Mechanism of the Long Range Anti-silencing Function of Targeted Histone Acetyltransferases in Yeast*

Qun Yu, Joseph Sandmeier, Hengping Xu, Yanfei Zou, and Xin Bi

From the Department of Biology, University of Rochester, Rochester, New York 14627

Transcriptionally silent chromatin in Saccharomyces cerevisiae is associated with histone hypoacetylation and is formed through the action of the Sir histone deacetylase complex. A histone acetyltransferase (HAT) targeted near silent chromatin can overcome silencing at a distance by increasing histone acetylation in a sizable region. However, how a tethered HAT acetylates distant nucleosomes has not been resolved. We demonstrate here that targeting the histone H3-specific HAT Gcn5p promotes acetylation of not only histone H3 but also histone H4 in a broad region. We also show that long range anti-silencing and histone acetylation by targeted HATs can be blocked by nucleosome-excluding sequences. These results are consistent with the contention that a tethered HAT promotes stepwise propagation of histone acetylation along the chromatin. Because histone hypoacetylation is key to the formation and maintenance of transcriptionally silent chromatin, it is believed that acetylation promoted by a targeted HAT disrupts silent chromatin thereby overcoming silencing. However, we show that the acetylated and transcriptionally active region created by a tethered HAT retains structural hallmarks of Sir-dependent silent chromatin and remains associated with Sir proteins indicating that tethered HATs overcome silencing without completely dismantling silent chromatin.

Eukaryotic DNA is packed into chromatin through the formation of nucleosomes composed of a histone octamer around which 147 bp of DNA is wrapped (1, 2). Chromatin plays a pivotal role in the regulation of gene expression. It is subject to various modifications that differentially affect its compaction and accessibility to transcriptional regulators. The two major types of modifications of chromatin structure are the remodeling of nucleosome patterning along the chromatin fiber and covalent modifications of histones, including acetylation, methylation, phosphorylation, and ubiquitination (3, 4). Increasing evidence indicates that histone modifications create a histone code (surface) that is read (recognized) by other proteins to bring about various downstream events (5–7).

Acetylation of the N-terminal lysine residues of histones neutralizes the positive charge they carry, which is believed to alter the property of DNA. Acetylation is thought to enable recognition of chromatin by transcriptional activators or the preinitiation complex. On the other hand, the spreading model proposes that local acetylation of histones carried out by a targeted HAT serves to recruit additional HATs thereby reaching the promoter. Such factors can be transcriptional activators or the preinitiation complex. Histone deacetylation may help form a more rigid chromatin structure thereby decreasing DNA accessibility to transcriptional factors bound at the promoter thereby reaching the promoter. Opposite to histone acetylation, histone deacetylation is often involved in the repression/silencing of gene expression. Histone deacetylation may help form a more rigid chromatin structure thereby decreasing DNA accessibility to transcriptional factors. For example, promoter-targeted deacetylation can inhibit the recruitment of transcription factors to promoters (19). HDACs are also involved in the establishment of heterochromatin/silent chromatin domains that underlie region-specific, gene nonspecific transcriptional silencing. In this case, a sizable region is associated with relatively uniform hypoacetylation of histones. Silent domains in Saccharomyces cerevisiae include the cryptic mating loci HML and HMR, as well as regions near telomeres (20, 21). Histone hypoacetylation in these regions depends on the NAD+-dependent HDAC Sir2p, which forms a silencing complex with Sir3p and Sir4p (20, 21). The Sir complex is recruited to silent loci through interactions with telomere-binding proteins or factors that bind to special sequences called silencers flanking HML and HMR. Importantly, Sir complexes

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1 To whom correspondence should be addressed: Dept. of Biology, University of Rochester, Rochester, NY 14627. Tel: 585-275-6922; Fax: 585-275-2070; E-mail: xnibi@mail.rochester.edu.

2 The abbreviations used are: HAT, histone acetyltransferase; HDAC, histone deacetylase; ChIP, chromatin immunoprecipitation; MNase, micrococcal nuclease; FOA, 5-fluoro-orotic acid; H3-Ac, acetylated H3.
interact with each other and bind to the N termini of histones H3 and H4 with a strong preference for their unacetylated forms (22–24). Based on these findings, a model for the establishment of silent chromatin has been proposed (20, 21, 25). In this model, a Sir complex is recruited to a silencer/telomere, and Sir2p deacetylates an adjacent nucleosome, which then binds another Sir complex with high affinity. The nucleosome-bound Sir complex then deacetylates the neighboring nucleosome enabling it to bind a new Sir complex. Subsequent cycles of this process lead to the sequential propagation of the Sir complex along an array of nucleosomes. Such a stepwise (nucleosome-by-nucleosome) spreading model for Sir complexes implies that a continuous array of closely spaced nucleosomes is needed. This is supported by our finding that disrupting the regularity of nucleosomes by nucleosome-excluding structures blocks the spread of silent chromatin (26). The above mechanism for Sir complex propagation provides a paradigm (in the opposite sense) for the spreading model for long range HAT action.

Because histone deacetylation is essential to the formation of transcriptionally silent chromatin, a HAT might counteract silencing if it is directed to the silenced domain. This has been proven to be the case as targeting either the HAT Gcn5p or Esa1p in between a silencer and a reporter prevented the silencing of the reporter (27, 28). Therefore, a tethered HAT apparently functions as a barrier to block the spread of silencing emanating from the silencer. Moreover, we showed, surprisingly, that Gcn5p or Esa1p could counteract silencing even when targeted at a site that was outside the region bracketed by the silencer and reporter (27). Importantly, the long distance anti-silencing function of tethered LexA-Gcn5p or LexA-Esa1p coincided with the creation of a sizable (>2 kb) chromatin domain with elevated acetylation of histone H3 or H4 that spanned the target site (27). It was not clear whether the tethered LexA-HAT employed the looping or spreading mechanism to acetylate nucleosomes at a distance from its target site. Concerning the anti-silencing function of HATs, it is thought that elevated acetylation of histones would evict the Sir proteins thereby disrupting silent chromatin structure characterized by densely and stably positioned nucleosomes (29–31). However, this has not been experimentally tested.

In this report we investigated how targeted HATs create extended regions of histone hyperacetylation and counteract transcriptional silencing at a distance and analyzed changes in silent chromatin induced by the HATs. We obtained evidence in accord with the spreading model for long range HAT function. We also found that targeted HATs did not induce significant changes in the Sir-dependent silent chromatin structure pertaining to the profile of nucleosome distribution along the DNA and the abundance of Sir proteins associated with chromatin. These results are not consistent with the all-or-none model for the state of silent chromatin but instead suggest that HATs tethered to silent loci create a state of chromatin different from the “fully silent” (hypocacylated and Sir-associated) and “fully activated” (acetylated and Sir-free) chromatin.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—Plasmids pXB323, pXB301, and pRQ12 encoding LexA, LexA-Esa1p, and LexA-Gcn5p, respectively, have been described previously (27). Plasmid pAR61 contains the HindIII-BamHI fragment of chromosome III (coordinates 14,838–16,263). The 1.1-kb BglII–URA3–BglIII fragment of plasmid pFL44 (32) was inserted at the EcoRV site of pAR61 to make plasmid pMB22-a. Four tandem copies of the CoEl operator was inserted at the EagI site of pMB22-a to make plasmid pXB306 (27). Each CoEl operator consists of two LexA-binding sites. A 388-bp Xmal-NogMIV fragment of bacterial origin from the plasmid pBLUESCRIPT was inserted at the NgoMIV site of pXB306 making pYC20. A 190-bp sequence containing (CCGNN)\(_{16}\) \((n = A/G/C/T)\) and a 340-bp sequence containing two copies of (T)\(_{10}\) were inserted at the NgoMIV site of pXB306 making plasmids pYZ73 and pYZ72, respectively. The sequence of (CCGNN)\(_{16}\) has been described before (26).

**Yeast Strains**—S. cerevisiae strains used in this study were derived from YXB76 (MATa ura3–52 leu2–3,112 ade2–1 lys1–1 his5–2 can1–100 E-HML-I\(_{\text{inv}}\)) (33). Strain c carrying the construct near the inverted HML-I as shown in Fig. 1A has been described before (27). Strains c1 and c2 were derived from c by replacing the open reading frames of Sir3 and Sas2, respectively, with kanMX. Strains h and h1–h3 were made by transforming YXB76 to Ura+ with HindIII and BamHI-digested plasmids pXB306, pYC20, pYZ73, and pYZ72, respectively. Strains c’, c”, and c’’ were made by transforming strain c to being Geneticin-resistant with a PCR-produced fragment encoding 9-Myc linked to kanMX embedded in a sequence spanning the 3’ region of the Sir3 and Sir2 open reading frames, respectively. The relevant genotypes of all strains were verified by Southern blotting.

**Chromatin Immunoprecipitation**—ChIP was carried out as described (27). The sequences of PCR primers used in ChIP are available upon request.

**Chromatin Analysis with Micrococcal Nuclease**—Spheroplasts were made from ~1 × 10\(^8\) log phase cells using zymolyase and were permeabilized with Nonidet P-40. MIIase was used at 150 and 300 units/ml, respectively, to treat 2 × 10\(^8\) spheroplasts at 37 °C for 5 min. DNA was then isolated and digested with EagI (Fig. 2C), AvaII (Fig. 4B), or NgolMIV (Fig. 4C). DNA samples were subjected to agarose gel electrophoresis and Southern blotting. Relevant fragments were detected by probes shown in Figs. 2A and 4A, respectively.

**RESULTS**

**Tethered Gcn5p Leads to Elevated Acetylation of Not Only Histone H3 but Also Histone H4 in a Sizable Region**—In our previous work on the anti-silencing effect of targeted HATs, we used strain c in which the URA3 reporter gene was inserted near the HML-I silencer and two tandem LexA-binding sites were inserted 3’ to URA3 (Fig. 1A) (27). The URA3 product can convert 5-fluoroorotic acid (FOA) into a toxic product thus rendering cells sensitive to FOA (34). Therefore, URA3 silencing can be measured by FOA resistance of the cell. URA3 was silenced in strain c bearing LexA (Fig. 1B, growth of strain c bearing LexA on –Leu–FOA medium) (27). As predicted, URA3 silencing was Sir-dependent, because it was abolished by deleting Sir3 (Fig. 1B, lack of growth of strain c1 bearing LexA). The LexA-Esa1p or LexA-Gcn5p fusion proteins abolished URA3 silencing in strain c thus exhibiting anti-silencing activity (Fig. 1B, strain c bearing LexA-Esa1p or LexA-Gcn5p). When the LexA–binding sites were deleted from strain c, LexA-Esa1p or LexA-Gcn5p did not have any effect on the silencing of URA3 (27), indicating that LexA-Esa1p or -Gcn5p overcame silencing only when they were tethered near the silent region.

To examine the mechanism(s) underlying the anti-silencing function of tethered HATs, we tested the spreading model for HAT function, which proposes that local acetylation carried out by a targeted HAT serves to recruit additional HATs to the silencing region (10, 11). In this model, the incoming HATs may or may not have the same substrate (histone) specificity as the tethered HAT. This implies that a histone H3-specific HAT might recruit one or more H4-specific HAT(s) to chromatin, and vice versa. Consequently, a targeted HAT regardless of its substrate specificity might generate a region of both H3 and H4 hyperacetylation. This could be tested by simultaneously examining the profiles of acetylation of both histones H3 and H4 around the targeted HAT.
We measured the acetylation of both H3 and H4 in a region of ~2.6 kb encompassing URA3 and the LexA-binding sites in strain c bearing LexA-Gcn5p by chromatin immunoprecipitation (ChIP) with antibodies against acetylated H3 (α-H3-Ac) or acetylated H4 (α-H4-Ac) (Fig. 1C). Strain c bearing LexA was examined as a control. PCR primers were designed to detect DNA fragments 1–9 (200–450 bp in length) in immunoprecipitated DNA (Fig. 1A). PCR products corresponding to these fragments were examined by agarose gel electrophoresis. Three independent ChIP experiments (on independent cultures of each strain) were performed, and a representative gel picture is presented (Fig. 1C, left). The intensity of each band was quantified and normalized against input control. LexA-Gcn5p-induced acetylation in a particular sequence (1–9) was esti-

FIGURE 1. Tethered Gcn5p induces acetylation of not only histone H3 but also histone H4 in a sizable region. A, construct for testing the anti-silencing effect of targeted HATs in strain c. The URA3 gene was inserted to the right (centromere-proximal) of the HML-I silencer, and two LexA-binding sites were inserted downstream from URA3. Note the direction of HML-I was inverted so that it could efficiently silence URA3 expression (33). Fragments 1–9 to be tested in ChIP are indicated. B, growth phenotypes of strains c, c1 (c deleted for SIR3), and c2 (c deleted for SAS2) bearing plasmid pXB23 (encoding LexA), pXB301 (LexA-Esa1p), or pRQ12 (LexA-Gcn5p) on synthetic medium lacking leucine (−Leu) and −Leu supplemented with 1 mg/ml FOA (−Leu + FOA) were shown. The 10-fold serial dilutions of a late log phase culture of each strain were spotted and grown for 3 days. C, targeted Gcn5p induces acetylation of both histones H3 and H4 in a sizable region. Left, results from one of three independent ChIP experiments on strain c bearing LexA or LexA-Gcn5p with antibodies a-H3-Ac for K9/K14-acetylated histone H3 and α-H4-Ac for multiple (penta-) acetyl-H4 isoforms, respectively. Sequences 1–9 (A) and control sequences from the ACT1 and TDH3 loci were examined by PCR and gel electrophoresis. Input, DNA from whole cell extract before chromatin-IP. No Ab, DNA from mock ChIP without using antibody. Right, quantification of results from three ChIP experiments. Each bar represents the mean of-fold increase in acetylation at a sequence in strain c bearing LexA-Gcn5p compared with that in strain c bearing LexA, which was calculated as the immunoprecipitation/input ratio for LexA-Gcn5p divided by the immunoprecipitation/input ratio for LexA. The standard deviation is also shown (error bars). Shaded bars, acetylated H3. Filled bars, acetylated H4. D, domain of histone acetylation generated by LexA-Esa1p. Shown were results of ChIP on strain c bearing LexA and LexA-Esa1p, respectively.
mated as the ratio of the intensity of the corresponding fragment in LexA-Gcn5p-expressing cells over that in LexA-expressing cells, and the mean of data from all the repeats (together with standard deviation) was graphed in the right panel of Fig. 1C. Also included as controls were results for the ACT1 and TDH3 loci that were not associated with LexA-Gcn5p. Tethered LexA-Gcn5p increased H3 acetylation 1.5- to 3-fold in a region of ~2 kb spanning the LexA sites (Fig. 1C, right panel, 2–9, H3-Ac bars). This was consistent with results from our previous experiments (27), but the -fold increase in H3 acetylation detected here was lower, perhaps because α-H3-Ac from a different source was used. In general, LexA-Gcn5p also increased histone H4 acetylation in the same region where H3 acetylation was elevated (Fig. 1C, right panel, 2–9, H4-Ac bars). Therefore, targeted Gcn5p induced the acetylation of not only histones H3 but also histone H4 in a sizable region. It is possible that, although native Gcn5p prefers histone H3 as substrate for acetylation (12), tethered LexA-fused Gcn5p might have an expanded spectrum of substrates that includes histone H4. An alternative explanation is that histone H3 acetylated by LexA-Gcn5p may help recruit one or more histone H4-specific HATs. The two known H4 HATs in yeast are Esalp and Sas2p (12). We wondered whether Esalp or Sas2p was involved in the function of tethered Gcn5p. We showed that deletion of SAS2 had no significant effect on the long range anti-silencing function of tethered Gcn5p or Esalp (Fig. 1B, strain c2, compare LexA-Gcn5p or -Esalp to LexA). Therefore, SAS2 is not required for the anti-silencing function of targeted Gcn5p. Because Esalp is essential for cell growth, it was not possible to use deletion analysis to test if Esalp is required for the function of tethered Gcn5p.

We also measured the acetylation of both histones H3 and H4 around the LexA-binding sites in strain c bearing LexA-Esalp. As predicted, LexA-Esalp increased histone H4 acetylation in a region of ~2.6 kb encompassing the LexA sites (Fig. 1D, right, 1–9, H4-Ac bars). Acetylation of histone H3, however, was not elevated in this region except for a 2-fold increase at 2- and a 1.5-fold increase at 9 (Fig. 1D, right panel, H3-Ac bars at 4 and 9). Therefore, unlike LexA-Gcn5p, LexA-Esalp only induced acetylation of histone H4 but not H3 in a broad region. One possible explanation was that histone H4 acetylation by LexA-Esalp only helps recruit additional H4-specific HATs.

We note that the apparent increase in acetylation of histones induced by tethered LexA-Gcn5p or -Esalp as described above might also have been the result of a change in the density and distribution of nucleosomes. However, this was unlikely, because we showed that LexA-Esalp or -Gcn5p did not induce detectable changes in the primary chromatin structure pertaining to the density and distribution of nucleosomes in the region between HML-I and the LexA sites in strain c (see Fig. 4 and corresponding descriptions in a later part under “Results”).

Long Distance Anti-silencing Function of a Tethered HAT Can Be Blocked by Nucleosome-excluding Sequences—In the spreading model for long distance HAT function, nucleosomes not only are the substrates but also serve to recruit HATs (10, 11). Therefore, a nucleosome-free region should hinder the putative spread of HAT complexes and histone acetylation. To test this, we examined the effect of known nucleosome-excluding sequences (CCGNN)n (n = A/G/C/T) and poly(dA:dT) (26, 35, 36) on the long range anti-silencing function of LexA-HAT. Strain h was identical to strain c except that LexA-binding sites were located farther (1.7 kb) from the IRA3 promoter (Fig. 2A) (27). LexA-Gcn5p abolished IRA3 silencing, whereas LexA-Esalp caused a sharp decrease in silencing in strain h (Fig. 2B, compare the growth phenotype of strain h bearing LexA-Gcn5p or -Esalp to that of strain h bearing LexA). The anti-silencing function of LexA-HAT was eliminated by inserting (CCGNN)n or (T)n between IRA3 and the LexA sites (Fig. 2B, compare strains h2 and h3 to h). This was not a distance effect, because a spacer sequence failed to significantly affect the anti-silencing function of LexA-HAT (Fig. 2B, compare strain h1 to h).

We used MNase digestion and indirect end labeling to verify that (CCGNN)n or (T)n actually led to nucleosome exclusion in the context of strains h2 or h3. We observed salient MNase-hypersensitive sites within and/or near (CCGNN)n in strain h2 and (T)n in strain h3 (Fig. 2C, lanes 8, 9, 11, and 12, note the MNase sites labeled by dots). This is reminiscent of previous results of chromatin mapping of regions spanning (CCGNN)n, or poly(dA:dT) in other genomic locations (37, 38) and suggests that the ability of (CCGNN)n or poly(dA:dT) to prevent nucleosome positioning is independent of its position in the genome. In summary, we conclude that (CCGNN)n and (T)n exclude nucleosomes and block the long distance anti-silencing function of targeted HATs.

The Long Range Acetylation Function of a Tethered HAT Can Be Blocked by Nucleosome-excluding Sequences—We examined whether the effect of (CCGNN)n on the anti-silencing function of LexA-HAT described above was correlated with a halt in the putative spreading of histone acetylation. Histone H4 or H3 acetylation at sequences 1–5 in a region of ~2 kb spanning (CCGNN)n, and the LexA sites (Fig. 3A) was measured by ChIP with α-H4-Ac or α-H3-Ac in strains h1 and h2 expressing LexA-Esalp or LexA-Gcn5p (Fig. 3, B and C). For each DNA segment, the mean of data from all three repeats was graphed in Fig. 3 (D or E). In strain h1 bearing LexA-Esalp, H4 acetylation was generally high in the 2-kb region examined (Fig. 3D, shaded bars), which coincided with the abolishment of URA3 silencing by LexA-Esalp (Fig. 2B, strain h1 bearing LexA-Esalp). In strain h2 bearing LexA-Esalp, however, H4 acetylation was greatly reduced at sequences 1 and 2 but remained relatively high at 3–5 (Fig. 3D, filled bars). The sharp reduction of histone acetylation at 1 and 2 coincided with 2-fold increase at 2 and 1.5-fold increase at 9 (Fig. 1D, right panel, H3-Ac bars at 4 and 9). Therefore, unlike LexA-Gcn5p, LexA-Esalp only induced acetylation of histone H4 but not H3 in a broad region. One possible explanation was that histone H4 acetylation by LexA-Esalp only helps recruit additional H4-specific HATs.

Long Distance Anti-silencing by a Targeted HAT Is Not Accompanied by Significant Changes in the Profile of Micrococcocal Nucleosome Sensitivity of Chromatin in the Affected Region—Transcriptional silencing at the HML loci or telomeres in yeast is mediated by the formation of a special silent chromatin whose structure is markedly different from its active counterpart with respect to nucleosome density and positioning in it (29–31). In strain c, the URA3 gene was silenced by the HML-I silencer (Fig. 1B, strain c bearing LexA). As predicted, strains c and c1 (ΔSir3) differed greatly in the configuration of chromatin spanning the URA3 gene as revealed by chromatin mapping by MNase digestion and indirect end labeling (Fig. 4, B and C, compare lanes 1 and 1*, note the presence of salient MNase-sensitive sites labeled by dots in lane 1, and sites labeled...
by diamonds in lane 1). Therefore, transcriptional silencing of URA3 in strain c was correlated with a special Sir-dependent chromatin structure.

URA3 was derepressed in strain c bearing LexA-HAT (Fig. 1B, strain c bearing LexA-Esa1p or -Gcn5p). Intuitively, one would think that the structure of chromatin in strain c bearing LexA-Esa1p or -Gcn5p was nearly identical to that in strains c bearing LexA (Fig. 4, B and C, compare lanes 2 and 3 to 1) and was Sir-dependent (Fig. 4, B and C, compare lanes 2 and 3 to 2' and 3', respectively). In other words, LexA-Esa1p and LexA-Gcn5p overcame URA3 silencing (Fig. 1B) and increased chromatin acetylation (Fig. 1, C and D) without significantly altering the distribution of nucleosomes in silent chromatin (Fig. 4, B and C). This poses the intriguing question of how targeted LexA-HAT overcomes the inhibitory effect of silent chromatin on transcription without significantly altering the primary chromatin structure in the silent region.

Association of Sir Proteins with Acetylated and Transcriptionally Active Chromatin—It was surprising that in strain c bearing LexA-HAT, the acetylated and transcriptionally active region between HML-I and the LexA sites apparently maintained a Sir-dependent configuration characteristic of silent chromatin (Fig. 4). One possibility was that Sir proteins remained associated with chromatin in this region (but were unable to prevent the transcription of URA3). To test this idea, we constructed strains c' and c'' that were identical to strain c except bearing Myc-tagged Sir3p and Sir2p in place of Sir3p and Sir2p, respectively. Sir3p-Myc and Sir2p-Myc were functional as URA3 was silenced in strains c' and c'' (Fig. 5A, growth of strain c' bearing LexA on −Leu+FOA, and data not shown). As predicted, LexA-Gcn5p abolished URA3 silencing in strains c' or c'' (Fig. 5A and data not shown). We used ChIP to simultaneously measure the levels of acetylated histone H3 (H3-Ac) and Sir3p-Myc across the URA3 gene in strain c' expressing LexA or LexA-Gcn5p. Levels of H3-Ac and Sir3p-Myc associated with the transcriptionally silent HMR locus and the constitutively active ACT1 locus were also measured as controls. As predicted, the abundance of H3-Ac was low at the silent HMR locus and high at the constitutively active ACT1 locus, which was not affected by LexA-Gcn5p (Fig. 5C, HMR and ACT1 bars), whereas Sir3p was associated with HMR but not ACT1 (Fig. 5D, HMR and ACT1 bars). The abundance of H3-Ac across URA3 in strain c' bearing LexA was low and comparable to that at HMR (Fig. 5C, compare shaded bars a–d to HMR and ACT1), which
coincided with the silencing of \( URA3 \) (Fig. 5A). The abundance of Sir3p-Myc near \( HML-I \) was high and comparable to that at \( HMR \) (Fig. 5D, compare shaded bars a and \( HMR \)) but was gradually reduced at increasingly distant sites from the silencer (Fig. 5D, compare shaded bars b–d to a), which was in accord with the notion that Sir proteins spread outward from the \( HML-I \) silencer. In strain c’ bearing LexA-Gcn5p, the abundance of H3-Ac across \( URA3 \) was significantly increased (Fig. 5C, compare filled and shaded bars at a–d), which was correlated with the derepression of \( URA3 \) (Fig. 5A). On the other hand, the profile of Sir3p-Myc distribution in strain c’ bearing LexA-Gcn5p was comparable to that in strain c’ bearing LexA (Fig. 5D, compare filled and shaded bars at a–d). We also obtained similar results regarding the distribution of Sir2p-Myc in strain c’ bearing LexA-Gcn5p versus LexA (Fig. 5E, lanes 14–18, compare LexA-Gcn5p bands to their corresponding LexA bands). These results indicate that targeted LexA-Gcn5p did not cause a detectable loss of Sir proteins from silent chromatin, despite the fact that it induced histone acetylation and counteracted \( URA3 \) silencing (Fig. 1, B and C). This provides an explanation for our finding that targeting LexA-Gcn5p failed to induce detectable changes in Sir-dependent features of primary chromatin structure in the region spanning \( URA3 \) (Fig. 4, B and C, compare 3 to 1).

**DISCUSSION**

Histone acetylation carried out by histone acetyltransferases (HATs) has long been linked to transcriptional activity in the genome (39). Targeted histone acetylation at promoters helps to activate individual genes, whereas coordinated acetylation of histones across a sizable chromosomal region serves to maintain a transcriptionally active/poised chromatin state. On the other hand, histone hypoacetylation is associated with transcriptionally silent chromatin and is maintained by histone deacetylases (HDACs). We have previously shown that a HAT targeted to a silent locus in yeast was able to acetylate histones in a sizable region and overcome transcriptional silencing at a distance (27). Two distinct models have been proposed for long range functions of tethered HATs (10, 11). The looping model suggests that a HAT complex recruited to a distant regulatory element contacts the promoter of a gene by interacting with promoter-bound factors such as transcriptional activators or the preinitiation complex, which enables the HAT to acetylate histones at the promoter region and help activate the gene. The spreading model proposes that local acetylation of histones carried out by a tethered HAT serves to recruit additional HATs thereby initiating the spread of HAT complexes along the chromatin. We have obtained evidence in this study supporting the spreading model for HAT function.

The spreading model proposes that acetylated histones serve to recruit additional HATs that may or may not have the same substrate (histone) specificity as the tethered HAT. This implies that a tethered HAT, regardless of its substrate specificity, may induce the acetylation of both histones H3 and H4. Consistent with this contention, we have demonstrated in this work that targeted Gcn5p, an H3-specific HAT, elevated the acetylation of both H3 and H4 in a broad region, indicating that both H3-specific and H4-specific HATs were recruited to the region acetylated by Gcn5p. However, given the lack of direct evidence for tethered Gcn5p recruiting H4-specific HAT, it is also formally possible that tethered Gcn5p acetylates histone H4 in the specific context of the construct used in our experiment. If one or more additional HAT complexes were recruited to nucleosomes acetylated by a tethered HAT, then what might mediate the recruitment of the HATs? Some HAT complexes contain conserved bromodomains and chromodomains that are implicated in interactions with chromatin. The bromo-
Long Range Functions of Histone Acetyltransferases

A diagram of sequences near HML-I in strains c or c1 examined by chromatin mapping. The filled bars a and b indicated the DNA probes used in B and C, respectively. B, shows are results of MNase digestion and indirect end labeling on strain c (SIR+) (lanes 1–3) and c1 (Asir3) (lanes 1′–3′) bearing LexA, LexA-Esa1p, and LexA-Gcn5p, respectively. Permeabilized cells were treated with MNase at 150 units/ml, a condition that had been experimentally determined to be able to clearly reveal MNase-sensitive sites in a broad region. DNA was isolated and digested with AvaII. An aliquot of permeabilized cells of each strain that were not treated by MNase was used to isolate naked DNA that was then treated with 7.5 units/ml of MNase and digested with AvaII. This sample was designated N (for strain c) or N′ (for c1). DNA samples were subjected to agarose gel electrophoresis and Southern blotting. Relevant DNA fragments were detected by probe a (200 bp) near the AvaII site as shown in A. The relative positions of HML-I, URA3, and LexA sites are shown on the left. Bands uniquely salient in lanes 1–3 but not 1′–3′ are indicated by dots, whereas bands present in lanes 1′–3′ but not 1–3 are indicated by diamonds. C, results from an experiment identical to that in B except that the restriction enzyme used was NgoMIV, and the probe used was b near the NgoMIV site. Note that results in B focused on HML-I and the region between HML-I and URA3, whereas those in C focused on the URA3 sequence.

domain interacts with acetyllysines on the N termini of histones H3 and H4 (9, 40), whereas the chromodomain recognizes methylated lysines in histone H3 (41, 42). These motifs in HAT complexes may recognize acetyllysines or secondary modifications in chromatin induced by acetylation. A clear example of these motifs being involved in HAT-chromatin interaction in yeast is Gcn5p bromodomain-mediated retention of SAGA on nucleosome arrays with H3 or H4 acetylation after the removal of the activator that first recruited it to DNA in vitro (43). Therefore, SAGA or other Gcn5p-containing HAT complexes could possibly be recruited to acetylated chromatin in vivo via the interaction between its bromodomain and acetyllysines. On the other hand, we showed that tethered Esa1p induced the acetylation of only histone H4 but not histone H3, indicating that H3-specific HATs such as Gcn5p was not recruited to nucleosomes acetylated by Esa1p in vivo. Further investigations are needed to resolve the discrepancy between the pattern of chromatin acetylation induced by tethered Gcn5p and that by Esa1p.

The spreading model for long distance HAT function implies that a regular array of closely positioned nucleosomes is key to the successful propagation of HATs and histone acetylation. This model closely resembles the mechanism for the spread of Sir complexes and histone hypoacetylation in the establishment of transcriptionally silent chromatin in that the spreading complex modifies the nucleosome and preferentially binds the modified nucleosome (10, 11, 20, 25). In support of the spreading mechanism for Sir proteins, we have recently demonstrated that disrupting the regularity of chromatin by sequences/structures that exclude nucleosomes blocks the propagation of transcriptionally silent chromatin (26). We showed in this report that nucleosome-excluding sequences can also block the long range acetylation and anti-silencing functions of targeted HATs (Figs. 2 and 3). This provides clear evidence of DNA sequence/chromatin structure dramatically affecting the function of a targeted HAT and supports the spreading model for long distance HAT function. In addition, this also provides an explanation for the apparent discrepancy concerning the range of action of targeted HATs measured in different studies. It has been shown that Gcn5p-induced histone H3 acetylation in only two to three nucleosomes when targeted to the HIS3 promoter (44) but acetylated six to seven nucleosomes when recruited to the HO promoter (45). On the other hand, Gcn5p (and Esa1p) created a sizable (>2 kb, or ~12 nucleosomes) region of histone hyperacetylation when targeted near the silent HML locus (27). We propose that a targeted HAT complex is intrinsically capable of acting on many nucleosomes in a relatively large region of chromatin via the spreading mechanism under optimal conditions such as the array of closely spaced nucleosomes in silent chromatin. However, we think HAT action is often negatively influenced by the genomic context of its target site. Factors that may restrict the range of action of a targeted HAT include low nucleosome density on the chromatin fiber, prior modifications of histone tails, as well as chromatin-associated proteins. In short, we think that the range of function of a tethered HAT is regulated by chromatin structure.

A commonly held view about silencing and silent chromatin is that there are only two states, all or nothing, as exemplified by the state of chromatin at the HM loci in a Sir+ versus sir− background (29, 31, 46). However, there is evidence against such a contention. For example, silencing of the heat shock-inducible HIS3 promoter (44) but acetylated six to seven nucleosomes when recruited to the HO promoter (45). On the other hand, Gcn5p (and Esa1p) created a sizable (>2 kb, or ~12 nucleosomes) region of histone hyperacetylation when targeted near the silent HML locus (27). We propose that a targeted HAT complex is intrinsically capable of acting on many nucleosomes in a relatively large region of chromatin via the spreading mechanism under optimal conditions such as the array of closely spaced nucleosomes in silent chromatin. However, we think HAT action is often negatively influenced by the genomic context of its target site. Factors that may restrict the range of action of a targeted HAT include low nucleosome density on the chromatin fiber, prior modifications of histone tails, as well as chromatin-associated proteins. In short, we think that the range of function of a tethered HAT is regulated by chromatin structure.
associated) and fully activated (hyperacetylated and Sir-free) structures of chromatin.

The existence of a mosaic state of chromatin that exhibits hallmarks of both silent and active chromatin raises the question of what feature(s) of silent chromatin is (are) directly responsible for gene silencing. Results from this work suggest that increasing the acetylation of histones in silent chromatin without completely eviction Sir proteins or disrupting the Sir-dependent chromatin configuration is sufficient for gene derepression. In light of this, it is likely that the most relevant function of the Sir complex or silent chromatin with respect to transcriptional silencing is histone hypoacetylation, not a particular “silent chromatin structure,” that is responsible for transcriptional silencing.

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FIGURE 5. Association of Sir proteins with highly acetylated and transcriptionally active chromatin. A, strain c' is identical to c except bearing SIR3-Myc instead of SIR3. Growth phenotypes of strain c' bearing LexA and LexA-Gcn5p, respectively, are shown. DNA sequences a–d examined by ChIP in B are indicated. B, measurement of the abundance of acetylated histone H3 and Sir3p-Myc around HMR loci was examined by PCR after chromatin IP. Lanes 1–6, input from whole cell extract before ChIP. Lanes 19–24, mock ChIP without using antibody. Shown were data from one of three independent experiments. C, data from three ChIP experiments with α-H3-Ac on strains c' bearing LexA and LexA-Gcn5p, respectively, were quantified and graphed. **Shaded bars** α-H3-Ac and Sir3p-Myc around HMR (designated **H**), ACT1 (designated **A**) loci was examined by PCR after chromatin IP. Lanes 1–6, input from whole cell extract before ChIP. Lanes 19–24, mock ChIP without using antibody. Shown were data from one of three independent experiments. D, data from three ChIP experiments with α-H3-Ac on strains c' bearing LexA and LexA-Gcn5p, respectively, were quantified and graphed. **Shaded bars** α-H3-Ac and Sir3p-Myc around HMR (designated **H**), ACT1 (designated **A**) loci was examined by PCR after chromatin IP. Lanes 1–6, input from whole cell extract before ChIP. Lanes 19–24, mock ChIP without using antibody. Shown were data from one of three independent experiments. E, data from three ChIP experiments with α-H3-Ac on strains c' bearing LexA and LexA-Gcn5p, respectively, were quantified and graphed. **Shaded bars** α-H3-Ac and Sir3p-Myc around HMR (designated **H**), ACT1 (designated **A**) loci was examined by PCR after chromatin IP. Lanes 1–6, input from whole cell extract before ChIP. Lanes 19–24, mock ChIP without using antibody. Shown were data from one of three independent experiments.
Long Range Functions of Histone Acetyltransferases

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