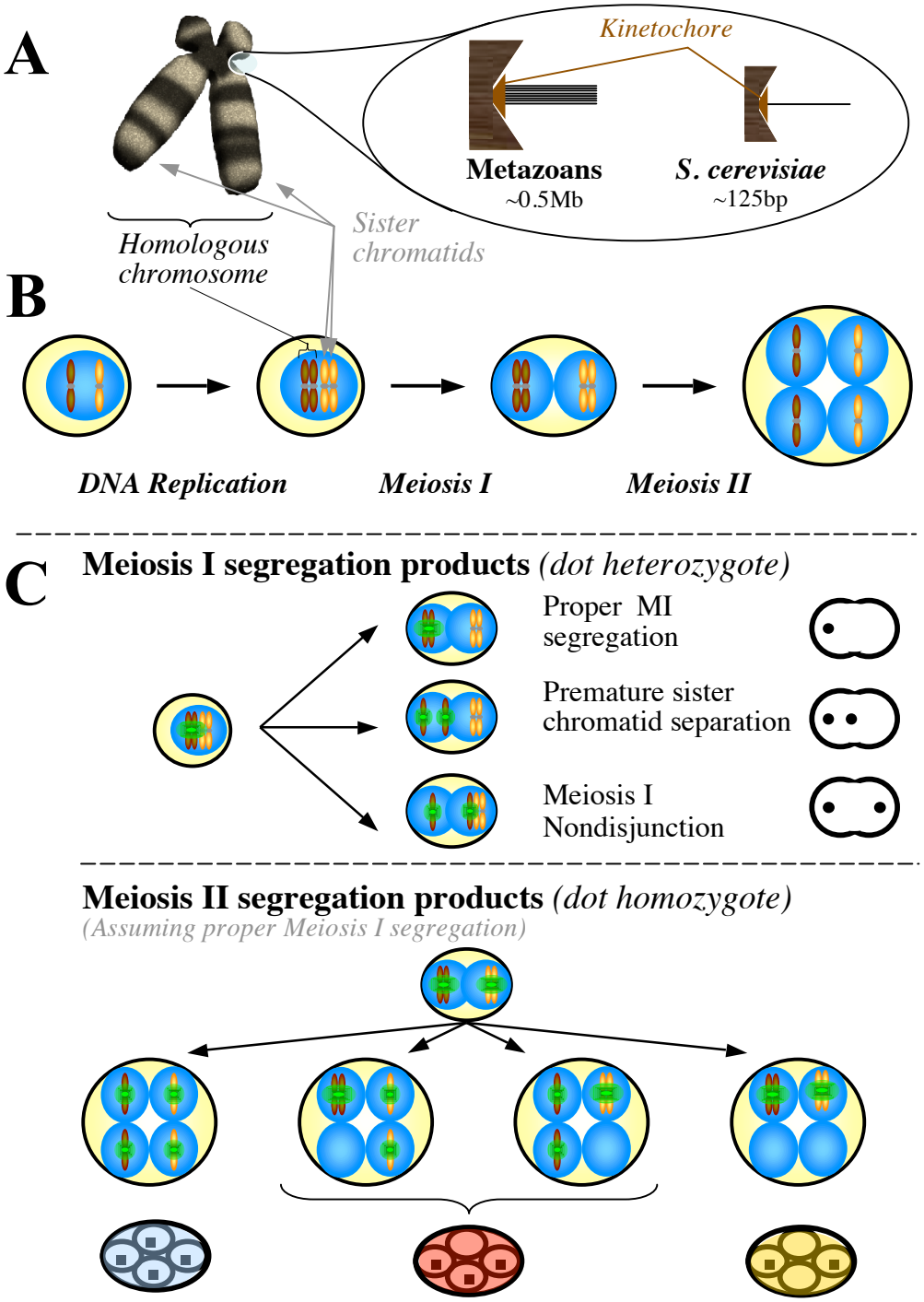


Figure 2: Meiotic chromosome segregation and GFP dot assay

(A) Schematic of metazoan and budding yeast (*S. cerevisiae*) centromeres, indicating differences in size and in the number of microtubules attached (black lines). **(B)** Overview of meiotic chromosome segregation: after DNA replication, sister chromatids remain associated. Following recombination, homologous chromosomes align and, upon loss of arm cohesion, segregate apart; protection of centromeric cohesion leads sister chromatids to co-segregate (meiosis I). Upon the subsequent loss of centromeric cohesion, the sister chromatids separate (meiosis II). **(C)** GFP dot patterns resulting from different meiosis I & II chromosome segregation events. GFP-labeled centromeres are represented in green; cartoon schematics are as used in Figures 6, 7.

and to be required for subsequent centromeric protection (Yamagishi *et al*., 2008). Aurora B kinase regulates heterochromatin stability by phosphorylating H3 and interfering with HP1 binding (Hirota *et al*., 2005; Fischle *et al*., 2005). Complementarily, CenH3 phosporylation by another Aurora kinase, Aurora A, promotes enrichment of Aurora B at the inner centromere (Kunitoku *et al*., 2003). The interactions between Sgo, Aurora B, and pericentric heterochromatin illustrate the intricate nature of cell division regulation and the prominent role of epigenetic factors in the process.

A role for H2AZ in cell division?

Several lines of evidence indicate that H2AZ may be important for chromosome segregation and genome integrity. Loss of H2AZ in budding and fission yeast and in mammals leads to chromosome segregation defects and genome instability (Krogan *et al*., 2004; Carr *et al*., 1994; Ahmed *et al*., 2007; Rangasamy *et al*., 2004; Greaves *et al*., 2006). Furthermore, acetylation of the *S. cerevisiae* homolog, Htz1, by the NuA4 complex has been shown to be important for proper chromosome transmission (Keogh *et al*., 2006). The fission yeast homolog (Pht1) is required for suppression of a temperature-sensitive CenH3 mutation, possibly by recruiting kinetochore components (Ahmed *et al*., 2007), also supports a functional role for H2AZ in cell division. Further genetic and biochemical interactions have been reported between kinetochore and spindle checkpoint components and Htz1, NuA4 complex components, and members of the H2AZ-dedicated loading complex SWR-C (Krogan *et al*., 2004; Keogh *et al*., 2006; Daniel *et al*., 2006; Uetz et al, 2000; Tong *et al*., 2004). Evidence for an interaction between mammalian H2AZ and the Chromosomal Passenger Complex component INCENP has also been

reported (Rangasamy *et al*., 2003), suggesting a functional connection between this histone variant and Aurora B function. H2AZ localizes to centromeric chromatin in yeast, mouse and human (Krogan *et al.*, 2004; Greaves *et al.*, 2007; Millar & Grunstein, 2006) and has been suggested to contribute to proper centromere structure (Greaves *et al*., 2007). In *Drosophila* and differentiating mouse embryos, proper HP1 localization and pericentric heterochromatin formation require H2AZ (Swaminathan *et al*., 2005; Greaves *et al*., 2006; Rangasamy *et al*., 2004), suggesting a role for this variant in proper centromere formation (Bernard & Allshire, 2002; Durand-Dubief & Ekwall, 2008) and centromeric cohesion (Bernard *et al*., 2001; Nonaka *et al*., 2002). Interestingly, H2AZ over-expression has been reported in various human cancers (Hua *et al*., 2008; Rhodes *et al*., 2004; Dunican *et al*., 2002), further supporting a role for the histone variant in maintaining genome stability. Thus, H2AZ appears to have a conserved role in contributing to stable genomic transmission.

Despite the extensive evidence across several organisms implicating H2AZ in the maintenance of genome stability and in centromere and kinetochore function, a possible function for the histone variant in meiosis has not been investigated. The budding yeast *Saccaromyces cerevisiae* provides a good model system for cell division studies, combining a wealth of genetic and molecular tools with the ability to independently observe all four products of meiosis. Therefore, I sought to investigate the meiotic function of the sole H2AZ homolog in *S. cerevisiae*, Htz1.

While the role of H2AZ in cell division appears to be conserved from mammals to yeast, some caveats must be considered. *S. cerevisiae* lacks H3K9 methylation, HP1, or RNAi, and therefore H2AZ-dependent pericentric chromatin akin to that in *S. pombe* and metazoans (Grewal & Jia, 2007; Morris & Moazed, 2007). Furthermore, despite a strong degree of conservation, Htz1 structure differs from that of mouse H2AZ in its N- and C-termini (Redon *et al*., 2002). Finally, in contrast with H2AZ in metazoans, Htz1 is dispensable for budding yeast viability (Santisteban *et al*., 2000; Krogan *et al*., 2004). These arguments seem to suggest that functional connections between H2AZ and cell division are not conserved in budding yeast. Htz1 deletion, however, results in mitotic chromosome segregation defects and genome instability phenotypes similar to those seen upon H2AZ deletion in other organisms (Krogan *et al*., 2004; Carr *et al*., 1994; Rangasamy *et al*., 2004; Keogh *et al*., 2006). Mutations in components of the deposition complex Swr1 and the histone acetyltransferase complex NuA4 responsible for acetylation of Htz1 replicate these phenotypes (Krogan *et al*., 2004). The function of these complexes is conserved through mammals, as the human Tip60 complex has recently been shown to be the functional equivalent of these two complexes (Auger *et al*., 2008). A conserved role is further supported by the full rescue of Htz1 deletion phenotypes upon expressing *T. thermophila* H2AZ in budding yeast (Jackson & Gorovsky, 2000). The functional conservation and phenotypic similarities argue that Htz1 shares at least some cell-division-related functions with other H2AZ homologs.

Here we show that the *S. cerevisiae* H2AZ homologue Htz1 is required for proper meiotic chromosome segregation, and that its deletion leads to a significant increase in

chromosome nondisjunction during both meiosis I and II. Htz1 deletion also results in premature sister chromatid separation, implicating the histone variant in centromeric protection and kinetochocore co-orientation. Although the deletion phenotype observed is significant, Htz1 in is not essential for meiosis indicating that its function is likely redundant with other factors. This part belongs in the discussion. While further investigation is required to fully understand the contribution of Htz1, these results suggest a novel role for H2AZ in regulating chromosome segregation during meiosis.

MATERIALS AND METHODS

Strain construction

HTZ1 (YOL012C) was deleted by homologous recombination in a SK1 background (Amon Lab strain 4842: MATα, ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG). The open reading frame was fully replaced with a KanMX6 cassette by a one-step PCR-based gene replacement method (Longtine *et al*., 1998).

Primers (HTZ1 homology regions capitalized; KanMX6 homology regions in lower case):

LB68f:
AATTTCGCACTATAGCCGCACGTAAAAATAACTTAACATAcggatccccgggttaattaa

LB68r:
AGGGAGAATTACGGGAAATGGGAAAGAAAAACTATTCTTCgaattcgagctcgtttaaac

Transformants were selected on G418 (100ug/mL on YPD-Agar) and back-crossed to dot-containing strain 18026 (MATa, ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG,

leu2::LEU2::tetR-GFP::TetO-HIS3). The diploids were sporulated, and haploid strains of the desired genotypes were obtained from mating independent transformants. These haploids were subsequently crossed, and the diploids obtained were screened for GFP 'dot' signal to noise prior to being used in synchronous meiosis assays. See **Supplement 6** for a complete list of strains used.

Sporulation conditions

Budding yeast sporulations were performed essentially as described in Lee *et al.*, 2004. Briefly, cells were grown to saturation in YPD (YEP+2% dextrose) for 24 hours at 24ºC (all subsequent cultures were at 30ºC). Cultures were diluted into YPA (YEP+2% KOAc) to OD600= 0.2-0.3, grown overnight, washed with sterilized water, and resuspended in SPO medium (0.3% KOAc, pH 7.0) to induce sporulation.

Sporulation efficiency was determined by scoring cells for the number of spores under light microscopy, 24 hours post-SPO medium addition. 3- and 4-spore cells were combined in a single category, due to the difficulty in distinguishing true 3-spore cells from 4-spore ones in which a spore is obscured due to depth or perspective.

Fluorescence imaging

SPO culture samples (700uL) were collected at 2 hour timepoints from 0 to 12 hours post-induction, fixed in 2.5% formaldehyde in Gomori buffer (KPi) pH6.4, washed twice in KPi pH6.6, and resuspended in KPi pH7.4. Immediately prior to imaging, cells were incubated in 80% ethanol for 10 minutes, stained with DAPI (1mg/mL final), and

sonicated. Cells were then scored for the number of DAPI-stained nuclear masses and for the distribution of GFP dots therein using a Zeiss Axioplan 2 microscope equipped with an Alpha-Plan-Fluar 100x/1.45 Oil M27 objective). Images were acquired on a Hamamatsu ORCA-ER C4742-80 CCD camera and processed for contrast adjustment and false color using OpenLab 4.0.2 (Improvision). Cells for which the GFP dot distribution could not be confidently determined were termed 'no calls' and were scored for nuclear masses alone.

Spore viability

24 hours after SPO induction, cells were collected from 250uL of SPO culture/sample, resuspended in zymolyase solution (Seikagaku), and digested at 37ºC for 10-15 minutes. Tetrads were dissected as per standard procedures, and spores were grown at 37ºC and scored after 2-3 days.

RESULTS

In order to investigate whether H2AZ plays a role in meiotic chromosome segregation, we investigated the effect of loss of its homolog in budding yeast *Saccharomyces cerevisiae*, a system amenable to cell division studies. We constructed mutant strains in which we deleted HTZ1 (YOL012C), the sole budding yeast H2AZ homolog, by targeted homologous recombination. In subsequent experiments, a wild-type strain[1] and an

[1] Throughout, 'wild-type' refers to the genotype of the loci for which deletions are being studied (HTZ1, SPO11). The wild-type strains are otherwise genotypically identical to the mutant strains, particularly with regard to the GFP dots and to nutritional markers.

analogous strain homozygous for a deletion of the homologous recombination-inducing endonuclease Spo11 served as negative and positive controls for meiotic defects, respectively. Deleting Spo11 precludes crossing over and therefore prevents homologous chromosomes from pairing but not from attaching to the spindle. This results in near-random chromosome segregation and significantly reduced viability without significantly impacting spore formation (Klein *et al*., 1999).

Htz1 deletion leads to reduced sporulation efficiency and impaired spore viability

To investigate a role for the histone variant Htz1 in meiosis, we measured the effects of loss of Htz1 on sporulation efficiency and spore viability, classical meiotic defect phenotypes. Approximately two-fold less sporulation products were observed in the htz1 homozygous deletion strain compared to the wild-type and *Spo11* control strains 24 hours post-induction of sporulation (**Figure 3**). This difference was primarily due to a significantly lower number of 4-spore asci (31% vs. 69% for the wild-type), as the proportion of 2-spore asci was unchanged. Htz1 is therefore required for proper sporulation.

We next analyzed spore viability by tetrad analysis and found that loss of Htz1 led to a marked reduction in four spore asci and a marked increase in the number of tetrads with only two or three viable spores. In contrast, all four spores were viable for the vast majority of wild-type tetrads analyzed, while *spo11* spores were inviable as expected (**Figure 4; Supplement 2**). *htz1*-deleted spores also displayed a slight growth defect compared to wild-type spores, in agreement with previously reported observations (Adam

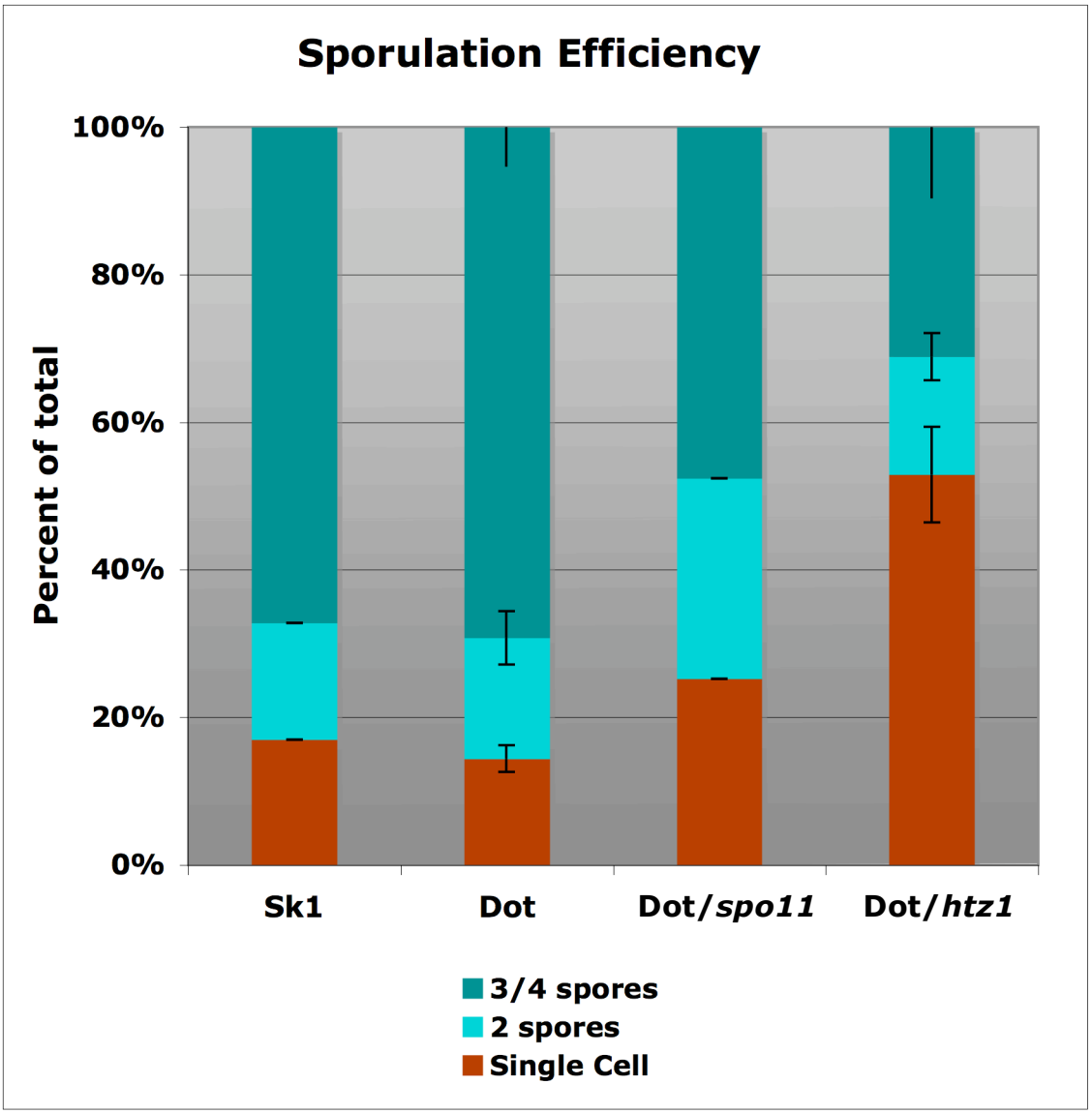
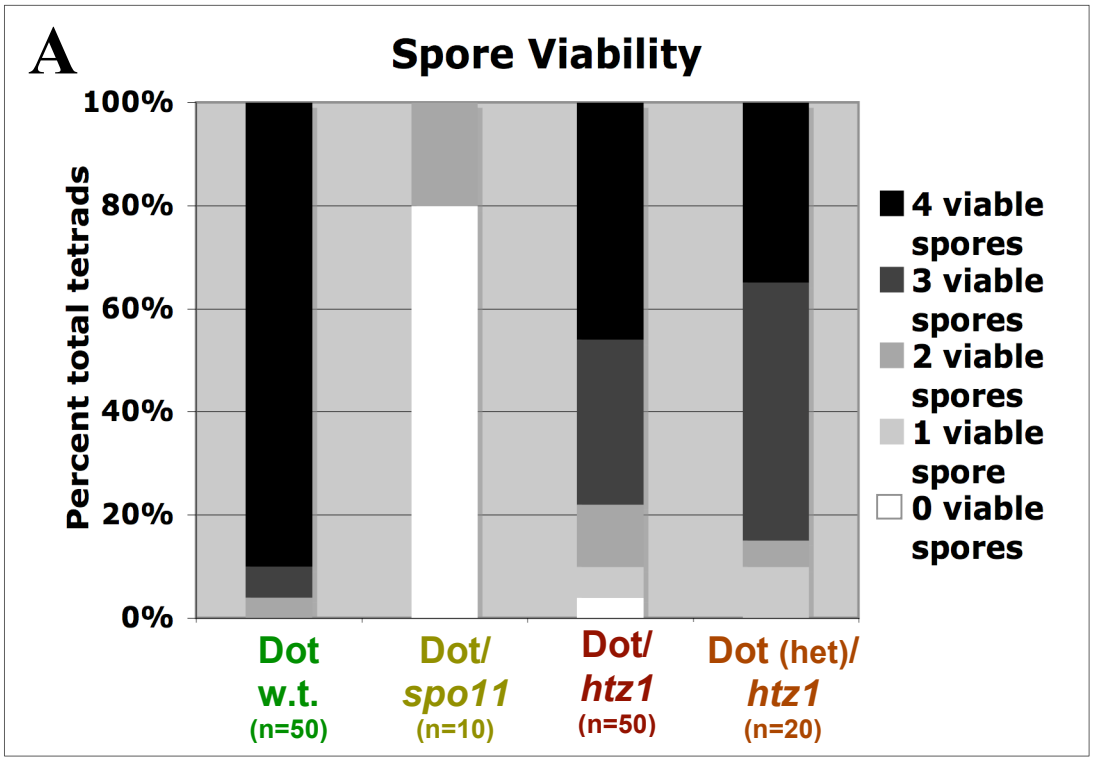


Figure 3: Htz1 deletion leads to reduced sporulation efficiency
Cells undergoing synchronous meiosis were scored for the number of spores under light microscopy, 24 hours after induction of sporulation**.** 'SK1' is the parental strain, without centromeric GFP dots. n=500 for each count; the 'Dot' and 'Dot/*htz1*' genotypes were performed in duplicate, with diploid strains resulting from two independent matings (and distinct *htz1* deletion transformants). *See* ***Supplement 1*** *for tabulated data.*



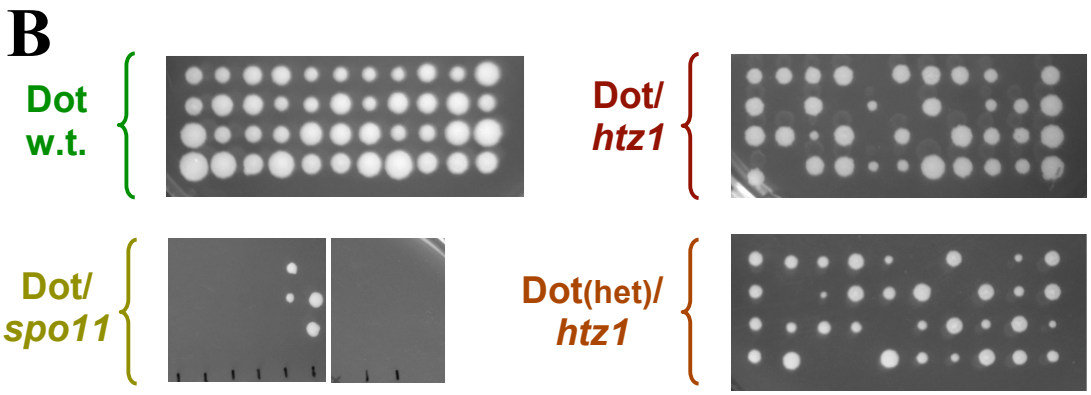


Figure 4: Htz1 deletion leads to impaired spore viability
(A) Cells were collected 24 hours after sporulation, zymolyase-treated, and tetrads were dissected. Spores were grown at 37ºC for 2-3 days and scored for viability. Unless otherwise noted, all strains are homozygous for centromeric GFP dots; all deletions are homozygous. The *htz1Δ* strains display a marked increase in tetrads with only 1, 2, and particularly 3 viable spores. This defect, however, is not so severe as that of the *spo11Δ* strain, which is defective in homologous recombination. *See* ***Supplement 2*** *for data in tabulated form.*
(B) Representative tetrads. Note that a substantial proportion of *htz1Δ* spores is delayed in growth compared with the wild-type control.

et al., 2001; Jackson & Gorovsky, 2000). The milder phenotype compared with that of the *spo11Δ* strain indicates that Htz1 either is not essential for meiosis or that it is redundant with other factors. Nonetheless, the significant reduction in sporulation efficiency and spore viability upon Htz1 deletion clearly suggests a role for the histone variant in meiosis.

Htz1 deletion causes a defect in progression through meiosis

The sporulation and viability phenotypes observed upon Htz1 deletion constitute endpoint readouts of meiosis. We therefore sought to investigate a specific meiotic defect more directly by monitoring the ability of the Htz1 deletion strain to progress through the cell division stages of meiosis. Wild-type and Htz1-deletion strains were induced to undergo synchronous meiosis, and cells were scored for the number of nuclear masses at regular time points (**Figure 5**). The wild-type and *spo11* control strains displayed a similar progression: two-cell (binucleate) meiosis I products accumulated and peaked around 6 hours. Four-cell (tetranucleate) meiosis II products appeared around 4-6 hours and accumulated steadily, reaching 50-60 % of the total by 12 hours. In contrast, at 6 hours the *htz1Δ* strain displayed only half as many binucleates as the control strains and the peak accumulation of binucleates was delayed to ~ 8-10 hours. By 12 hours only ~30 % of total cells were tetranucleates, compared with 50-60% for the controls. The lack of the histone variant may be causing a delay in progression through meiosis I, as indicated by the 2-hour lag in reaching maximal binucleate accumulation, yet, the number of *htz1Δ* binucleates remained lower as compared to wild-type. The overall accumulation of total

cell division products is also drastically reduced (**Figure 5B**). Thus, absence of Htz1 results in a partially penetrant phenotype of impaired meiosis I completion.

Htz1 deletion results in increased nondisjunction events during meiosis I and meiosis II

Since Htz1 has been implicated in chromosome segregation and because loss of Htz1 resulted in a defect in the progression through meiosis, we sought to measure nondisjunction events directly in mutant and control strains. To do so we utilized strains expressing a tetracycline repressor-GFP fusion protein (TetR::GFP), along with a Tet operon array inserted near the centromere of chromosome V (Marston *et al*., 2004). The TetR::GFP fusion binds the array, fluorescently labeling the centromere. This allowed monitoring of chromosome segregation *in vivo* by tracking these GFP 'dots' throughout cell division *via* fluorescent microscopy (**Figure 2**). Strains homozygous for the GFP dots (Dot wild-type), for dots and *htz1* deletion (Dot/*htz1*), and for dots and *spo11* deletion (Dot/*spo11*) were generated for this study. The wild-type dot strains showed sporulation efficiency similar to that of the background strain that did not contain GFP dots (**Figure 3**), and *htz1* strains homo- and heterozygous for the GFP dot displayed similar tetrad viabilities (**Figure 4**), indicating that neither the Tet operon array nor the TetR::GFP fusion impacted either sporulation efficiency or spore viability.

To assay for chromosome nondisjunction events, diploid strains homozygous for the GFP dots and for the respective deletions were induced to undergo synchronous meiosis (Lee *et al*., 2004) and, twelve hours after sporulation, cells were scored for the number of

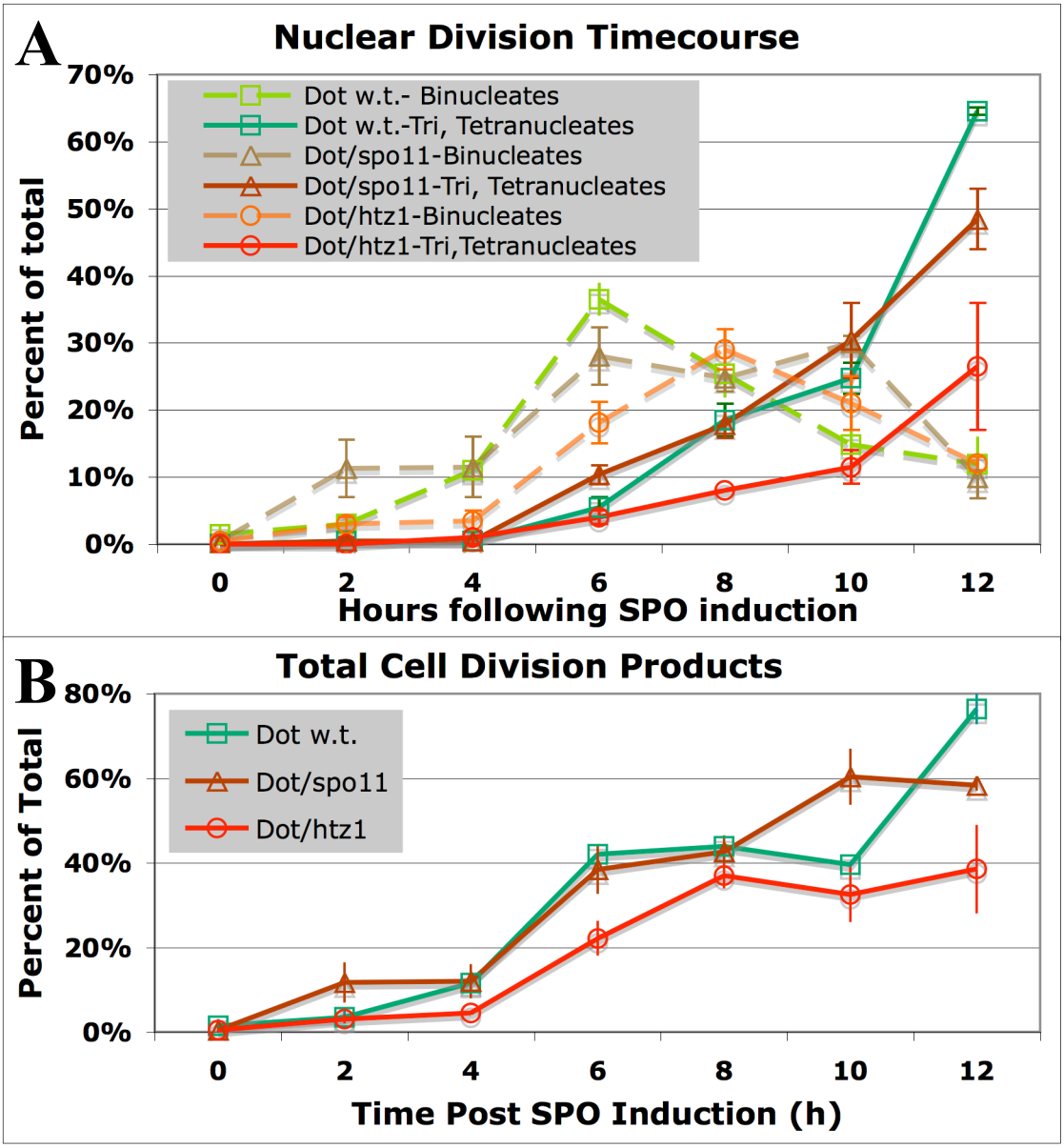


Figure 5: Htz1 deletion leads to a defect in progress through Meiosis
Diploid strains homozygous for centromeric GFP dots were collected at 2-hour intervals after induction of sporulation, fixed, and subsequently scored for the number of nuclear masses per cell as determined by DAPI staining. Two independent strains/genotype were assayed; for each sample, n ~ 100. **(A)** The proportion of binucleate meiosis I products is indicated in dashes, and that of tri/tetranucleate meiosis II products, in solid lines. The remaining percentage consists of mononucleate cells that presumably have not sporulated. **(B)** The lower panel represents the combined cell meiosis I and meiosis II products, ie, the total of bi- and tri/tetranucleate cells per strain/timepoint.. *See **Supplement 3** for tabulated data*.

nuclear masses and for the distribution of GFP dots (**Figure 6**). The majority of wild-type tetranucleate meiosis products (96%) displayed four distinct GFP dots, one in each nuclear mass. This is as expected upon proper chromosome segregation, as all four chromosome V sister chromatids have their centromeres tagged with a GFP dot. Also as expected, in the *spo11Δ* control strain GFP dots distributed randomly in the nuclear masses. In contrast, the *htz1Δ* strain displayed a marked increase in asci containing GFP dots in three out of four nuclear masses relative to wild-type (22.4% vs. 2.2%) and asci containing dots in only two out of four nuclear masses (8.4% vs. 1.4%). These dot segregation patterns correspond to meiosis II and I nondisjunction products, respectively (**Figure 2**). These results implicate Htz1 in proper meiotic chromosome segregation. Furthermore, the increase in both meiosis I and II nondisjunction products upon Htz1 deletion indicates that the histone variant's role is not specific to either meiotic stage. A role for Htz1 in proper centromere structure and/or kinetochore assembly would explain these observations.

Htz1 deletion causes premature sister chromatid separation during meiosis I

Because the observations implicate Htz1 in meiosis 1 and Meiosis II, we sought to further investigate its contributions to each stage. In order to examine a role for Htz1 in meiosis I, we investigated the effects of a homozygous *htz1* deletion in the context of a strain heterozygous for the GFP 'dot.' In this and an analogous wild-type control strain, only one of the two chromosome V homologues is tagged at its centromere. Centromeric cohesion should be maintained through meiosis I in order for sister chromatids to co-segregate and only separate at Anaphase II. Thus, if the homologues are properly

segregated a single GFP dot should be visible in the binucleate meiosis I products. The dot heterozygous strains underwent synchronized meiosis, and the GFP dot distribution in binucleates was scored at the end of meiosis I (6 hours post-sporulation for wild-type control, 8 hours for *Htz1*; **Figure 7**). Accordingly, 92% of wild-type meiosis I products displayed a single GFP dot corresponding to properly cosegregating sister chromatids. This proportion was reduced to 64% upon HTZ1 deletion, as the mutant strains displayed a greater than five-fold increase in nondisjunction products with two distinct bi-oriented GFP dots. Furthermore, a significant proportion of *htz1* binucleates (14%, vs. 4% of wild-type) contained two distinct GFP dots which, rather than being fully bi-oriented, were either co-segregating or displayed one dot remaining at the cell equatorm indicating a lagging chromosome (Ekwall *et al*., 1995; Rangasamy *et al*., 2004). This significant increase in binucleates with two distinct GFP dots indicates an increased frequency of prematurely separated sister chromatids in the absence of Htz1. Htz1 may therefore be required for proper centromeric cohesion, sister kinetochore mono-orientation, or both.

DISCUSSION

Htz1 is necessary for proper meiotic chromosome segregation

The faithful transmission of genetic information is crucial for species viability. Given the body of evidence implicating H2AZ in maintenance of genome stability, centromere structure and function, and chromosome segregation in a variety of organisms, we investigated a potential role for H2AZ during meiosis in budding yeast. Ablation of the

S. cerevisiae H2AZ homologue Htz1 resulted in reduced sporulation efficiency, impaired spore viability, and a defect in meiotic progression, indicating that Htz1 function is necessary for proper meiosis. Loss of the histone variant resulted in increased meiosis I and meiosis II nondisjunction products, indicating that Htz1 also may play a role in chromosome segregation. Consistent with this, premature separation of sister chromatids was also observed upon Htz1 abrogation, implicating the histone variant in maintaining centromeric cohesion and/or kinetochocore co-orientation during meiosis I.

Htz1 may be required to establish proper chromatin architecture at the centromere and surrounding areas and thereby impacting kinetochore assembly. The mis-segregation phenotypes observed upon Htz1 deletion indicate defects in spindle attachment, chromosome cohesion, or kinetochore attachment, all of which can result from impaired centromere structure and kinetochore function. The increased nondisjunction events observed during both meiosis I and meiosis II upon Htz1 loss, as well as the impaired sister kinetochore co-orientation, further support a role for the histone variant at the centromere. Thus, Htz1 may be contributing to the formation of functional centromeric structure in a role similar to that of H2AZ in establishing pericentric heterochromatin formation in *S. pombe* and metazoans.

H2AZ function during meiosis may extend beyond a purely structural role. Htz1 deletion does not substantially impact the centromeric recruitment or distribution of various kinetochore components and cohesins during mitosis (Keogh *et al*., 2006). The finding that loss of *S. pombe* heterochromatin abolishes centromeric cohesion but does not affect

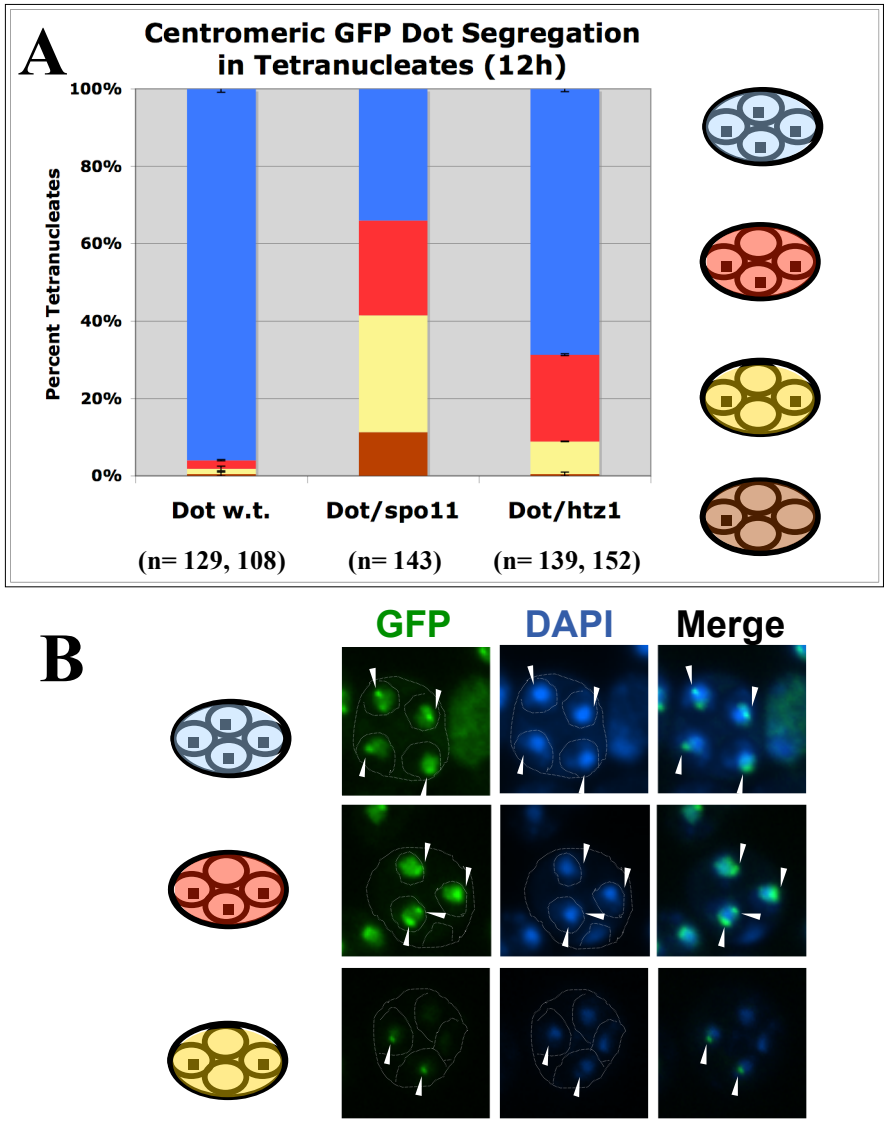


Figure 6: Htz1 deletion leads to increased meiosis I & meiosis II nondisjunction
(A) Homozygous strains containing centromere-proximal GFP 'dots' underwent synchronous meiosis. 12 hours post-sporulation, cells were collected, fixed, and subsequently DAPI-stained and scored by fluorescent microscopy for the number of nuclear masses and the distribution of GFP dots. The wild-type and *htz1*-deleted strains were assayed in duplicate, with diploids resulting from independent *htz1Δ* transformant strain matings. For simplicity, multiple dots within a same nuclear mass are represented as a single dot in legend cartoons. *See **Supplement 4** for tabulated data*. **(B)** Microscopy images illustrating representative samples; asci of interest and individual meiotic progeny are outlined in the GFP and DAPI panels; arrowheads indicate individual dots.
Proper segregation results in one GFP dot localizing to each nuclear mass *(blue)*. *htz1*-deleted strains displayed an increase in both meiosis I *(yellow)* and meiosis II *(red)* nondisjunction products relative to wild-type control: 8.4% vs. 1.4% for the former and 22.4% vs. 2.2% for the latter. GFP dots distributed near-randomly in the *spo11Δ* strain, a positive control for mis-segregation, in agreement with previously reported results (Keeley *et al*., 1997).

monopolar attachment (Kitajima *et al*., 2003) further supports this argument. Furthermore, the premature sister chromatid separation we observed in the absence of Htz1 suggests impairment of the Monopolin complex, whose function is sufficient to promote kinetochore co-orientation (Monje-Casas *et al*., 2007; Marston & Amon 2004). Therefore, in addition to a structural contribution by contributing to proper centromere and kinetochore formation, H2AZ likely plays a more direct role in recruiting specific meiosis regulators or otherwise modulating their function.

Sgo1 as a primary effector of Htz1 meiotic function

Among the potential interaction partners for Htz1, Sgo1 and Ipl1 are prime candidates. Nocodazole-treated *htz1*-deleted strains can bypass the Spindle Assembly Checkpoint, in contrast with wild type strains and reminiscent of *sgo1* and *ipl1* mutants (Keogh *et al*., 2006; Ruchaud *et al*., 2006). Furthermore, Monopolin and Sgo1 both depend on the same gene, Spo13 for proper localization and function (Monge-Casas *et al*., 2007; Lee *et al*., 2004). Spo13, in turn, depends in part on Ipl1 and the CPC for its own localization. Finally, the nondisjunction and loss of chromatid cohesion phenotypes we observed upon Htz1 deletion strongly resemble those resulting from ablating Ipl1 or Sgo1 (Monge-Casas *et al*., 2007): nondisjunction frequencies are similar in *htz1*- or *Ipl1*-deleted strains but not as severe as those in *Sgo1*-deleted strains, indicating that Sgo1 is epistatic to both. Thus, impaired Sgo1 function seems to be the principal cause for the meiotic phenotypes observed upon deletion of Htz1, which may be acting parallel to or with Ipl1 upstream of Sgo1.

Several observations in budding yeast and other organisms support functional interactions between H2AZ and Sgo1 and argue for its conservation across species. While mammalian H2AZ and Sgo have both been shown to bind the Chromosome Passenger Complex member INCENP (In mammals and *Drosophila*, respectively; Rangasamy *et al*., 2003; Resnick *et al*., 2006), INCENP mutations in *Drosophila* result in defects in Shugoshin localization and in maintenance of meiotic sister chromatid cohesion. Defective Sgo function would also result in mitotic segregation defects such as those reported upon RNAi-mediated knockdown of mammalian H2AZ (Rangasamy *et al*., 2004), by impairing Aurora B localization and function. Accordingly, the unequal distribution of H2AZ in mitotic daughter nuclei upon knockdown is reminiscent of biased sister chromatid segregation towards the old spindle pole body upon Ipl1 deletion in yeast (Monje-Casas *et al*., 2007; Pereira *et al*., 2001; Tanaka *et al*., 2002).

The interactions of pericentric heterochromatin with both Shugoshin and H2AZ provide further support for a functional connection between the two. In fission yeast, the meiosis-specific Sgo1 binds Swi6/HP1, and maintaining centromeric cohesion requires heterochromatin (Yamagishi *et al*., 2008). HP1 localization and pericentric heterochromatin formation, in turn, are dependent on H2AZ. Although budding yeast lacks such an H2AZ-dependent, H3K9me- and HP1-containing chromatin domain, *S. cerevisiae* Sgo1 as well as Htz1 have been shown to localize to a 50kb region surrounding centromeres - the former presumably to maintain cohesion in face of the force exerted by microtubules (Kiburz 2005, He et al. 2000). Thus, these data together

with a number of observations in organisms ranging from yeast to man support a conserved link between Shugoshin and H2AZ function.

Future Directions

Our observations open several potential avenues of future inquiry. Primary among them is to investigate whether Sgo1 is responsible for the observed phenotype. This can be addressed by investigating whether Htz1 is necessary for centromeric localization of Sgo1 in meiosis I-arrested cells, and also whether overexpression of Sgo1 or Ipl1 in an *htz1*-deleted background rescues the phenotype. Furthermore, testing whether an *htz1Δ /ipl1Δ* double mutant exhibits a synthetic phenotype will clarify whether they act in concert or in parallel to promote Sgo1 function. It will be of further interest to ask whether Htz1 associates with Sli15, the *S. cerevisiae* INCENP homolog. If so, this avenue of investigation could provide mechanistic insight into the interactions of Htz1 and Ipl1 in regulating meiosis or cell division.

Another mechanistic approach is to investigate what structural features of Htz1/H2AZ are required for its function in meiosis. The dot segregation assay, using strains carrying point mutations or partial deletions rather than a full deletion, would provide an ideal system to test this. For example, the Htz1 C-terminal acidic patch is essential for viability in *Drosophila* and *Xenopus laevis* (Clarkson *et al*., 1999; Ridgway *et al*., 2004), and likely serves as a binding platform for *trans*-acting factors (Larochelle & Gaudreau, 2003). Lysine 14, a site of acetylation by the NuA4 complex, is also of particular interest. Point mutations preventing such acetylation fully recapitulate chromosome loss

and benomyl sensitivity phenotypes of a Htz1 deletion strain (Keogh et al., 2006). It would therefore be expected that these mutants would display similar nondisjunction events as observed in this study. In addition to providing further mechanistic insight regarding the functions of Htz1 in meiosis, such structural investigations may also indicate the extent to which these functions are conserved across different species.

Conclusions

With the sequencing of various genomes complete, the importance of epigenetics in gene expression and other essential cell processes has increasingly come to the fore (Reviewed in Bernstein *et al*., 2007; Goldberg *et al*., 2007). We report results implicating H2AZ in a novel role regulating meiosis in budding yeast, linking histone variant function with the fidelity of chromosome segregation during cell division. We propose a model by which H2AZ functions in meiosis by promoting the proper function of the cohesion protector Shugoshin. While further experiments are required to elucidate the role of the histone variant, this model provides a mechanistic explanation for various H2AZ-related phenotypical observations in multiple organisms. Despite the differences between budding yeast and metazoans regarding H2AZ and pericentric heterochromatin, this suggests that the relationship is conserved throughout. Finally, the misregulation of cell division is a major source of genome error and instability. The link between H2AZ and cell division may therefore provide important insights into how defects in chromatin organization and function may contribute to human disease processes such as malignancy.

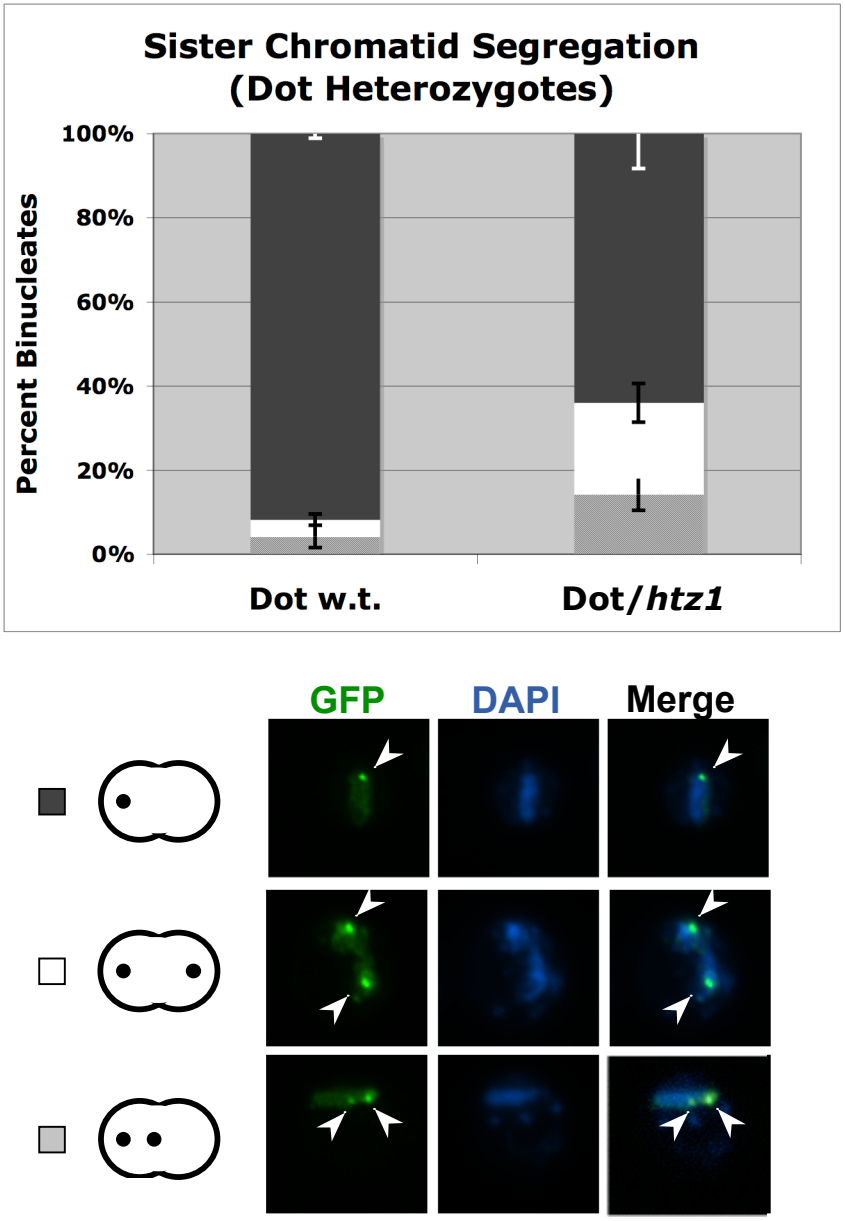


Figure 7: Htz1 deletion leads to premature sister chromatid separation during meiosis I

Strains heterozygous for GFP dots underwent synchronous meiosis, and dot number and distribution was scored in binucleate meiosis I products (6h post sporulation for wild-type, 8h for *htz1* deletion). The experiment was performed in duplicate with independent strains. Microscopy images illustrate representative samples.

92% of wild-type meiosis I products displayed a single GFP dot corresponding to properly co-segregating sister chromatids (■). This proportion was reduced to 64% upon *htz1* deletion.The mutant displayed a substantial increase in nondisjunction (□; 22% vs 4%) and also a significant proportion (14%) of lagging or co-segregating yet distinct GFP dots (▢). *See* ***Supplement 5*** *for tabulated data*.

BIBLIOGRAPHY

1. Adam M, Robert F, Larochelle M, Gaudreau L. H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. Mol Cell Biol. 2001 Sep ;21(18):6270—9.

2. Ahmed S, Dul B, Qiu X, Walworth NC. Msc1 acts through histone H2A.Z to promote chromosome stability in Schizosaccharomyces pombe. Genetics. 2007 Nov ;177(3):1487—97.

3. Allis CD, Glover CV, Bowen JK, Gorovsky MA. Histone variants specific to the transcriptionally active, amitotically dividing macronucleus of the unicellular eucaryote, Tetrahymena thermophila. Cell. 1980 Jul ;20(3):609—17.

4. Auger A, Galarneau L, Altaf M, Nourani A, Doyon Y, Utley RT, et al. Eaf1 is the platform for NuA4 molecular assembly that evolutionarily links chromatin acetylation to ATP-dependent exchange of histone H2A variants. Mol Cell Biol. 2008 Apr ;28(7):2257—70.

5. Ausió J, Abbott DW. The many tales of a tail: carboxyl-terminal tail heterogeneity specializes histone H2A variants for defined chromatin function. Biochemistry. 2002 May ;41(19):5945—9.

6. Babiarz JE, Halley JE, Rine J. Telomeric heterochromatin boundaries require NuA4-dependent acetylation of histone variant H2A.Z in Saccharomyces cerevisiae. Genes Dev. 2006 Mar ;20(6):700—10.

7. Barski A, Cuddapah S, Cui K, Roh T, Schones DE, Wang Z, et al. High-resolution profiling of histone methylations in the human genome. Cell. 2007 May ;129(4):823—37.

8. Bergerat A, Massy BD, Gadelle D, Varoutas PC, Nicolas A, Forterre P. An atypical topoisomerase II from Archaea with implications for meiotic recombination. Nature. 1997 Mar ;386(6623):414—7.

9. Bernstein E, Hake SB. The nucleosome: a little variation goes a long way. Biochem. Cell Biol. 2006 Aug ;84(4):505—17.

10. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell. 2007 Feb ;128(4):669—81.

11. Black BE, Bassett EA. The histone variant CENP-A and centromere specification. Curr Opin Cell Biol. 2008 Feb ;20(1):91—100.

12. Blower MD, Sullivan BA, Karpen GH. Conserved organization of centromeric chromatin in flies and humans. Developmental Cell. 2002 Mar ;2(3):319—30.

13. Carr AM, Dorrington SM, Hindley J, Phear GA, Aves SJ, Nurse P. Analysis of a histone H2A variant from fission yeast: evidence for a role in chromosome stability. Mol Gen Genet. 1994 Dec ;245(5):628—35.

14. Cheeseman IM, Desai A. Molecular architecture of the kinetochore-microtubule interface. Nat Rev Mol Cell Biol. 2008 Jan ;9(1):33—46.

15. Choudhary P, Varga-Weisz P. ATP-dependent chromatin remodelling: action and reaction. Subcell Biochem. 2007 Jan ;4129—43.

16. Clarkson MJ, Wells JR, Gibson F, Saint R, Tremethick DJ. Regions of variant histone

His2AvD required for Drosophila development. Nature. 1999 Jun ;399(6737):694—7.

17. Cosgrove MS, Boeke JD, Wolberger C. Regulated nucleosome mobility and the histone code. Nat Struct Mol Biol. 2004 Nov ;11(11):1037—43.

18. Daniel JA, Keyes BE, Ng YPY, Freeman CO, Burke DJ. Diverse functions of spindle assembly checkpoint genes in Saccharomyces cerevisiae. Genetics. 2006 Jan ;172(1):53—65.

19. Dhillon N, Oki M, Szyjka SJ, Aparicio OM, Kamakaka RT. H2A.Z functions to regulate progression through the cell cycle. Mol Cell Biol. 2006 Jan ;26(2):489—501.

20. Dunican DS, McWilliam P, Tighe O, Parle-McDermott A, Croke DT. Gene expression differences between the microsatellite instability (MIN) and chromosomal instability (CIN) phenotypes in colorectal cancer revealed by high-density cDNA array hybridization. Oncogene. 2002 May ;21(20):3253—7.

21. Dunn RK, Kingston RE. Gene regulation in the postgenomic era: technology takes the wheel. Mol Cell. 2007 Dec ;28(5):708—14.

22. Durand-Dubief M, Ekwall K. Heterochromatin tells CENP-A where to go. Bioessays. 2008 Jun ;30(6):526—529.

23. Eir\'ın-López J, Ausió J. H2A.Z-Mediated Genome-Wide Chromatin Specialization. Curr Genomics. 2007 Mar ;8(1):59—66.

24. Ekwall K. Epigenetic control of centromere behavior. Annu Rev Genet. 2007 Jan ;4163—81.

25. Faast R, Thonglairoam V, Schulz TC, Beall J, Wells JR, Taylor H, et al. Histone variant H2A.Z is required for early mammalian development. Curr Biol. 2001 Aug ;11(15):1183—7.

26. Fernius J, Hardwick KG. Bub1 kinase targets Sgo1 to ensure efficient chromosome biorientation in budding yeast mitosis. PLoS Genet. 2007 Nov ;3(11):e213.

27. Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, et al. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature. 2005 Dec ;438(7071):1116—22.

28. Folco HD, Pidoux AL, Urano T, Allshire RC. Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres. Science. 2008 Jan ;319(5859):94—7.

29. Fraser P, Bickmore W. Nuclear organization of the genome and the potential for gene regulation. Nature. 2007 May ;447(7143):413—7.

30. Gieni RS, Chan GKT, Hendzel MJ. Epigenetics regulate centromere formation and kinetochore function. J Cell Biochem. 2008 Aug ;104(6):2027—39.

31. Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. Cell. 2007 Feb ;128(4):635—8.

32. Greaves IK, Rangasamy D, Devoy M, Graves JAM, Tremethick DJ. The X and Y chromosomes assemble into H2A.Z-containing [corrected] facultative heterochromatin [corrected] following meiosis. Mol Cell Biol. 2006 Jul ;26(14):5394—405.

33. Greaves IK, Rangasamy D, Ridgway P, Tremethick DJ. H2A.Z contributes to the unique 3D structure of the centromere. Proc Natl Acad Sci USA. 2007 Jan ;104(2):525—30.

34. Grewal SIS, Jia S. Heterochromatin revisited. Nat Rev Genet. 2007 Jan ;8(1):35—46.

35. Guillemette B, Gaudreau L. Reuniting the contrasting functions of H2A.Z. Biochem. Cell Biol. 2006 Aug ;84(4):528—35.

36. Guillemette B, Bataille AR, Gévry N, Adam M, Blanchette M, Robert FC, et al. Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. PLoS Biol. 2005 Dec ;3(12):e384.

37. He X, Asthana S, Sorger PK. Transient sister chromatid separation and elastic deformation of chromosomes during mitosis in budding yeast. Cell. 2000 Jun ;101(7):763—75.

38. Hua S, Kallen CB, Dhar R, Baquero MT, Mason CE, Russell BA, et al. Genomic analysis of estrogen cascade reveals histone variant H2A.Z associated with breast cancer progression. Mol Syst Biol. 2008 Jan ;4188.

39. Indjeian VB, Murray AW. Budding yeast mitotic chromosomes have an intrinsic bias to biorient on the spindle. Curr Biol. 2007 Nov ;17(21):1837—46.

40. Indjeian VB, Stern BM, Murray AW. The centromeric protein Sgo1 is required to sense lack of tension on mitotic chromosomes. Science. 2005 Jan ;307(5706):130—3.

41. Iouzalen N, Moreau J, Méchali M. H2A.ZI, a new variant histone expressed during Xenopus early development exhibits several distinct features from the core histone H2A. Nucleic Acids Res. 1996 Oct ;24(20):3947—52.

42. Jackson JD, Gorovsky MA. Histone H2A.Z has a conserved function that is distinct from that of the major H2A sequence variants. Nucleic Acids Res. 2000 Oct ;28(19):3811—6.

43. Kamakaka RT, Biggins S. Histone variants: deviants? Genes Dev. 2005 Feb ;19(3):295—310.

44. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, et al. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes Dev. 2005 Feb ;19(4):489—501.

45. Kawashima SA, Tsukahara T, Langegger M, Hauf S, Kitajima TS, Watanabe Y. Shugoshin enables tension-generating attachment of kinetochores by loading Aurora to centromeres. Genes Dev. 2007 Feb ;21(4):420—35.

46. Keeney S, Giroux CN, Kleckner N. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell. 1997 Feb ;88(3):375—84.

47. Keogh M, Mennella TA, Sawa C, Berthelet S, Krogan NJ, Wolek A, et al. The Saccharomyces cerevisiae histone H2A variant Htz1 is acetylated by NuA4. Genes Dev. 2006 Mar ;20(6):660—5.

48. Kiburz BM, Reynolds DB, Megee PC, Marston AL, Lee BH, Lee TI, et al. The core centromere and Sgo1 establish a 50-kb cohesin-protected domain around centromeres during meiosis I. Genes Dev. 2005 Dec ;19(24):3017—30.

49. Kiburz BM, Amon A, Marston AL. Shugoshin Promotes Sister Kinetochore Biorientation in Saccharomyces cerevisiae. Mol Biol Cell. 2008 Mar ;19(3):1199—209.

50. Kitajima TS, Yokobayashi S, Yamamoto M, Watanabe Y. Distinct cohesin complexes organize

meiotic chromosome domains. Science. 2003 May ;300(5622):1152—5.

51. Kouzarides T. Chromatin modifications and their function. Cell. 2007 Feb ;128(4):693—705.

52. Krogan NJ, Keogh M, Datta N, Sawa C, Ryan OW, Ding H, et al. A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol Cell. 2003 Dec ;12(6):1565—76.

53. Krogan NJ, Baetz K, Keogh M, Datta N, Sawa C, Kwok TCY, et al. Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4. Proc Natl Acad Sci USA. 2004 Sep ;101(37):13513—8.

54. Kunitoku N, Sasayama T, Marumoto T, Zhang D, Honda S, Kobayashi O, et al. CENP-A phosphorylation by Aurora-A in prophase is required for enrichment of Aurora-B at inner centromeres and for kinetochore function. Developmental Cell. 2003 Dec ;5(6):853—64.

55. Larochelle M, Gaudreau L. H2A.Z has a function reminiscent of an activator required for preferential binding to intergenic DNA. EMBO J. 2003 Sep ;22(17):4512—22.

56. Lee BH, Kiburz BM, Amon A. Spo13 maintains centromeric cohesion and kinetochore coorientation during meiosis I. Curr Biol. 2004 Dec ;14(24):2168—82.

57. Lee J, Kitajima TS, Tanno Y, Yoshida K, Morita T, Miyano T, et al. Unified mode of centromeric protection by shugoshin in mammalian oocytes and somatic cells. Nat Cell Biol. 2008 Jan ;10(1):42—52.

58. Liu X, Bowen J, Gorovsky MA. Either of the major H2A genes but not an evolutionarily conserved H2A.F/Z variant of Tetrahymena thermophila can function as the sole H2A gene in the yeast Saccharomyces cerevisiae. Mol Cell Biol. 1996 Jun ;16(6):2878—87.

59. Longtine MS, McKenzie A, Demarini DJ, Shah NG, Wach A, Brachat A, et al. Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast. 1998 Jul ;14(10):953—61.

60. Marston AL, Amon A. Meiosis: cell-cycle controls shuffle and deal. Nat Rev Mol Cell Biol. 2004 Dec ;5(12):983—97.

61. Marston AL, Tham W, Shah H, Amon A. A genome-wide screen identifies genes required for centromeric cohesion. Science. 2004 Feb ;303(5662):1367—70.

62. Meneghini MD, Wu M, Madhani HD. Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell. 2003 Mar ;112(5):725—36.

63. Millar CB, Grunstein M. Genome-wide patterns of histone modifications in yeast. Nat Rev Mol Cell Biol. 2006 Sep ;7(9):657—66.

64. Millar CB, Xu F, Zhang K, Grunstein M. Acetylation of H2AZ Lys 14 is associated with genome-wide gene activity in yeast. Genes Dev. 2006 Mar ;20(6):711—22.

65. Mizuguchi G, Shen X, Landry J, Wu W, Sen S, Wu C. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science. 2004 Jan ;303(5656):343—8.

66. Monje-Casas F, Prabhu VR, Lee BH, Boselli M, Amon A. Kinetochore orientation during

meiosis is controlled by Aurora B and the monopolin complex. Cell. 2007 Feb ;128(3):477—90.

67. Morris CA, Moazed D. Centromere assembly and propagation. Cell. 2007 Feb ;128(4):647—50.

68. Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol. 2007 May ;8(5):379—93.

69. Nonaka N, Kitajima T, Yokobayashi S, Xiao G, Yamamoto M, Grewal SIS, et al. Recruitment of cohesin to heterochromatic regions by Swi6/HP1 in fission yeast. Nat Cell Biol. 2002 Jan ;4(1):89—93.

70. Okada M, Hori T, Fukagawa T. The DT40 system as a tool for analyzing kinetochore assembly. Subcell Biochem. 2006 Jan ;4091—106.

71. Pereira G, Schiebel E. The role of the yeast spindle pole body and the mammalian centrosome in regulating late mitotic events. Curr Opin Cell Biol. 2001 Dec ;13(6):762—9.

72. Pidoux AL, Allshire RC. The role of heterochromatin in centromere function. Philos Trans R Soc Lond, B, Biol Sci. 2005 Mar ;360(1455):569—79.

73. Pinsky BA, Kung C, Shokat KM, Biggins S. The Ipl1-Aurora protein kinase activates the spindle checkpoint by creating unattached kinetochores. Nat Cell Biol. 2006 Jan ;8(1):78—83.

74. Polo SE, Almouzni G. Chromatin assembly: a basic recipe with various flavours. Current Opinion in Genetics & Development. 2006 Apr ;16(2):104—11.

75. Raisner RM, Hartley PD, Meneghini MD, Bao MZ, Liu CL, Schreiber SL, et al. Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. Cell. 2005 Oct ;123(2):233—48.

76. Rangasamy D, Berven L, Ridgway P, Tremethick DJ. Pericentric heterochromatin becomes enriched with H2A.Z during early mammalian development. EMBO J. 2003 Apr ;22(7):1599—607.

77. Rangasamy D, Greaves I, Tremethick DJ. RNA interference demonstrates a novel role for H2A.Z in chromosome segregation. Nat Struct Mol Biol. 2004 Jul ;11(7):650—5.

78. Resnick TD, Satinover DL, MacIsaac F, Stukenberg PT, Earnshaw WC, Orr-Weaver TL, et al. INCENP and Aurora B promote meiotic sister chromatid cohesion through localization of the Shugoshin MEI-S332 in Drosophila. Developmental Cell. 2006 Jul ;11(1):57—68.

79. Revenkova E, Jessberger R. Keeping sister chromatids together: cohesins in meiosis. Reproduction. 2005 Dec ;130(6):783—90.

80. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression. Proc Natl Acad Sci USA. 2004 Jun ;101(25):9309—14.

81. Ridgway P, Brown KD, Rangasamy D, Svensson U, Tremethick DJ. Unique residues on the H2A.Z containing nucleosome surface are important for Xenopus laevis development. J Biol Chem. 2004 Oct ;279(42):43815—20.

82. Ruchaud S, Carmena M, Earnshaw WC. Chromosomal passengers: conducting cell division.

Nat Rev Mol Cell Biol. 2007 Oct ;8(10):798—812.

83. Santisteban MS, Kalashnikova T, Smith MM. Histone H2A.Z regulats transcription and is partially redundant with nucleosome remodeling complexes. Cell. 2000 Oct ;103(3):411—22.

84. Sarcinella E, Zuzarte PC, Lau PNI, Draker R, Cheung P. Monoubiquitylation of H2A.Z distinguishes its association with euchromatin or facultative heterochromatin. Mol Cell Biol. 2007 Sep ;27(18):6457—68.

85. Sart DD, Cancilla MR, Earle E, Mao JI, Saffery R, Tainton KM, et al. A functional neo-centromere formed through activation of a latent human centromere and consisting of non-alpha-satellite DNA. Nat Genet. 1997 Jun ;16(2):144—53.

86. Schneider R, Grosschedl R. Dynamics and interplay of nuclear architecture, genome organization, and gene expression. Genes Dev. 2007 Dec ;21(23):3027—43.

87. Sullivan BA, Karpen GH. Centromeric chromatin exhibits a histone modification pattern that is distinct from both euchromatin and heterochromatin. Nat Struct Mol Biol. 2004 Nov ;11(11):1076—83.

88. Swaminathan J, Baxter EM, Corces VG. The role of histone H2Av variant replacement and histone H4 acetylation in the establishment of Drosophila heterochromatin. Genes Dev. 2005 Jan ;19(1):65—76.

89. Tanaka TU, Rachidi N, Janke C, Pereira G, Galova M, Schiebel E, et al. Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. Cell. 2002 Feb ;108(3):317—29.

90. Tang Z, Shu H, Qi W, Mahmood NA, Mumby MC, Yu H. PP2A is required for centromeric localization of Sgo1 and proper chromosome segregation. Developmental Cell. 2006 May ;10(5):575—85.

91. Tong AHY, Lesage G, Bader GD, Ding H, Xu H, Xin X, et al. Global mapping of the yeast genetic interaction network. Science. 2004 Feb ;303(5659):808—13.

92. Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, et al. A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature. 2000 Feb ;403(6770):623—7.

93. Updike DL, Mango SE. Temporal regulation of foregut development by HTZ-1/H2A.Z and PHA-4/FoxA. PLoS Genet. 2006 Sep ;2(9):e161.

94. Vader G, Lens S. The Aurora kinase family in cell division and cancer. Biochim Biophys Acta. 2008 Jul ;

95. Vagnarelli P, Ribeiro SA, Earnshaw WC. Centromeres: old tales and new tools. FEBS Letters. 2008 Jun ;582(14):1950—9.

96. Vanoosthuyse V, Prykhozhij S, Hardwick KG. Shugoshin 2 regulates localization of the chromosomal passenger proteins in fission yeast mitosis. Mol Biol Cell. 2007 May ;18(5):1657—69.

97. Varga-Weisz PD, Becker PB. Regulation of higher-order chromatin structures by nucleosome-remodelling factors. Current Opinion in Genetics & Development. 2006 Apr ;16(2):151—6.

98. Verdel A, Moazed D. RNAi-directed assembly of heterochromatin in fission yeast. FEBS Letters. 2005 Oct ;579(26):5872—8.

99. Watanabe Y. Shugoshin: guardian spirit at the centromere. Curr Opin Cell Biol. 2005 Dec ;17(6):590—5.

100. Westermann S, Drubin DG, Barnes G. Structures and functions of yeast kinetochore complexes. Annu Rev Biochem. 2007 Jan ;76563—91.

101. Workman JL. Nucleosome displacement in transcription. Genes Dev. 2006 Aug ;20(15):2009—17.

102. Yamagishi Y, Sakuno T, Shimura M, Watanabe Y. Heterochromatin links to centromeric protection by recruiting shugoshin. Nature. 2008 Aug ;

103. Yu H, Koshland D. The Aurora kinase Ipl1 maintains the centromeric localization of PP2A to protect cohesin during meiosis. J Cell Biol. 2007 Mar ;176(7):911—8.

104. Zhang H, Roberts DN, Cairns BR. Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell. 2005 Oct ;123(2):219—31.

105. Zlatanova J, Thakar A. H2A.Z: view from the top. Structure. 2008 Feb ;16(2):166—79.

Supplement 1:
Table: Htz1 deletion leads to reduced sporulation efficiency

Cells underwent synchronous meiosis, and asci were scored for their number of spores under light microscopy, 24 hours after induction of sporulation**.** 3- and 4-spore cells were combined in a single category, due to the difficulty in distinguishing true 3-spore cells from 4-spore ones in which one is obscured due to depth or perspective. 'SK1' is the parental strain, without centromeric GFP dots. n=500 for each count. Two strains each of 'Dot' and 'Dot/*htz1*' genotypes, obtained from two independent matings (and distinct *htz1* deletion transformants), are represented. Frequencies are in italics below absolute count values.
Tabulation of data illustrated in ***Figure 3***

	Single Cells	**Binucleate asci**	**Tri, Tetra nucleate Asci**	**Bi/Tri/Tetra nucleate Asci** ***(Sporulation Efficiency)***
Sk1	85 *17.00%*	79 *15.80%*	336 *67.20%*	415 ***83.00%***
Dot w.t. 1	63 *12.60%*	64 *12.80%*	373 *74.60%*	437 ***87.40%***
Dot w.t. 2	81 *16.20%*	100 *20.00%*	319 *63.80%*	419 ***83.80%***
Dot w.t. Avg.	14.40%	16.40%	69.20%	***85.60%***
Dot/*spo11*	126 *25.20%*	136 *27.20%*	238 *47.60%*	374 ***74.80%***
Dot/*htz1* 1	232 *46.40%*	64 *12.80%*	204 *40.80%*	268 ***53.60%***
Dot/*htz1* 2	297 *59.40%*	96 *19.20%*	107 *21.40%*	203 ***40.60%***
Dot/ htz1 Avg.	52.90%	16.00%	31.10%	***47.10%***

Supplement 2
Table: Tetrad viability - spore survival counts

Cells were collected 24 hours after sporulation, zymolyase-treated, and tetrads were dissected. Spores were grown at 37ºC for 2-3 days and scored for viability. "Het" and "Hom" indicate hetero- and homozygosity, respectively, for the GFP dots; all deletions are homozygous.

Tabulation of data illustrated in ***Figure 4***.

	Viable Spores				
	0	**1**	**2**	**3**	**4**
Dot w.t.	0 *0%*	0 *0%*	2 *4%*	3 *6%*	45 *90%*
Dot/ *spo11*	8 *80%*	0 *0%*	2 *20%*	0 *0%*	0 *0%*
Dot (Hom)/ *htz1*	2 *4%*	3 *6%*	6 *12%*	16 *32%*	23 *46%*
Dot (Het)/ *htz1*	0 *0%*	2 *10%*	1 *5%*	10 *50%*	7 *35%*

Supplement 3

Table: Progression Through Meiosis - Nuclear Division Timecourse

Fixed cell samples along a synchronized meiosis timecourse were scored for the number of nuclear masses per cell as determined by DAPI staining. Two independent strains of each genotype were assayed; n ~100 for each sample. Timepoint' refers to hours after induction of sporulation. Tri & tetranucleates were combined in a single category due to the difficulty in distinguishing true trinucleates from tetranucleates from which one product cell is obscured due to depth or perspective.

Tabulation of data illustrated in ***Figure 5****.*

	Time point (h)	Strain	Raw Counts, *(Frequency)* Mono	Bi	Tri/ Tetra	Combined Frequencies Mono *(Error)*	Bi *(Error)*	Tri/Tetra *(Error)*	Sporul'n *(Error)*
Dot wild-type	0	1	100 *100%*	0 *0%*	0 *0%*	99% *2%*	2% *2%*	0% *0%*	***2%*** *2%*
		2	97 *97%*	3 *3%*	0 *0%*				
	2	1	95 *95%*	4 *4%*	1 *1%*	97% *2%*	3% *1%*	1% *1%*	***4%*** *2%*
		2	98 *98%*	2 *2%*	0 *0%*				
	4	1	86 *86%*	14 *14%*	0 *0%*	89% *3%*	11% *3%*	1% *1%*	***12%*** *3%*
		2	91 *91%*	8 *8%*	1 *1%*				
	6	1	59 *59%*	34 *34%*	7 *7%*	58% *1%*	37% *3%*	6% *2%*	***42%*** *1%*
		2	57 *57%*	39 *39%*	4 *4%*				
	8	1	55 *55%*	29 *29%*	16 *16%*	56% *1%*	25% *4%*	18% *2%*	***44%*** *1%*
		2	63 *57%*	24 *22%*	23 *21%*				
	10	1	61 *61%*	12 *12%*	27 *27%*	60% *1%*	15% *3%*	25% *2%*	***40%*** *1%*
		2	64 *60%*	19 *18%*	24 *22%*				
	12	1	28 *27%*	8 *8%*	67 *65%*	24% *4%*	12% *4%*	65% *1%*	***76%*** *4%*
		2	20 *20%*	16 *16%*	64 *64%*				

Supplement 3

Table: Progression Through Meiosis - Nuclear Division Timecourse

*Tabulation of data illustrated in **Figure 5**.*

	Time point (h)	Strain	Raw Counts, (Frequency) Mono	Bi	Tri/Tetra	Combined Frequencies Mono (Error)	Bi (Error)	Tri/Tetra (Error)	Sporul'n (Error)
Dot / spo11	0	1	99 99%	1 1%	0 0%	100%	1%	0%	**1%**
		2	100 100%	0 0%	0 0%	1%	1%	0%	1%
	2	1	91 83%	17 16%	1 1%	88%	11%	0%	**12%**
		2	93 93%	7 7%	0 0%	5%	4%	0%	5%
	4	1	84 84%	16 16%	0 0%	88%	12%	1%	**12%**
		2	92 92%	7 7%	1 1%	4%	5%	1%	4%
	6	1	57 56%	33 32%	12 12%	62%	28%	10%	**38%**
		2	68 67%	24 24%	9 9%	6%	4%	1%	6%
	8	1	54 53%	27 27%	20 20%	57%	25%	18%	**43%**
		2	62 61%	23 23%	16 16%	4%	2%	2%	4%
	10	1	33 33%	31 31%	36 36%	40%	30%	30%	**60%**
		2	43 46%	27 29%	23 25%	7%	1%	6%	7%
	12	1	41 40%	7 7%	54 53%	42%	10%	48%	**58%**
		2	43 43%	13 13%	44 44%	1%	3%	4%	1%

Supplement 3

Table: Progression Through Meiosis - Nuclear Division Timecourse

*Tabulation of data illustrated in **Figure 5**.*

	Time point (h)	Strain	Raw Counts, (Frequency) Mono	Bi	Tri/Tetra	Combined Frequencies Mono (Error)	Bi (Error)	Tri/Tetra (Error)	Sporul'n (Error)
Dot / htz1	0	1	99 99%	1 1%	0 0%	100% 1%	1% 1%	0% 0%	**1%** 1%
		2	100 100%	0 0%	0 0%				
	2	1	96 96%	4 4%	0 0%	97% 1%	3% 1%	0% 0%	**3%** 1%
		2	99 98%	2 2%	0 0%				
	4	1	95 95%	5 5%	0 0%	96% 1%	4% 2%	1% 1%	**5%** 1%
		2	96 96%	2 2%	2 2%				
	6	1	82 82%	15 15%	3 3%	78% 4%	18% 3%	4% 1%	**22%** 4%
		2	73 74%	21 21%	5 5%				
	8	1	66 66%	26 26%	8 8%	63% 3%	29% 3%	8% 0%	**37%** 3%
		2	60 60%	32 32%	8 8%				
	10	1	74 74%	17 17%	9 9%	68% 6%	21% 4%	12% 3%	**33%** 7%
		2	61 61%	25 25%	14 14%				
	12	1	72 72%	11 11%	17 17%	62% 11%	12% 1%	27% 10%	**39%** 11%
		2	51 51%	13 13%	36 36%				

Time Point	Sample	Frequency- Nuclear Masses				Nuclear Masses / GFP dot segregation							
						Binucleates			Tetranucleates				
		Mono	Bi	Tri/ Tetra	Sporul'n (2+3/4)	1	2	No Call	1	2	3	4	No call
12h	Dot w.t. (Strain 1)	59	12	129	141	12	0	0	0	1	3	125	0
		30%	6%	65%	**71%**	100%	0%		0%	1%	2%	97%	
12h	Dot w.t. (Strain 2)	91	9	108	117	1	8	0	1	2	2	96	7
		44%	4%	52%	**56%**	11%	89%		1%	2%	2%	95%	
	Combined	**37%**	**5%**	**58%**	**63%**	**56%**	**44%**		**0%**	**1%**	**2%**	**96%**	
	(Error)	7%	1%	6%	**7%**	44%	44%		0%	1%	0%	1%	
12h	Dot/ *spo11*	115	29	143	172	8	13	8	12	32	26	36	37
		40%	**10%**	**50%**	**60%**	**38%**	**62%**		**11%**	**30%**	**25%**	**34%**	
12h	Dot/*htz* (Strain 1)	568	58	139	197	11	39	8	0	9	24	75	31
		74%	8%	18%	**26%**	22%	78%		0%	8%	22%	69%	
12h	Dot/*htz* (Strain 2)	79	13	152	165	0	12	1	1	9	24	72	46
		32%	5%	62%	**68%**	0%	###		1%	8%	23%	68%	
	Combined	**53%**	**6%**	**40%**	**47%**	**11%**	**89%**		**0%**	**8%**	**22%**	**69%**	
	(Error)	21%	1%	22%	**21%**	11%	11%		0%	0%	0%	1%	

Supplement 4

Table: GFP 'Dot' Assay Counts – Dot Homozygotes

Homozygous strains containing centromere-proximal GFP 'dots' underwent synchronous meiosis. 12 hours post-sporulation, cells were collected, fixed, and scored for the number of nuclear masses and the distribution of GFP dots therein. Headers in green denote the number of nuclear masses containing one or more GFP dots. Replicates constitute strains constructed from independent transformants. "No calls" are cells that could be scored for nuclear masses, but whose GFP dot distribution could not be confidently surmised. Note the significantly higher frequency of No-calls in *spo11Δ*and *htz1Δ* strains, due to either low signal to noise or to the presence of lagging chromosomes. *The highlighted section of the table tabulates data illustrated in* ***Figure 6****.*

Time Point	Sample	DAPI Masses Compiled: Mono	Bi	Tri/ Tetra	Sporul'n Efficiency (2+3/4)	DAPI Masses / GFP dot segregation: Binucleates: 1	2 (*--*)	2 (*-*-)	No Call	Tetranucleates: 1	2	No call
6h	Dot w.t.	98	109	18	127	97	3	7	2	0	17	1
	(Strain 1)	44%	48%	8%	56%	91%	3%	7%		0%	100%	
6h	Dot w.t.	184	134	60	194	119	7	2	6	0	55	5
	(Strain 2)	49%	35%	16%	51%	93%	5%	2%		0%	100%	
	Combined	46%	42%	12%	54%	92%	4%	4%		0%	100%	
	(Error)	3%	6%	4%	3%	1%	1%	2%		0%	0%	
8h	Dot/*htz*	140	119	33	152	59	28	19	13	0	24	9
	(Strain 1)	48%	41%	11%	52%	56%	26%	18%		0%	100%	
8h	Dot/*htz*	161	130	9	139	76	18	11	25	0	7	2
	(Strain 2)	54%	43%	3%	46%	72%	17%	10%		0%	100%	
	Combined	51%	42%	7%	49%	64%	22%	14%		0%	100%	
	(Error)	3%	1%	4%	3%	8%	5%	4%		0%	0%	

Supplement 5

Table: GFP 'Dot' Assay Counts – Dot Heterozygotes

Strains heterozygous for GFP dots underwent synchronous meiosis, and dot number and distribution was scored in binucleate Meiosis I products At the end of Meiosis I (6h post sporulation for wild-type, 8h for *htz1* deletion) samples were collected, stained, and scored for the number of nuclear masses and the distribution of GFP dots therein. Due to prior observations we scored cells with two resolvable GFP dots that were not clearly segregating apart as a distinct category. Headers in green denote the number of distinct GFP dots observed, along with their positions relative to the segregating nuclear masses *(*--* = segregating; *-*- = distinct but not necessarily segregating)* . Replicates constitute strains constructed from independent transformants. The highlighted section of the table contains data illustrated in ***Figure 7***.

Supplement 6: Strains generated and used

Strain #	Description	Back ground	Mating Type	Genotype	Notes
y01	Tester Strain		MatA		Amon lab
y02	Tester Strain		Matalpha		Amon lab
y03	"Wild Type"	SK1	MatA	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG	Amon lab- strain 4841
y04	"Wild Type"	SK1	Matalpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG	Amon lab- strain 4842
y05	Dot	SK1	MatA	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, leu2::LEU2::tetR-GFP::TetO-HIS3	Amon lab- strain 18026
y06	Dot	SK1	Matalpha	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, HIS pdr5::KanMX6, leu2::LEU2::tetR-GFP::TetO-HIS3	Amon lab- strain16028
y07	Dot/*spo11*	SK1	MatA	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, HIS+ ura3::pGPD1-GAL4(848).ER::URA3, spo11::URA3 leu2::LEU2::tetR-GFP::TetO-HIS3	Amon lab- strain 17186
y08	Dot/*spo11*	SK1	Matalpha	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, HIS+? ura3::pGPD1-GAL4(848).ER::URA3, spo11::URA3 leu2::LEU2::tetR-GFP::TetO-HIS3	Amon lab- strain 17206
y09-A	*htz1*	SK1	MatA	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 4, spore 1
y09-B	*htz1*	SK1	MatA	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 7, spore 1

Supplement 6: Strains generated and used *(Continued)*

Strain #	Description	Back ground	Mating Type	Genotype	Notes
y10-A	*htz1*	SK1	Matalpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 4, spore 3
y10-B	*htz1*	SK1	Matalpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 7, spore 2
y11-A	Dot/*htz1*	SK1	MatA	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 2, spore 3
y11-B	Dot/*htz1*	SK1	MatA	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 6, spore 2
y11-C	Dot/*htz1*	SK1	MatA	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 9, spore 1
y12-A	Dot/*htz1*	SK1	Matalpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 3, spore 3
y12-B	Dot/*htz1*	SK1	Matalpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 10, spore 4

Supplement 6: Strains generated and used *(Continued)*

Strain #	Description	Back ground	Mating Type	Genotype	Notes
y12-C	Dot/*htz1*	SK1	Matalpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3	(KanMX6 cassette replaces Htz1 ORF in y03) x y06 Tetrad 10.5, spore 1
y13-1	SK1	SK1	MatA/ alpha	ho::LYS2, (lys2?), leu2, ura3, his4X, trp1::hisG, promURA3::TetR::GFP::LEU2, tetOx224-URA3 containing AMp54 = YEPac112(pDMC1-SGO1):TRP1	Amon Lab-strain 13923
y14-A	Dot homozygous/ w.t.	SK1	MatA/ alpha	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, leu2::LEU2::tetR-GFP::TetO-HIS3 ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, HIS pdr5::KanMX6, leu2::LEU2::tetR-GFP::TetO-HIS3	y05 x y06
y14-B	Dot homozygous/ w.t.	SK1	MatA/ alpha	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, leu2::LEU2::tetR-GFP::TetO-HIS3 ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, HIS pdr5::KanMX6, leu2::LEU2::tetR-GFP::TetO-HIS3	y05 x y06
y15-A	Dot Homozygous/ *spo11*	SK1	MatA/ alpha	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, HIS+ ura3::pGPD1-GAL4(848).ER::URA3, spo11::URA3 leu2::LEU2::tetR-GFP::TetO-HIS3	y07 x y08
y15-B	Dot Homozygous/ *spo11*	SK1	MatA/ alpha	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, HIS+ ura3::pGPD1-GAL4(848).ER::URA3, spo11::URA3 leu2::LEU2::tetR-GFP::TetO-HIS3 *Homozygous*	y07 x y08

Supplement 6: Strains generated and used *(Continued)*

Strain #	Description	Back ground	Mating Type	Genotype	Notes
y16-A	*htz1* homozygous (No dot)	SK1	MatA/ alpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 *Homozygous*	y09A x y10A
y16-B	*htz1* homozygous (No dot)	SK1	MatA/ alpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 *Homozygous*	y09B x y10B
y17-A	Dot homozygous/ *htz1*	SK1	MatA/ alpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3 *Homozygous*	y11A x y12A
y17-B	Dot homozygous/ *htz1*	SK1	MatA/ alpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3 *Homozygous*	y11B x y12B
y18-A	Dot heterozygous/ w.t.	SK1	MatA/ alpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, HIS pdr5::KanMX6, leu2::LEU2::tetR-GFP::TetO-HIS3	y03 x y06
y18-B	Dot heterozygous/ w.t.	SK1	MatA/ alpha	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, leu2::LEU2::tetR-GFP::TetO-HIS3 ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG	y05 x y04
y18-C	Dot heterozygous/ w.t.	SK1	MatA/ alpha	ho::LYS2, ura3, leu2::hisG, his3::hisG, trp1::hisG, leu2::LEU2::tetR-GFP::TetO-HIS3 ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG	y05 x y04

Supplement 6: Strains generated and used *(Continued)*

Strain #	Description	Back ground	Mating Type	Genotype	Notes
y19-A	Dot heterozygous/ *htz1*	SK2	MatA/ alpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3 ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, htz1::KanMX6	y11A x y10A
y19-B	Dot heterozygous/ *htz1*	SK3	MatA/ alpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3 ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, htz1::KanMX6	y11B x y10B
y19-C	Dot heterozygous/ *htz1*	SK4	MatA/ alpha	ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, htz1::KanMX6 ho::LYS2, lys2, ura3, leu2::hisG, his3::hisG, trp1::hisG, htz1::KanMX6 leu2::LEU2::tetR-GFP::TetO-HIS3	y09A x y12A

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