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The *Saccharomyces cerevisiae* histone H2A variant Htz1 is acetylated by NuA4

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The histone H2A variant H2A.Z (*Saccharomyces cerevisiae* Htz1) plays roles in transcription, DNA repair, chromosome stability, and limiting telomeric silencing. The Swr1-Complex (SWR-C) inserts Htz1 into chromatin and shares several subunits with the NuA4 histone acetyltransferase. Furthermore, mutants of these two complexes share several phenotypes, suggesting they may work together. Here we show that NuA4 acetylates Htz1 Lys 14 (K14) after the histone is assembled into chromatin by the SWR-C. K14 mutants exhibit specific defects in chromosome transmission without affecting transcription, telomeric silencing, or DNA repair. Function-specific modifications may help explain how the same component of chromatin can function in diverse pathways.

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Two classes of enzymes have been implicated in regulating chromatin structure and access to the underlying DNA template. The ATP-dependent chromatin remodeling enzymes use ATP hydrolysis to induce nucleosome mobility or disrupt histone–DNA interactions. The second class of enzymes covalently modify (e.g., lysine acetylation, serine phosphorylation, lysine and arginine methylation, ubiquitylation, or ADP ribosylation) various histones, usually on their N-terminal tails (Strahl and Allis 2000; Jenuwein and Allis 2001). Acetylation is carried out by histone acetyltransferases (HATs), which in *Saccharomyces cerevisiae* include the Gcn5-containing ADA and SAGA complexes, Hat1, Elongator, NuA3, and NuA4 (for review, see Bottomley 2004). These typically have specificity for distinct lysine residues on certain histone N-terminal tails. The acetylation of lysine residues on the N-terminal tails of histones H3 and H4 neutralizes their positive charge, possibly decreasing their affinity for DNA and facilitating chromatin decompaction and disassembly (Eberharter and Becker 2002). Perhaps more important than simple charge neutralization is the specific pattern of acetylation at individual lysine residues, at least some of which recruit bromodomain-containing proteins (Matangkasombut and Buratowski 2003, and references therein).

Further chromatin specialization can be introduced by incorporation of variant histones. The major histones are assembled during DNA replication, but can be replaced by variants at specific locations (for review, see Malik and Henikoff 2003). The histones with known variants are H3 and H2A, both of which self-interact within a single nucleosome core particle (Malik and Henikoff 2003). Among the H2A variants is H2A.Z (Htz1 in *S. cerevisiae*), which is inserted into chromatin by the Swr1–ATPase complex, SWR-C (Krogan et al. 2003; Kobor et al. 2004; Mizuguchi et al. 2004). Htz1 plays roles in multiple processes, including transcription (Santisteban et al. 2000; Krogan et al. 2003; Meneghini et al. 2003; Kobor et al. 2004; Mizuguchi et al. 2004), limiting telomeric silencing (Krogan et al. 2003; Meneghini et al. 2003), and chromosome segregation (Krogan et al. 2004).

The SWR-C shares several subunits with the NuA4 HAT complex (Kobor et al. 2004; Krogan et al. 2004; Mizuguchi et al. 2004), and expression microarray analysis shows the two complexes have common regulatory targets (Krogan et al. 2004). NuA4 is required for the majority of histone H4 acetylation on Lys 5 (K5), K8, and K12 and some on histone H2A K7 (Smith et al. 1998; Allard et al. 1999). Htz1, SWR-C, and NuA4 have each been implicated in the maintenance of chromosome stability (Krogan et al. 2004). This function for H2A.Z is conserved in the fission yeast *Schizosaccharomyces pombe* (Carr et al. 1994) and metazoans (Rangasamy et al. 2004).

How NuA4 and SWR-C are functionally connected remains unclear. Htz1 incorporation into chromatin is dependent on SWR-C, but independent of NuA4 (Krogan et al. 2004). Therefore, acetylation of histone H4 by NuA4 is not required to recruit Htz1. Another possibility is that the HAT acetylates Htz1 after its incorporation into chromatin. H2A.Z N-terminal tails are acetylated in mammals (Pantazis and Bonner 1981, 1982; Bruce et al. 2005), chicken (Bruce et al. 2005), and *Tetrahymena* (Ren and Gorovsky 2001), although the mediating HATs and biological relevance of these modifications is unknown. Here we show that NuA4 acetylates *S. cerevisiae* Htz1 on K14 after it is assembled into chromatin and that this modification plays a role in maintaining stable propagation of chromosomes.

[*Keywords*: H2A.Z; NuA4; acetylation; chromosome segregation]
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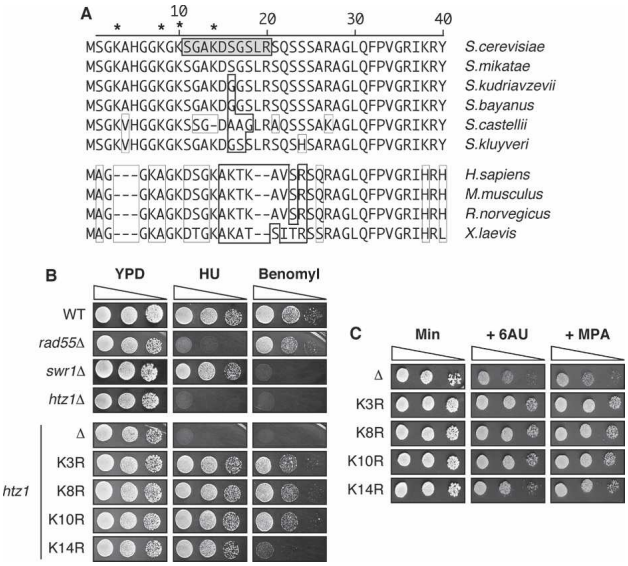

Figure 1. The Htz1-K14R mutant is selectively sensitive to benomyl. (*A*) Cross-species alignment of the H2A.Z N-terminal tail; *upper* set is multiple *Saccharomyces* species (Cliften et al. 2003), *lower* set is metazoans. Residues different from *S. cerevisiae* Htz1 are boxed. The four modifiable lysines in the N-terminal tail of *S. cerevisiae* Htz1 (K3, K8, K10, and K14) are indicated with asterisks. The peptide used to raise the Htz1 K14^{Ac} antibody is boxed and shaded. (*B*) Htz1 K3, K8, K10, and K14 were individually mutated to arginine (R). Mutants were expressed as the sole source of Htz1 and 10-fold serial dilutions of each strain were spotted onto the indicated plates (HU [100mM]; benomyl [15 µg/mL] in YPD). Plates were incubated for 3 d at 30°C. (*C*) As in *B*, except the sensitivity of the indicated strains to 6AU (75 µg/mL) or MPA (15 µg/mL) was examined after 72 h.

Results and Discussion

S. cerevisiae Htz1 has four lysine sites on its N-terminal tail: K3, K8, K10, and K14 (Fig. 1A). These were mutated individually to arginine (R) and expressed as the sole source of the histone in an *htz1*Δ background. Mutant strains were analyzed for phenotypes associated with *htz1*Δ or mutants of SWR-C or NuA4 (Kobor et al. 2004; Krogan et al. 2004). Phenotypes tested included sensitivity to the genotoxins methane methyl-sulfonate (MMS) and hydroxyurea (HU), the microtubule (MT) destabilizing agent methyl-1-(butylcarbamoyl)-2-benz-imidazolecarbamate (benomyl), and the transcription elongation inhibitors 6-azauracil (6AU) and mycophenolic acid (MPA). While the *htz1*Δ strain is sensitive to all of these agents, the *htz1-K14R* mutant displays selective sensitivity to benomyl (Fig. 1B,C). None of the other point mutants exhibited any sensitivity in these assays. We also tested silencing of telomere-proximal reporter genes and found that while heterochromatin may spread in *htz1*Δ cells, silencing in the *htz1-K14R* and *htz1-K14Q* mutants is comparable to wild type (Supplementary Fig. 1).

To test for acetylation of Htz1-K14, antibodies were raised against a peptide consisting of Htz1 amino acids 11–20 in which K14 was acetylated (Htz1-K14^{Ac}) (Fig. 1A). Affinity purification of the antibody produced selective recognition of the Htz1-K14^{Ac} peptide relative to unmodified peptide (Fig. 2A). On immunoblots of *S. cerevisiae* whole-cell extracts (WCEs) the antibody recognized a protein of the appropriate size for Htz1. This reactivity was not seen in extracts from *htz1*Δ, *htz1-K14R*, or *htz1-K14Q* cells (Fig. 2B). Htz1 levels and K14 acetylation were unaffected when K3, K8, or K10 were individually mutated to arginine (Fig. 2B).

Of multiple HATs tested, Htz1-K14 acetylation was exclusively dependent on NuA4 (Fig. 2C; Supplementary Fig. 2). Htz1-K14^{Ac} levels were strongly reduced in strains with conditional alleles of the NuA4 catalytic subunit Esa1 (Fig. 2C) or deletions of other NuA4 subunits required for acetylation of H4 (Fig. 2D; Supplementary Fig. 2A). To determine if NuA4 acts directly on Htz1, in vitro acetylation was tested. Esa1-TAP complexes display robust HAT activity toward Htz1-K14. In contrast, Elp3-TAP- or Gcn5-TAP-containing complexes do not acetylate the substrate under the same conditions (Fig. 2E).

SWR-C is required for efficient insertion of Htz1 into chromatin (Krogan et al. 2003; Kobor et al. 2004; Mizu-

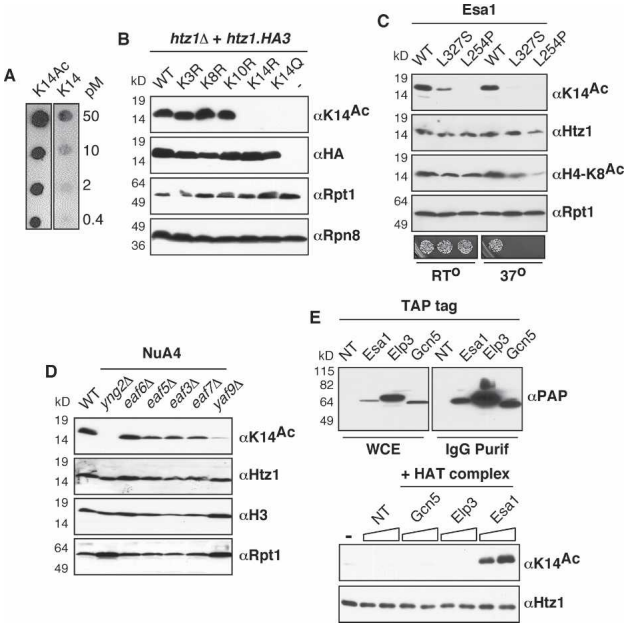

Figure 2. Htz1-K14 is acetylated by NuA4. (*A*) Specificity of the Htz1-K14^{Ac} antibody for the acetylated (K14Ac) relative to the unmodified (K14) Htz1 peptide. Indicated amounts (in picomoles) of each peptide were spotted onto nitrocellulose before immunoblotting. (*B*) Htz1-K14 is acetylated in vivo. HA-tagged forms of the indicated Htz1 proteins were individually expressed as the sole source of the histone and strains were analyzed by immunoblotting. Deletion of Htz1 or mutation of K14 to arginine (K14R) or glutamine (K14Q) abolishes recognition by the anti-K14^{Ac} antibody. In contrast, mutation of residues K3, K8, or K10 has no effect on K14^{Ac} levels. Total Htz1 was detected with anti-HA. Anti-Rpt1 and anti-Rpn8 were used as loading controls. (*C*) Esa1 is required for acetylation of Htz1-K14 in vivo. Strains containing *ESA1* or the temperature-sensitive mutants *esa1-L327S* or *esa1-L254P* were grown at room temperature (RT°) or for 4 h at the nonpermissive temperature (37°C). (*Bottom*) Growth was assayed after 2 d at room temperature (RT°) or 37°C. Cell extracts were assayed by immunoblotting with the indicated antibodies. (*D*) Htz1-K14 acetylation is differentially dependent on subunits of NuA4. Extracts from indicated deletion strains were immunoblotted with the antibodies shown to the *right* of each panel. (*E*) NuA4 efficiently acetylates Htz1-K14 in vitro. (*Top*) Partial TAP purifications of indicated HATs were monitored by reactivity of the protein A tag (αPAP). (*Bottom*) An untagged strain (NT) was used as a control. IgG-purified HATs were added to reactions containing recombinant Htz1/H2B dimer and acetyl CoA. Htz1-K14^{Ac} and Htz1 were detected by immunoblotting.

guchi et al. 2004). In cells lacking SWR-C, total Htz1 levels are unaffected while Htz1-K14^{Ac} levels are significantly reduced (Fig. 3A). Fractionation experiments show reduced total and acetylated Htz1 in chromatin from *swr1*Δ cells (Supplementary Fig. 2C). Therefore, Htz1 acetylation is likely to occur after assembly into chromatin. Unacetylatable Htz1-K14 mutants (*htz1-K14R* or *htz1-K14Q*) are expressed at levels similar to wild type (Fig. 2B) and efficiently assemble into chromatin (Fig. 3B), so acetylation is not required for Htz1 incorporation. Furthermore, the distribution of K14 mutants throughout the genome is similar to wild type at all positions tested by chromatin immunoprecipitation (ChIP), suggesting that acetylation is not specifically correlated with insertion at a particular location (Fig. 3C).

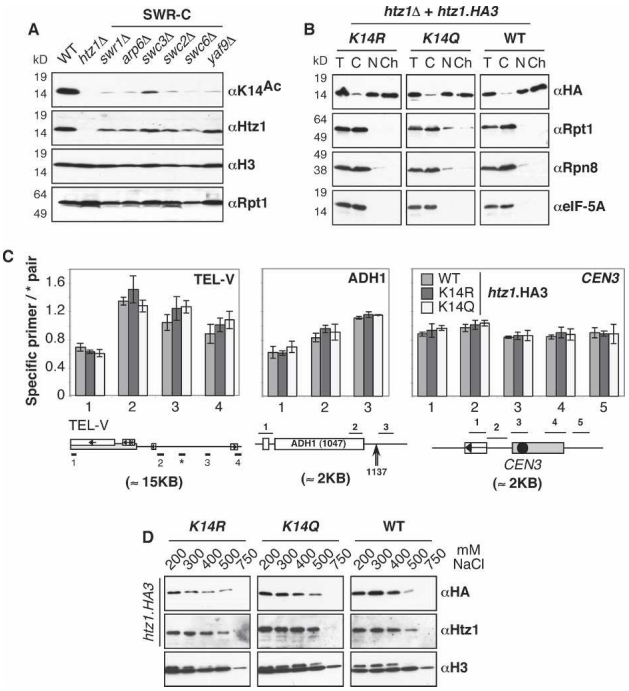

Figure 3. Htz1-K14 is acetylated after assembly into chromatin (*A*) Htz1-K14 acetylation is reduced in SWR-C mutants. Extracts from the indicated deletion strains were assayed for total and acetylated Htz1 by immunoblotting. Histone H3 and Rpt1 were used as loading controls. (*B*) Htz1 localization to chromatin does not require K14 acetylation. Cells containing HA-tagged forms of the indicated Htz1 proteins were separated into total (T), cytoplasmic (C), nuclear (N), or chromatin (Ch) fractions and immunoblotted with the indicated antibodies. Cytoplasmic segregation of the proteasome components Rpt1 and Rpn8 and the translation initiation factor eIF-5A demonstrates efficient fractionation. (*C*) Htz1 K14 acetylation does not regulate genomic distribution of the histone variant. HA-tagged Htz1 proteins (wild type [WT], K14R, or K14Q) were individually expressed as the sole source of the histone. The relative recruitment of each at the telomere of chromosome V (TEL-V), the highly transcribed ADH1 gene, and the centromere of chromosome III (*CEN3*) was then determined by ChIP. A schematic of each location is shown (size in kilobases is indicated), along with the relative location of the primer pairs used (see Supplementary Table 3; Krogan et al. 2003, 2004). In each case, occupancy is expressed relative to *, a subtelomeric region of chromosome V (9716–9823; see TEL-V schematic) (Krogan et al. 2003). Results are the mean ± standard deviation (SD) of three independent ChIPs. (*D*) Htz1 K14 mutants in chromatin have normal stability. The chromatin pellets from *B* were extracted with increasing salt concentrations as indicated and proteins remaining in the pellet assayed by immunoblotting.

Unfortunately, the Htz1-K14^{Ac}-specific antibody did not work in ChIP (data not shown), so we were unable to compare the genomic distribution of Htz1-K14^{Ac} to total Htz1. To determine whether acetylation affects the stability of Htz1 in the nucleosome, chromatin preparations from wild-type, *htz1-K14R*, and *htz1-K14Q* cells were subjected to washes of increasing ionic concentrations (Raisner et al. 2005). No differences were observed, suggesting that K14 acetylation does not dramatically affect interactions between Htz1 and other histones (Fig. 3D).

Acetylation is a reversible modification (Eberharter and Becker 2002), but a screen of several known histone deacetylase (HDAC) mutants failed to find any that affected levels of Htz1-K14^{Ac} (Supplementary Fig. 2B). In addition, total Htz1-K14^{Ac} levels are unchanged throughout the cell cycle (Supplementary Fig. 3B) or in response to MMS-induced DNA damage (Supplementary Fig. 3C). It should be noted, however, that all these studies examined the total cellular pool of Htz1-K14^{Ac} and thus could miss the removal of the modification at a specific location or time.

Accurate chromosome transmission requires the coordination of many events. During S phase the chromosomes are duplicated and the resultant sister chromatids held together by the cohesin complex (for review, see Uhlmann 2003). The centromere (*CEN*) is the assembly site of a multiprotein kinetochore complex that links the chromosomes with spindle MTs (Fig. 4B; McAinsh et al. 2003; Measday and Hieter 2004). Once all chromosomes have attached to the spindle, the metaphase-to-anaphase transition proceeds by degradation of the cohesin complex and chromosome segregation. If kinetochores do not attach properly to spindle MTs, spindle checkpoint proteins halt cell cycle progression at the metaphase-to-anaphase transition (Cleveland et al. 2003). Defects in any of these processes can result in chromosome imbalance, or aneuploidy. Although chromatin impacts many aspects of chromosome transmission, including *CEN* function, the specific regulators that impact chromosome transmission fidelity (CTF) have not been comprehensively identified, nor are the mechanisms understood at the molecular level.

Sensitivity to benomyl is a common phenotype of kinetochore and spindle checkpoint mutants. Like deletions of *SWR1* or *HTZ1*, the *htz1-K14R* mutant is benomyl sensitive (Fig. 1B). To further characterize the role of Htz1 acetylation in genome stability, we quantified chromosome missegregation in *htz1-K14R* and *htz1-K14Q* diploid strains by colony half-sector analysis (Koshland and Hieter 1987; Krogan et al. 2004). The *htz1-K14R* strain shows an increase in the rate of chromosome loss comparable to an *htz1*Δ strain (6.6- vs. 7.7-fold greater than wild type) (Fig. 4A). Interestingly, the *htz1-K14Q* strain, which might mimic constitutive acetylation of Htz1, has normal segregation.

Comprehensive synthetic genetic array (SGA) screening of spindle checkpoint mutants (*mad1*, *mad2*, *mad3*, and *bub3*) identified genetic interactors, one of which was *HTZ1*, that may have roles in regulating MTs, kinetochores, or sister chromatid cohesion (SCC) (Daniel et al. 2006). We also observed genetic interactions between *htz1*Δ and components of the kinetochore and spindle checkpoint machinery (Krogan et al. 2004). Like other chromatin components (Sharp and Kaufman 2003), K14 acetylation might contribute to proper centromere

A

Plasmid	Rate of Nondisjunction 2:0 events	Rate of Loss 1:0 events	Total Colonies
Vector	1.58×10^{-4} (1.4)	2.74×10^{-3} (7.7)	18,972
pHTZ1	1.07×10^{-4} (1)	3.58×10^{-4} (1)	27,950
phtz1-K14R	2.96×10^{-4} (2.8)	2.37×10^{-3} (6.6)	23,626
phtz1-K14Q	1.79×10^{-4} (1.1)	4.02×10^{-4} (1.1)	22,369

B

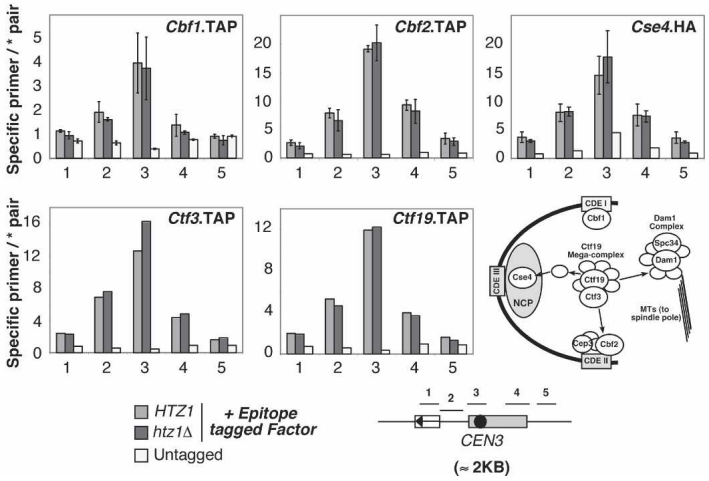

Figure 4. Chromosome missegregation rates are increased in *htz1-K14R* mutants. (*A*) Diploid *htz1*Δ/*htz1*Δ cells containing empty vector (pRS313) or Htz1 plasmids (wild type [WT], K14R, or K14Q) were plated to single colonies, with selection maintained for the plasmids. Segregation of a reporter chromosome fragment was scored visually by counting sectored colonies (Koshland and Hieter 1987; Krogan et al. 2004). Frequencies of events are listed, with relative rate (normalized to the pHTZ1 control) shown in parentheses. (*B*) Htz1 is not required for kinetochore localization at the centromere. Epitope-tagged forms of the centromere-specific histone H3 variant Cse4, or representatives of the inner (Cbf1, Cbf2/Ndc10) or central (Ctf3, Ctf19) kinetochore were analyzed by ChIP at *CEN3*. Schematic shows a model of kinetochore components; arrows indicate interactions between complexes. Location of the ChIP primers at *CEN3* and means of calculating relative occupancy are as in Figure 3C.

function. However, by ChIP analysis, *htz1*Δ (Fig. 4B) or unacetylatable *htz1-K14* alleles (Supplementary Fig. 4) have no obvious effect on the distribution of kinetochore components at *CEN3*. Furthermore, *htz1*Δ cells show no defects in cohesin recruitment or arm SCC (data not shown). Htz1 may regulate spindle function. Haploid *htz1*Δ cells do exhibit an appropriate proper checkpoint in response to nocodazole, but *htz1*Δ cells escape this arrest at a slightly higher frequency than wild-type cells (data not shown).

NuA4 mutants have greater chromosome stability defects than *htz1*Δ (Supplementary Table 1; Krogan et al. 2004) or *htz1-K14R*, suggesting that Htz1 acetylation accounts for only part of the NuA4 phenotype. Histone H4, the primary acetylation target of NuA4, has also been implicated in chromosome stability (Saunders et al. 1990; Meluh et al. 1998; Glowczewski et al. 2000; Choy et al. 2001; Measday et al. 2005). While many of these H4 effects are likely to be acetylation-independent (Saunders et al. 1990; Smith et al. 1996; Glowczewski et al. 2000), unacetylatable H4 alleles are sensitive to benomyl (Le Masson et al. 2003) and display defects in genome integrity (Megee et al. 1995). NuA4 is the only essential HAT in yeast (Smith et al. 1998; Allard et al. 1999), perhaps because it acts in multiple pathways that include transcription and chromosome stability. Our work suggests that this latter function of NuA4 also involves the acetylation of Htz1.

Materials and methods

Materials

Yeast strains used in this study are listed in Supplementary Table 2 and oligonucleotides are listed in Supplementary Table 3. Htz1 point mutants were created by the megapriming method (Keogh et al. 2003) and confirmed by sequencing. Peroxidase anti-peroxidase (αPAP) from Sigma was used in immunoblotting to recognize the TAP tag. Monoclonal antibody 12CA5 recognizing the HA-epitope tag was from Covance. Polyclonal rabbit anti-Clb2 was from A. Rudner (Harvard Medical School, Boston, MA), rabbit anti-Rpt1 and anti-Rpn8 from D. Finley (Harvard Medical School, Boston, MA), and rabbit anti-eIF-5A (YEL034W) from R.S. Zitomer (State University of New York at Albany, Albany, NY). Polyclonal rabbit anti-H3 (ab1791) was from Abcam. Polyclonal affinity purified anti-Htz1 antibodies were produced in rabbits by Upstate. The C-terminal antibody (07-718) was raised against amino acids 116–133 of Htz1 (C-PHINKALLLKVEKKGSKK), the anti-K14^{Ac} antibody (07-719) against amino acids 11–20 [SGA(K^{Ac})DSGSLR-KC] (Fig. 1A). The terminal C residue on each peptide was used to couple to KLH for immunization or a sulfolink resin for affinity purification.

Phenotypic analyses

For spotting analyses, cells were resuspended at 10^7/mL, subjected to 10-fold serial dilutions and 10 µL of each dilution spotted per plate. Growth was assayed at 48 or 72 h as indicated. Camptothecin (20 µM), HU (100 mM), MMS (0.05%), or benomyl (15 µg/mL) plates were in yeast extract–peptone–dextrose (YPD). Sensitivity to 6AU (75 µg/mL) or MPA (15 µg/mL) was assayed on minimal media lacking uracil; when needed, *ura3*Δ cells were transformed with a CEN/ARS URA3 plasmid. The telomeric silencing assay (Supplementary Fig. 1) employed strains containing a *URA3* reporter gene integrated from 1 to 2.5 kb proximal to the telomere of chromosome V (Renauld et al. 1993). Strains were grown to $OD_{600} \approx 0.2$ before plating at 10-fold serial dilutions on YPD or SC medium with 5-Fluoroorotic acid (5-FOA) as indicated. Growth on YPD plates was analyzed after 48 h and 5-FOA after 96 h.

ChIP

ChIPs were performed as described (Keogh et al. 2003; Krogan et al. 2003). Two-hundred-fifty milliliters of each strain were grown to $OD_{600} \approx 0.6$ in minimal medium before formaldehyde cross-linking and further processing.

Recombinant histone purification

Recombinant *S. cerevisiae* histones (Htz1, H2A, H2B) were expressed as individual proteins in *Escherichia coli* (strain BL21 CodonPlus [DE3] RIL; Stratagene) from plasmids supplied by C. Wu (National Institutes of Health, Bethesda, MD). For each sample, 2-L cultures were grown to $OD_{600} \approx 0.4$ in LB plus kanamycin, and histone expression induced with 0.2 mM IPTG. After 4 h, cells were harvested at 4°C and washed with W buffer (WB: 50 mM Tris.HCL at pH 7.6, 100 mM NaCl, 1 mM DTT, 5 mM βME, 0.2 mM PMSF). Cells were resuspended in 30 mL TW buffer (WB + 1% Triton X-100) and lysed by sonication on ice. The pellet was collected by centrifugation (16,000*g*, 20 min, 4°C), the supernatant discarded, and three further TW washes performed. To solubilize histones the pelleted inclusion bodies were resuspended in 120 µL DMSO for 30 min at room temperature, and 5 mL guanidine buffer (GB: 20 mM Tris.HCL at pH 7.6, 7 M guanidine.HCL, 10 mM DTT) added. The pellet was disrupted by sonication, the histones completely solubilized by rotation for 1 h at room temperature, and cell debris removed by centrifugation (16,000*g*, 20 min). The resulting supernatant was dialyzed at room temperature overnight against freshly made 8 M urea. Precipitates were removed by centrifugation and the supernatant bound in batch to 8 mL Q-Sepharose 4 Fast flow (Pharmacia) for 1 h at room temperature with rotation. The unbound supernatant (+10 mL wash) was pooled and bound

to 8 mL SP-Sepharose 4 Fast flow (Pharmacia) as above. The resin was placed in an Econo-Pak column and successively washed with 20 mL U-200 (7 M urea, 200 mM NaCl) and 20 mL U-400. Histones were eluted by U-600, with positive fractions pooled and dialyzed overnight against ddH_2O (with 5 mM βME, 0.2 mM PMSF). Precipitates were removed by centrifugation and the histones were lyophilized and stored at –80°C. For each sample purity was estimated by 15% SDS-PAGE with colloidal Coomassie staining, and the absence of DNA contamination confirmed by analysis on agarose gels containing ethidium bromide.

In vitro HAT assays

WCEs (untagged, Esa1-TAP, Gcn5-TAP, or Elp3-TAP) were prepared as described (Keogh et al. 2003). Each HAT complex was affinity purified from 10 mg WCE with IgG agarose (Sigma) (5 µL resin/mg WCE). After overnight incubation at 4°C the bead complexes were extensively washed with Lysis Buffer (LB: 20 mM Tris.Cl at pH 7.6, 10% glycerol, 200 mM KoAc, 1 mM EDTA, 1 mM DTT + protease inhibitors), resuspended to 200 µL total volume in TEV buffer (TB: 50 mM Tris.HCL at pH 8.0, 1 mM DTT, 0.5 mM EDTA) and complexes cleaved from the beads with recombinant TEV protease (4 h, 4°C). The supernatant was collected, dialyzed for 1 h against HAT buffer (HB: 50 mM Tris.HCL at pH 8.0, 10% glycerol, 10 mM butyric acid, 1 mM DTT, 1 mM PMSF), and stored in aliquots at –80°C. In vitro HAT reactions were performed for 1 h at 30°C (25 µL reactions containing 100 ng histone substrates, 2 µM acetyl CoA, and 2 or 5 µL of immunopurified HAT complexes; conditions derived from Mizzen et al. 1999), resolved by 15% SDS-PAGE, and specific acetylation of Htz1-K14 determined by immunoreactivity with anti-$K14^{Ac}$.

S. cerevisiae fractionation

Cells from 50-mL cultures (OD_{600} < 1.0) were collected by centrifugation, successively washed with ddH_2O, PSB (20 mM Tris.Cl at pH 7.4, 2 mM EDTA, 100 mM NaCl, 10 mM β-ME) and SB (1 M sorbitol, 20 mM Tris.Cl at pH 7.4), and transferred to a 2-mL test tube. Cells were suspended in 1mL SB, 125 µL Zymolase 20T (10 mg/mL in SB) added, and samples incubated at 30°C with rotation until >85% spheroblasts (60–90 min). Spheroblasts were collected by centrifugation in a benchtop microfuge (2K, 5 min, 4°C), washed twice with SB, and suspended in 500 µL EBX (20 mM Tris.Cl at pH 7.4, 100 mM NaCl, 0.25% Triton X-100, 15 mM β-ME, 50 mM Na-butyrate + protease inhibitors). Triton X-100 was added to 0.5% final to lyse the outer cell membrane, and the samples kept on ice for 10 min with gentle mixing. An aliquot was taken for immunoblotting (Total), and the remainder of the lysate layered over 1 mL NIB (20 mM Tris.Cl at pH 7.4, 100 mM NaCl, 1.2 M sucrose, 15 mM β-ME, 50 mM Na-butyrate + protease inhibitors) and centrifuged (13,000*g*, 15 min, 4°C). A sample of the upper layer cytoplasmic fraction was taken (Cyto) and the rest of the supernatant discarded. The glassy white nuclear pellet was suspended in 500 µL EBX and Triton X-100 added to 1% final to lyse the nuclear membrane. Samples were kept on ice for 10 min with gentle mixing and an aliquot taken (Nuclear) and chromatin and nuclear debris collected by centrifugation (16,000*g*, 10 min, 4°C). Chromatin was washed three times with EBX and suspended in 50 µL 1 M Tris (pH 8.0) (Chromatin). To each fraction an equal volume of 2× SDS-PAGE loading buffer (60 mM Tris at pH 6.8, 2% SDS, 10% glycerol, 0.2% bromophenol blue, 200 mM DTT) was added, samples were incubated at 95°C for 5 min, centrifuged (16,000*g*, 5 min, room temperature) and the supernatant collected. Samples were analyzed by SDS-PAGE and immunoblot analyses.

Chromatin salt stability analyses

EBX-100-isolated chromatin from above was split into aliquots and each washed three times with EBX-100 to EBX-750 (all millimoles NaCl) (Raisner et al. 2005). Pellets were then resuspended and analyzed by immunoblotting.

Chromosome stability analyses

Quantitative half-sector analysis was performed as previously described (Koshland and Hieter 1987; Krogan et al. 2004). In brief, homozygous diploid strains were created containing an $ade2^{ochre}$ allele at the endogenous locus and the *SUP11* ochre suppressor on a single chromosome fragment (CFIII [CEN3.L] *URA3 SUP11*). Strains were plated to single colonies on solid SC-HIS and two-fifths adenine and grown at 25°C for 3 d before the plates were placed at 4°C for optimal red pigment development. Efficient chromosome stability/transmission results in pink colonies. Chromosome loss or 1:0 events were scored as colonies that were half red and half pink, and nondisjunction or 2:0 events were scored as colonies that were half red and half white.

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