# Insight Into the Mechanism of Nucleosome Reorganization From Histone Mutants That Suppress Defects in the FACT Histone Chaperone

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**ABSTRACT** FACT (FAcilitates Chromatin Transcription/Transactions) plays a central role in transcription and replication in eukaryotes by both establishing and overcoming the repressive properties of chromatin. FACT promotes these opposing goals by interconverting nucleosomes between the canonical form and a more open reorganized form. In the forward direction, reorganization destabilizes nucleosomes, while the reverse reaction promotes nucleosome assembly. Nucleosome destabilization involves disrupting contacts among histone H2A-H2B dimers, $(H3\text{-}H4)_2$ tetramers, and DNA. Here we show that mutations that weaken the dimer:tetramer interface in nucleosomes suppress defects caused by FACT deficiency *in vivo* in the yeast *Saccharomyces cerevisiae*. Mutating the gene that encodes the Spt16 subunit of FACT causes phenotypes associated with defects in transcription and replication, and we identify histone mutants that selectively suppress those associated with replication. Analysis of purified components suggests that the defective version of FACT is unable to maintain the reorganized nucleosome state efficiently, whereas nucleosomes with mutant histones are reorganized more easily than normal. The genetic suppression observed when the FACT defect is combined with the histone defect therefore reveals the importance of the dynamic reorganization of contacts within nucleosomes to the function of FACT *in vivo*, especially to FACT's apparent role in promoting progression of DNA replication complexes. We also show that an H2B mutation causes different phenotypes, depending on which of the two similar genes that encode this protein are altered, revealing unexpected functional differences between these duplicated genes and calling into question the practice of examining the effects of histone mutants by expressing them from a single plasmid-borne allele.

FACT (FAcilitates Chromatin Transcription/Transactions) is a highly conserved histone chaperone with roles in both transcription and DNA replication (Reinberg and Sims 2006; Formosa 2008). In *Saccharomyces cerevisiae*, FACT is an Spt16-Pob3 heterodimer whose activity is supported by the High Mobility Group B (HMGB)-like DNA-binding protein Nhp6 (Brewster *et al.* 2001; Formosa *et al.* 2001). Susceptibility of nucleosomal DNA to digestion by some restriction endonucleases *in vitro* increases dramatically in the presence of FACT (Xin *et al.* 2009), indicating that FACT either induces a structural change or stabilizes an existing alternative nucleosomal structure (Winkler and Luger 2011). We have called this activity "nucleosome reorganization" (Formosa 2008). FACT enhances binding of TATA sequences within nucleosomes by TATA-binding protein (TBP/Spt15) (Biswas *et al.* 2005), suggesting that increasing access to DNA through reorganization is a physiologically important role of FACT.

FACT can induce displacement of H2A-H2B dimers from nucleosomes under some conditions *in vitro* (Belotserkovskaya *et al.* 2003; Xin *et al.* 2009). However, increased nuclease sensitivity does not require H2A-H2B loss (Xin *et al.* 2009). Reorganized nucleosomes instead appear to have the same composition as canonical nucleosomes, but the components are associated with one another in a substantially different manner. This change in structure increases the probability of H2A-H2B dimer loss, but dimer loss appears to be just one possible result of reorganization, not its mechanism. The effect of FACT on nucleosomes is not common to other histone chaperones, and FACT-mediated reorganization does not require ATP hydrolysis so it is unlike ATP-dependent

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chromatin remodeling (Orphanides *et al.* 1998; Clapier and Cairns 2009). FACT is essential for viability (Formosa 2008; Lolas *et al.* 2010), but the detailed nature of reorganized nucleosomes, the role of FACT in forming and resolving alternative nucleosome forms, and the importance of H2A-H2B dimer displacement to FACT activity *in vivo* remain poorly understood.

Partial loss-of-function mutations in the genes encoding FACT subunits cause a range of defects in transcription, replication, and other processes (Lycan *et al.* 1994; O'Donnell *et al.* 2004, 2009; Formosa 2008). Some of these defects can be enhanced by decreasing histone acetylation (Formosa *et al.* 2002), by blocking histone H3-K4 methylation (Biswas *et al.* 2006), by mutating histone genes themselves (Vandemark *et al.* 2008), or by inactivating the Hir/Hpc complex that is involved in regulating histone gene expression and depositing nucleosomes outside of S phase (Formosa *et al.* 2002). Some features of chromatin therefore support FACT activity *in vivo*, and their loss makes FACT defects more difficult to tolerate. In contrast, other chromatin factors oppose FACT activity, as the phenotypes caused by some FACT gene mutations can be suppressed by preventing methylation of H3-K36 (Biswas *et al.* 2006) or by inactivating the chromodomain-helicase Chd1 (Biswas *et al.* 2008). FACT gene mutations can also either enhance or suppress defects caused by mutating other chromatin factors such as the histone H3 or the Swi/Snf remodeling complex (Malone *et al.* 1991; Duina *et al.* 2007). Furthermore, phenotypes caused by FACT gene mutations can be either enhanced or suppressed by altering the ratio of expression of H2A-H2B relative to H3-H4 (Formosa *et al.* 2002). The properties of chromatin therefore affect the efficiency of FACT function *in vivo*, but it remains unclear how these properties influence the central activity of FACT.

To examine the relationship between FACT and nucleosome reorganization *in vivo*, we sought histone gene mutations that could compensate for FACT defects. We reasoned that deficiency for FACT activity could be counterbalanced by mutating the histones in a way that makes nucleosomes easier to reorganize. Such mutations would provide insight into both the functions of FACT *in vivo* and the nature of nucleosome reorganization. Here we report that the temperature sensitivity (Ts) and hydroxyurea sensitivity caused by the *spt16-11* allele of FACT can be suppressed by weakening the H2A-H2B dimer interface with $(H3\text{-}H4)_2$ tetramers within nucleosomes, but this does not significantly affect the $Spt^-$ phenotype also caused by *spt16-11*. Purified nucleosomes with these mutated histones display elevated rates of spontaneous reorganization using nuclease sensitivity as an assay, and this partially compensates for Spt16-11 protein's defect in achieving stable reorganization *in vitro*. These results provide new insights into the mechanism of FACT activity *in vivo*, supporting an important role for the stability of the histone dimer:tetramer interface in nucleosome reorganization and revealing a potential role for reorganization in replication fork progression.

## Materials and Methods

Strains are listed in Table 1 and in the supporting information, Table S1. W303 strains were derived from MSY1905 (kindly provided by M. Mitchell Smith, University of Virginia) by conversion of the KanMX marker replacing *HHT1-HHF1* with *HIS3* followed by standard crosses within the W303 background to introduce other mutations. A364a strains were constructed by integrating markers downstream of the histone genes and then amplifying the marked genomic locus by PCR using an upstream primer containing the desired mutation ~30 nucleotides from the 5′ end and ~25 nucleotides from the 3′ end and a downstream primer ~200 bp distal to the marker (the strategy is outlined in Figure 3; the primers used are listed in Table S2; Toulmay and Schneiter 2006). The PCR product was used to transform a wild-type strain, selecting for transfer of the marker and then screening for cotransfer of the mutation by sequencing the entire histone gene. Standard crosses within the A364a background were then performed to obtain combinations of mutations.

The *S. cerevisiae* genome contains two copies of each of the genes that encode the four core histones (Osley 1991). Plasmids pJH33, M4958, and M4959 carrying the genes *HTA1-HTB1* and *HHT2-HHF2* encoding histones H2A, H2B, H3, and H4 in vectors pRS316 (*URA3*), pRS315 (*LEU2*), or pRS314 (*TRP1*), respectively (Sikorski and Hieter 1989), were kindly provided by M. Mitchell Smith (University of Virginia) (Ahn *et al.* 2005). Mutagenized histone genes were obtained by amplifying M4958 using primers that align ~400 bp into the vector sequences flanking the histone gene insert, resulting in a 5279-bp product with ~400 bp of homology at each end to pRS414 or pRS415 (Figure 1). PCR was performed under standard conditions (100-μl reactions containing 20 pmol of each primer, 10 ng template, 0.2 mM each deoxynucleoside triphosphate (dNTP), 10 mM Tris–Cl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl; 30 cycles of 1 min at 94°, 1 min at 54°, 5 min at 72°) using *Pfu* polymerase (a small number of candidates were generated with a 1:200 mixture of *Pfu:Taq* polymerases, but most of these contained multiple mutations). Yeast strains DY10003 and DY10004 with the *spt16-11* allele (Table 1) and carrying pJH33 were transformed with this PCR product mixed with vectors pRS414 (*TRP1*) or pRS415 (*LEU2*) (Christianson *et al.* 1992) that had been linearized with *Bam*HI and *Hind*III. This yielded pLM04 (*TRP1*) and pTF238 (*LEU2*) derivatives by recombination *in vivo*. About 70,000 transformants were obtained and replica-plated to medium containing 5′-FOA to select for cells lacking the original wild-type histone plasmid (Boeke *et al.* 1987), thus demanding that the mutagenized plasmids expressed histones adequate for supporting viability. Strains surviving with only the mutagenized plasmids were replica-plated to 37° or to medium containing 120 mM hydroxyurea (HU), conditions nonpermissive for growth of the parent strains. About 2000 candidates were chosen for retesting, yielding several hundred suppressed strains.

**Table 1 Strains used**

| Strain | Genotype |
|---|---|
| Figure 1 | W303 background |
| DY10003 | *MAT**a** ade2 can1 his3 leu2 trp1 ura3 spt16-11 hht1-hhf1-Δ(::HIS3) hht2-hhf2-Δ(::KanMX) hta1-htb1-Δ(::NatMX) hta2-htb2-Δ(::HphMX) pJH33 (YCp URA3 HHT2-HHF2, HTA1-HTB1)* |
| DY10004 | *MATα ade2 can1 his3 leu2 trp1 ura3 spt16-11 hht1-hhf1-Δ(::HIS3) hht2-hhf2-Δ(::KanMX) hta1-htb1-Δ(::NatMX) hta2-htb2-Δ(::HphMX) pJH33 (YCp URA3 HHT2-HHF2, HTA1-HTB1)* |
| Figure 3 | A364a background |
| 8127-7-4 | *MAT**a** ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂* |
| 8500-10-2 | *MAT**a** ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ HTA1(220, His3MX) HTA2(30, URA3)* |
| 8541-3-2 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ hta1-V101I(220, His3MX) hta2-V101I(30, URA3)* |
| 8262-11-4 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ spt16-11* |
| 8554-5-3 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ spt16-11 HTA1(220, His3MX) HTA2(30, URA3)* |
| 8541-4-2 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ hta1-V101I(220, His3MX) hta2-V101I(30, URA3) spt16-11* |
| 8127-7-4 | *MAT**a** ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂* |
| 8500-10-2 | *MAT**a** ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ HTA1(220, His3MX) HTA2(30, URA3)* |
| 8541-3-2 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ hta1-V101I(220, His3MX) hta2-V101I(30, URA3)* |
| 8324-2-2 | *MAT**a** ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ pob3-Q308K(LEU2)* |
| 8500-2-2 | *MAT**a** ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ HTA1(220, His3MX) HTA2(30, URA3) pob3-Q308K(LEU2)* |
| 8555-4-2 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ hta1-V101I(220, His3MX) hta2-V101I(30, URA3) pob3-Q308K(LEU2)* |
| Figure 4 | A364a background |
| 8483-9-1 | *MAT**a** ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ HTB1(30, URA3) HTB2(30, His3MX)* |
| 8606-3-4 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ htb1-A84D(30, URA3) HTB2(30, His3MX)* |
| 8868-5-1 | *MAT**a** ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ HTB1(30, URA3) htb2-A84D(30, His3MX)* |
| 8442-4-3 | *MAT**a** ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ htb1-A84D(30, URA3) htb2-A84D(30, His3MX)* |
| 8482-5-3 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ HTB1(30, URA3) HTB2(30, His3MX) spt16-11* |
| 8606-2-1 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ htb1-A84D(30, URA3) HTB2(30, His3MX) spt16-11* |
| 8607-1-1 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ HTB1(30, URA3) htb2-A84D(30, His3MX) spt16-11* |
| 8455-8-4 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ htb1-A84D(30, URA3) htb2-A84D(30, His3MX) spt16-11* |
| 8625-3-4 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ HTB1(30, URA3) hta2-htb2-Δ(::HIS3) spt16-11* |
| 8608-5-4 | *MAT**a** ura3 leu2 trp1 his3 lys2-128∂ htb1-A84D(30, URA3) hta2-htb2-Δ(::KanMX) spt16-11* |
| Table 2 | W303 background |
| DY9999 | *MAT**a** ade2 can1 his3 leu2 trp1 ura3 hht1-hhf1-Δ(::HIS3) hht2-hhf2-Δ(::KanMX3) hta1-htb1-Δ(::NatMX) hta2-htb2-Δ(::HphMX) pJH33 (YCp URA3 HHT2-HHF2, HTA1-HTB1)* |
| 8264-17-3 | *MAT**a** ade2 can1 his3 leu2 trp1 ura3 pob3-Q308K hht1-hhf1-Δ(::HIS3) hht2-hhf2-Δ(::KanMX3) hta1-htb1-Δ(::NatMX) hta2-htb2-Δ(::HphMX) pJH33 (YCp URA3 HHT2-HHF2, HTA1-HTB1)* |

Plasmids were recovered from ~100 of the strongest candidates, screened for the expected restriction digestion pattern, and used to transform DY10004 pJH33 to establish linkage of the suppressing mutation with the plasmid. Twenty-six of these plasmids were found to contain suppressor mutations and were sequenced using four primers to fully cover all four histone genes on each plasmid (Table S2). Eighteen of the plasmids had single mutations affecting 1 of 10 residues in H2A or H2B, distributed as shown in Table 2. Some of the 8 plasmids with multiple mutations also affected these same residues, and all plasmids had at least one mutation in H2A or H2B that mapped to the interface between H2A-H2B and H3-H4, but plasmids with complex mutations were not analyzed further. The screens for suppression of temperature sensitivity and HU sensitivity yielded overlapping results, so the resulting plasmids were combined for further analysis (Figure 1B).

Nucleosomes were reconstituted *in vitro* with recombinant histones, labeled with fluorescent dyes, and tested for binding affinity with FACT, sensitivity to *Dra*I digestion, and retention of H2A-H2B dimers as described previously (Ruone *et al.* 2003; Rhoades *et al.* 2004; Xin *et al.* 2009). The *spt16-11* (T828I, P859S) (Formosa *et al.* 2002), *hta1-V101I*, and *htb1-A84D* mutations were introduced into expression constructs using the Quikchange strategy (Stratagene), and the proteins were purified as previously described (Ruone *et al.* 2003; Rhoades *et al.* 2004; Xin *et al.* 2009).

## Results

### *Defects caused by FACT mutants can be suppressed by histone mutants*

The *spt16-11* allele causes sensitivity to elevated temperatures, sensitivity to the replication toxin HU, and the Spt$^-$ phenotype (Formosa *et al.* 2001). The amount of Spt16 protein in an *spt16-11* mutant drops about fivefold after a 3-hr incubation at 37° (Vandemark *et al.* 2008), so the Ts$^-$ phenotype probably reflects simple loss of FACT activity. HU inhibits ribonucleotide reductase (RNR), causing DNA replication to stall due to the shortage of dNTPs, but also

leading to increased transcription of the genes encoding RNR. At least some FACT mutants retain normal induction of RNR gene transcription (Biswas *et al.* 2008; Formosa 2008), so HU sensitivity often reflects a defect in DNA replication, but indirect effects due to flawed transcription are also possible. The Spt$^-$ phenotype results from inappropriate transcription initiation start-site selection (Clark-Adams *et al.* 1988), indicating that *spt16-11* causes a defect in transcription even at temperatures permissive for growth where Spt16 protein levels are normal. *spt16-11* was chosen for the suppressor analysis described here because it causes a broad range of phenotypes associated with transcription and replication.

To detect histone gene mutations that can compensate for the defects caused by *spt16-11*, we constructed a strain with this mutation that lacked genomic versions of histone genes but instead carried a single copy of the genes encoding H2A, H2B, H3, and H4 on a plasmid (*Materials and Methods*). PCR amplification of the insert containing all four histone genes was used to introduce random mutations, and alleles capable of suppressing the Ts$^-$ or HU sensitivity phenotypes caused by *spt16-11* were identified. This approach yielded 26 suppressing plasmids, 18 of which carried single mutations that altered 1 of 10 residues in H2A or H2B (Table 2, Figure 1B). H2B-A84 mutants were isolated five times (A84D four times and A84V once), but mutations affecting other residues were recovered only once or twice. The screen was therefore not saturated, but all of the mutated residues matched the same physical profile in that they affected residues that are buried within the histone octamer core on or near the surface of the H2A-H2B dimer that contacts the $(H3\text{-}H4)_2$ tetramer (indicated in Figure 2 as either magenta or red residues). These results therefore strongly suggest that FACT activity *in vivo* involves disruption of the dimer:tetramer interface, that the *spt16-11* mutation causes a defect in this function, and that weakening the interface by mutating residues in the interface reduces the requirement for efficient FACT. It should also be possible to interfere with this interface by mutating H3 or H4, so it is puzzling that no such mutations were identified, but it is possible that mutating the interface from the more highly conserved H3 or H4 protein side leads to inviability.

Suppressors were isolated for the ability to reverse either the Ts$^-$ or the HU sensitivity caused by *spt16-11*, but each suppressor was found to suppress both phenotypes to variable but similar extents (Figure 1B). This suggests that both phenotypes have a common underlying defect. Suppression of *spt16-11* was partially allele-specific, as some of the mutants weakly suppressed a *pob3-Q308K* mutation affecting the Pob3 subunit of FACT, but others had no effect and some even enhanced the defects caused by *pob3-Q308K* (Table 2, Figure S1A, Figure S4, and Figure 3). *spt16-11* and *pob3-Q308K* therefore cause distinct defects in FACT function, and weakening the dimer:tetramer interface is strongly beneficial only to the cells with the *spt16-11* deficiency. The histone gene mutations had little or no effect

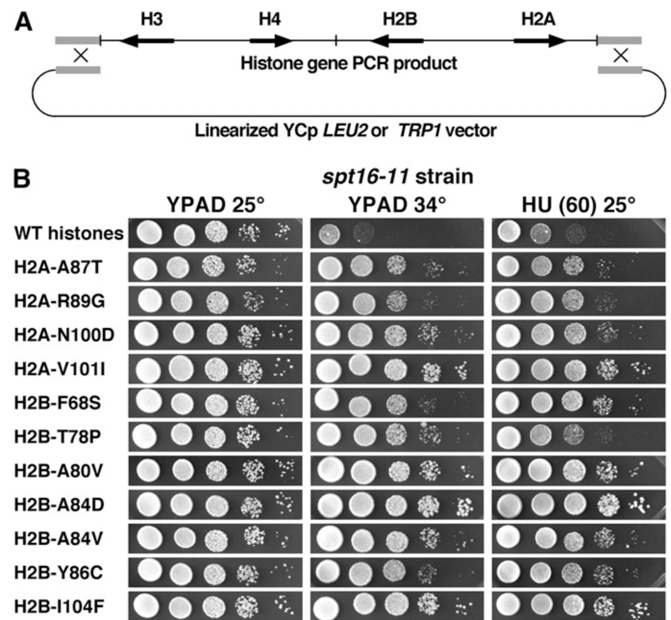


**Figure 1** Histone gene mutations can suppress defects caused by an *spt16-11* mutation. (A) Scheme for mutagenizing histone genes. A plasmid carrying wild-type *HHT2-HHF2* (H3-H4) and *HTA1-HTB1* (H2A-H2B) was used as the template for PCR using primers TF04-25 and TF05-28 in the vector sequence flanking the histone gene insert (Table S2). The product was mixed with linearized vector DNA and used to transform DY10003 or DY10004 (Table 1). Recombination *in vivo* produced mutagenized histone gene plasmids. (B) Candidate plasmids with the single mutations indicated were recovered and used to transform strain DY10004 (Table 1), and then isolates lacking the wild-type histone gene plasmid were derived. Strains with only the mutated plasmid were grown to saturation, and aliquots of 10-fold serial dilutions were tested on YPAD (rich medium; Yeast Extract, Peptone, Adenine, Dextrose) at 25° or 34° or on HU (60) (YPAD with 60 mM hydroxyurea) at 25°.

in an *SPT16* wild-type strain (Table 2, Figure S1B). Altering the histones therefore caused a significant enough effect on nucleosome structure that an *spt16-11* mutation was strongly suppressed, but the change was not sufficient to cause an obvious defect in an otherwise normal cell.

#### *Integration of histone gene mutations reveals differences among expression contexts*

FACT mutants are sensitive to histone gene copy-number variation (Formosa *et al.* 2002), and it is difficult to maintain plasmids at uniform copy number throughout a population of cells. Furthermore, *HTA1-HTB1* and *HTA2-HTB2* encode slightly different amino acid sequences (Figure S3), transcription of each gene is regulated by different factors (Osley and Lycan 1987; Xu *et al.* 1992; Dollard *et al.* 1994; Hess and Winston 2005), and *HTA1-HTB1* is essential for viability but *HTA2-HTB2* is not (Formosa *et al.* 2002; Libuda and Winston 2006). Either the sequence differences or their transcription profiles under different conditions are therefore functionally important. To test the effects of the suppressing mutations in a more native context, we integrated two of the mutations into the genome at the endogenous

**Table 2 Effects of *spt16-11* suppressors in wild-type and *pob3-Q308K* strains**

| Histone | Mutation | No. of isolates | Phenotypes with *pob3-Q308K* | Phenotypes in wild type |
|---|---|---|---|---|
| H2A | *hta1-A87T* | 2 | | |
| H2A | *hta1-R89G* | 2 | Ts$^-$ HUs (enhanced defect) | Slight Ts$^-$, Slight HUs, NaCl-s |
| H2A | *hta1-N100D* | 1 | Ts+ HUr (weak suppression) | |
| H2A | *hta1-V101I* | 1 | Ts+ HUr (weak suppression) | Slight Fmd-s |
| H2B | *htb1-T55S* | 2 | | Fmd-s |
| H2B | *htb1-N66S* | 1 | | Fmd-s |
| H2B | *htb1-F68S* | 2 | Ts$^-$ HUs (enhanced defect) | |
| H2B | *htb1-T78P* | 1 | Ts$^-$ HUs (enhanced defect) | |
| H2B | *htb1-A80V* | 2 | Ts$^-$ HUs (enhanced defect) | |
| H2B | *htb1-A84V* | 4 | Ts+ HUr (weak suppression) | |
| H2B | *htb1-A84D* | 1 | Ts$^-$ HUs (enhanced defect) | Mild Spt$^-$ |
| H2B | *htb1-Y86C* | 1 | Mixed weak effects | Slight Fmd-s, Slight HUs |
| H2B | *htb1-I104F* | 1 | Ts+ HUr (weak suppression) | |

Strains 8264-17-3 (*pob3-Q308K*) and DY9999 (wild type) were transformed with *LEU2* plasmids carrying the mutation indicated, and then transformants lacking the wild-type histone gene plasmid were screened for growth at elevated temperatures or on media containing 30–150 mM hydroxyurea, 1.2 M NaCl, 3% formamide, 10 mM caffeine, or 10 μg/ml camptothecin. The *pob3-Q308K* mutation causes Ts$^-$ and HUs phenotypes, and these were unaffected, enhanced, or suppressed as indicated. In all cases, the effects were small compared to other synthetic interactions observed with this allele. Moderate formamide sensitivity was observed where indicated (Fmd-s), with all other effects in the wild-type strain being weak. The number of times each mutation was isolated in the original screen for *spt16-11* suppression is indicated as "No. of isolates." HUs, hydroxyurea sensitive; HUr, hydroxyurea resistant; NaCl-s, NaCl sensitive.

loci. We chose H2A-V101I and H2B-A84D for this as they were two of the strongest suppressors of *spt16-11* but they had opposite effects on the *pob3-Q308K* allele (Table 2).

We inserted selectable markers downstream of each gene that encodes H2A or H2B in separate wild-type strains (Figure S2). We then used genomic DNA from these strains to amplify each gene with the targeted mutation incorporated into one PCR primer (Figure 3A) (Toulmay and Schneiter 2006). The product carrying the desired mutation was used to transform a wild-type yeast strain, and transformants were screened by sequencing the entire histone gene to find strains in which the desired mutation but no unexpected additional mutation had been integrated into the genome along with the selectable marker. Finally, standard crosses were performed to obtain combinations of mutations.

Figure 3B shows the results obtained with the H2A-V101I expressed from both *HTA1* and *HTA2*. Neither the integrated markers themselves (tags) nor the histone mutations affected the phenotypes tested in an *SPT16* strain, but the tags themselves slightly enhanced the HU sensitivity and Spt$^-$ phenotypes (but not the Ts$^-$) caused by *spt16-11* (Figure 3B; compare rows 4 and 5: the Spt$^-$ phenotype is revealed by growth of these *lys2-128∂* strains on medium lacking lysine). Marking the wild-type H2A genes therefore causes a minor defect in their expression; as noted previously and consistent with these results, a decrease in H2A-H2B expression is detrimental to some *spt16* mutants (Formosa *et al.* 2002). To control for this effect, subsequent experiments always include marked versions of the H2A genes, whether wild type or mutant. This ensures that all comparisons are between strains that are as genetically matched as possible, although it remains possible that the markers could have a differential effect on mutant and wild-type alleles.

The integrated H2A-V101I mutation suppressed both the Ts$^-$ and hydroxyurea sensitivity phenotypes caused by *spt16-11*, but had only a slight effect on the Spt$^-$ phenotype (Figure 3B; compare rows 5 and 6). H2A-V101I therefore significantly corrects the *spt16-11* defect most closely associated with DNA replication, but it has less effect on the transcription defect. Integrated H2A-V101I had no effect in a wild-type strain (Table 2, Figure S1B), but partially suppressed the Ts$^-$ and HU sensitivity caused by *pob3-Q308K* (Table 2, Figure S1A, Figure 3C), generally recapitulating the results from the plasmid-based assays.

In contrast, the locus expressing H2B-A84D expression significantly influenced the effect of this mutant. In a wild-type strain, expressing H2B-A84D from *HTB1* caused a very mild Spt$^-$ phenotype (Figure 4A; compare the medium lacking lysine (−lys) growth at 2 and 6 days with the 2-day incubation of an *spt16-11* mutant in Figure 4B). Expressing H2B-A84D from *HTB2* did not cause this phenotype and did not enhance the effect of *htb1-A84D* (Figure 4A; compare rows 2 and 4). In an *spt16-11* strain, the *htb1-A84D* allele suppressed the Ts$^-$ and hydroxyurea sensitivity phenotypes but did not affect the Spt$^-$ phenotype; this was true in both an *HTA1 htb1-A84D HTA2 HTB2* strain (normal H2B available from the second copy of the gene) and an *HTA1 htb1-A84D hta2-htb2-Δ* strain (only H2B-A84D available; Figure 4B; compare row 1 with 2 or 6). However, the *htb2-A84D* allele had no effect when paired with *HTB1* (Figure 4B, row 3) and caused variable effects when paired with *htb1-A84D* (Figure 4B, rows 3 and 4). (*HTB1* is essential for viability so the effect of *htb1-Δ htb2-A84D* could not be assessed.) Relative to *htb1-A84D HTB2 spt16-11*, the *htb1-A84D htb2-A84D spt16-11* strain displayed markedly reduced suppression of the Ts$^-$ phenotype, increased suppression of the Spt$^-$ phenotype, and enhanced sensitivity to low concentrations of HU (Figure 4B, rows 2 and 4).

The effects of the source of the H2B-A84D mutation are therefore quite complex and vary with the phenotype being observed. When challenged with HU or elevated

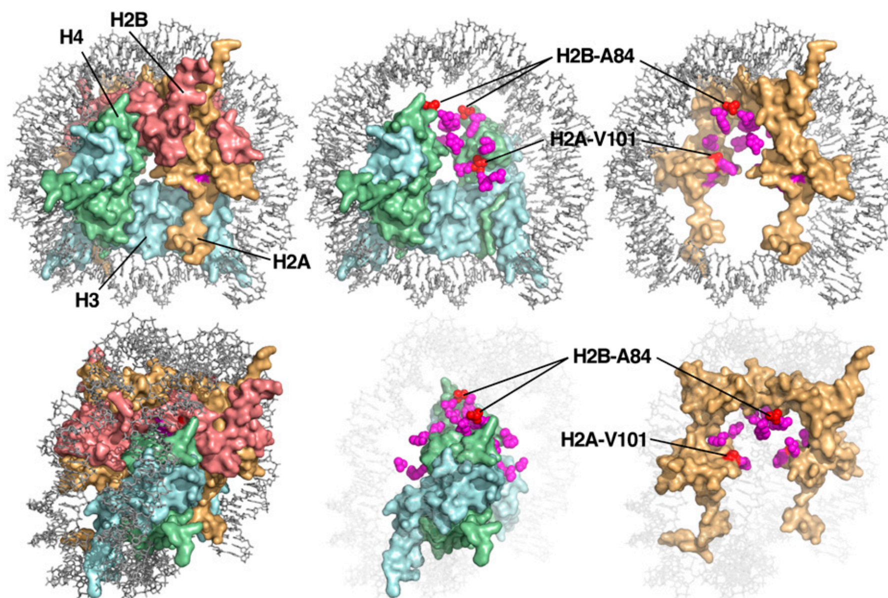


**Figure 2** Histone gene mutations that suppress *spt16-11* map to the dimer:tetramer interface. Histone residues identified in the suppressor screen are shown within the structure of a yeast nucleosome (PDB 1ID3) (White *et al.* 2001) as rendered in MacPyMOL (DeLano Scientific). Two orientations are shown (top and bottom panels) with a full nucleosome (left), H2A-H2B removed (center), or only H2A shown (right) to reveal buried sites. Sites of suppressor mutants are shown in magenta or red, with the latter indicating residues H2A-V101 and H2B-A84 that were chosen for further analysis.

temperature, expressing H2B-A84D only from the *HTB1* locus was strongly advantageous to an *spt16-11* strain (Figure 4B) but had no apparent effect in a wild-type strain (Figure 4A). However, supplying H2B-A84D only from the *HTB2* locus under these stress conditions had no effect on either *spt16-11* or wild-type strains. *htb1-A84D* caused the same effects whether normal *HTB2* was available or not, suggesting that *HTB2* expression is irrelevant in these tests. However, cells with both *htb1-A84D* and *htb2-A84D* mutations displayed distinct phenotypes compared to the single mutants, so *HTB2* expression is important in this context. While both *HTB1* and *HTB2* are transcribed at similar levels under standard growth conditions (David *et al.* 2006), these results show that the two loci have distinct functions under some circumstances, as observed previously for the heat-shock response (Norris and Osley 1987) and the ability to suppress the effects of different Ty1 delta-element insertions (Clark-Adams *et al.* 1988). These differences may result from the slightly different proteins produced by the two genes (Figure S3), or they may indicate that the two genes are differentially activated by stress conditions. In any case, this shows that testing variants only from a plasmid-borne copy of *HTB1* can produce an incomplete picture.

### *Histone mutations that suppress spt16-11 form unstable nucleosomes in vitro*

The clustering of *spt16-11* suppressors in the nucleosome structure (Figure 2) suggests that the Spt16-11 protein is defective in a process that includes disruption of the H2A-H2B interface with $(H3\text{-}H4)_2$ tetramers. To investigate this possibility as well as other potential mechanisms, we reconstituted nucleosomes containing H2A-V101I or H2B-A84D and tested their stability *in vitro*. A 181-bp DNA fragment including the 146-bp sea urchin 5S rDNA nucleosome positioning sequence was labeled with Cy5 and assembled into nucleosomes using recombinant yeast histones expressed in bacteria as described previously (Xin *et al.* 2009). The H2A-Q114C mutation was introduced into the *HTA1* gene to provide a unique cysteine residue for labeling this subunit with a maleimide derivative of the fluorescent dye Oregon Green 488 prior to assembly of octamers. The resulting nucleosomes therefore contained two fluorescent dyes that could be detected independently, allowing us to follow the DNA and H2A-H2B dimer components of the nucleosome separately.

To test whether suppressor mutations affect the physical stability of nucleosomes, we used native polyacrylamide gel electrophoresis to measure the amount of H2A-H2B displaced from the nucleosomes, the amount of tetrasome formed, and the amount of free DNA released under various conditions. Over 90% of a sample of wild-type nucleosomes remained intact by all three measurements after a 1-hr incubation at 30° in 550 mM NaCl (Figure 5, A and B). In contrast, nucleosomes constructed with H2A-V101I lost dimers and formed tetrasomes more readily than wild type, and these effects were even more pronounced with H2B-A84D. In the latter case, even nucleosomes not exposed to high salt migrated aberrantly in native polyacrylamide gels, consistent with the observed spontaneous loss of dimers during preparation and storage (Figure 5A, lane 5 and not shown). Incubation of nucleosomes for 1 hr at 65° also caused low levels of tetrasome formation with wild-type nucleosomes, but substantially elevated levels for each mutant nucleosome (Figure 5, C and D). Thus, both H2A-V101I and H2B-A84D cause nucleosome instability *in vitro*, including an increase in dimer displacement.

FACT might either induce nucleosomes to reorganize or selectively bind to and stabilize spontaneously reorganized nucleosomes. In the first case, a weakened dimer:tetramer interface would make it easier to overcome resistance to reorganization, and in the second case, it would provide a larger subpopulation of complexes for binding. In either

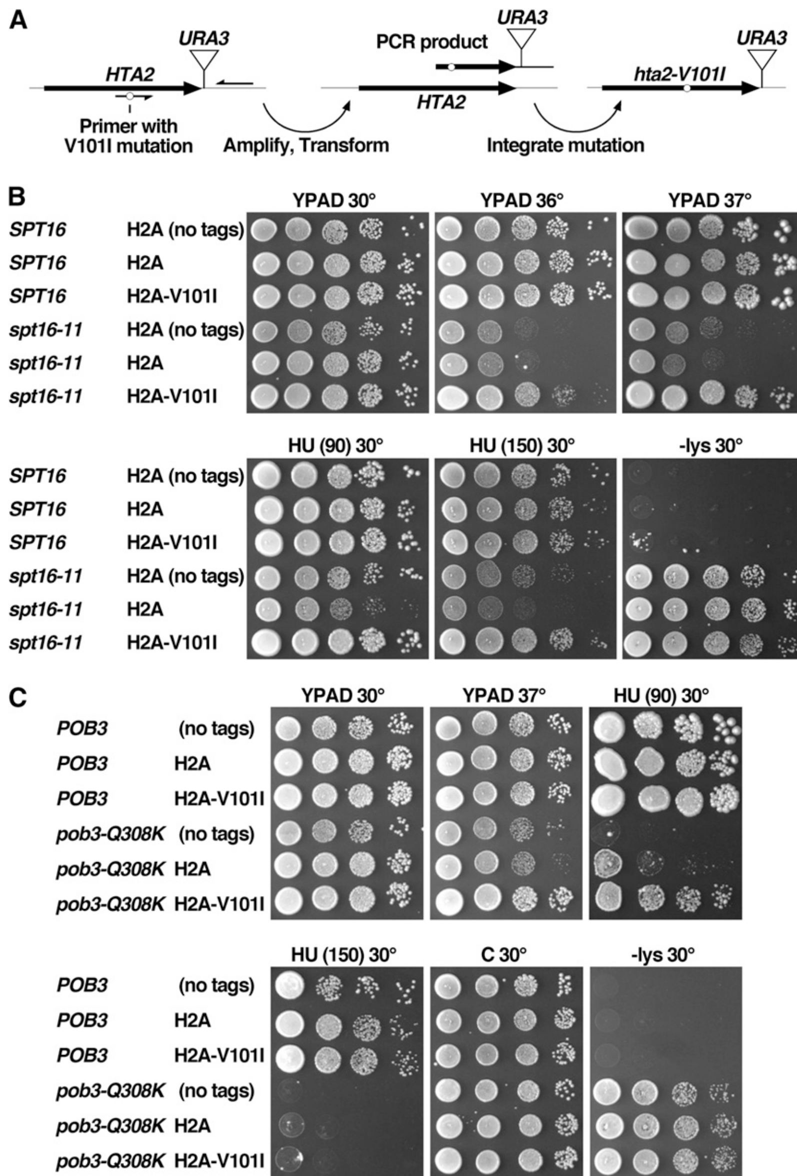


**Figure 3** H2A-V101I suppresses some phenotypes caused by two distinct FACT gene mutations. (A) Schematic showing the method used for integrating the *hta2-V101I* mutation into the genome (strategies for other genes shown in Figure S2 and Table S2). (B and C) Cultures of strains isogenic with the A364a genetic background and the additional genotypes shown (see Table 1 for the full genotypes) were tested as in Figure 1). "C" is complete synthetic medium; HU (90) and HU (150) are YPAD with 90 or 150 mM hydroxyurea. Growth on medium lacking lysine (−lys) reveals the Spt$^{-}$ phenotype in the strains with the *lys2-128∂* allele (Simchen *et al.* 1984). Unless noted, all strains have marker genes (tags) inserted adjacent to the normal or mutated histone genes (Figure S2). Strains labeled "H2A-V101I" express this mutant protein from both *HTA1* and *HTA2* loci. Note that the severity of the Ts$^{-}$ and hydroxyurea sensitivity phenotypes caused by *spt16-11* here is lower than in Figure 1 because the initial screen (Figure 1) was performed in the W303 genetic background where FACT gene mutations routinely cause stronger defects, whereas the integrants shown here were constructed in the A364a genetic background.

case, these results show that the stability of the dimer: tetramer interface is an important element of the core reaction promoted by FACT *in vivo*.

### *FACT(Spt16-11) has a nucleosome-binding defect in vitro*

We used purified nucleosomes mixed with normal and mutant FACT complexes to examine the nature of the suppression observed *in vivo*. We first measured the apparent affinity of FACT for nucleosomes using an electrophoretic mobility shift assay (EMSA) (Rhoades *et al.* 2004). The DNA-binding protein Nhp6 is required for complex formation in this assay (Formosa *et al.* 2001; Xin *et al.* 2009), with 50% saturation occurring at ~460 nM Nhp6 (Ruone *et al.* 2003). This same value was observed for both Spt16-Pob3 and (Spt16-11)-Pob3 with wild-type and mutant nucleosomes (not shown), so Spt16-11 and Spt16 proteins each require about the same amount of Nhp6 to support stable complex formation. In contrast, Spt16-11 was three- to five-fold less effective than Spt16 in this assay (Figure 6A). This could mean that Spt16-11 has a lower affinity for nucleosomes, that it is less effective in performing reorganization, or that it is unable to maintain the reorganized state stably enough for detection by EMSA. Nucleosomes with H2A-V101I or H2B-A84D were the same as wild-type H2A in this assay (Figure S5), so while this reveals a defect in Spt16-11 activity, it does not reveal the mechanism of genetic suppression.

### *spt16-11 and histone mutants have opposing effects on reorganization rates*

Reorganized nucleosomes are more prone to losing one or both H2A-H2B dimers *in vitro* (Belotserkovskaya *et al.* 2003; Xin *et al.* 2009). Nuclease sensitivity can be detected in complete octameric nucleosomes, so dimer loss is not a necessary feature of reorganization, but reorganization can lead

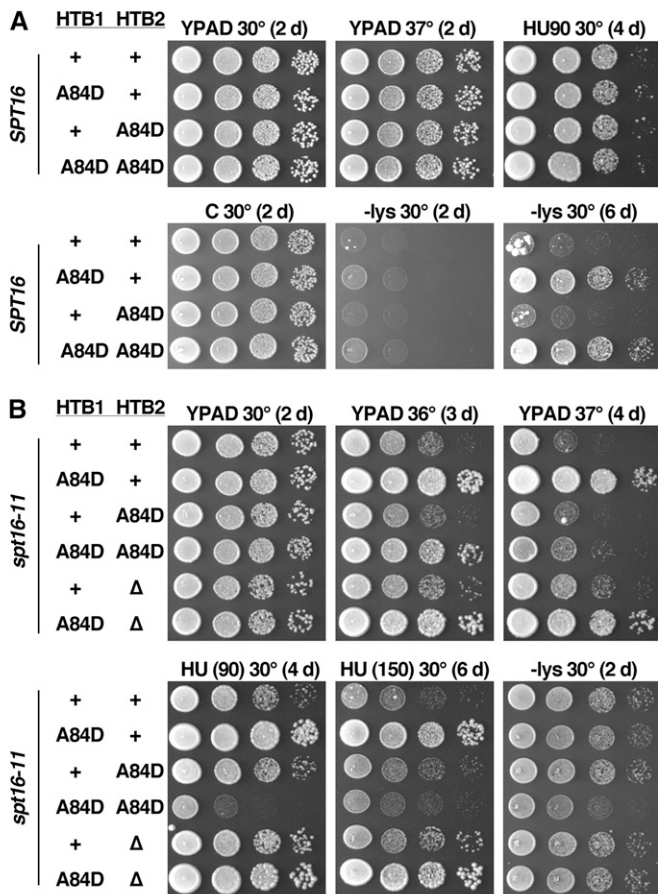


**Figure 4** The source of expression of H2B-A84D affects the resulting phenotypes. Isogenic strains from the A364a genetic background with the *spt16-11* mutation (Table 1) were tested as in Figure 1, with the concentration of HU indicated. Panel A shows strains with the WT *SPT16* allele and B shows strains with the *spt16-11* mutation. Incubation times are listed in days to allow comparison of the level of growth at comparable times. In particular, *htb1-A84D* causes a weak Spt$^-$ phenotype, as very little growth on medium lacking lysine is observed after 2 days but substantial growth is visible after 6 days. This is weak relative to the effect of *spt16-11*, which causes substantial growth on medium lacking lysine after 2 days. Both *HTB1* and *HTB2* are tagged in all cases, whether wild-type (+) or mutant (A84D). "Δ" indicates deletion of both *HTA2* and *HTB2*.

to dimer loss so it serves as an assay for the ability of FACT to promote this structural change.

We measured the total dimer displacement after a 10-min incubation. As expected, FACT caused more dimer displacement than Nhp6 alone, but the levels for Spt16 and Spt16-11 were comparable (Figure 6B). Histone mutants displayed increased dimer loss in the presence of Nhp6 alone, consistent with the results described above showing inherent instability of these nucleosomes. Nucleosomes with wild-type histones or with the H2A-V101I protein displayed slightly lower dimer displacement with Spt16-11 than with Spt16, but nucleosomes with H2B-A84D had a similarly small but opposite effect. Spt16-11 therefore appears to have a small effect on dimer loss after a 10-min incubation.

We next examined the rate of dimer displacement. Instead of the 181-bp 5S rDNA nucleosomes containing recombinant yeast histones used above, we used a 255-bp MMTV (Mouse Mammary Tumor Virus) sequence (Flaus *et al.* 2004), recombinant *Xenopus laevis* histones, and low $Mg^{2+}$ ion concentrations. These conditions increase the total dimer loss caused by FACT (Xin *et al.* 2009) and increase the electrophoretic separation of octameric and tetrameric nucleosomes. These nucleosomes migrate as one major band in native gels (Flaus *et al.* 2004) (Figure 6C, "Nuc") along with several minor translational variants, while reconstructions show that tetrasomes migrate to four main bands (Figure 6C, "Tet"). FACT does not appear to promote translocation of nucleosomes (Rhoades *et al.* 2004), so we infer that the multiple forms observed with this larger MMTV fragment are corresponding pairs of octasomal and tetrasomal species occupying the same preferred translational positions.

Samples taken during a 5-min incubation show that FACT promotes tetrasome formation more rapidly with mutant histones than with wild-type histones (Figure 6, C–E). Conversely, FACT with Spt16-11 caused displacement more slowly than FACT with wild-type Spt16 protein (Figure 6E, Figure S6). Combining histone mutants with the FACT mutant resulted in a slight suppression of the Spt16-11 defect (Figure 6E, Figure S6), but the amount of compensation was minimal.

Together, these results show that FACT containing Spt16-11 protein has significant defects *in vitro* in forming stable complexes with nucleosomes and in promoting the normal rapid rate of dimer loss. Mutant histones identified as suppressors of the *spt16-11* allele are lost from nucleosomes at an abnormally high rate, but do not strongly reverse the Spt16-11 defects in these assays.

### *Nuclease sensitivity reveals a potential mechanism of suppression*

We have proposed that FACT promotes equilibration of nucleosomes between canonical and reorganized forms, with the rate of digestion by nucleases being proportional to the fraction of time that the nucleosomes spend in the reorganized state (Xin *et al.* 2009). We therefore used restriction endonuclease sensitivity to examine whether Spt16-11 and histone mutants alter the persistence of the reorganized state.

We measured the rates of *Dra*I digestion of 181-bp 5S rDNA nucleosomes with yeast histones to probe two distinct physical contexts [*Dra*I-78, near the dyad of symmetry, and *Dra*I-140, near an entry/exit point, as described in Xin *et al.* (2009)]. Both FACT and FACT(Spt16-11) enhanced the rate of digestion near the dyad significantly, although FACT (Spt16-11) consistently produced less of an effect (Figure 6F). The histone mutants had the opposite effect of increasing the rate of *Dra*I digestion. The same overall pattern was observed near the entry/exit points, although the rates of digestion were generally higher (Xin *et al.* 2009) and the

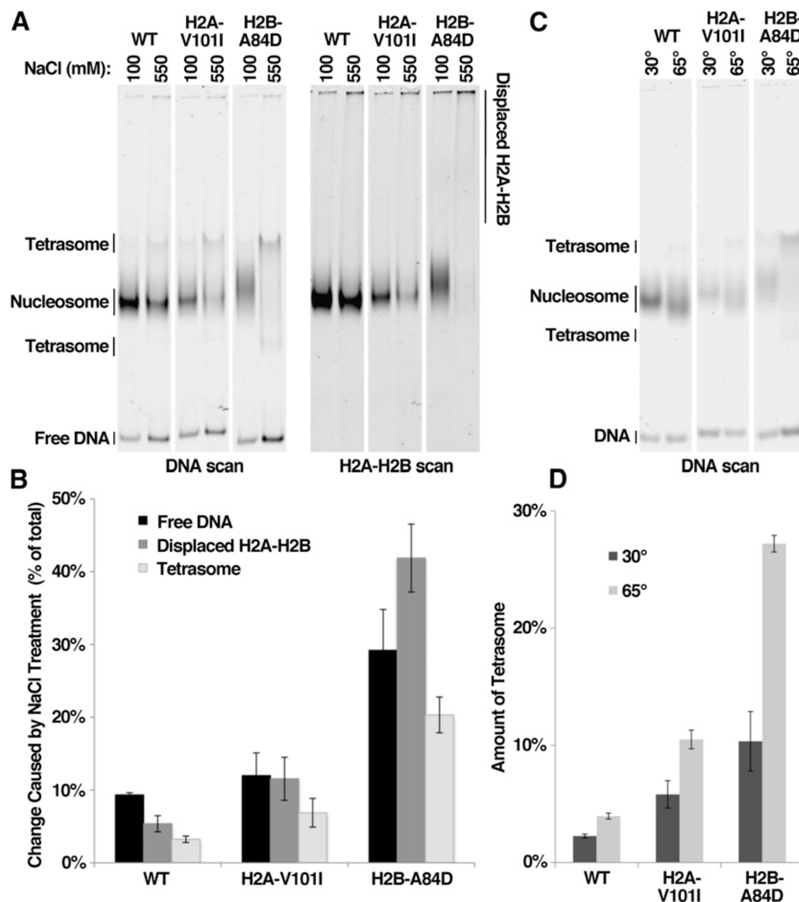


**Figure 5** Histone gene mutations destabilize nucleosomes *in vitro*. (A) Nucleosomes were constructed using recombinant yeast histones (normal or the mutant indicated) and a 181-bp 5S rDNA fragment with Cy5 at the 5′ end and Oregon Green 488 (Molecular Probes) attached to H2A residue C114 (originally Q114) (Xin *et al.* 2009). Samples were prepared in triplicate and incubated for 1 hr at 30° in 100 or 550 mM NaCl and then separated by electrophoresis through a native 4% polyacrylamide gel in 0.25× TBE as described (Xin *et al.* 2009). The single gel was then scanned to detect the fluorescent dyes on the DNA and the H2A independently using a Typhoon scanner (GE). One of the three repeats for each condition is shown. (B) Each lane was scanned and the amount of signal corresponding to free DNA, displaced H2A-H2B, or tetrasome forms was determined as a percentage of the total signal in the lane. The regions assigned as free DNA and tetrasomes are shown in the DNA scan in A, as determined using pure reference samples (tetrasomes migrate as two main bands using this combination of DNA and histones). The amount of displaced H2A-H2B was determined by calculating the signal in the top 40% of the H2A-H2B scan in A, as described previously (Xin *et al.* 2009). The change in the level of each form caused by treatment with high salt is plotted, with error bars indicating the standard deviation among the three samples. (C and D) As in A and B, except samples were incubated at 30° or 65° for 1 hr and only the total amount of tetrasomes detected is displayed.

defect of FACT(Spt16-11) was less significant. These results show that FACT(Spt16-11) can reorganize nucleosomes, but it either produces a structure with less accessibility to the DNA or fails to achieve or maintain the reorganized state for the normal length of time.

Notably, the combination of FACT(Spt16-11) with H2B-A84D nucleosomes produced a rate of digestion that is as high or higher than the rate with wild-type FACT combined with wild-type nucleosomes (Figure 6F). This also appears to be true for FACT(Spt16-11) with H2A-V101I nucleosomes at the entry/exit point, but not near the dyad. These two histones behave differently *in vivo* and may use overlapping but distinct mechanisms of suppression. These results suggest that FACT with Spt16-11 protein fails to achieve or maintain the open reorganized nucleosome form long enough to allow the normal rate of restriction endonuclease digestion, but this defect is counterbalanced by histone mutants that achieve this state more rapidly or maintain it for a longer amount of time than usual.

## Discussion

We have shown that histone gene mutations can compensate for defects in FACT activity, with the *spt16-11* mutation being suppressed by H2A-H2B mutants that destabilize the interface between these dimers and the $(H3\text{-}H4)_2$ tetramer. In tests with purified components, these histone mutants caused increased dimer displacement in the absence of FACT and more rapid or more persistent nucleosome reorganization in the presence of FACT. Genetic suppression therefore appears to result from combining a FACT complex that is inefficient at nucleosome reorganization with nucleosomes that are more rapidly or more easily reorganized. By comparing the properties of the same FACT and histone gene mutations *in vivo* and *in vitro*, these results provide insight into the mechanism of FACT activity in its physiological settings. Initial reports indicated that FACT removes one H2A-H2B dimer from a nucleosome (Belotserkovskaya *et al.* 2003), and our experiments showed that dimer displacement can be an outcome of FACT action but is not a necessary feature of the mechanism of reorganization (Xin *et al.* 2009). Both sets of *in vitro* investigations indicate that FACT activity involves disruption of the H2A-H2B:$(H3\text{-}H4)_2$ interface, and the results presented here confirm that this is an important feature of FACT function *in vivo*.

FACT activity has been implicated in both DNA replication and transcription (Formosa 2008). FACT's ability to promote equilibration between stable and dynamic forms of histone:DNA complexes could reduce the barrier to polymerase progression posed by existing nucleosomes, but it could also promote establishment of chromatin through the reverse reaction by converting loosely associated components into

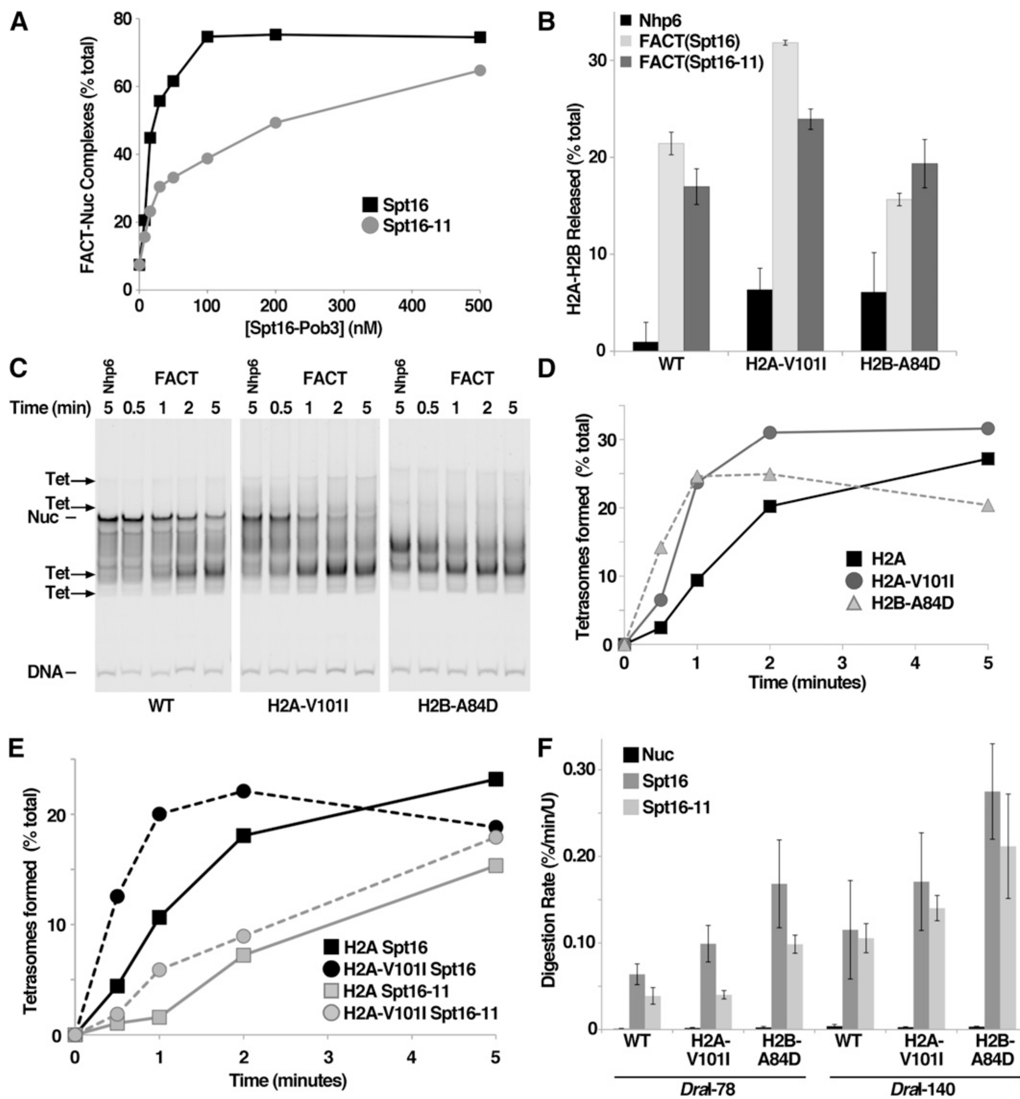


**Figure 6** Spt16-11 protein and its suppressors alter the rates of reactions on the basis of reorganization *in vitro*. (A) Nucleosomes constructed as in Figure 5 were mixed with different concentrations of Spt16-Pob3 heterodimers containing normal Spt16 or Spt16-11 protein, along with 10 μM Nhp6. Samples were incubated for 10 min at 30° and then separated by native PAGE, and the fraction of the total DNA signal in FACT–nucleosome complexes was determined as described previously (Xin *et al.* 2009). The nucleosomes shown contained the H2B-A84D mutation and are typical of results obtained with wild type and H2A-V101I. Multiple preparations of wild-type and mutant FACT were tested in independent experiments; values for half saturation varied somewhat between experiments, but the defect for Spt16-11 protein was reproducible. (B) Nucleosomes constructed as in Figure 5 were treated for 10 min at 30° with 5 μM Nhp6 alone, 5 μM Nhp6 and 200 nM Spt16-Pob3 ["FACT (Spt16)"], or 5 μM Nhp6 and (Spt16-11)-Pob3 ["FACT(Spt16-11)"]. Three independent samples for each condition were analyzed as in Figure 5, A and B, with the average and standard deviation of the three measurements presented. (C and D) Nucleosomes were constructed using recombinant histones from *X. laevis* (with normal histones or the mutation indicated) and a 255-bp MMTV DNA fragment (Flaus *et al.* 2004) labeled with Cy5. Samples were treated with FACT for the amount of time indicated, excess unlabeled genomic DNA was added to disrupt the FACT–nucleosome complexes, and then products were separated by native PAGE (Rhoades *et al.* 2004). Conversion from initial forms ("Nuc") to the tetrasomal products (indicated by arrows) was quantitated and plotted in D. (E) As in D, except FACT(Spt16) or FACT(Spt16-11) were mixed with nucleosomes containing H2A or H2A-V101 as indicated. (F) Nucleosomes were constructed using wild-type or mutant yeast histones and 181-bp 5S rDNA fragments with *Dra*I recognition sites 78 or 140 bp from the left edge of the nucleosome (Xin *et al.* 2009). The initial rate of digestion by *Dra*I in the absence of other factors or with wild-type or mutant FACT was determined by examining samples taken at 8-min intervals with denaturing PAGE (Xin *et al.* 2009). Each condition was tested in three independent experiments with the average and standard deviation shown.

mature nucleosomes. Consistent with a nucleosome assembly function, FACT has been implicated in the deposition of new nucleosomes after DNA replication (Belotserkovskaya *et al.* 2003; Vandemark *et al.* 2006) as well as in the reestablishment of repressive chromatin after transcription (Jamai *et al.* 2009). Defects in this nucleosome deposition activity are likely to be the cause of cryptic promoter activation (Kaplan *et al.* 2003), failed repression of *SER3* expression (Hainer *et al.* 2010), and the Spt$^-$ phenotype (Malone *et al.* 1991; Rowley *et al.* 1991). Most FACT gene mutations (including *spt16-11*) cause phenotypes associated with chromatin quality defects, indicating that maintaining appropriately repressive chromatin throughout the genome requires optimal levels of FACT activity. The histone mutants described in this report did not suppress the Spt$^-$ phenotype caused by *spt16-11*, so by this assay they do not enhance the ability of the Spt16-11 protein to assemble normal chromatin.

Essentially all known mutations in FACT genes cause the Spt$^-$ phenotype, but only a subset of these mutations causes the HU sensitivity that is likely to reflect a defect in replication fork progression or stability. The results described here suggest that *spt16-11* causes HU sensitivity because Spt16-11 protein is unable to promote nucleosome reorganization rapidly enough to allow a normal rate of fork progression

through a normal chromatin template. The additional delay that results from decreased availability of dNTPs when RNR is inhibited by HU might be intolerable when FACT is defective. Histone mutants that promote faster nucleosome reorganization by destabilizing the histone dimer:tetramer interface then diminish the impaired progression and restore viability under HU stress, but do not resolve the defect in chromatin quality, so the Spt$^{-}$ phenotype persists.

Our tests of an H2B mutation integrated into the genome reveal a clear functional difference between *HTB1* and *HTB2*. In normal cells, each gene is transcribed at about the same level on about the same cell cycle schedule. However, the A84D mutant is effective only at suppressing the HU sensitivity (likely to be a replication defect) caused by *spt16-11* if it is expressed from *HTB1* alone. Curiously, the effects of *htb1-A84D* are similar when it is paired with normal *HTB2* or a deletion of *HTB2*. In contrast, expressing H2B-A84D from both loci begins to suppress the Spt$^{-}$ phenotype (transcription defect) caused by *spt16-11*, but enhances the HU sensitivity (probable replication defect). These differences could be due to the different primary sequences of Htb1 and Htb2 proteins, or they could indicate that the two genes have different expression profiles under some circumstances such as nucleosome replacement outside of S phase. In either case, Htb1 and Htb2 proteins have different functional roles, and this becomes particularly important in an *spt16-11* strain. It is therefore necessary to consider the expression context when examining the effects of histone gene mutations, especially when they affect H2B.

The results presented here provide support for the model that FACT activity promotes or requires destabilization of histone dimer:tetramer interaction. The analysis suggests that promoting the reversible, rapid oscillation of nucleosomes between a stable canonical form and a more open reorganized form is a key function of FACT *in vivo*, highlighting the dynamic nature of nucleosomes under physiological conditions.

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GENETICS

**Supporting Information**
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# Insight Into the Mechanism of Nucleosome Reorganization From Histone Mutants That Suppress Defects in the FACT Histone Chaperone

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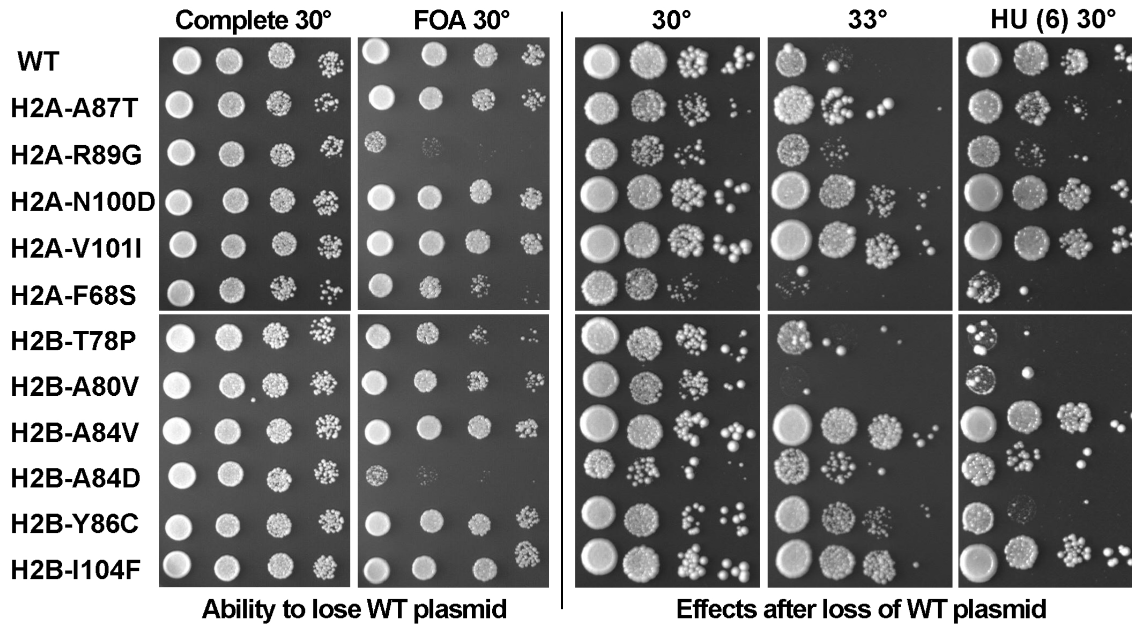


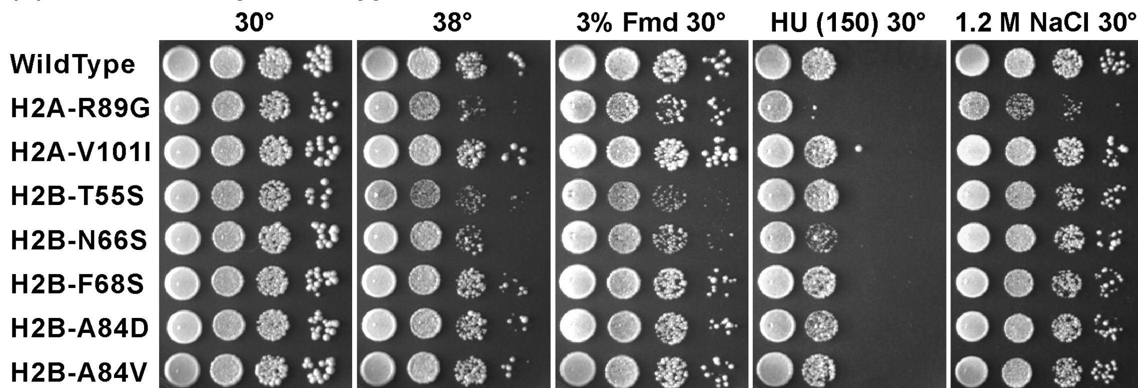


**Figure S1** Effects of histone mutations in *pob3-Q308K* and WT strains.

(A) Strain 8264-17-3 pTF237 (*pob3-Q308K* histone-Δ, YCp *URA3* WT histones; Table 1) was transformed with YCp plasmids carrying the WT *HHT2-HHF2* and the H2A or H2B mutation noted. Cultures were grown to saturation in rich medium to allow loss of pTF237 carrying WT histone genes and the *URA3* marker, then 10-fold serial dilutions were placed on complete synthetic medium or medium containing 5-FOA to determine how often cells lost the WT histone gene plasmid during growth without selection for this plasmid. Plasmid loss is assumed to be random, so a decrease in the yield of 5-FOA resistant cells indicates that the *pob3-Q308K* mutation displays a synthetic growth defect with the mutation on the incoming plasmid, as observed for H2A-R89G and H2B-A84D. FOA resistant clones were then chosen and tested for growth at 33° or on medium containing 6 mM hydroxyurea. This temperature is semi-permissive for growth of this *pob3-Q308K* strain with WT histones, and both weak suppression and weak enhancement of the Ts- phenotype was noted for different histone mutations. None of the histone mutations that suppressed the HUs caused by *spt16-11* also suppressed the HUs caused by *pob3-Q308K*, although about half enhanced this phenotype. (The effects of H2A-R89G and H2B-A84D in this assay are difficult to interpret as these strains represent rare survivors of FOA selection and therefore may include additional mutations or alterations of expression). Mutations that ameliorate a defect in Spt16 therefore generally exacerbate a defect in Pob3 when tested from a plasmid context, although H2A-V101I displayed mild suppression when integrated alleles were tested (main text, Fig 3).

(B) Strain DY9999 pTF237 (FACT WT, histone-Δ ,YCp *URA3* WT histones; Table 1) was transformed with YCp plasmids carrying various histone mutations, isolates lacking the WT plasmid were obtained by selection on FOA, and serial dilutions were tested as above. Strains were also tested for their response to exposure to camptothecin, bleomycin, 0.03% MMS, and ultraviolet light

without effect (not shown). All of the *spt16-11* suppressors were tested as described in Table 2; only a representative sample is shown here. Some effects of the histone mutations in this otherwise WT strain were noted (Table 2), but no general trend was observed that would indicate that suppression of *spt16-11* correlates with development of some other phenotype in a WT strain. Destabilization of the dimer-tetramer interface is therefore tolerated surprisingly well in the presence of WT FACT. Importantly, the histone mutations did not cause a general resistance to hydroxyurea or to growth at elevated temperatures, indicating that the suppression of these phenotypes in an *spt16-11* strain is not due to a general ability to resist these stresses.

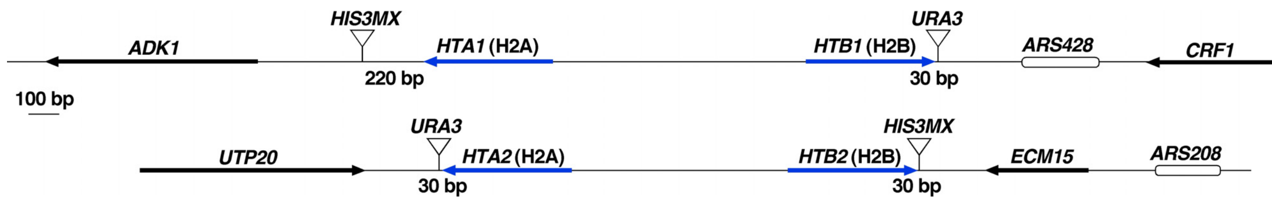


**Figure S2** Locations of selectable markers inserted downstream of histone genes. The eight histone genes are found in four sets, with each set expressing either H2A-H2B or H3-H4 gene pairs divergently. *HIS3*MX or *URA3* cassettes were integrated downstream of each of the genes expressing H2A or H2B as shown using primers described in Table S2. The distance from the marker insertion to the end of the histone gene ORF is indicated in the figure. Integration of a marker 76 bp downstream of *HTA1* caused mild defects, so one at 220 bp downstream was used instead. The locations of adjacent features for each locus are shown, drawn to the scale indicated.

```
HTA1   MSGGKGGKAGSAAKASQSRSAKAGLTFPVGRVHRLLRRGNYAQRIGSGAPVYLTAVLEYLAAEILE (65)
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
HTA2   MSGGKGGKAGSAAKASQSRSAKAGLTFPVGRVHRLLRRGNYAQRIGSGAPVYLTAVLEYLAAEILE (65)

                                                  V101I
HTA1   LAGNAARDNKKTRIIPRHLQLAIRNDDELNKLLGNVTIAQGGVLPNIHQNLLPKKSAKATKASQEL (131)
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::  ::::::
HTA2   LAGNAARDNKKTRIIPRHLQLAIRNDDELNKLLGNVTIAQGGVLPNIHQNLLPKKSAKTAKASQEL (131)


HTB1p  MSAKAEKKPASKAPAEKKPAAKKTSTSTDGKKRSKARKETYSSYIYKVLKQTHPDTGISQKSMSIL (65)
       ::  ::::::::::::::::::::::: ::::::: ::::::::::::::::::::::::::::::
HTB2p  MSSAAEKKPASKAPAEKKPAAKKTSTSVDGKKRSKVRKETYSSYIYKVLKQTHPDTGISQKSMSIL (65)

                          A84
HTB1p  NSFVNDIFERIATEASKLAAYNKKSTISAREIQTAVRLILPGELAKHAVSEGTRAVTKYSSSTQA (130)
       :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
HTB2p  NSFVNDIFERIATEASKLAAYNKKSTISAREIQTAVRLILPGELAKHAVSEGTRAVTKYSSSTQA (130)
```

**Figure S3** Protein sequence variation between the two copies of the genes expressing H2A and H2B. The sequences of the proteins expressed by *HTA1* and *HTA2* are compared in the top panel, and those expressed by *HTB1* and *HTB2* are compared in the lower panel. Sites of variation are shown in red, with the sites of the V101I and A84D mutants highlighted in yellow. Traditional numbering of histones excluding the initiating methionine is used here. Integration of the V101I mutation into the genome used primers TF0751 and TF0752 for *HTA1* and TF0751 and TF0738 for *HTA2* (Table S2). The resulting integrants at *HTA1* therefore have the same sequence as *HTA1* except for the mutation leading to the V101I change, and the integrants at *HTA2* have the same sequence as *HTA2* except for the mutation leading to the V101I change and 3 silent mutations in the nearby DNA sequence. Thus, *hta1-V101I* has a single mutation leading to a single amino acid change, but *hta2-V101* has four mutations leading to a single amino acid change, as confirmed by sequencing each gene after integration. The mutagenic primers are upstream of the variation (protein sequence AT or TA) between the two genes, so this variation is not affected by the strategy used for the mutagenesis. The A84D mutations were integrated using TF0749 and TF0750 for *HTB1* and TF0747 and TF0748 for *HTB2* (Table S2). These primers are matched to each allele, and produce only a single change that is downstream of the variation between the four *HTB1* and *HTB2* protein sequence differences. Mutagenesis therefore alters only the single target residue, leaving the variation between the two copies of the genes encoding H2B intact.

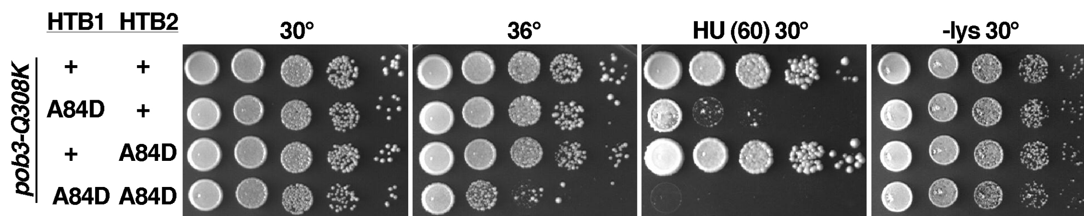


**Figure S4** A *pob3-Q308K* strain is primarily affected by integrated *htb1-A84D* and not by *htb2-A84D*. Isogenic strains with the *pob3-Q308K* mutation and the WT or A84D mutation in *HTB1* or *HTB2* were grown to saturation, then aliquots of 10-fold serial dilutions were tested for growth under the conditions indicated. Rich media without or with 60 mM hydroxyurea (30°, 36°, HU (60) 30°) or synthetic medium lacking lysine (to assay the Spt$^-$ phenotype caused by *pob3-Q308K*) were used. The *htb1-A84D HTB2* combination enhances the HU sensitivity caused by *pob3-Q308K* (as observed in the plasmid-based test, Table 2 and Fig S1), but the *HTB1 htb2-A84D* combination does not. Thus, expression of the A84D mutation from *HTB1* is more detrimental to a *pob3-Q308K* strain than expression from *HTB2* is, just as it was more beneficial to an *spt16-11* strain (main text, Figure 4). H2B-A84D expression from *HTB2* remains detrimental, as mutating both genes results in additive effects on both temperature sensitivity and HU sensitivity. However, H2B-A84D does not cause a global enhancement of the defects caused by *pob3-Q308K*, as the Spt$^-$ phenotype is unaffected.

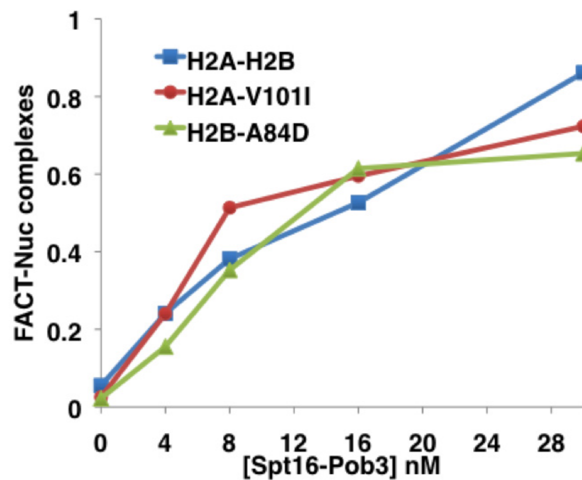


**Figure S5** Histone mutations don't significantly affect the affinity of FACT for nucleosomes. Nucleosomes were constructed with a fluorescently labeled 181 bp DNA fragment containing the 5S rDNA sequence and yeast histones (XIN *et al.* 2009) with either the WT H2A-H2B sequences or the mutation shown. These were incubated with varying concentrations of Spt16-Pob3 with 5 μM Nhp6, incubated 10 minutes at 30°, then separated by native polyacrylamide gel electrophoresis as described (RHOADES *et al.* 2004;RUONE *et al.* 2003). The fraction of DNA signal in the region of the gel associated with complexes was determined and plotted for the concentrations near the half-maximal binding region. Some variation was observed, but no repeatable pattern was detected in multiple repeats of similar experiments, indicating that FACT binds to nucleosomes with these histone mutations with the same apparent affinity as nucleosomes with WT histones.

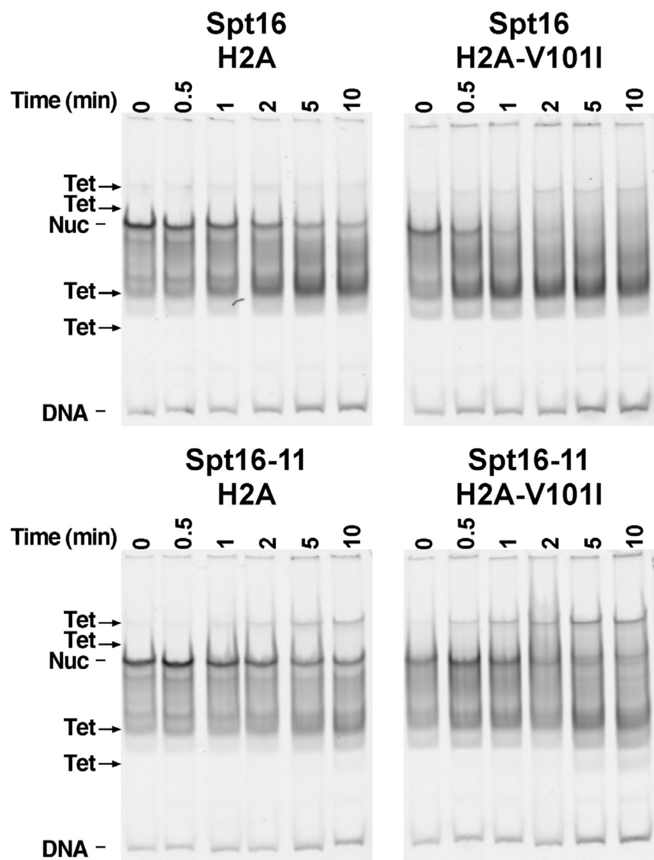


**Figure S6** The rates of dimer displacement using nucleosomes with the H2A-V101I mutant and FACT with the Spt16-11 mutant. The gels used for the experiment shown in Figure 6E of the main text are shown. The experiment is described in the legend for Figure 6E.

**Table S1  Strains used in Figure S4 (A364a background)**

| Fig S4 | |
|---|---|
| 8483-1-4 | *MATa ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ HTB1(30, URA3) HTB2(30, His3MX) pob3-Q308K(LEU2)* |
| 8869-2-4 | *MATa ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ htb1-A84D(30, URA3) HTB2(30, His3MX) pob3-Q308K(LEU2)* |
| 8868-4-2 | *MATa ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ HTB1(30, URA3) htb2-A84D(30, His3MX) pob3-Q308K(LEU2)* |
| 8867-6-4 | *MATa ura3-Δ0 leu2-Δ0 trp1-Δ2 his3 lys2-128∂ htb1-A84D(30, URA3) htb2-A84D(30, His3MX) pob3-Q308K(LEU2)* |

**Table S2 Oligonucleotides Used**

| Name | Purpose | Oligonucleotide sequence (5' to 3') |
|---|---|---|
| TF04-25 | 1 | TGATTCTGTGGATAACCGTA |
| TF05-28 | 1 | GTCGAGGTGCCGTAAAGCACT |
| TF05-29 | 2 | CACGCGCTTAATGCGAAGTGC |
| TF05-30 | 2 | GCACTTCGCATTAAGCGCGTG |
| TF05-31 | 2 | TGGTTAGACGCTCAATGTCG |
| TF05-32 | 2 | GAGCACAATAGTTAACTCGT |
| TF0726 | 4 | AAGCTTCTCAAGAACTGTAAGAACTGAGTTGAAAAGAAACAAAGCAAATCAGATTGTACTGAGAGTGCAC |
| TF0727 | 4 | CAGTCTTCCTCATATGACCTACTTTAAAACCCCAAATGACAAGAATGTTTCTGTGCGGTATTTCACACCG |
| TF0728 | 5 | CTTCCTCTACTCAAGCATAATGAAATCACTTCCTTTGGTTATAATTAATAAGATTGTACTGAGAGTGCAC |
| TF0729 | 5 | ACAATTTTTTTTACTTTACTTTAATTTTTATATACCCATATAAATAATAACTGTGCGGTATTTCACACCG |
| TF0730 | 6 | TCCTCCTCTACTCAAGCCTAAGTCACTCACTAGGTATTGTGATTTAGTCACAGCTGAAGCTTCGTACGCTGCAGGTCG |
| TF0731 | 6 | GAACCCTAATGTTACAATATATAAAAAATGCCACTAATAAAAAGAAAACAGAACGACCGAGCGCAGCGAGTCAGTGAG |
| TF0738 | 8 | CAAAAGAAAGAGAGCCTAGCTG |
| TF0747 | 10 | GAAAGAATTGCTACTGAAGCTTCTAAATTGGaCGCTTATAACAAGAAATCCA |
| TF0748 | 10 | CTCAATGAATGCTCGTGTAGTGAACC |
| TF0749 | 9 | GAAAGAATCGCTACTGAAGCTTCTAAATTGGacGCGTATAACAAGAAGTCTAC |
| TF0750 | 9 | CACACGAGCCAATGCTCTGAAGTTTCG |
| TF0751 | 7, 8 | GATGACGAATTGAACAAGCTATTGGGTAACaTTACCATTGCCCAAGGTGGTG |
| TF0752 | 7 | ATGGGCGAATAAGGACAGAAGCCGC |
| TF0791 | 3 | TGTGTCTTCCTGTTTTCTCTGTTGCTCTGTTTAGGTTCATTGGGCACTGTCAGCTGAAGCTTCGTACGCTGCAGGTCG |
| TF0792 | 3 | GTGAACAAAGCACAGAATGTGTTTGCTACTCAATCATGTTCAAGTAAGCAGAACGACCGAGCGCAGCGAGTCAGTGAG |

1 Amplify histone genes for PCR-based mutagenesis (Fig 1)
2 Sequencing primers for histone genes
3 Tag *HTA1* by inserting a marker 220 bp downstream of the ORF
4 Tag *HTA2* by inserting a marker 30 bp downstream of the ORF
5 Tag *HTB1* by inserting a marker 30 bp downstream of the ORF
6 Tag *HTB2* by inserting a marker 30 bp downstream of the ORF
7 Integrate *hta1-V101I* into the genome
8 Integrate *hta2-V101I* into the genome
9 Integrate *htb1-A84D* into the genome
10 Integrate *htb2-A84D* into the genome

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