Kinetochore function and chromosome segregation rely on critical residues in Histones H3 and H4 in Budding Yeast

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ABSTRACT

Accurate chromosome segregation requires that sister kinetochores biorient and attach to microtubules from opposite poles. Kinetochore biorientation relies on the underlying centromeric chromatin, which provides a platform to assemble the kinetochore and to recruit the regulatory factors that ensure the high fidelity of this process. To identify the centromeric chromatin determinants that contribute to chromosome segregation, we performed two complementary unbiased genetic screens using a library of budding yeast mutants in every residue of histone H3 and H4. In one screen, we identified mutants that lead to increased loss of a non-essential chromosome. In the second screen, we isolated mutants whose viability depends on a key regulator of biorientation, the Aurora B protein kinase. Nine mutants were common to both screens and exhibited kinetochore biorientation defects. Four of the mutants map near the unstructured nucleosome entry site and their genetic interaction with reduced IPL1 can be suppressed by increasing the dosage of SGO1, a key regulator of biorientation. In addition, the composition of purified kinetochores was altered in six of the mutants. Together, this work identifies previously unknown histone residues involved in chromosome segregation and lays the foundation for future studies on the role of the underlying chromatin structure in chromosome segregation.
INTRODUCTION

It is critical to understand the mechanisms that ensure accurate chromosome segregation because errors are associated with diseases such as cancer and can lead to cell death (Williams and Amon 2009; Compton 2011). Proper chromosome segregation is a highly regulated process that requires the coordination of a number of events. After DNA replication, sister chromatids are physically linked by cohesion (Oliveira and Nasmyth 2010; Nasmyth 2011). Kinetochores, the macromolecular complexes that assemble on centromeric DNA, must biorient and attach to microtubules from opposite poles. Once bioriented attachments are made, sister kinetochores come under tension due to microtubule-pulling forces on linked sister chromatids. Kinetochores lacking tension trigger the spindle checkpoint until proper attachments are made (Nezi and Musacchio 2009). Once every pair of sister chromatids has made bioriented attachments at metaphase, cohesion is dissolved, allowing sister chromatids to segregate to opposite poles at anaphase.

A key regulator of biorientation is the conserved chromosomal passenger complex (CPC), an essential protein kinase complex that detects and corrects microtubule-kinetochore attachments that are not under tension (Lampson and Cheeseman 2011). Phosphorylation of kinetochore substrates by the CPC protein kinase Aurora B destabilizes such aberrant attachments, giving the cell another opportunity to make proper, bioriented attachments (Liu and Lampson 2009). The CPC localizes to the inner centromere (Cooke et al. 1987), consistent with the model that tension between sister kinetochores stabilizes bioriented attachments by moving key substrates at the outer kinetochore away from the CPC (Tanaka et al. 2002; Fuller et
al. 2008; Liu et al. 2009). However, the precise mechanism by which the CPC acts on attachments not under tension is still unclear (Maresca and Salmon 2010). The Aurora B kinase is also required for the spindle checkpoint when kinetochores lack tension (Biggins and Murray 2001), although it is controversial whether this function is due to its role in destabilizing kinetochore-microtubule attachments (Musacchio 2011).

Another conserved protein implicated in biorientation and the tension checkpoint is shugoshin. Although the shugoshin family is well known for its meiotic role in protecting centromeric cohesion (Watanabe 2005; Gutierrez-Caballero et al. 2012), some family members also facilitate kinetochore biorientation and the checkpoint response to the lack of tension during mitosis (Indjeian et al. 2005; Vaur et al. 2005; Indjeian and Murray 2007; Kiburz et al. 2008). A conserved requirement for shugoshin localization to centromeres and pericentromeres is the phosphorylation of H2A S121 by the Bub1 protein kinase (Kawashima et al. 2010). In budding yeast, shugoshin (Sgo1) recruitment to nucleosomes also requires residue G44 in H3, which resides near H2A S121 in the nucleosome structure (Luger et al. 1997; Luo et al. 2010). In many organisms, there is an interdependence between shugoshin and Aurora B localization and activity (Dai et al. 2006; Resnick et al. 2006; Kawashima et al. 2007; Vanoosthuyse et al. 2007; Kawashima et al. 2010; Kelly et al. 2010; Wang et al. 2010; Yamagishi et al. 2010; Storchova et al. 2011), consistent with their close association with chromatin.

The underlying foundation of kinetochores is a specialized chromatin structure that creates the epigenetic mark for kinetochores and contributes to their assembly and function. While the bulk of the genome contains nucleosomes with ~147 bp of DNA
wrapped around two copies each of histone H2A, H2B, H3, and H4, the centromere contains a specialized histone H3 variant called Cenp-A (MADDOX et al. 2012). In most organisms, Cenp-A nucleosomes are interspersed with H3 nucleosomes in the core centromere and flanked by H3 nucleosomes in heterochromatin (BLOWER et al. 2002; CAM et al. 2005). In budding yeast, there is a single Cenp-A nucleosome positioned at the centromere (MELUH et al. 1998; FURUYAMA and BIGGINS 2007; KRASSOVSKY et al. 2012), as well as additional Cenp-A in the flanking pericentromeric chromatin (LAWRIMORE et al. 2011; HENIKOFF and HENIKOFF 2012). While budding yeast pericentromereres lack heterochromatin, a conserved feature is the enrichment of cohesin and Sgo1 to promote kinetochore biorientation (BLAT and KLECKNER 1999; TANAKA et al. 1999; KIBURZ et al. 2005; ECKERT et al. 2007; KIBURZ et al. 2008). In addition, evidence suggests that the pericentromeric chromatin adopts a specialized intramolecular structure that is organized by Sgo1 and facilitates biorientation in budding yeast (YEH et al. 2008; HAASE et al. 2012). Consistent with this, changes in pericentromeric chromatin composition lead to defects in the organization of inner kinetochore proteins and chromosome segregation (CHAMBERS et al. 2012; VERDAASDONK et al. 2012).

While it is clear that a specialized chromatin structure facilitates the assembly and function of more than 38 core kinetochore proteins and additional regulatory proteins (STELLFOX et al. 2012; VALENTE et al. 2012), the key determinants of this chromatin structure have still not been fully elucidated. We therefore set out to identify histone H3 and H4 residues that contribute to chromosome segregation and kinetochore biorientation by performing two systematic genetic screens in budding yeast. Our work identifies key residues in both histones that were previously not known
to regulate segregation, some of which contribute to Sgo1 function. This work lays the foundation for future studies aimed at understanding the roles of centromeric and pericentromeric chromatin in chromosome segregation and genomic stability.

MATERIALS AND METHODS

Screen to identify mutants sensitive to decreased IPL1 function

Individual mutations were integrated at the endogenous HHT2-HHF2 locus as described previously (Dai et al. 2008). H3 mutations were integrated into SBY9120 and H4 mutations into SBY9119. Correct integration was verified by PCR using the primers SB2409 and SB2410 for H3 mutants, and SB2409 and SB2411 for H4 mutants. Integrations were attempted at least three times before a given mutant was not pursued (Supplementary Table S1). The absence of the endogenous WT locus was also confirmed using the primers SB2409 and SB2412. Five-fold serial dilutions of asynchronously growing cells were grown for 2-3 days on YPD plates in the presence and absence of 25 µg/mL doxycycline, or 15 µg/mL benomyl. Primer sequences are available upon request.

Chromosome loss assays

The yeast strain (JDY176) used for testing chromosome loss was derived from SBY8053, which contains an artificial chromosome III fragment with SUP11 and HIS3 markers (Hieter et al. 1985). The HHT1-HHF1 coding fragment including the promoter was knocked out to generate JDY168. The ura3-1 mutation was corrected to obtain URA3 strains followed by deletion of the ORF to generate an ura3Δ0 strain as described
(BRACHMANN et al. 1998). The resultant strain, JDY176, was used in subsequent studies.

Individual histone mutations were integrated at the endogenous HHT2-HHF2 locus as described previously (DAI et al. 2008). Correct integration was confirmed by PCR and at least two independent isolates were obtained for each mutant (primers SB2409, SB2410, SB2411, SB2412). Each mutant was streaked onto synthetic complete agar plates containing 48µM adenine and grown at 30˚C for 4 days. The plates were stored at 4˚C for three days before evaluating the percentage of colonies with red sectors. For quantification of chromosome loss, the yeast strains were grown in liquid medium overnight. The cell density in the culture was measured and approximately 200 cells were plated onto synthetic complete agar plates containing 48µM adenine. The number of colonies containing at least half red sectors was quantified and divided by the total number of colonies to calculate the percentage of chromosome loss in the first generation.

**Microbial Techniques and Plasmid Construction**

Media and microbial techniques were as described (SHERMAN et al. 1974; ROSE et al. 1990). All experiments were performed at 23˚C unless otherwise noted. In all synchronous cell cycle experiments reported, 1 or 10 µg/mL α-factor (custom synthesized by United Biochemical Research, Inc., Seattle, WA) was used to arrest bar1-1 and BAR1 cells in G1, respectively. Doxycycline (Sigma-Aldrich, St. Louis, MO) was used at 25 µg/mL. Yeast strains are listed in Supplementary Table S3. High copy SGO1 (pSB1780, see construction notes below) or control (pRS425, (SIKORSKI and
HIETER 1989)) plasmids were introduced into the histone mutant strains by transformation.

To generate a high copy \textit{SGO1} plasmid with the \textit{LEU2} marker (pSB1780), the \textit{URA3} two micron plasmid pMK573 (LUO \textit{et al.} 2010) was digested with Hpal and AatII to remove \textit{URA3}. The \textit{LEU2} gene was isolated from YEplac181 (GIETZ and SUGINO 1988) by digestion with the same restriction enzymes and ligated to the digested plasmid pMK573 to create pSB1780.

**Flow Cytometry**

For the \textit{orc2-1} experiment, WT and \textit{orc2-1} strains were shifted to 37 degrees for 3 hours. For the histone mutant strains, cells were grown at room temperature. After harvesting cells, they were fixed with 70% ethanol at room temperature. Fixed cells were then incubated in 0.2 mg/mL RNase A (Sigma-Aldrich, St. Louis, MO) in 50mM TrisHCl, pH 8.0 for four hours at 37˚C and 2 mg/mL Proteinase K (Roche, Indianapolis, IN) in 50mM Tris, pH 7.5 for one hour at 50˚C. Cells were then incubated with 5mM Sytox Green (Molecular Probes, Eugene, OR) in 50mM Tris, pH 7.5. Data were collected and analyzed using Cell Quest software (BD Biosciences, San Jose, CA).

**Microscopy, Protein and Immunological Techniques**

Analysis of GFP-LacI was performed as described (BIGGINS \textit{et al.} 1999). For all microscopy experiments, greater than 200 cells were scored. The Bernoulli distribution was used to assess statistical significance at 95% confidence. Anaphase was analyzed by staining cells with 4’,6-diamidino-2-phenylindole (DAPI, obtained from Sigma-Aldrich)
and identifying cells with two separated DNA masses. Protein extracts were made and immunoblotted as described (Minshull et al. 1996). Quantitative immunoblotting was performed with IRDye secondary antibodies from LI-COR at a 1:15,000 dilution. The immunoblots were imaged on a LI-COR imaging system and the protein levels were quantified using the ImageJ program. The mean of three independent experiments is reported. Loading controls for all experiments were either anti-tubulin (Accurate Chemical and Scientific Corp.) used at 1:1000, or anti-PGK1 (Invitrogen) at a 1:10,000 dilution. Centromeric minichromosomes were purified and analyzed by immunoblotting as described previously (Akiyoshi et al. 2009). Anti-Spc105 polyclonal antibodies were used at a 1:1,000 dilution (Akiyoshi et al. 2010), anti-FLAG monoclonal antibodies (Sigma-Aldrich) were used at 1:3,000 and anti-Cse4 polyclonal antibodies at 1:500 (Pinsky et al. 2003). Anti-Ndc80 (OD4, 1:10,000), anti-Ndc10 (OD1, 1:5,000), anti-Mif2 (OD2, 1:6,000), and anti-Ctf19 (OD10, 1:15,000) polyclonal antibodies were a generous gift from Arshad Desai (Akiyoshi et al. 2009).

ChIP Assays and Quantitative Real-Time PCR

ChIP was performed using antibodies against Cse4 as described previously (Collins et al. 2005) and samples were quantified by quantitative real-time PCR (7900HT, ABI Prism). DNA samples were amplified using a SYBR PCR mix (Applied Biosystems) at 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 55°C for 1 minute using CEN3 (SB1253 and SB1254) and PHO5-specific primers (SB3063 and SB3064). PCR amplification efficiency and linearity were determined using serial dilutions of samples. Standard curves were generated for every PCR reaction and used
for quantification of bound DNA that was expressed as the percentage of input DNA. Sequences of PCR primers are available upon request.

**Nucleosome Structures**

Nucleosome structures were prepared with PyMOL ([http://www.pymol.org/](http://www.pymol.org/)).

**Expression profiling**

Each mutant strain (derivatives of JDY86 as previously described (Dai et al. 2008)) was profiled four times from two independently inoculated cultures and harvested in early mid-log phase in SC medium with 2% glucose. Sets of mutants were grown alongside corresponding H3 and H4 WT cultures (single copy H3 or H4) and processed in parallel. Dual-channel 70-mer oligonucleotide arrays were employed with a common reference WT RNA. All steps after RNA isolation were automated using robotic liquid handlers. These procedures were first optimized for accuracy (correct fold-change) and precision (reproducible result), using spiked-in RNA for calibration (Van de PeppeL et al. 2003). After quality control, normalization, and dye-bias correction (Margaritis et al. 2009), statistical analysis for mid-log cultures was performed for each mutant versus the WT cells grown alongside using Limma. The reported fold-change (FC) is an average of the replicate mutant profiles versus the H3 or H4 WT. Microarray data have been deposited in ArrayExpress under accession number E-MTAB-1242, as well as in GEO under accession number GSE39903.

**RESULTS**
Identification of H3 and H4 residues important for chromosome segregation

To identify histone residues involved in the regulation of chromosome segregation, we screened for budding yeast H3 and H4 mutants that exhibit chromosome loss. We used a previously constructed library of mutants where every H3 and H4 amino acid was systematically changed to alanine, and the modifiable residues were changed to both alanine and a charged residue (DAi et al. 2008). Deletions within the amino-terminal tails of each histone were also assayed. A cassette containing the mutants was introduced into the primary H3 and H4 locus, HHT2-HHF2, in a strain containing a deletion of the secondary H3 and H4 locus, hht1-hhf1Δ. The strain also contained an artificial chromosome fragment that allowed us to assay the frequency of chromosome loss using a colony color-sectoring assay (HIETER et al. 1985). Mutants that exhibited increased sectoring were quantified for the percent chromosome loss per generation. Together, 26 mutants in H3 and 15 mutants in H4 exhibited an increased frequency of chromosome loss compared to WT (Figure 1A and 1B).

Histone mutations can cause pleiotropic effects, so we performed a second screen to identify the histone residues that might specifically contribute to kinetochore biorientation. We isolated mutants that require the full activity of the Ipl1/Aurora B protein kinase for viability. To do this, we used a previously characterized doxycycline-repressible degron allele, deg-ipl1, that targets the protein for degradation by the proteasome (Ng et al. 2009). Although IPL1 is essential, doxycycline addition does not severely inhibit the growth of deg-ipl1 cells (Figure 1C), indicating that these cells retain enough Ipl1 function to support viability. However, deg-ipl1 is lethal when combined with other non-essential mutants such as the mcm21 kinetochore mutant, indicating that
it is a hypomorphic allele (Ng et al. 2009). We therefore introduced each alanine substitution mutation in H3 and H4 into a deg-ipl1 strain containing a deletion of the secondary copy of H3 and H4, hht1-hhf1Δ. We note that 24 of the alanine mutants, including six of the residues identified in the chromosome loss screen (H3 Y41, H3 Q68, H3 L103, H3 I112, H3 I119, H4 L97), could not be generated in the deg-ipl1 strain background, suggesting synthetic lethality, and could not be further pursued (Supplementary Table S1). The remaining mutants were analyzed for growth in the presence and absence of doxycycline and 29 mutants were identified that exhibited some degree of sensitivity to downregulation of IPL1 compared to WT strains (Table 1).

We focused on the mutants that were identified in both screens and are therefore most likely to be important for chromosome segregation. The corresponding residues are H3 Q5, H3 R40, H3 G44, H3 T45, H3 R53, H3 N108, H3 L109, H3 K115, and H4 K44, H4 V81 and H4 Y98. However, the H3 K115A mutant cells grew extremely slowly (data not shown) and the H3 T45A mutant cells were previously reported to have replication defects (Baker et al. 2010), so we did not continue to analyze them. The remaining nine mutants were assayed for the severity of their growth defect with or without the deg-ipl1 allele by plating serial dilutions in the absence and presence of doxycycline (Figure 1C). In the absence of IPL1 downregulation, all of the mutants grew well except H4 K44A and H4 Y98A. In the presence of doxycycline, the H3 R40A, H3 G44A, H3 R53A, H3 N108A, H3 L109A, and H4 K44A, H4 V81A mutants exhibited a strong or complete loss of viability, whereas the H3 Q5A and H4 Y98A mutants showed a weak dependence on full IPL1 function. All of the identified residues are conserved, and mapping them onto the nucleosome structure shows that H3 R40, H3 G44, H3 R53,
and H4 K44 cluster near the nucleosome entry/exit site, whereas H3 N108, H3 L109, H4 V81 and H4 Y98 are buried residues (Figure 1D) (Luger et al. 1997).

**Analysis of replication and segregation in the histone mutant strains**

Because chromosome loss phenotypes can either be a result of replication or segregation defects, we performed fluorescence-activated cell sorting (FACS) on the histone mutants to analyze replication. As a control, we analyzed the *orc2-1* temperature sensitive mutant that is defective in replication and shows an accumulation of cells with DNA content between 1N and 2N (Figure 2A). The *deg-ipl1 hht1-hhf1Δ* strains containing WT or mutant H3 or H4 were grown in the absence of doxycycline and processed for FACS (Figure 2B). None of the mutants exhibited a strong delay in S-phase, although subtle replication defects may exist that cannot be detected due to the resolution of this assay.

We next directly assayed chromosome segregation in each mutant strain by analyzing a fluorescently marked chromosome (Straight et al. 1996). Asynchronous cultures of *deg-ipl1* strains containing GFP-marked ChrIV and the H3 and H4 mutations were grown in doxycycline to repress *IPL1* for six hours. Cells that had proceeded through anaphase (segregated DNA to opposite poles) were scored for segregation of ChrIV to a single pole (misseggregation) or opposite poles (accurate segregation) (Figure 3A). The strongest segregation defects occurred in the H3 R40A, H3 G44A, H3 L109A, H4 K44A and H4 V81A mutant strains, which all exhibited greater than 15% misreggregation of ChrIV within six hours of *IPL1* downregulation. The H3 R53A and H3 N108A mutant strains showed a greater than 10% misreggregation defect after 6 hours,
whereas there were no significant segregation defects in the H3 Q5A or H4 Y98A strains. Strikingly, the levels of chromosome missegregation in each mutant strain parallel the growth defects when *IPL1* is downregulated (see Figure 1B), suggesting that the loss of viability is due to chromosome missegregation.

We next attempted to analyze segregation in a synchronous cycle by releasing cells from a G1 arrest into doxycyline. However, many of the histone mutants exhibited a transient delay in the onset of anaphase when released from G1 as indicated by the reduced percentage of cells with DNA masses at opposite poles relative to WT cells (data not shown). We reasoned that this delay could be due to spindle checkpoint activation, which would give sister chromatids additional time to biorient. We therefore examined ChrIV segregation in *deg-ipl1 mad3Δ* strains containing the histone mutations during a synchronous cell cycle. As expected, the absence of the spindle checkpoint reduced the delay in anaphase onset because greater than 50% of the cells segregated their DNA to opposite poles within 80 minutes post-G1 release, similar to WT cells. ChrIV segregation was monitored at the time point (100 or 120 min after G1 release) when the highest percentage of cells had DNA masses at opposite poles (Figure 3B). Similar to our findings on asynchronous cells, there were significant segregation defects (greater than 8%) in the H3 R40A, H3 N108A, H3 L109A, and the H4 K44A mutant strains. By extrapolation, a missegregation frequency of 8% for a single chromosome means that less than 26% of the mutant cells would be able to segregate all 16 chromosomes properly, consistent with the strong growth defects observed in these mutant strains. There were minor defects in the H3 Q5A and H3 R53A mutant strains, and no observable defect in the H4 Y98A strain. Whereas the H4 V81A strain appeared
to have a missegregation defect, greater than 4% of G1-arrested cells exhibited two GFP foci, indicative of aneuploidy, as compared to less than 1.3% in all other strains. *H3 G44A* mutant cells were not quantified in this experiment because greater than 5% of cells exhibited two GFP foci in the G1 arrest, indicating pre-existing aneuploidy that makes accurate quantification impossible.

**The expression of segregation genes is not significantly altered in the histone mutants**

To determine whether the segregation defects may be due to altered transcription of segregation genes, we performed DNA microarray expression analysis on each mutant. RNA was prepared from asynchronous cultures of WT or histone mutant strains (without *deg-ipl1*). The cRNA was then labeled and hybridized to 70-mer oligonucleotide microarrays. Each mutant was analyzed for gene expression changes greater than 1.7-fold up or down and with P-value less than 0.01. We analyzed the list (Supplementary Table S2) for genes known to be involved in kinetochore function or chromosome segregation and did not find any mutant that significantly altered any chromosome segregation genes. We note that H3 G44A, H4 K44A and H4 V81A exhibit aneuploidy based on their gene expression profile, consistent with their segregation defects. Together, the microarray data suggest that the segregation defects in the histone mutants are not due to the altered transcription of one or more genes required for kinetochore function.

**Cse4 localization to centromeres is normal in the histone mutants**
Because the kinetochore assembles on a specialized chromatin structure, we reasoned that the histone mutants might alter the chromatin at and/or around centromeres, thus disrupting chromosome segregation. Yeast centromeres contain a single well-positioned nucleosome that contains the specialized histone H3 variant Cse4 (STOLER et al. 1995; MELUH et al. 1998; FURUYAMA and BIGGINS 2007; KRASSOVSKY et al. 2012). We therefore tested whether total Cse4 levels are altered by any of the histone mutants by performing quantitative immunoblotting on crude lysates from WT and histone mutant strains with antibodies against Cse4. We compared the ratio of Cse4 to Pgk1, a loading control, and found that Cse4 levels are close to WT (+/- 10%) in most of the strains. However, Cse4 levels were decreased by 13% in the H3 L109A mutant and 29% in the H4 V81A mutants, and increased by 17% in the H3 R53A mutant strain (Figure 4A). The microarray data did not reveal any significant change in the transcription of CSE4 in these mutants, indicating that the lower protein levels are likely due to a post-transcriptional effect. Cse4 levels are tightly regulated by proteolysis, so the H4 V81A mutant may alter the function or accessibility of the ubiquitin ligase that regulates Cse4 (COLLINS et al. 2004; HEWAWASAM et al. 2010; RANJITKAR et al. 2010). Although it is unclear how H3 mutants would alter Cse4 levels, they may affect the ability of Cse4 to incorporate into euchromatin, which could affect the accessibility of Psh1 to degrade Cse4.

We next asked whether Cse4 incorporation at the centromere was affected in any of the histone mutant strains. Although most of the mutants we identified are in H3, changes in the nucleosomes surrounding the centromere could lead to changes in Cse4 incorporation at centromeres. In addition, it was recently reported that H3 also localizes
to centromeres (LOCHMANN and IVANOV 2012), although the resolution of the assay used cannot discriminate between localization at the core centromere and surrounding nucleosomes. To analyze Cse4 localization at centromeres, we performed chromatin immunoprecipitation on the deg-ipl1 histone mutant strains grown in doxycycline for six hours. The cells contained a non-essential minichromosome so that we could analyze Cse4 in the context of a plasmid in addition to the endogenous centromere (AKIYOSHI et al. 2009). Cse4 was immunoprecipitated and the amount of DNA bound to Cse4 was analyzed by standard PCR with primers to the centromere. There was no obvious change in any of the strains (data not shown), so we analyzed a subset of them using quantitative real-time PCR with primers to the centromeres or a control locus, PHO5 (Figure 4B). This revealed a slight decrease in Cse4 bound to the centromere in the H4 V81A mutant that had lower Cse4 levels in the lysate. There was also a slight decrease in the level of Cse4 at the endogenous centromere in the H3 R53A mutant that had higher levels of total Cse4, although this was not apparent on the minichromosome. We have not previously detected changes in centromere-bound Cse4 when the gene is overexpressed (COLLINS et al. 2004), so this result is likely related to changes in the chromatin due to the H3 mutation rather than the altered Cse4 levels. The remaining mutants showed no significant differences in the level of Cse4 at either the endogenous or minichromosome centromere when compared to WT. Together, these data demonstrate that the histone mutants do not significantly alter Cse4 localization to the centromere.

**Kinetochore stability is altered in the histone mutant strains**
We next asked whether overall kinetochore integrity is normal in the mutants by purifying centromeric minichromosomes (Akiyoshi et al. 2009). In contrast to chromatin immunoprecipitation techniques that require a cross-linking step prior to immunoprecipitation, the minichromosome purification technique isolates native material and can therefore reveal subtle changes in kinetochore stability. A centromeric minichromosome containing LacO sequences was introduced into each deg-ipl1 histone mutant that also expressed lacI-Flag and the cells were grown asynchronously in doxycycline for six hours. We did not detect major alterations in the total protein levels of representative core kinetochore proteins in the lysates prepared from each histone mutant strain other than a slight reduction in Ndc10 levels in the H3 L109A mutant strain (Figure 5A). We therefore immunoprecipitated LacI-Flag and analyzed the purified minichromosome samples for the levels of co-purifying kinetochore proteins. The Ndc10 protein is a component of the CBF3 complex that binds directly to the CDEIII element of the yeast centromere (Lechner and Carbon 1991). Because Ndc10 binding is unlikely to be affected by changes in neighboring nucleosomes (Cho and Harrison 2012), we compared the relative levels of representative components spanning the inner (Mif2, Ctf19, and Cse4) and outer kinetochore (Spc105 and Ndc80) to Ndc10 levels (Figure 5B). In the H3 Q5A, H3 N108A, and H4 Y98A histone mutant strains, the levels of kinetochore proteins bound to minichromosomes were similar to WT. However, in the H3 G44A mutant, there was a decrease in all components, consistent with previous work that showed that this residue contributes to segregation (Luo et al. 2010). The H4 K44A and H4 V81A mutants appeared to co-purify somewhat lower levels of the inner kinetochore components Cse4, Mif2 and Ctf19, revealing an overall
defect in inner kinetochore stability. Because the levels of Cse4 at the centromere were not significantly different than WT in these mutants when assayed by chromatin immunoprecipitation (see Figure 4B), our data are consistent with the possibility that there is a subtle defect in kinetochore stability that is revealed during the purification of the minichromosome. Consistent with this, the Spc105 outer kinetochore protein was also reduced in these two H4 mutants. We note that although H4 K44 is a key residue in Set2 methylation of H3 K36 (Du et al. 2008), the latter residue was not identified in our screens. Surprisingly, the H3 R40A, H3 R53A and H3 L109A mutants appeared to have a stronger association of one or more kinetochore proteins relative to Ndc10 than WT. While the underlying mechanism is not clear, it was recently reported that a deletion of the Cnn1 kinetochore protein leads to a more robust association between outer kinetochore proteins (Bock et al. 2012). Cnn1 is the budding yeast ortholog of the chromatin-associated Cenp-T protein (Nishino et al. 2012; Schleiffer et al. 2012), raising the possibility that its function is altered in these histone mutants. Regardless of the mechanism, the kinetochore appears to be more robust to purification in the presence of these mutations.

**High copy SGO1 can alleviate defects in some histone mutant strains**

Pericentromeric chromatin recruits the Sgo1 protein to facilitate biorientation and the tension checkpoint. Because Ipl1 and Sgo1 both have roles in kinetochore biorientation and the tension checkpoint (Biggins et al. 1999; Biggins and Murray 2001; Indjeian et al. 2005; Indjeian and Murray 2007), we considered the possibility that the histone mutants have defects in Sgo1 function. Consistent with this, we
identified the H3 G44 residue near the nucleosome entry/exit site that is required for Sgo1 localization to pericentromeric chromatin in our screen (Luo et al. 2010). In addition, we also identified two other residues near the nucleosome entry exit site (H3 R40 and H4 K44) as well as two buried residues that could affect this region (H3 N108 and H3 L109). High copy SGO1 can suppress the mitotic defects in an H3 G44S mutant (Luo et al. 2010), so we tested whether SGO1 overexpression has an effect on the histone mutants we identified. We analyzed the growth of deg-ipl1 histone mutant strains containing a high copy SGO1 plasmid in the presence and absence of doxycycline (Figure 6A). We found that H3 R40A, H3 G44A, H3 N108A, H3 L109A, H4 K44A were all suppressed to varying degrees while the growth of the other mutants were not affected. These data strongly suggest that Sgo1 function is compromised in these histone mutants and may be the underlying mechanism that leads to defects in segregation and biorientation. Strikingly, all of these residues are close to the nucleosome/entry exist site, suggesting that we have further defined structural constraints of the nucleosome required for Sgo1 localization and/or function at centromeres.

**DISCUSSION**

In sum, we utilized an H3 and H4 mutant library to identify residues that ensure the fidelity of chromosome segregation. Although many screens for histone mutations that affect genomic stability have been reported (Smith et al. 1996; Hyland et al. 2005; Matsubara et al. 2007; Dai et al. 2008; Sakamoto et al. 2009; Kawashima et al. 2011),
none have been specifically directed at measuring chromosome segregation frequencies or sensitivity to Ipl1/Aurora downregulation. While this work was in progress, Kawashima et al. reported a systematic screen of all histone mutants for sensitivity to the microtubule-depolymerizing agents benomyl and TBZ (KAWASHIMA et al. 2011). There was little overlap of mutants identified in their study with those identified here, with the exception of H3 G44A and H4 Y98A. The lack of overlap could potentially be due to off-target effects of the drugs.

Our work identified H3 residues Q5, R40, G44, R53, N108, and L109, and H4 residues K44, V81, and Y98 as important for segregation and biorientation. While it is unclear how all of the residues we identified contribute to these processes, five of the mutants reside near the nucleosome entry/exit site and can be suppressed by increasing the dosage of SGO1, consistent with the Sgo1 binding site spanning this region of the nucleosome. An attractive hypothesis is that the DNA at the entry/exit site of the nucleosome may come under tension when kinetochores biorient, thus signaling to the cell that the kinetochores have achieved biorientation. Consistent with this, tension-dependent changes in budding yeast pericentromeric chromatin structure have been observed by microscopy (HAASE et al. 2012; VERDAASDONK et al. 2012). The localization of Sgo1 to this region may therefore be coupled to its ability to trigger the spindle checkpoint when the pericentromeric chromatin is not under tension. Sgo1 and Bub1 modulate pericentromeric chromatin structure in response to microtubule dynamics (HAASE et al. 2012), so it is possible that the histone mutations we have identified alter a specific structural property associated with pericentromeres. We attempted to analyze chromatin structure in the pericentromere region in the absence of
tension and Sgo1, but the resolution of the assay we used was not sensitive enough to
detect any changes (data not shown). In addition, we were not able to detect significant
changes in Sgo1 localization to pericentromeres by ChIP (data not shown). An
important future direction will be to determine how the interaction between Sgo1 and
nucleosomes mechanistically contributes to biorientation. It will also be important to
understand how the other histone mutants we identified contribute to chromosome
segregation and thus maintain genomic stability. It was recently shown that histones in
the pericentromere are turned over at a higher rate than the arms, so one possibility is
that the mutants we identified alter histone dynamics within the pericentromere
(VERDAASDONK et al. 2012). Our work provides a foundation for further mechanistic
studies aimed at understanding the role of centromeric and pericentromeric chromatin in
chromosome segregation.

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comments on the manuscript. Author contributions were as follows: chromosome loss
assays and analysis were performed by SJ, JD and JDB, microarrays and
corresponding analyses were performed by TLL, SB and FCPH, the deg-ipl screen and
all further characterization of mutants in the manuscript were performed by TMN with
help from ND, SC, and SB on the kinetochore protein quantification and kinetochore
purifications. TMN and SB wrote the manuscript and analyzed the data. This work was
supported by the following funding sources: TMN was supported in part by Public Health Service, National Research Service Award, T32 GM07270, from the National Institute of General Medical Sciences. TLL and FCPH are supported by the Netherlands Organization of Scientific Research (NWO). This work was supported by NIH grant GM078079 to SB and U54RR020839 to JDB.
LITERATURE CITED


BIGGINS, S., and A. W. MURRAY, 2001 The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. Genes Dev 15: 3118-3129.


HEWAWASAM, G., M. SHIVARAJU, M. MATTINGLY, S. VENKATESH, S. MARTIN-BROWN \textit{et al.}, 2010 Psh1 is an E3 ubiquitin ligase that targets the centromeric histone variant Cse4. Mol Cell \textbf{40}: 444-454.


KELLY, A. E., C. GHENOIU, J. Z. XUE, C. ZIERHUT, H. KIMURA et al., 2010 Survivin reads phosphorylated histone H3 threonine 3 to activate the mitotic kinase Aurora B. Science 330: 235-239.


SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122**: 19-27.


FIGURE LEGENDS

Figure 1. Identification of H3 and H4 mutants with chromosome segregation defects. (A and B) The frequency of chromosome loss for the indicated histone H3 (A) and H4 (B) mutants identified in the colony sectoring assay is plotted (all strains are derived from JDY176, Supplementary Table S3). (C) Five-fold serial dilutions of strains containing the indicated histone mutant in the presence or absence of deg-ipl1 (SBY719, SBY9837-SBY9847, SBY9880-SBY9888) were plated with (+) or without (-) doxycycline. Note that two different parent cassettes (WT H3 and H4) were used to generate mutants in the corresponding histone, and various pairs of images were cropped to assemble the figure. (D) The H3 (blue) and H4 residues (magenta) identified in both screens are highlighted on the nucleosome structure (Luger et al. 1997). The H3 Q5 residue in the unstructured tail and buried residues of H4 V81 and H4 Y98 are not shown. Front and side views are shown.

Figure 2. The histone mutants do not exhibit replication defects. A. WT (SBY4) and orc2-1 (SBY11682) strains were shifted to 37 degrees and then subjected to FACS analysis. B. FACS profiles of histone mutants that exhibit segregation defects. Asynchronous cultures of the indicated histone mutants (SBY9119, SBY9120, SBY9625, SBY9660, SBY9664, SBY9665, SBY9673, SBY9724, SBY9725, SBY9786, SBY9818, SBY9832) were processed for FACS analyses.

Figure 3. Analysis of sister chromatid segregation in the histone mutant strains. (A) Asynchronous cultures of deg-ipl1 strains (SBY9837 - SBY9847) were grown in
doxycycline for zero or six hours and ChrIV segregation was monitored in anaphase cells that had DNA masses at opposite poles. (B) deg-ipl1 mad3Δ strains containing WT H3 and H4 or the mutations indicated (SBY9848 - SBY9858) were released from G1 in the presence of doxycycline. ChrIV segregation was monitored in anaphase cells with DNA masses at opposite poles.

Figure 4. Analysis of Cse4 levels and localization to the centromere in the H3 and H4 mutants. (A) The indicated deg-ipl1 histone mutant strains (SBY10182-10192) were grown in doxycycline for six hours and crude lysates were immunoblotted with anti-Cse4 and anti-Pgk1 antibodies for quantitative analysis. A representative immunoblot is shown, and the mean quantified Cse4 to Pgk1 ratio relative to the corresponding WT parent is reported under each lane. (B) Cse4 chromatin immunoprecipitation was performed on deg-ipl1 H3 (top) and H4 (bottom) mutant strains (SBY10182-10192) containing centromeric minichromosomes grown in doxycycline for six hours. Quantitative real-time PCR was carried out using oligos specific to endogenous CEN3, the minichromosome CEN, and a control locus, PHO5.

Figure 5. Analysis of kinetochore composition on minichromosomes purified from H3 and H4 mutant strains. (A) The indicated deg-ipl1 histone mutant strains (SBY10182-10192) were grown asynchronously in doxycycline for six hours and immunoblotted with antibodies against the indicated kinetochore proteins. Tubulin or Pgk1 are shown as loading controls. (B) Centromeric minichromosomes were immunoprecipitated from deg-ipl1 H3 (left) and H4 (right) mutant strains (SBY10182-10192) grown in doxycycline
for six hours. The purifications were immunoblotted for the indicated outer kinetochore proteins (Spc105, Ndc80) and inner (Mif2, Ctf19, Cse4) kinetochore proteins. Ndc10 directly binds to the centromere and was used as a loading control.

Figure 6. Analysis of SGO1 overexpression in the deg-ipl1 histone mutant strains. Five-fold serial dilutions of deg-ipl1 histone mutant strains containing a control (-) or high copy SGO1 plasmid (+)(SBY9870, SBY9871, SBY9874 SBY9875, and SBY10284-SBY10297) were analyzed for growth in the presence and absence of doxycycline.
Table 1. Summary of mutant phenotypes identified in the *deg-ipl1* and chromosome loss screens.

<table>
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<tr>
<th>Histone residue</th>
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<th>Dox sens</th>
<th>Increased temp sens</th>
<th>Ben Sens</th>
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<td>+</td>
<td>R</td>
</tr>
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<td>+</td>
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<td>H4 G99</td>
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<td></td>
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<tr>
<td>H4 Δ1-24</td>
<td>T</td>
<td></td>
<td>N/D</td>
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<tr>
<td>H4 Δ4-14</td>
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</table>

1. All histone residues assayed were mutated to alanine. In some cases, additional mutants were also assayed as indicated in Chr loss column.

2. Locations as defined in (DAI et al. 2008) (T = Tail; L = Lateral; B = Buried; D=Disk).

3. Chromosome Loss (Chr loss). The indicated histone mutant exhibited increased chromosome loss relative to WT.


5. Indicated mutants were crossed to ipl1-321 and assayed for genetic interaction based on increase temperature sensitivity.

6. Benomyl sensitivity (Ben sens). + indicates sensitivity and – indicates no sensitivity. R indicates resistance compared to WT.

N/A* = Not applicable because the histone mutant could not be generated in the deg-ipl1 strain background.
A

B

CEN3

Minichromosome CEN

PHO5

CEN3

Minichromosome CEN

PHO5

Percent IP

Percent IP
<table>
<thead>
<tr>
<th></th>
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<th>+Dox</th>
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