RESEARCH COMMUNICATION

SAS-mediated acetylation of histone H4 Lys 16 is required for H2A.Z incorporation at subtelomeric regions in *Saccharomyces cerevisiae*

Wei-Jong Shia,^{1,2} Bing Li,¹ and Jerry L. Workman^{1,3}

¹Stowers Institute for Medical Research, Kansas City, Missouri 64110, USA; ²Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, USA

The yeast SAS (Something About Silencing) complex and the histone variant H2A.Z have both previously been linked to an antisilencing function at the subtelomeric regions. SAS is an H4 Lys 16-specific histone acetyltransferase complex. Here we demonstrate that the H4 Lys 16 acetylation by SAS is required for efficient H2A.Z incorporation near telomeres. The presence of H4 Lys 16 acetylation and H2A.Z synergistically prevent the ectopic propagation of heterochromatin. Overall, our data suggest a novel antisilencing mechanism near telomeres.

Supplemental material is available at <http://www.genesdev.org>.

Received April 11, 2006; revised version accepted July 27, 2006.

The eukaryotic genome is packaged with histones to form higher order chromatin structure. This compact state of chromatin renders the DNA poorly accessible for many essential cellular processes including transcription, DNA recombination, repair, and replication (Workman and Kingston 1998). To overcome the naturally repressive nucleosome impediment, cells have devised three mechanisms to alter chromatin architecture: post-translational histone modifications, ATP-dependent chromatin remodeling, and histone variant incorporation (Berger 2002; Henikoff et al. 2004; Cairns 2005).

The formation of highly condensed heterochromatin results in transcription repression. In budding yeast, *Saccharomyces cerevisiae*, this type of transcriptional silencing occurs at several genomic locations including the *HML* and *HMR* mating-type loci, telomeres, and rDNA in a gene-independent, position-dependent manner (Rusche et al. 2003). Among the numerous silencing regulatory factors, Sir2 plays a crucial role at all silent loci. It is generally believed that the NAD⁺-dependent histone deacetylase activity of Sir2 is the driving force behind the formation of silenced heterochromatin (Blander and Guarente 2004). Yet, how the spreading of

this heterochromatin is blocked remains poorly understood.

Several hypotheses have been proposed to explain how the propagation of heterochromatin is halted. One model suggests that specific DNA elements function as silencing barriers either by interacting with perinuclear substrates to separate chromosomal regions, or by recruiting histone modification machinery to alter the underlying chromatin structure (Oki et al. 2004; West et al. 2004). At yeast telomeres, there is no clear evidence of such barrier elements; competition between histone acetylation and deacetylation forms the euchromatin–heterochromatin boundary. One very important histone acetyltransferase, the SAS (Something About Silencing) complex, plays a crucial role in antisilencing in this subtelomeric region (Kimura et al. 2002; Suka et al. 2002).

Sas2 is the catalytic subunit of the yeast histone acetyltransferase SAS complex. The yeast SAS complex is a small trimeric protein complex consisting of Sas2, Sas4, and Sas5 (Osada et al. 2001; Sutton et al. 2003; Shia et al. 2005). Genetic studies suggest that all of the SAS subunits are essential for maintaining the proper silencing at all loci (Reifsnyder et al. 1996; Ehrenhofer-Murray et al. 1997; Xu et al. 1999; Meijnsing and Ehrenhofer-Murray 2001). The highly conserved MYST domain in the Sas2 catalytic subunit is absolutely required for both the silencing function and enzymatic activity of the SAS complex (Osada et al. 2001; Shia et al. 2005). The function of the Sas4 and Sas5 subunits has yet to be determined. SAS is a histone H4 Lys 16-specific acetyltransferase complex (Sutton et al. 2003; Shia et al. 2005). Mutation at Lys 16 of histone H4 phenocopies the silencing defects of *sas2Δ* (Meijnsing and Ehrenhofer-Murray 2001), suggesting a functional link between the histone acetyltransferase (HAT) activity of SAS and its role in regulating transcription silencing. Notably, the unique lysine preference of SAS has been shown to antagonize the deacetylation function of Sir2 at telomeres, thereby preventing Sir proteins from spreading into subtelomeric regions. This dynamic balance between Sas2 and Sir2 is responsible for the establishment of euchromatin–heterochromatin boundaries at telomeres.

In addition to its global antisilencing effects at telomeres, SAS also plays a role at the *HMR* silent locus. The SAS complex is thought to be targeted to a unique tRNA^{Thr} gene close to the *HMR* I silencer, and may help establish a heterochromatic barrier, presumably through acetylating histone H4 Lys 16 (Oki et al. 2004). Together, these observations suggest that the SAS complex plays an important role in blocking the propagation of heterochromatin.

Histone H2A.Z is one of the evolutionarily conserved histone variants found from yeast to human. Unlike the canonical histone H2A, which is expressed and assembled exclusively in the S phase, the variant H2A.Z is constitutively expressed and deposited into chromatin throughout the cell cycle (Kamakaka and Biggins 2005). The H2A.Z-containing nucleosomes are only slightly different from H2A nucleosomes in surface charge and structure (Suto et al. 2000). There is considerable evidence, however, to suggest that incorporation of H2A.Z into nucleosomes has significant effects on cell cycle

[Keywords: SAS; H2A.Z; H4 Lys 16 acetylation; antisilencing]

³Corresponding author.

E-MAIL jlw@stowers-institute.org; FAX (816) 926-4692.

Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.1439206>.

regulation, chromosome segregation, DNA repair, genomic stability, heterochromatin barrier formation, and transcription (both activation and repression) (Kamakaka and Biggins 2005; Dhillon et al. 2006). H2A.Z is deposited by the SWR1 complex, which specifically exchanges H2A/H2B dimers with variant H2A.Z/H2B dimers in an ATP-dependent manner (Mizuguchi et al. 2004). Studies from different groups have demonstrated a role for several SWR1 subunits, including Bdf1, Swr1, and Yaf9, in antisilencing at telomeres (Ladurner et al. 2003; Kobor et al. 2004; Mizuguchi et al. 2004; Zhang et al. 2004).

Interestingly, transcription activation of many genes near telomeres is dependent on deposition of H2A.Z, possibly due to the ability of this histone variant to limit the ectopic spreading of Sir proteins into the nearby euchromatin (Meneghini et al. 2003). In the absence of either the SAS complex or histone variant H2A.Z (encoded by *HTZ1*), Sir proteins are capable of spreading further into neighboring euchromatic regions both at *HMR* and telomeres (Kimura et al. 2002; Suka et al. 2002; Meneghini et al. 2003; Kobor et al. 2004; Oki et al. 2004). This finding suggests a possible role for both Sas2 and H2A.Z in an antisilencing mechanism.

Here we present evidence demonstrating that SAS-mediated acetylation at histone H4 Lys 16 is required for H2A.Z incorporation at telomeres. *SAS2* and *HTZ1* regulate transcriptional activation of a similar set of genes near telomeres. In the absence of SAS, H4 Lys 16 acetylation and H2A.Z occupancy are reduced specifically at the subtelomeric genome in a SAS-dependent manner. Consistent with these observations, a point mutation at histone H4 Lys 16 results in the loss of histone variant H2A.Z. Moreover, when recruited to a genomic locus where H4 AcK16 and H2A.Z are not detectable, the SAS complex can boost the acetylation at H4 Lys 16, which subsequently leads to enrichment of H2A.Z. Overall, our findings suggest a novel, SAS-dependent, signaling pathway for H2A.Z incorporation. The coordinate presence of H4 Lys 16 acetylation and H2A.Z establishes a mechanism for antisilencing at telomeres.

Results and Discussion

SAS2 and HTZ1 synergistically regulate transcription of telomere-proximal genes

The yeast SAS complex has long been implicated in the maintenance of transcription silencing in yeast. At telomeres, SAS counteracts the deacetylation function of Sir2, and consequently blocks the spread of silenced heterochromatin (Kimura et al. 2002; Suka et al. 2002). Interestingly, the histone H2A variant, H2A.Z, exerts a similar antisilencing function through SWR1-mediated deposition (Meneghini et al. 2003; Kobor et al. 2004; Mizuguchi et al. 2004). We sought to investigate a possible interplay between histone acetylation and variant incorporation in the regulation of silencing at telomeres.

It has been shown that many genes near telomeres are dependent on SAS or H2A.Z for transcription activation (Kimura et al. 2002; Meneghini et al. 2003). In order to determine whether SAS and H2A.Z regulate the same set of telomere-proximal genes, we performed microarray analysis in *sas2Δ*, *htz1Δ*, and *sas2Δhtz1Δ* yeast strains. Our results indicated that very few genes were activated in the absence of *SAS2* and/or *HTZ1*. We did observe, however, that genes requiring *SAS2* or *HTZ1* for activa-

tion clustered at subtelomeric regions. Interestingly, the telomere bias for transcription regulation was exceedingly obvious in *sas2Δhtz1Δ* double-knockout yeast, especially for genes residing within 20 kb from the chromosome ends (Fig. 1A). In fact, >30% of genes within this range were highly repressed when both *SAS2* and *HTZ1* were deleted. Upon comparison of the transcription profiles of telomere-proximal genes in all three strains, we noted that transcription was similarly regulated by *SAS2* and *HTZ1*. A slight repression of telomere-proximal genes was noted when either *SAS2* or *HTZ1* was deleted, but these same genes became highly repressed in the *sas2Δhtz1Δ* double-deletion mutant strain (e.g., see Fig. 1B). We concluded that *SAS2* and *HTZ1* synergistically regulated transcription activation of a subset of genes near telomeres.

The greater-than-additive repression on telomere-proximal genes observed in *sas2Δhtz1Δ* double-knockout yeast suggests the possibility that the presence of both SAS-mediated acetylation and H2A.Z in the subtelomeric region is necessary to prevent heterochromatin spreading. A recently published study indicated that formation of telomeric heterochromatin boundaries required acetylated H2A.Z (Babiarz et al. 2006). Since nucleosomes containing H2A.Z are structurally similar to canonical nucleosomes (Suto et al. 2000), the physical presence of unmodified H2A.Z might not be sufficient to prevent heterochromatin spreading. Instead, H2A.Z that has been modified by acetylation appears to play a crucial role in telomeric boundary formation. In fact, the N-terminal of H2A.Z shows significant similarity to the N-terminal tail of histone H4 (Schaper et al. 2005). It has been shown that, after its deposition into chromatin,

Figure 1. Microarray analysis using *htz1Δ* and/or *sas2Δ* yeast strains. (A) Histogram showing the number of H2A.Z and/or Sas2-activated genes (>1.5-fold) plotted as a function of their distance to the nearest telomere. Genes were categorized at 5-kb intervals for up to 50 kb from telomeres. The inset table shows the percentage of genes that require H2A.Z and/or Sas2 for activation in either genome-wide scale 50-kb or 20-kb region from telomeres. Strains used were YJW100, YJW253, YJW491, and YJW493. (B) Expression profiles for telomere-proximal genes (50 kb from both chromosome ends) at chromosome XII. These results are representative of the whole genome.

H2A.Z is acetylated by NuA4 at Lys 14 (Babiarz et al. 2006; Keogh et al. 2006; Millar et al. 2006), which is the consensus residue to Lys 16 of histone H4. This fact raises the possibility that Sir proteins might need to overcome the acetylation of both H4 Lys 16 and H2A.Z Lys 14 in order to propagate toward euchromatin.

Acetylation of H4 Lys 16 at telomeres is dramatically reduced in the absence of SAS

SAS is a histone H4 Lys 16-specific acetyltransferase complex; however, it is not the only HAT that is capable of acetylating H4 Lys 16. In order to determine where in the genome SAS specifically acetylated this residue, we took advantage of the genome-wide chromatin immunoprecipitation (ChIP)-chip technique using an antibody against acetylated Lys 16 of H4. In wild-type yeast, we did not observe any obvious pattern of H4 AcK16 distribution. In *sas2Δ* cells, however, the acetylation of H4 Lys 16 was specifically lowered near telomeres (data not shown). We then merged our data sets from both cell types to determine the genomic loci where the H4 Lys 16 acetylation was reduced in the mutant (Fig. 2A). We found that the SAS-mediated acetylation was extremely specific to the regions neighboring telomeres. Among the genomic loci where we observed over twofold reduction in H4 Lys 16 acetylation, more than two-thirds were located within 20 kb from chromosome ends (Fig. 2B). It should be noted that the loss of acetylation was not limited to either open reading frames or intergenic regions. This restricted and limited acetylation pattern of SAS coincides with its function in transcription. In fact, previous studies reported that mutation at H4 Lys 16 specifically results in the repression of telomere-proximal

genes (Dion et al. 2005). Given the fact that Sas2 and H2A.Z also show a synergistic effect on transcription activation near telomeres, these findings suggest possible cooperation between SAS and H2A.Z in antisilencing function at telomeres.

Acetylation of H4 Lys 16 at telomeres is required for H2A.Z incorporation

We next investigated whether there was a relationship between SAS-mediated H4 Lys 16 acetylation and SWR1-mediated H2A.Z incorporation in telomeric anti-silencing. In vitro HAT assays demonstrated that the SAS complex showed similar HAT activity on wild-type and H2A.Z-containing nucleosome substrates (Supplementary Fig. 1). We also carried out ChIP experiments to examine whether H2A.Z incorporation influenced the in vivo H4 Lys 16 acetylation near a telomere (Fig. 3A). The results showed that the H4 Lys 16 acetylation was unaffected by *HTZ1* deletion (Fig. 3B). Collectively, these results suggest that H2A.Z incorporation is not the upstream signal for SAS acetylation.

It has been proposed that histone acetylation targets the Bdf1 subunit of SWR1 to chromatin, leading to H2A.Z deposition (Zhang et al. 2005). Perhaps H4 Lys 16 acetylation by SAS directly affects the presence of H2A.Z at the subtelomeric regions. ChIP assays on the right telomere of chromosome VI were used to monitor TAP-tagged H2A.Z occupancy in vivo. In wild-type cells, the amount of H2A.Z gradually increased from the telomere end toward the euchromatin (Fig. 3C), which correlated with the pattern of H4 Lys 16 acetylation (Fig. 3B). Following deletion of *SWR1*, H2A.Z was no longer detected, due to the lack of deposition machinery. Interestingly, we observed a dramatic reduction in the amount of H2A.Z in *sas2Δ* cells (Fig. 3C). This loss of H2A.Z was restored by expression of a plasmid containing wild-type Sas2, but not the HAT-deficient Sas2 mutant (Fig. 3D; Osada et al. 2001; Sutton et al. 2003). It has been demonstrated that the deletion of *SAS2* causes the ectopic spreading of Sir2 at the subtelomeric regions, which might interfere with the H2A.Z incorporation. To address this concern, we compared the H2A.Z incorporation in *sas2Δ* and *sir2Δsas2Δ* yeast strains (Fig. 3C). Notably, no noticeable difference in H2A.Z amount was detected in these two mutant yeasts, suggesting that the reduction of H2A.Z is the direct consequence of *SAS2* deletion. These results were reproducible when we examined the left telomere of chromosome XV (data not shown), suggesting a universal role for SAS at telomeres. We also examined the distribution of H2A.Z in H4 mutant cells using yeast strains that expressed either wild-type or mutant H4 solely from a plasmid (Fig. 3E). In these strains, the H4 K16R mutation, but not the H4 K5R mutation, caused a loss of H2A.Z. This pattern was similar to that seen in *SAS2* deletion strains, indicating that Lys 16 acetylation was specifically required for H2A.Z incorporation at telomeres. Notably, the overall amount of chromatin-bound H2A.Z remained the same in these yeast strains (Supplementary Fig. 2). This finding suggests that the relatively low H2A.Z occupancy near telomeres was not a result of a more efficient incorporation of H2A.Z across the entire genome.

We next established a direct connection between SAS-mediated acetylation and H2A.Z incorporation by integrating a UASgal1 sequence containing four Gal4-bind-

Figure 2. ChIP-chip analysis for H4 AcK16 in wild-type and *sas2Δ* yeast strains. (A) Shown is the result reflecting the genome-wide difference in the H4 AcK16 level between wild-type (YWJS001) and *sas2Δ* (YWJS002) yeast strains. Each data set was first normalized to its individual input, followed by merging to generate the final result. The data shown represents the H4 AcK16 ratio of *sas2Δ* over the wild type. Each bar represents either an ORF or intergenic locus. The loci where H4 AcK16 is decreased upon the deletion of *SAS2* are in green color; in contrast, red loci have more H4 AcK16 in the *sas2Δ* strain. (B) Diagram showing the number of ORF or intergenic loci, either in the whole genome scale or within 20 kb from telomeres, whose loss of H4 AcK16 is greater than twofold in the absence of Sas2. The dark-blue bars represent data gathered from whole yeast genome, and the light-blue bars represent data gathered specifically from loci within 20 kb from telomeres.

Shia et al.

Figure 3. SAS-mediated acetylation of H4 Lys 16 is required for H2A.Z incorporation at the telomeres. (A) Location of PCR primer sets used in the ChIP assay (shown in B–E). Each primer set is ~2.5 kb apart, interspersed along the 20-kb region from the right telomere of chromosome VI. (B) The α -H4 AcK16 antibody was used in ChIP assays to determine the level of H4 AcK16. Strains used were YWJS001, YWJS002, and YWJS046. (C,D) Rabbit IgG was used to pull down TAP-tagged H2A.Z to determine its enrichment by ChIP assays. Strains used in C included YWJS056, YWJS069, YWJS075, and YWJS139 to examine the effects upon deletion of genes indicated. Strains used in D included YWJS101, YWJS102, and YWJS103 to demonstrate the rescue by *SAS2* plasmid. (E) H2A.Z occupancy was determined by ChIP assays using the α -Myc antibody in H4 mutant yeasts. Strains used were YWJS132, YWJS133, and YWJS134. Data shown are the average of three independent experiments. Error bars represent standard deviation.

ing sites into a genomic locus, iYDR381W, where our ChIP-chip data indicated that both H4 Lys 16 acetylation and H2A.Z were barely detectable (Supplementary Fig. 3A; Li et al. 2005). By performing ChIP analysis, we confirmed the low abundance of H4 AcK16 and H2A.Z at this locus, and showed that the integration of the UASgal1 sequence did not influence the amount of H4 AcK16 and H2A.Z (Supplementary Fig. 3B). By expressing Gal4-fused Sas2 in these cells, we were able to monitor the recruitment of SAS to the Gal4-binding sites and determine whether SAS-mediated acetylation would result in the enrichment of H2A.Z. Indeed, ChIP analysis confirmed that SAS was bound to this locus. The recruited SAS was able to raise the local acetylation level at histone H4 Lys 16. More importantly, we also observed the enrichment of H2A.Z at this particular locus. In contrast, recruitment of the HAT-deficient Sas2 (SAS2 M1) failed to elevate the H2A.Z incorporation at this locus. H3 ChIP showed that overall histone amounts remained unchanged in these yeast strains. Thus, we provided direct evidence that SAS-mediated acetylation of H4 Lys 16 could facilitate H2A.Z incorporation.

We demonstrated that acetylation of H4 K16 by SAS was largely a prerequisite for H2A.Z incorporation (Figs. 3, 4). We also showed that SAS and H2A.Z synergistically regulate transcription of telomere-proximal genes (Fig. 1). Our data did, however, indicate that in the ab-

sence of *SAS2* there was marginal transcriptional repression near telomeres. This result suggested that a small subset of H2A.Z might not be deposited in a SAS-dependent manner. The residual H2A.Z in *sas2Δ* cells, as shown in Figure 3C, might partially antagonize the Sir-mediated transcriptional repression of telomere-proximal genes.

The requirement of SAS for H2A.Z incorporation appears to be specific to telomere-proximal genes since the enrichment of H2A.Z beyond the 15-kb region did not seem to be affected by either SAS or H4 Lys 16 acetylation. Our ChIP-chip analysis (Fig. 2) suggested that SAS specifically functioned near telomeres. Outside the subtelomeric region, the acetylation of H4 Lys 16 did not correlate well with H2A.Z occupancy (Li et al. 2005). Our artificial recruitment assays demonstrated that a euchromatic locus acetylated at H4 Lys 16 by SAS subsequently became enriched with H2A.Z (Fig. 4). Interestingly, we did not observe this enrichment of H2A.Z when we tethered SAS to a locus where H4 Lys 16 was already highly acetylated by a SAS-independent mechanism (Supplementary Fig. 4). Taken together, these findings suggest that SAS-dependent acetylation at histone H4 Lys 16 can facilitate H2A.Z deposition by the SWR1 complex. On the other hand, SAS-independent H4 Lys 16 acetylation alone (as seen throughout euchromatin) might not be sufficient for SWR1 targeting. Several ly-

Figure 4. Artificial recruitment of SAS results in H4 Lys 16 acetylation followed by H2A.Z enrichment. (A) ChIPs were performed in UASgal1-integrated strains expressing various GBD fusion proteins. PCR primers are specific to a genomic locus (iYDR381W) where both H4 AcK16 and H2A.Z signals are low (PCR-A in Supplementary Fig. 3B). Strains used were YWJS148, YWJS149, and YWJS161. (B) Quantification for ChIP results from A. Data shown are the average of three independent experiments. Error bars represent standard deviation.

sines in other histones have shown much higher genome-wide correlation with H2A.Z (Raisner et al. 2005; Zhang et al. 2005). Besides H4 Lys 16, the SWR1 complex may target other histones outside the subtelomeric regions since the Bdf1 subunit responsible for targeting is associated with other acetylated lysine residues (Kurdistani et al. 2004). Therefore, the enrichment of H2A.Z induced by SAS-mediated H4 K16 acetylation is restricted to telomere-proximal regions, and a distinct mechanism appears to be responsible for H2A.Z deposition throughout the rest of the genome.

The origin of the telomeric antisilencing mechanism remains to be determined. The recruitment of Sir proteins to telomeric silencers by Rap1 and Ku70 initiates heterochromatin formation. The deacetylase activity of Sir2, as well as the binding ability of Sir3 and Sir4 to hypoacetylated histones, then enables the silent chromatin to propagate (Rusche et al. 2003). We have shown that SAS and H2A.Z cooperate to form a barrier to Sir spreading. Nevertheless, it remains to be shown how SAS is specifically targeted to the subtelomeric regions. Given that the newly deposited histone H4 is not acetylated at Lys 16 (Sobel et al. 1995), it has been proposed that SAS is recruited to newly replicated DNA through the interaction with histone chaperon Asf1 or CAF-I, thus maintaining the epigenetic mark (Ehrenhofer-Murray 2004). Understanding whether this replication and chromatin assembly-coupled model for SAS recruitment applies specifically to subtelomeric regions will certainly better our knowledge of this important antisilencing mechanism.

Materials and methods

Yeast strains and plasmids

The strains used in this study are listed in Supplementary Table S1. All genomically tagged or deleted strains were generated by a one-step PCR-based integration method (Longtine et al. 1998) and confirmed by PCR or Western blots. Sequences of primers used in strain construction can be

found in Supplementary Table S2. YWJS069 was generated through a cross between YWJS038 and YWJS056. YWJS075 was generated through a cross between YWJS060 and YWJS056. Strains YWJS098–100 were generous gifts from M. Smith (Megee et al. 1990). Plasmids pS-15, pS-126, and pS-236 were published previously (Osada et al. 2001). Plasmids pS-236 and pS-237 were generous gifts from R. Kamakaka (Oki et al. 2004). To generate pS-238, a SAS2 M1 fragment with *AscI* and *NotI* restriction sites was PCR-amplified, followed by subcloning into pS-237. pS-235 (pBS–LoxP–URA3–LoxP–UASgal1) was constructed as follows: LoxP–URA3 (with *BamHI* and *EcoRI* sites) and LoxP–UASgal1 (with *EcoRI* and *HindIII* sites) were PCR-amplified from pRS406–URA3 and yeast genomic DNA, respectively, followed by subcloning into *BamHI*/*HindIII*-digested pBlueScript. Using pS-235 as a template, the LoxP–URA3–LoxP–UASgal1 fragment was then PCR-amplified and integrated to a genomic locus of our interest to generate YWJS141.

ChIP assay and DNA microarray analysis

ChIP analysis was performed as described (Li and Reese 2001). Antibodies used in ChIPs include α -Histone H3 (Abcam), α -Histone H4 AcK16 (Se-rotec), α -Sas2 (rabbit, generated against the N terminus of Sas2, amino acids 1–20), α -cMyc (clone 9E10, Roche), and rabbit IgG (Sigma). Reactions were resolved on 1.5% agarose gels, scanned by Typhoon 9400 (Amersham), and quantified by ImageQuant TL software. Fold enrichment was determined as the ratio of normalized ChIP DNA to the input DNA using *PRP8* ORF as an internal control. DNA microarray analysis was done as previously described (Li et al. 2005). For expression microarray in Figure 1, arrays used contain ~6300 spots (70-mer oligos) including all ORFs in the *S. cerevisiae* genome. For ChIP–chip experiments, arrays used contain ~14,000 spots (PCR products) including all ORFs and intergenic regions.

Acknowledgments

We thank R. Kamakaka, M. Smith, and C. Wu for the generous supply of materials, and Samantha Pattenden for critical reading of the manuscript. We also thank members of the Workman laboratory for instructive discussions and valuable suggestions. Work in our laboratory is supported by NIGMS Grant R37 GM047867 and the Stowers Institute for Medical Research.

References

- Babiarz, J.E., Halley, J.E., and Rine, J. 2006. Telomeric heterochromatin boundaries require NuA4-dependent acetylation of histone variant H2A.Z in *Saccharomyces cerevisiae*. *Genes & Dev.* **20**: 700–710.
- Berger, S.L. 2002. Histone modifications in transcriptional regulation. *Curr. Opin. Genet. Dev.* **12**: 142–148.
- Blander, G. and Guarente, L. 2004. The Sir2 family of protein deacetylases. *Annu. Rev. Biochem.* **73**: 417–435.
- Cairns, B.R. 2005. Chromatin remodeling complexes: Strength in diversity, precision through specialization. *Curr. Opin. Genet. Dev.* **15**: 185–190.
- Dhillon, N., Oki, M., Szyjka, S.J., Aparicio, O.M., and Kamakaka, R.T. 2006. H2A.Z functions to regulate progression through the cell cycle. *Mol. Cell. Biol.* **26**: 489–501.
- Dion, M.F., Altschuler, S.J., Wu, L.F., and Rando, O.J. 2005. Genomic characterization reveals a simple histone H4 acetylation code. *Proc. Natl. Acad. Sci.* **102**: 5501–5506.
- Ehrenhofer-Murray, A.E. 2004. Chromatin dynamics at DNA replication, transcription and repair. *Eur. J. Biochem.* **271**: 2335–2349.
- Ehrenhofer-Murray, A.E., Rivier, D.H., and Rine, J. 1997. The role of Sas2, an acetyltransferase homologue of *Saccharomyces cerevisiae*, in silencing and ORC function. *Genetics* **145**: 923–934.
- Henikoff, S., Furuyama, T., and Ahmad, K. 2004. Histone variants, nucleosome assembly and epigenetic inheritance. *Trends Genet.* **20**: 320–326.
- Kamakaka, R.T. and Biggins, S. 2005. Histone variants: Deviants? *Genes & Dev.* **19**: 295–310.
- Keogh, M.C., Mennella, T.A., Sawa, C., Berthelet, S., Krogan, N.J., Wolek, A., Podolny, V., Carpenter, L.R., Greenblatt, J.F., Baetz, K., et al. 2006. The *Saccharomyces cerevisiae* histone H2A variant Htz1 is acetylated by NuA4. *Genes & Dev.* **20**: 660–665.

Shia et al.

- Kimura, A., Umehara, T., and Horikoshi, M. 2002. Chromosomal gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. *Nat. Genet.* **32**: 370–377.
- Kobor, M.S., Venkatasubrahmanyam, S., Meneghini, M.D., Gin, J.W., Jennings, J.L., Link, A.J., Madhani, H.D., and Rine, J. 2004. A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biol.* **2**: E131.
- Kurdistani, S.K., Tavazoie, S., and Grunstein, M. 2004. Mapping global histone acetylation patterns to gene expression. *Cell* **117**: 721–733.
- Ladurner, A.G., Inouye, C., Jain, R., and Tjian, R. 2003. Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. *Mol. Cell* **11**: 365–376.
- Li, B. and Reese, J.C. 2001. Ssn6-Tup1 regulates RNR3 by positioning nucleosomes and affecting the chromatin structure at the upstream repression sequence. *J. Biol. Chem.* **276**: 33788–33797.
- Li, B., Pattenden, S.G., Lee, D., Gutierrez, J., Chen, J., Seidel, C., Gerton, J., and Workman, J.L. 2005. Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. *Proc. Natl. Acad. Sci.* **102**: 18385–18390.
- Longtine, M.S., McKenzie III, A., Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* **14**: 953–961.
- Megee, P.C., Morgan, B.A., Mittman, B.A., and Smith, M.M. 1990. Genetic analysis of histone H4: Essential role of lysines subject to reversible acetylation. *Science* **247**: 841–845.
- Meijsing, S.H. and Ehrenhofer-Murray, A.E. 2001. The silencing complex SAS-I links histone acetylation to the assembly of repressed chromatin by CAF-I and Asf1 in *Saccharomyces cerevisiae*. *Genes & Dev.* **15**: 3169–3182.
- Meneghini, M.D., Wu, M., and Madhani, H.D. 2003. Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell* **112**: 725–736.
- Millar, C.B., Xu, F., Zhang, K., and Grunstein, M. 2006. Acetylation of H2AZ Lys 14 is associated with genome-wide gene activity in yeast. *Genes & Dev.* **20**: 711–722.
- Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S., and Wu, C. 2004. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* **303**: 343–348.
- Oki, M., Valenzuela, L., Chiba, T., Ito, T., and Kamakaka, R.T. 2004. Barrier proteins remodel and modify chromatin to restrict silenced domains. *Mol. Cell. Biol.* **24**: 1956–1967.
- Osada, S., Sutton, A., Muster, N., Brown, C.E., Yates III, J.R., Sternglanz, R., and Workman, J.L. 2001. The yeast SAS [something about silencing] protein complex contains a MYST-type putative acetyltransferase and functions with chromatin assembly factor ASF1. *Genes & Dev.* **15**: 3155–3168.
- Raisner, R.M., Hartley, P.D., Meneghini, M.D., Bao, M.Z., Liu, C.L., Schreiber, S.L., Rando, O.J., and Madhani, H.D. 2005. Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. *Cell* **123**: 233–248.
- Reifsnyder, C., Lowell, J., Clarke, A., and Pillus, L. 1996. Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. *Nat. Genet.* **14**: 42–48.
- Rusche, L.N., Kirchmaier, A.L., and Rine, J. 2003. The establishment, inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae*. *Annu. Rev. Biochem.* **72**: 481–516.
- Schaper, S., Franke, J., Meijsing, S.H., and Ehrenhofer-Murray, A.E. 2005. Nuclear import of the histone acetyltransferase complex SAS-I in *Saccharomyces cerevisiae*. *J. Cell Sci.* **118**: 1473–1484.
- Shia, W.J., Osada, S., Florens, L., Swanson, S.K., Washburn, M.P., and Workman, J.L. 2005. Characterization of the yeast trimeric-SAS acetyltransferase complex. *J. Biol. Chem.* **280**: 11987–11994.
- Sobel, R.E., Cook, R.G., Perry, C.A., Annunziato, A.T., and Allis, C.D. 1995. Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc. Natl. Acad. Sci.* **92**: 1237–1241.
- Suka, N., Luo, K., and Grunstein, M. 2002. Sir2p and Sas2p oppositely regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat. Genet.* **32**: 378–383.
- Suto, R.K., Clarkson, M.J., Tremethick, D.J., and Luger, K. 2000. Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. *Nat. Struct. Biol.* **7**: 1121–1124.
- Sutton, A., Shia, W.J., Band, D., Kaufman, P.D., Osada, S., Workman, J.L., and Sternglanz, R. 2003. Sas4 and Sas5 are required for the histone acetyltransferase activity of Sas2 in the SAS complex. *J. Biol. Chem.* **278**: 16887–16892.
- West, A.G., Huang, S., Gaszner, M., Litt, M.D., and Felsenfeld, G. 2004. Recruitment of histone modifications by USF proteins at a vertebrate barrier element. *Mol. Cell* **16**: 453–463.
- Workman, J.L. and Kingston, R.E. 1998. Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu. Rev. Biochem.* **67**: 545–579.
- Xu, E.Y., Kim, S., and Rivier, D.H. 1999. SAS4 and SAS5 are locus-specific regulators of silencing in *Saccharomyces cerevisiae*. *Genetics* **153**: 25–33.
- Zhang, H., Richardson, D.O., Roberts, D.N., Utley, R., Erdjument-Bromage, H., Tempst, P., Cote, J., and Cairns, B.R. 2004. The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation, and Htz1 replacement near telomeres. *Mol. Cell. Biol.* **24**: 9424–9436.
- Zhang, H., Roberts, D.N., and Cairns, B.R. 2005. Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. *Cell* **123**: 219–231.

SAS-mediated acetylation of histone H4 Lys 16 is required for H2A.Z incorporation at subtelomeric regions in *Saccharomyces cerevisiae*

Wei-Jong Shia, Bing Li and Jerry L. Workman

Genes Dev. 2006, **20**:

Access the most recent version at doi:[10.1101/gad.1439206](https://doi.org/10.1101/gad.1439206)

Supplemental Material

<http://genesdev.cshlp.org/content/suppl/2006/09/18/20.18.2507.DC1>

References

This article cites 39 articles, 20 of which can be accessed free at:
<http://genesdev.cshlp.org/content/20/18/2507.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

Focused on your science.

USC
SCIENTIFIC
SIDE BY SCIENCE