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Histone H2A.Z acid patch residues required for deposition and function

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Abstract

The incorporation of histone variants is one mechanism used by the eukaryotic cell to alter the generally repressive chromatin template. However, the exact molecular mechanisms that direct this incorporation are not well understood. The SWR1 chromatin remodeling complex that binds to and directs incorporation of histone variant H2A.Z into chromatin has been characterized, but significantly less information is available concerning the requirements on the H2A.Z target molecule. We performed an unbiased mutagenic screen designed to elucidate the function of H2A.Z in *Saccharomyces cerevisiae*. The screen identified residues within the conserved acidic patch of H2A.Z as being important for the function of the variant. We characterized single point mutations in the patch that are phenotypically sensitive to a variety of growth conditions and are expressed at lower protein levels, but are functionally defective (*htz1-D99A*, *htz1-D99K*, and *htz1-E101K*). The mutants were significantly less detectable by chromatin immunoprecipitation at *PHO5*, a gene previously described to be enriched for H2A.Z. These results identify acidic patch residues of H2A.Z that are critical for mediating deposition and function in chromatin, and represent potential candidates for the interaction of H2A.Z with its deposition and/or targeting machinery.

Keywords

H2A.Z; Histone variant; Chromatin; *Saccharomyces*; Acid patch

Introduction

Histones compact the immense eukaryotic genome down to a manageable state. However, these histones obstruct access of cellular processes that require access to the DNA. Eukaryotic cells have evolved several mechanisms to overcome the repressive chromatin structure imposed by the presence of histones, including replacement of the major histones by histone variants with specialized functions. Variants of histone H2A have been among the most widely studied; in particular, the histone H2A.F/Z variant family represents an extremely conserved chromatin component (Guillemette and Gaudreau 2006; Raisner and

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Madhani 2006). The gene encoding H2A.Z is essential for viability in most organisms studied, and is even more evolutionarily conserved than the major histone H2A (White et al. 1988), indicating an important role in the cell. Research into H2A.Z has pointed to key roles in a wide variety of sometimes contrasting cellular processes in a variety of organisms. In *Tetrahymena thermophila*, H2A.Z has been implicated in gene transcription (Allis et al. 1980, 1986; Stargell et al. 1993). In mammalian cells, H2A.Z has been shown to be required for early development (Faast et al. 2001), appropriate chromosome segregation (Rangasamy et al. 2004), and the establishment of constitutive hetero-chromatin (Fan et al. 2004). In *Saccharomyces cerevisiae*, H2A.Z has been proposed to be involved in gene activation (Adam et al. 2001; Larochelle and Gaudreau 2003), protection from heterochromatin spreading (Meneghini et al. 2003), chromosome segregation (Krogan et al. 2004), and cell cycle progression (Dhillon et al. 2006). Despite these contrasts, incorporation of H2A.Z increases the potential diversity of eukaryotic chromatin. However, the exact molecular mechanisms that direct this incorporation are not well understood. The SWR1 chromatin remodeling complex that binds to and directs incorporation of H2A.Z into chromatin has been characterized, but significantly less information is available concerning the detailed requirements on H2A.Z. We performed an unbiased genetic screen designed to characterize the deposition function of H2A.Z in *Saccharomyces cerevisiae*. This study was intended to identify specific residues responsible for conferring H2A.Z deposition.

The X-ray crystal structure of the nucleosome core particle containing H2A.Z instead of H2A has been resolved (Suto et al. 2000). These data indicate that the overall structure of the histone octamer and path of the DNA superhelix are not seriously affected by the substitution of H2A.Z in place of H2A. However, the carboxy-terminal “docking domain” of H2A, which contacts the H3/H4 tetramer, contains amino acid differences in H2A.Z that are predicted to subtly alter the interaction with the tetramer. H2A.Z introduces localized changes, including the extension of a patch of acidic residues in the α C helix. This acidic patch in budding yeast H2A is composed of the three-residue sequence D99–D100–E101. In H2A.Z, this patch is extended to four acidic residues out of five, to form D99–D100–E101–L102–D103. Importantly, the acidic patch lies within a region of H2A.Z, referred to as M6, which is essential for embryonic development in *Drosophila melanogaster* (Clarkson et al. 1999). Amino acid domain swapping experiments indicated that M6 is the only region of H2A.Z that does not allow transgenic flies to survive to pupation when replaced with the equivalent region of the major H2A (Clarkson et al. 1999), demonstrating its functional importance.

Unlike H2A, H2A.Z is incorporated into chromatin through the action of the SWR1 chromatin remodeling complex (Kobor et al. 2004; Krogan et al. 2003; Mizuguchi et al. 2004). The SWR1 complex is a multi-subunit complex of 13 proteins that contains the Swr1 protein as its core. Swr1 is a Snf2/Swi2-related ATPase that catalyzes deposition of the variant H2A.Z into chromatin in exchange for the canonical H2A. Further characterization by domain swapping revealed that the C-terminal region of H2A.Z corresponding to *Drosophila* M6 is essential for its interaction with the complex (Wu et al. 2005). As in *Drosophila* development, replacement of the H2A.Z M6 region with the equivalent region of the major H2A abolishes the ability of H2A.Z to associate with the complex. Also, a histone chaperone, Chz1, has been discovered which preferentially binds to H2A.Z–H2B dimers and delivers them to the SWR1 complex for deposition into chromatin (Luk et al. 2007). The structure of the SWR1 complex has also been conserved during evolution, as complexes analogous to SWR1 have been described in human cells (Ruhl et al. 2006) as well as *Arabidopsis thaliana* (March-Díaz et al. 2007, 2008). These complexes are conserved at the functional level as well, and catalyze a similar histone exchange reaction (Deal et al. 2007; Wong et al. 2007). These observations highlight the SWR1 complex as a crucial mediator of H2A.Z function in many organisms.

Chromatin occupancy of H2A.Z in yeast and other organisms that lack Swr1 is reduced to background levels (Deal et al. 2007; Kobor et al. 2004; Krogan et al. 2003; Mizuguchi et al. 2004; Wong et al. 2007), pointing to the histone exchange reaction performed by Swr1 as its essential function. Interaction with H2A.Z is a crucial first step in this reaction. The M6 domain of H2A.Z mediates this interaction, but the detailed requirements of this domain for function have not been studied. Here, we present the results of experiments designed to characterize the genetic functions of H2A.Z, and show that specific residues contained within the acidic patch of H2A.Z confer the ability to be successfully deposited into chromatin to provide normal function.

Materials and methods

Yeast strains, media, and genetic methods

Yeast strains and plasmids utilized in this work are summarized in Table 1. YEPD, SC, and 5-FOA media were made as described (Amberg et al. 2005). To induce the *PHO5* gene for chromatin immunoprecipitations (ChIPs), YEPD lacking inorganic phosphate was generated as described (Ohnishi and Gall 1978). Camptothecin from a 4 mg/ml stock in dimethylsulfoxide (DMSO) was added to YEPD media at a final concentration of 30 µg/ml along with 26.4 mM HEPES (pH 7.2). Benomyl from a 1.5 mg/ml stock in DMSO was added to YEPD media at a final concentration of 15 µg/ml. Canavanine from a 10 mg/ml stock was added to SC-arginine media at a final concentration of 3 µg/ml. Hydroxyurea was added to YEPD media at a final concentration of 100 mM. Triple-HA tagging and gene disruption of *HTZ1* have been described previously (Santisteban et al. 2000). Transformation of yeast was performed essentially as described (Gietz and Woods 2002).

Mutagenesis

S. cerevisiae HTZ1 was mutagenized by PCR misincorporation and targeted to its genomic location by one-step co-transformation with the *URA3* gene of *Kluyveromyces lactis* to minimize inappropriate targeting to the endogenous *ScURA3* locus. Mutagenic amplification of *HTZ1* was carried out in PCRs with *Taq* DNA polymerase (Promega), 0.04 mM dATP and dGTP, 2 mM dCTP and dTTP, and 5.5 mM MgCl₂ using primer #1 (GGAAAAGCTCATGGAGGTAAAGG) and primer #2 (CATGGCAAGTCCCGTGGATC/TCATGCTATATATTATAGTAAAAACAT AAGAGATTAGACG). The *KlURA3* selectable marker was amplified under standard-fidelity reaction conditions using primer #3 (GATCCACGGGACTTGCCATG/TGTGATTCTGGGTAGAAGATC) and primer #4 (AATGATACAGACGTTAATATGGATAGAATGAGGATACAGG/CGATGATGTAGTTTCTGGTT). Primers #2 and #3 have 20 bp of homology to each other, linking the *HTZ1* and *KlURA3* fragments by a recombination event. Primers #1 and #4 have homology with the *HTZ1* locus itself, targeting the recombined fragments to the chromosome. Transformants were selected on SC-ura to verify successful co-transformation and recombination. Ura⁺ transformants were re-streaked onto SC-ura, tested for the *htz1Δ* phenotypes (first by patching and then serial spot dilutions to confirm), tested for rescue of those phenotypes by transforming each mutant candidate with a plasmid containing wild-type *HTZ1*, and then sequenced. To excise the *KlURA3* gene, mutants were plated onto 1 µg/ml 5-fluoro-orotic acid (5-FOA) for counter selection. The acidic patch of *HTZ1* was mutated in a site-directed fashion according to the megaprimer PCR method (Kammann et al. 1989).

Cell extract preparation

Logarithmically growing 10 ml yeast cultures were centrifuged, washed with dH₂O, and re-suspended in 0.25 ml HSB buffer (45 mM HEPES–KOH pH 7.4, 150 mM NaCl, 10%

glycerol, 1 mM EDTA pH 8.0, 20 mM sodium butyrate, 2 μ M DTT) with protease inhibitors (100 μ M PMSF, 1 μ g/ml each of aprotinin, leupeptin, chymostatin, and pepstatin A). Cells were lysed with ~0.25 ml of 425–600 μ m diameter acid-washed glass beads in a mini-BeadBeater (ThermoSavant, FastPrep FP120). The recovered cell lysate was centrifuged at 4,600g for 2 min to clear cell debris.

Immunodetection

Approximately 50 μ g of total protein from whole cell extracts were used in 12% SDS-PAGE gels. All antibody blotting steps were performed in 5% milk–PBS for 1 h at room temperature. Mouse monoclonal anti-HA (12CA5) was used at 1:2,000 dilution. Rabbit polyclonal anti-glucose-6-phosphate dehydrogenase (GPDH) was purchased from Sigma (cat. #A9521) and used at 1:10,000 dilution. Secondary antibodies consisted of IRDye700DX-conjugated goat anti-rabbit (Rockland #611-130-122, used at 1:50,000 dilution), and IRDye800-conjugated goat anti-mouse (Rockland, cat. #610-132-121; used at 1:20,000 dilution).

Chromatin immunoprecipitations

Yeast cells were grown in 50 ml of media to log phase, in media containing or lacking inorganic phosphate for 10 h. Cells were fixed in 1% formaldehyde for 1 h. Subsequent steps were performed as described (Santisteban et al. 2000). 1 ml aliquots of sheared chromatin solution (1 mg total protein) were incubated overnight at 4°C with IgG purified monoclonal anti-HA (12CA5) at a final concentration of 6 μ g/ml. Twofold dilutions of the immunoprecipitated material (1:1) and the total input (1:10) from a wild-type strain grown in the presence of inorganic phosphate were analyzed by PCR to establish a maximum quantity to apply to all the samples that would remain within the linear range of amplification. For example 12, 6, 3, 1.5, and 0.75 μ l of each would be tested, and 3 μ l IP and total would be used for all the samples if it was determined that 6 μ l or more would be outside the linear range of amplification. After an initial 3 min at 95°C, the precipitated DNA was amplified by *Taq* DNA polymerase in 28 cycles of 95°C for 30 s, 45°C for 45 s, and 72°C for 1 min, followed by a final extension of 5 min at 72°C. A 0.4 fraction of the reactions was analyzed on 2% agarose gels. In all cases, H2A.Z localization was analyzed at three locations of *PHO5*: fragments 5, 9, and 13 (Santisteban et al. 2000). The sequences of the primers used for amplifying regions of *PHO5* are available upon request. Each ChIP was performed three times to demonstrate consistency.

Results

Genetic screen for putative Htz1 deposition mutations

In order to gain a more detailed insight into specific residues required for Htz1 function, we screened for *htz1* deposition mutants in an unbiased forward genetic screen. As the complete deletion of *HTZ1* causes a wide range of different phenotypes (Kobor et al. 2004; Krogan et al. 2004; Mizuguchi et al. 2004; Santisteban et al. 2000), we reasoned that *htz1* mutants defective for nucleosome deposition might be identified among those that simultaneously confer multiple strong phenotypes. We first targeted mutations to *HTZ1* in vitro under error-prone PCR conditions to facilitate nucleotide misincorporation. This library of mutagenized alleles was then integrated in vivo at the *HTZ1* genomic location by homologous recombination, using a PCR-mediated *K. lactis URA3* gene as a linked selectable marker (see “Materials and methods” section). Finally, the resulting Ura⁺ transformants were screened for temperature sensitivity at 37°C and hypersensitivity to the drugs camptothecin, hydroxyurea, benomyl, and canavanine. This screen identified two mutant alleles, *htz1-21* and *htz1-27*, which exhibited strong phenotypes under at least four of the five assay conditions (Fig. 1). Sequence analysis revealed that *htz1-21* contains a single base

substitution resulting in a change of glutamic acid at position 101 to lysine (E101K) within the M6 domain (Fig. 2). This point mutation is within the same crucial region identified in earlier domain swapping experiments (Clarkson et al. 1999). The *htz1-27* allele is more complex and contains three mutations resulting in two amino acid substitutions, one of which affects the same amino acid residue as *htz1-21*, plus a nonsense mutation predicted to truncate the protein by 10 amino acids. Thus, both mutant alleles define E101 as an essential amino acid within the M6 domain with a potential role in Htz1 nucleosome deposition.

Different acid patch residues have different roles

Since E101 is part of the conserved acid patch domain shared by H2A.Z and canonical H2A, we reasoned that the other residues within the patch would also confer global defects in Htz1 function. To test this prediction, each of the four acidic residues in the patch was mutated to either neutral alanine or basic lysine and we integrated these mutant alleles at the chromosomal locus as before (Fig. 3). Surprisingly, several of these point mutations caused little or no phenotype in our assays. Phenotypic analysis showed that the residues in the acid patch could be grouped into three categories: two amino acid residues that lacked strong phenotypes for either substitution (D100, D103), one position that demonstrated strong phenotypic sensitivity for both substitutions (D99), and one position that caused the strong phenotypic sensitivity only for the lysine substitution (E101) (Fig. 3a). It is particularly striking that both point mutations to D103, the residue unique to the acid patch of the H2A.Z family, did not display strong phenotypic defects. Studies targeting the acid patch have been performed in *Xenopus laevis*, in which mutations have been constructed using the equivalent residues of D103 and S104 in yeast (Fan et al. 2004; Ridgway et al. 2004). These residues demonstrated effects on chromatin structure and development when mutated to the equivalent H2A residues. To determine whether our initial mutational analysis tested the importance of D103 (and S104) correctly, we also constructed the equivalent H2A residues in our mutant panel as single and double substitutions and tested them for phenotypes (Fig. 4). Neither of these additional mutants displayed any strong phenotypes. These results clearly identify D99 and E101 as candidates for residues important for Htz1 nucleosome deposition.

Htz1 D99A/K and E101K mutant proteins are functionally defective

We next tested the expression of each of the mutant Htz1 proteins, taking advantage of a triple-hemagglutinin (HA) epitope tag included at the amino-terminus of H2A.Z during strain construction (see “Materials and methods” section). Whole cell extracts were prepared from exponentially growing cultures and analyzed by Western blotting with anti-HA antibody (Fig. 3b). All of the mutant Htz1 proteins were expressed at detectable levels, but the three mutants that displayed the strong phenotypes (D99A, D99K, and E101K) were each expressed at lower levels than those with wild-type activity. These results suggested that the strong phenotypes of these three mutants could be due solely to their decreased expression, rather than any functional defect in the proteins themselves. To test this hypothesis, we increased the cellular pools of the mutant Htz1 proteins by increasing the gene dosage of the mutant alleles. Each mutant was transformed with a low-copy *CEN/ARS* plasmid expressing either the same endogenous *htz1* allele or one of the other defective mutant alleles (see “Materials and methods” section), and analyzed by Western blotting as before. This increased gene dosage resulted in mutant Htz1 expression levels comparable to that of wild-type cells (Fig. 5a). However, when these increased gene dosage strains were assayed for phenotypes, they retained their sensitivities to all of the conditions tested (Fig. 5b). These results demonstrate that the three Htz1 acid patch mutants are defective for function and not just for protein expression.

Htz1 D99A/K and E101K proteins have lower nucleosome occupancy at *PHO5*

The decreased levels of steady-state Htz1 proteins observed in the D99A/K and E101K mutants were consistent with a defect in nucleosome deposition. To challenge this interpretation, we examined the distribution of Htz1 over the *PHO5* locus by ChIP using the antibody against the triple-HA tag. In wild-type cells, Htz1 preferentially occupies the promoter of *PHO5* under repressing conditions in the presence of inorganic phosphate. When transcription is activated in the absence of inorganic phosphate, the promoter occupancy of Htz1 decreases. Low levels of Htz1 are detected over the open reading frame under both conditions (Santisteban et al. 2000). We prepared chromatin solutions from *HTZ1*, *htz1-D99K*, and *htz1-E101K* strains grown in the presence or absence of inorganic phosphate. Immunoprecipitations (IPs) were performed against the HA tags of the Htz1 alleles, and *PHO5* DNA was amplified using three sets of PCR primer pairs, referred to as fragments 5, 9, and 13 (Fig. 6a; Santisteban et al. 2000). As shown in Fig. 6b, wild-type Htz1 was distributed over *PHO5* as expected, with preferential occupancy of the promoter under repressing conditions and lesser amounts under activating conditions. However, in the acid patch mutants, the level of Htz1 occupancy over the *PHO5* promoter was severely reduced. Furthermore, the loss of H2A.Z at one region was not compensated for by increased aberrant deposition globally across *PHO5*.

These results were consistent with a defect in Htz1 nucleosome deposition of the acid patch mutants, and predicted that increased expression of mutant Htz1 proteins would nevertheless fail to produce increased occupancy at *PHO5*. To test this prediction, we performed ChIP assays using the increased gene dosage strains that restored cellular Htz1 protein to wild-type levels (Fig. 5). As shown in Fig. 6c, increased expression of wild-type *HTZ1* in wild-type cells gave increased occupancy of Htz1 at *PHO5*, with the expected preference for the promoter region. In contrast, increased expression of the D99A/K and E101K acid patch *htz1* mutants did not increase the level of Htz1 occupancy. Thus, even at equivalent levels of expression in whole cells, D99A/K and E101K acidic patch mutations result in greatly reduced accumulation of Htz1 at *PHO5*.

Discussion

The incorporation of histone variants into chromatin is one of the mechanisms used by the eukaryotic cell to increase the potential diversity of the conformation of and recruitment to the chromatin template. Though the SWR1 complex has been identified as the primary effector by which histone variant H2A.Z is deposited into chromatin, the exact nature of the binding interface between H2A.Z and the SWR1 complex is poorly understood. In this work, we have shown that specific residues contained within the acidic patch of H2A.Z confer the ability to be deposited into chromatin to provide normal function. Through an unbiased genetic screen, we have shown that two evolutionarily conserved amino acid residues of the acidic patch of H2A.Z are important for its correct function. Mutation of two of these residues confers strong phenotypes that stem from defective function, because these mutant histones are not deposited in appropriate patterns in chromatin at the *PHO5* locus. These findings can be thought of as a fine-structure mapping of the deposition function of the H2A.Z molecule. D99 and E101 represent candidate residues for direct interaction with the SWR1 complex, either contacting the scaffold Swr1 directly or possibly the Swc2 subunit (Wu et al. 2005). Further studies aimed at confirming and characterizing this interaction will shed considerable light on the nature of the histone exchange reaction carried out by the SWR1 complex.

A common technique for identifying important amino acid residues of a protein is to systematically mutate each individual residue to alanine. Alanine is a neutral amino acid and is fairly small, so it would not be expected to cause serious ionic or structural distortions. It

should be noted that if alanine scanning had been applied to the acidic patch to uncover important residues, it would have identified D99 as being important for Htz1 function. However, it would have missed E101 as an important residue due to its lack of serious effect in the alanine mutant. Our results are in concordance with the general genetic concept that site-directed and random methods are most successful when utilized together. We first performed a random screen for *HTZ1* mutations, and then applied thorough systematic techniques to the region highlighted by that screen. The obvious charge difference of the E101K mutation originally identified in *htz1-21* (as well as the presence of a more conservative mutation in *htz1-27*) suggested to us to construct our mutant panel taking into account mutating each acidic residue to a neutral as well as a basic amino acid. This allowed us to characterize the importance of both D99 and E101.

Previous domain swapping experiments have established the importance of the M6 domain of H2A.Z for function (Clarkson et al. 1999; Wu et al. 2005). Through the use of an unbiased genetic screen, we have also defined a region of functional importance in H2A.Z even more specific than the M6 domain. In H2A.Z, three consecutive conserved acidic residues of H2A (DDE) are extended to four out of five residues (DDELD). Given the importance of H2A.Z function as being distinct from H2A (Jackson and Gorovsky 2000), it might be expected that a crucial residue in the acidic patch that specifies a function for H2A.Z would be D103, since there is no corresponding residue in H2A (Fig. 2). Surprisingly, mutation of D103 was without effect in our studies (Figs. 3a, 4); we have shown that the important residues of the acidic patch are actually among those shared with the major H2A (D99 and E101). Importantly, not every residue in the patch conferred the strong phenotypes. Singly mutating two of three conserved residues (D99 or E101) was sufficient to generate phenotypes, but mutating the conserved residue immediately between them (D100) was without serious effect. In addition, changing acidic E101 to neutral alanine (a more conservative change) was without serious effect, but changing it to basic lysine (a less conservative change) was sufficient to generate the phenotypes in question. These results essentially describe a fine-structure mapping of the acidic patch region of H2A.Z within a sequence of amino acid residues that are crucial for binding.

Studies in the Tremethick laboratory have been performed on the acid patch region of H2A.Z in higher eukaryotes. In one study, mutations in the acidic patch region of *X. laevis* H2A.Z including the equivalent of yeast D103 were sufficient to confer an embryonic developmental defect (Ridgway et al. 2004). In the other study, those mutations conferred to H2A.Z nucleosomal arrays a folding property not different than that of H2A-containing arrays, pointing to a role for the acid patch residues of H2A.Z in the compaction of chromatin (Fan et al. 2004). The patch itself is highly conserved, but these studies are not incompatible with our findings, particularly the absence of any serious effect in our D103 yeast mutant strains. These other experiments were performed using H2A.Z from a multicellular organism in which H2A.Z is essential for viability. Since yeast does not require H2A.Z to survive, D103 likely acts in a pathway in *X. laevis* that is distinct from that of yeast.

There is also considerable structural support for the array of effects we have observed. The NMR structure of H2A.Z-specific histone chaperone Chz1 in a heterotrimer complexed with histones H2A.Z and H2B (the CZB complex) was recently published (Zhou et al. 2008). As H2A.Z–H2B dimers are expected to interact with the SWR1 complex in a chaperone-bound form (Luk et al. 2007), the structural predictions made are directly applicable to our observations. The structure indicates that D99 and E101, the two acidic residues that demonstrated strong phenotypes when mutated, are exposed in a groove on one side of the CZB complex (Zhou et al. 2008). In addition, D100 and D103, the two acidic residues that did not demonstrate strong phenotypes when mutated, are not in this groove. D100 is located

to the side of this groove, and D103 is behind the groove. We do not know the exact locations of the binding partners on the SWR1 complex, but it is tempting to speculate that this groove represents the binding surface for the SWR1 complex on the H2A.Z molecule. The differential locations of the four acidic residues could explain why D99 and E101 specifically demonstrate strong effects, and D100 and D103 do not. It is also possible that if D99 interacted with its binding partner through short-range hydrophobic interactions, and E101 interacted with its binding partner through long-range electrostatic interactions, then this could potentially explain the different effects of the alanine substitutions in these two residues. Mutation to alanine would then be better tolerated at E101, but still clash with its binding partner at D99. It should be noted that the disruption of SWR1 complex binding in the D99A/K and E101K mutations might also be due to an indirect effect, as D99 and E101 do not appear to be completely solvent-accessible. These mutations, rather than directly disrupting the interface between H2A.Z and the SWR1 complex, could instead simply alter the internal structure of the Chz1–H2A.Z–H2B heterotrimer so that the SWR1 complex becomes unable to bind to it.

Finally, while this manuscript was in revision, another study on H2A.Z incorporation by the SWR1 complex was published (Luk et al. 2010). This elegant series of experiments dissected the nature of the histone exchange reaction carried out by the SWR1 complex in great detail. Using immobilized nucleosome arrays, the Wu lab determined that the SWR1 complex deposits H2A.Z–H2B dimers into chromatin in a stepwise, unidirectional fashion (Luk et al. 2010). They observed that one canonical H2A–H2B dimer is removed from the nucleosome, generating a heterotypic intermediary nucleosome containing one H2A.Z–H2B dimer and the remaining H2A–H2B dimer. The second H2A–H2B dimer is then finally exchanged, generating a homotypic nucleosome containing two H2A.Z–H2B dimers as an end product. H2A-containing nucleosomes, H2A.Z–H2B dimers, and ATP are all required for this exchange reaction (Luk et al. 2010). However, these experiments do not address the molecular determinants of the H2A.Z–H2B dimer that are specifically recognized by the SWR1 complex. Specific acidic residues within H2A.Z may represent this molecular determinant.

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Fig. 1. Identification of a single point mutation in *HTZ1* that confers conditional lethality. Wild-type, *htz1Δ*, *htz1-21*, and *htz1-27* strains (MSYs 786 and 4108-10) were grown into log phase, and 10-fold serial dilutions of each were spotted onto plates and incubated with the conditions described

Fig. 2.

The conserved acidic patch of H2A.Z. Histones of the H2A.Z family were aligned (SeqWeb, Accelrys). Alignment includes *S. cerevisiae* H2A and Htz1, *Homo sapiens* H2A.Z, *D. melanogaster* H2Av, and *T. thermophila* hv1. Orange boxes corresponding to the secondary α -helical regions of *D. melanogaster* H2Av adapted from (Clarkson et al. 1999) have been placed underneath the primary sequence alignment. The region corresponding to M6 of H2Av has been boxed in brown. Acidic amino acids mutated in our analysis are indicated in boldface. The final two residues of *T. thermophila* hv1 have been omitted for simplicity. The numbering system is arbitrary and does not correspond exactly to any of the specific molecules indicated, due to their varying lengths.

Fig. 3.

Analysis of the full panel of *htz1* acid patch mutations. **a** Strains containing the indicated point mutations in the acidic patch of *HTZ1* (MSYs 1154, 4135, 4137, 4139, 4141, 4143, 4145, 4147, 4149, and 4151) were spotted as in Fig. 1. **b** Strains containing the indicated mutations in *HTZ1* (MSYs 1215, 4137, 4139, 4141, 4143, 4145, 4147, 4149, 4134, 4670, and 4674) were grown to mid-log phase and whole cell extracts prepared (see “Materials and methods” section). 50 μg of extract were loaded into each lane of a polyacrylamide gel. After transfer to a polyvinylidene difluoride membrane, the membrane was probed with α-HA (green, lower panel, to detect HA-Htz1) and α-GPDH (red, upper panel) as a loading control and analyzed by Odyssey software. Htz1 levels were calculated as the ratio of HA signal to GPDH signal, and relative Htz1 levels were computed by normalizing each ratio to that of the wild-type. $N = 3$ for all genotypes except MSYs 4670 and 4674, where $N = 2$. Standard deviations (SD) are listed below the relative Htz1 levels

Fig. 4.

Analysis of H2A-like mutations. Strains containing the indicated point mutations in D103 of Htz1 (MSYs 590, 1154, 4149, 4151, 4670, and 4674) were grown, spotted, and incubated as in Fig. 1

Fig. 5.

Analysis of the *htz1* increased gene dosage strains. **a** Wild-type, *htz1-D99A*, *htz1-D99K*, and *htz1-E101K* strains (MSYs 4135, 4137, 4139, and 4147) were transformed with empty plasmids (pRS316), plasmids containing the wild-type *HTZ1* gene (pJM1), plasmids containing the same mutation (self-complementing), and with plasmids containing different mutations (cross-complementing). Whole cell extracts from these transformants were prepared and analyzed as in Fig. 3b. Relative *Htz1* levels were computed by normalizing each ratio to the empty plasmid (pRS316) negative control. **b** Transformants from **a** were grown into log phase in liquid SC-ura, and spotted and incubated as in Fig. 2

Fig. 6.

Chromatin immunoprecipitation analysis of *HTZ1* alleles. **a** Schematic showing *PHO5* and its surrounding region. The locations of 18 PCR primer pairs (from Santisteban et al. 2000) are shown as off-white boxes underneath the locus. Fragments 5, 9, and 13 were analyzed by ChIP in **b** and **c**. **b** Wild-type, *htz1-D99K*, and *htz1-E101K* strains (MSYs 4135, 4139, and 4147) were grown in the absence or presence of inorganic phosphate to induce or repress the *PHO5* gene, respectively. Strains were harvested and analyzed as described in “Materials and methods” section. α -HA was used to determine HA-Htz1 occupancy at three regions of *PHO5*: fragments 5 (intergenic region), 9 (early coding region), and 13 (late coding region). **c** Wild-type, *htz1-D99A*, *htz1-D99K*, and *htz1-E101K* strains (MSYs 4135, 4137, 4139, and 4147) were transformed with empty plasmids (pRS316) or plasmids containing the same allele of *HTZ1* (pJM1-3, and 7) as in Fig. 4, and were harvested and analyzed for the presence of Htz1 over *PHO5* as in **a**

Table 1

Yeast strains and plasmids used in this study

Strain/plasmid	Genotype
MSY 590	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112</i>
MSY 786	<i>MATa ade2-101 ura3-52 lys2-801 trp1-Δ63 his3Δ200 leu2-Δ1</i>
MSY 1154	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 htz1Δ</i>
MSY 1215	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-HTZ1</i>
MSY 4108	<i>MATa ade2-101 ura3-52 lys2-801 trp1-Δ63 his3Δ200 leu2-Δ1 htz1::kan^r</i>
MSY 4109	<i>MATa ade2-101 ura3-52 lys2-801 trp1-Δ63 his3Δ200 leu2-Δ1 htz1-21::K1URA3</i>
MSY 4110	<i>MATa ade2-101 ura3-52 lys2-801 trp1-Δ63 his3Δ200 leu2-Δ1 htz1-27::K1URA3</i>
MSY 4135	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-HTZ1::K1URA3</i>
MSY 4137	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-D99A::K1URA3</i>
MSY 4139	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-D99K::K1URA3</i>
MSY 4141	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-D100A::K1URA3</i>
MSY 4143	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-D100K::K1URA3</i>
MSY 4145	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-E101A::K1URA3</i>
MSY 4147	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-E101K::K1URA3</i>
MSY 4149	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-D103A::K1URA3</i>
MSY 4151	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-D103K::K1URA3</i>
MSY 4670	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-D103N::K1URA3</i>
MSY 4674	<i>MATa Δ(HHT2-HHF2) ura3-52 lys2-Δ201 leu2-3,112 HA-htz1-D103N,S104K::K1URA3</i>
pRS316	<i>DH5α amp^r lacZ CEN6 ARSH4 URA3</i>
pJM1	<i>DH5α amp^r lacZ CEN6 ARSH4 URA3 HA-HTZ1</i>
pJM2	<i>DH5α amp^r lacZ CEN6 ARSH4 URA3 HA-htz1-D99A</i>
pJM3	<i>DH5α amp^r lacZ CEN6 ARSH4 URA3 HA-htz1-D99K</i>
pJM7	<i>DH5α amp^r lacZ CEN6 ARSH4 URA3 HA-htz1-E101K</i>