

11-1-2005

Multiple bromodomain genes are involved in restricting the spread of heterochromatic silencing at the *Saccharomyces cerevisiae* HMR-tRNA boundary

Nithya Jambunathan
Louisiana State University

Adam W. Martinez
Louisiana State University

Elizabeth C. Robert
Louisiana State University

Nneamaka B. Agochukwu
Louisiana State University

Megan E. Ibos
Louisiana State University

See next page for additional authors

Follow this and additional works at: https://digitalcommons.lsu.edu/biosci_pubs

Recommended Citation

Jambunathan, N., Martinez, A., Robert, E., Agochukwu, N., Ibos, M., Dugas, S., & Donze, D. (2005). Multiple bromodomain genes are involved in restricting the spread of heterochromatic silencing at the *Saccharomyces cerevisiae* HMR-tRNA boundary. *Genetics*, 171 (3), 913-922. <https://doi.org/10.1534/genetics.105.046938>

This Article is brought to you for free and open access by the Department of Biological Sciences at LSU Digital Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Digital Commons. For more information, please contact ir@lsu.edu.

Authors

Nithya Jambunathan, Adam W. Martinez, Elizabeth C. Robert, Nneamaka B. Agochukwu, Megan E. Ibos, Sandra L. Dugas, and David Donze

Multiple Bromodomain Genes Are Involved in Restricting the Spread of Heterochromatic Silencing at the *Saccharomyces cerevisiae* *HMR*-tRNA Boundary

Nithya Jambunathan, Adam W. Martinez, Elizabeth C. Robert, Nneamaka B. Agochukwu, Megan E. Ibos, Sandra L. Dugas and David Donze¹

Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803

Manuscript received June 17, 2005
Accepted for publication July 20, 2005

ABSTRACT

The transfer RNA gene downstream from the *HMR* locus in *S. cerevisiae* functions as part of a boundary (barrier) element that restricts the spread of heterochromatic gene silencing into the downstream region of chromosome III. A genetic screen for identifying additional genes that, when mutated, allow inappropriate spreading of silencing from *HMR* through the tRNA gene was performed. *YTA7*, a gene containing bromodomain and ATPase homologies, was identified multiple times. Previously, others had shown that the bromodomain protein Bdf1p functions to restrict silencing at yeast euchromatin-heterochromatin boundaries; therefore we deleted nonessential bromodomain-containing genes to test their effects on heterochromatin spreading. Deletion of *RSC2*, coding for a component of the RSC chromatin-remodeling complex, resulted in a significant spread of silencing at *HMR*. Since the bromodomain of *YTA7* lacks a key tyrosine residue shown to be important for acetylsine binding in other bromodomains, we confirmed that a GST-Yta7p bromodomain fusion was capable of binding to histones *in vitro*. Epistasis analysis suggests that *YTA7* and the *HMR*-tRNA function independently to restrict the spread of silencing, while *RSC2* may function through the tRNA element. Our results suggest that multiple bromodomain proteins are involved in restricting the propagation of heterochromatin at *HMR*.

PROPER regulation of gene expression is of utmost importance for development and survival of all forms of life, as regulated transcription of complex genomes is necessary for cellular energy economy and the precise patterns of gene expression required for cellular differentiation. Transcription of genomes is regulated at multiple levels, which can involve large regions of chromosomes, individual nucleosomes, or direct effects on the transcription machinery at individual promoters (KIMURA and HORIKOSHI 2004). At the level of chromatin in eukaryotes, active euchromatic and predominately inactive heterochromatic chromosomal domains alternate along chromosomes, and mechanisms must exist to prevent regulatory elements from each domain from influencing gene expression patterns of adjacent domains. Chromatin boundary elements (DONZE and KAMAKAKA 2002; WEST *et al.* 2002; DONZE 2004; KIMURA and HORIKOSHI 2004) serve this purpose, as boundaries can prevent either a distal enhancer from activating gene expression inappropriately (insulators) or the unrestricted spreading of heterochromatin (barriers).

The budding yeast *Saccharomyces cerevisiae* contains limited heterochromatic regions at its telomeric and silent mating loci, and these chromosomal regions are important model systems for studying the establishment and propagation of silenced chromatin (RUSCHE *et al.* 2003) and also for studying chromatin boundaries and global mechanisms that restrict heterochromatic propagation (BI and BROACH 1999; DONZE *et al.* 1999; FOUREL *et al.* 1999; KIMURA *et al.* 2002; SUKA *et al.* 2002; LADURNER *et al.* 2003; MENEGHINI *et al.* 2003; TACKETT *et al.* 2005). Silenced chromatin in yeast is maintained by the targeted regional action of the silent information regulator (Sir) proteins, where the Sir2p histone deacetylase establishes the histone acetylation state required for heterochromatin formation as part of a proposed histone code (JENUWEIN and ALLIS 2001), although the specificity of such a code is currently being debated (KURDISTANI *et al.* 2004; DION *et al.* 2005). Deacetylation of histones allows higher affinity binding of Sir3p (CARMEN *et al.* 2002), which then recruits the Sir4p/Sir2p complex, allowing heterochromatin to propagate along nucleosomes (HOPPE *et al.* 2002; RUSCHE *et al.* 2002).

At the *HMR* silent mating locus, a transfer RNA gene and its associated RNA polymerase III complex functions as part of a boundary element that prevents the spreading of Sir protein-mediated heterochromatin

¹Corresponding author: Department of Biological Sciences, Louisiana State University, 202 Life Sciences Bldg., Baton Rouge, LA 70803.
E-mail: ddonze@lsu.edu

(DONZE *et al.* 1999; DONZE and KAMAKAKA 2001). However, we found that while the *HMR*-tRNA alone is sufficient to act as a barrier to silencing in a plasmid-based assay (DONZE and KAMAKAKA 2001), when integrated back into the chromosome, the tRNA alone shows only partial barrier function (our unpublished results), suggesting that additional proteins are required to completely block silencing. Other genes coding for chromatin-associated proteins that can lead to a partial spreading of silencing through the tRNA have been identified and include *HTZI*, *BDF1*, and *YTA7* (LADURNER *et al.* 2003; MENEGHINI *et al.* 2003; TACKETT *et al.* 2005), and the global level of histone acetylation regulated by *SIR2* and *SAS2* influences silencing spread (KIMURA *et al.* 2002; SUKA *et al.* 2002). Taken together, these results suggest that both local boundary-associated factors and global chromatin modifications affect the formation of a true euchromatin-heterochromatin boundary (KIMURA and HORIKOSHI 2004).

To assess what other chromatin proteins participate in boundary formation, we used transposon-mediated mutagenesis and direct gene knockouts in a yeast boundary-element-dependent reporter strain to identify mutations that led to a spread of silencing through the *HMR*-tRNA boundary element, which included ~400 bp at either side of the tRNA. In addition to identifying several factors known to be involved in regulating the spread of silencing, we identified a novel bromodomain-containing gene, *YTA7*, which additionally contains two separate AAA family ATPase domains. We also find that mutation of several different bromodomain genes leads to a general loss of boundary function. This suggests that bromodomains may be key effectors that recognize and maintain the euchromatic histone modification patterns necessary for boundary integrity. Epistasis analysis implicates independent roles for *YTA7* and *HMR*-tRNA in restricting silencing, while *RCS2* may be functioning through the RNA polymerase III complex.

MATERIALS AND METHODS

All yeast strains used in this study (Table 1) were isogenic to W303-1a. The parent boundary reporter strain used in the mating assays (DDY277) was derived from a cross of a similar strain, ROY962, previously described (DONZE *et al.* 1999). The yeast transposon mutagenized library was obtained from Mike Snyder (Yale University), and mutagenesis was performed as described (BURNS *et al.* 1994; ROSS-MACDONALD *et al.* 1997, 1999). All transposon insertion strains were created in DDY277, and identification of mutagenized genes was performed using the vectorette PCR method as described (ROSS-MACDONALD *et al.* 1999). Mating assay strains containing specific mutations were constructed by direct homologous recombination-mediated replacement using standard methods or by crossing previously constructed mutant alleles into the DDY277 background. Mating assays were performed as described (DONZE *et al.* 1999).

The schematic of *YTA7* and amino acid positions of AAA ATPase and bromodomains was based on query of the

Conserved Domain Database (MARCHLER-BAUER *et al.* 2005). The alignment of *S. cerevisiae* bromodomains was created using ClustalW (CHENNA *et al.* 2003) and was manually adjusted to fit a structurally based alignment of bromodomains (ZENG and ZHOU 2002). The GST-Yta7p bromodomain fusion was made by PCR cloning the coding sequence of *YTA7* from amino acids 1000 to 1101 into the glutathione-S-transferase (GST) vector pGEX-2TK (Amersham Biosciences), and the fusion protein was expressed and purified according to the manufacturer's instructions. Purified chicken core histones were purchased from Upstate (Lake Placid, NY). Histone pull-down assays were performed as described (LADURNER *et al.* 2003), and the recovered samples were run on an 18% SDS-PAGE gel and stained with Bio-Safe Coomassie stain (Bio-Rad Laboratories, Hercules, CA). Briefly, core histones were dissolved in water at 1 mg/ml, and 500- μ l binding reactions contained 1 \times HEMG buffer (25 mM HEPES-KOH, pH 7.6, 0.2 mM EDTA, 12.5 mM MgCl₂, 10% glycerol, diluted from a 5 \times stock), 50- μ g core histones, 150 mM KCl, 15 μ l glutathione-Sepharose (Amersham Biosciences) equilibrated in 1 \times HEMG plus 150 mM KCl, and 15 μ g GST-Yta7p fusion protein or an equimolar amount of GST in the controls. Binding reactions were incubated for 4 hr at 4 $^{\circ}$, and beads were collected by centrifugation and then washed four times in 500 μ l 1 \times HEMG, 150 mM KCl, 0.01% NP-40. A total of 125 μ l 1 \times Laemmli loading buffer was added directly to the beads for gel analysis, and unbound fractions were precipitated with trichloroacetic acid, redissolved in 25 μ l 0.1 N NaOH, and brought to 125 μ l in 1 \times Laemmli loading buffer.

To construct the *HMR-ADE2* reporter strains, a plasmid containing the 8.2-kb *SacI-SalI* *HMR* fragment was mutagenized to create a *BamHI* site 180 bp downstream of the *HMR*-tRNA (pDD657). The 2.5-kb *BglII* fragment of *ADE2* (STOTZ and LINDER 1990) was cloned into this site such that *ADE2* was transcribed away from *HMR* to create plasmid pDD659. *HMR*-trna Δ -*ADE2* was constructed by site-directed mutagenesis of pDD659 to delete the tRNA coding sequence (pDD661). Plasmid pDD833, *hmr* Δ -*ADE2* was created by subcloning the *SacI-SalI* fragment of pDD659 into Bluescript cut with *SacI* and *XhoI* to destroy the polylinker *XhoI* site creating pDD833. The resulting plasmid was digested with *SnaBI* and *XhoI* to remove *HMR*, ends blunted with Klenow polymerase, and then religated to create pDD837. Each of these *ADE2* plasmids was linearized and transformed into yeast strain DDY142, which contains an *hmr* Δ ::*URA3* allele. Transformants were selected on minimal media lacking adenine, and individual colonies were patched and replica plated onto minimal media lacking uracil and media containing 5-FOA to identify isolates that had become Ade⁺ and uracil auxotrophic. Proper integration was then confirmed by Southern blot analysis.

Mutant alleles of genes affecting the spread of silencing through the boundary were then crossed into each of these *ADE2* parent strains (DDY811, DDY814, and DDY2114). Colony color assays were performed by streaking representative strains onto minimal media containing 3 μ g/ml adenine, which is 10% of the normal level in this medium. Colonies were grown for 3 days and held at 4 $^{\circ}$ for 1 week prior to photography on a dissecting microscope equipped with a digital imaging system. Colony color was assessed for three independent isolates for each mutant; one representative strain is shown for each.

RESULTS

We used a *MAT α* boundary reporter strain (DDY277) that harbors a modified *HMR* locus deleted for the *HMR-I* silencer and that also contains the downstream

TABLE 1
Strains of *S. cerevisiae* generated for this study

Strain	Genotype
DDY277	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1</i>
DDY282	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMRΔI</i>
DDY799	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:yta7</i>
DDY687	<i>MATα ADE2 his3 leu2 LYS2 trp1 ura3 HMR-E-Boundary-a1 sas2Δ::TRP1</i>
DDY695	<i>MATα ADE2 his3 leu2 LYS2 trp1 ura3 HMR-E-Boundary-a1 gen5::TRP1</i>
DDY800	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:sas5</i>
DDY802	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:sas5</i>
DDY803	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:yta7</i>
DDY811	<i>MATa ade2 his3 leu2 LYS2 trp1 ura3 HMR-trnaΔ-ADE2</i>
DDY814	<i>MATa ade2 his3 leu2 LYS2 trp1 ura3 HMR-ADE2</i>
DDY1174	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:yta7</i>
DDY1309	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:yta7</i>
DDY1313	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:sas4</i>
DDY1316	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:yta7</i>
DDY1318	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:yta7</i>
DDY1340	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:yta7</i>
DDY1344	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:rpd3</i>
DDY1345	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:rpd3</i>
DDY1347	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:yta7</i>
DDY1665	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 rsc2Δ::TRP1</i>
DDY1893	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 rsc1Δ::URA3</i>
DDY1997	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 yta7Δ::TRP1</i>
DDY2078	<i>MATα ADE2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 Tn::LEU2:yta7 sir2Δ::TRP1</i>
DDY2093	<i>MATα ade2 his3 leu2 lys2Δ trp1 ura3 HMR-ADE2 Tn:LEU2:rpd3</i>
DDY2106	<i>MATα ade2 his3 leu2 lys2Δ trp1 ura3 HMR-ADE2 Tn:LEU2:sas5</i>
DDY2114	<i>MATa ade2 his3 leu2 LYS2 trp1 ura3 hmrΔ::ADE2</i>
DDY2128	<i>MATa ade2 his3 leu2 lys2Δ trp1 ura3 Tn:LEU2:rpd3 hmrΔ::ADE2</i>
DDY2142	<i>MATa ade2 his3 leu2 LYS2 trp1 ura3 Tn:LEU2:sas5 hmrΔ::ADE2</i>
DDY2143	<i>MATa ade2 his3 leu2 LYS2 trp1 ura3 Tn:LEU2:rpd3 HMR trnaΔ-ADE2</i>
DDY2156	<i>MATa ade2 his3 leu2 LYS2 trp1 ura3 Tn:LEU2:sas5 HMR trna Δ-ADE2</i>
DDY2198	<i>MATa ade2 his3 leu2, LYS2 trp1 ura3 hmrΔ::ADE2 yta7Δ::TRP1</i>
DDY2200	<i>MATa ade2 his3 leu2, LYS2 trp1 ura3 HMR trnaΔ-ADE2 yta7Δ::TRP</i>
DDY2205	<i>MATα ade2 his3 leu2, lys2Δ trp1 ura3 HMR-ADE2 yta7Δ::TRP1</i>
DDY2450	<i>MATα ade2 his3 leu2 LYS2 trp1 ura3 hmrΔ::ADE2 rsc2Δ::TRP1</i>
DDY2489	<i>MATa ade2 his3 leu2 LYS2 trp1 ura3 HMR trna Δ-ADE2 rsc2::TRP1</i>
DDY2496	<i>MATα ade2 his3 leu2 lys2Δ trp1 ura3 HMR-ADE2 rsc2Δ::TRP1</i>
DDY2514	<i>MATα ade2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 spt7Δ::URA3</i>
DDY2596	<i>MATα ade2 his3 leu2 LYS2 trp1 ura3 HMR-E-Boundary-a1 bdf1Δ::HIS3</i>
DDY2601	<i>MATα ade2 his3 leu2 lys2Δ trp1 ura3 HMR-E-Boundary-a1 bdf2Δ::LEU2</i>
DDY2659	<i>MATα ade2 his3 leu2 LYS2 trp1 ura3 HMR-E-Boundary-a1 snf2::LEU2</i>
DDY2716	<i>MATα ADE2 his3 leu2 LYS2 trp1 ura3 HMR-E-Boundary-a1 htz1Δ::KanMX</i>

boundary sequence cloned into the *HMRa2* gene (Figure 1, top). This strain is nonmating, as the boundary blocks the spread of silencing from *HMR-E*, allowing the *a1* gene to be expressed in the *MATα* background (DONZE *et al.* 1999). To identify genes involved in restricting the spread of silencing, we subjected this strain to a transposon mutagenesis protocol (ROSS-MACDONALD *et al.* 1999) and selected transposon insertion mutants that acquired an α -mating phenotype, indicating that silencing was now able to spread through the boundary into *a1*. A total of 20,000 Leu⁺ transformants were replica plated onto *MATa* tester lawns to identify mutants that lost boundary function at the modified *HMR* locus as judged by their ability to mate.

α -Mating isolates were confirmed by backcrossing to a *MATa* strain containing the same reporter construct, and only tetrad isolates in which the transposon-encoded *LEU2* marker completely cosegregated with the mating phenotype (20 tetrads analyzed) were studied further. After this analysis, mutations in only four separate genes were confirmed as leading to a spread of silencing: *SAS4* and *SAS5*, encoding subunits of the something about silencing (SAS) acetyltransferase complex; *RPD3*, a histone deacetylase; and *YTA7*, a little studied gene containing a weak bromodomain homology and two domains of AAA family ATPase homology. The mating phenotypes of each independent mutant isolate are shown in Figure 1. A single mutant of the

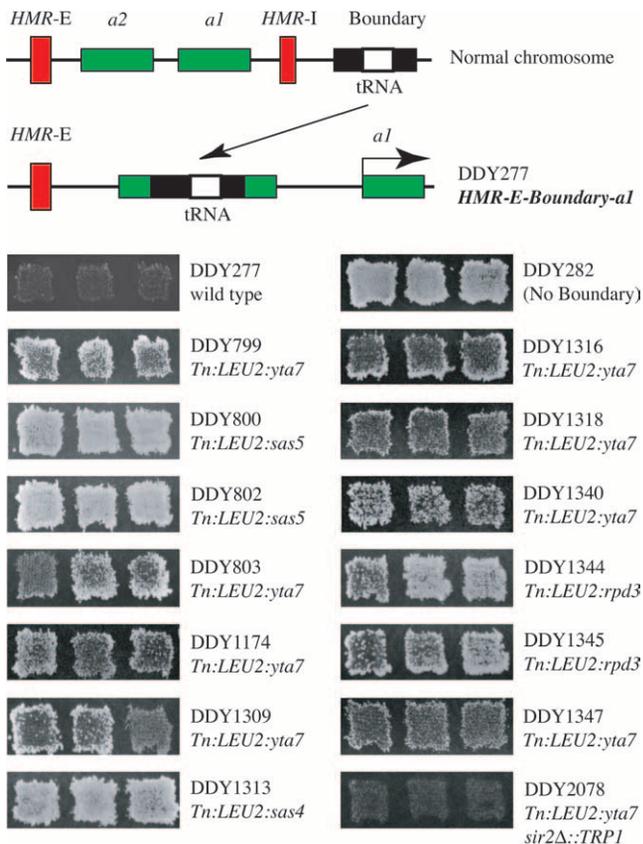


FIGURE 1.—Mating assay to identify extragenic mutations that lead to spreading of silencing through the *HMR* right boundary. (Top) The 1.0-kb region downstream of *HMR* containing the boundary tRNA was cloned into the *a2* gene, and the resulting construct was integrated back into chromosome III in a *MAT α* strain. The boundary element blocks the spread of silencing into *a1*, resulting in a nonmating phenotype (DDY277). The mating assay was performed essentially as described previously (DONZE *et al.* 1999). DDY277 was mutagenized by transformation with a yeast genomic DNA library containing random transposon-*LEU2* insertions (ROSS-MACDONALD *et al.* 1999), and Leu⁺ recombinants were tested for mating. (Bottom) Thirteen independent isolates that showed increased silencing were confirmed, representing mutations in four different genes, *YTA7*, *SAS5*, *SAS4*, and *RPD3*. All strain genotypes are listed in Table 1.

SAS4 gene resulted in increased mating, along with two independent insertions into *SAS5* and two independent insertions into *RPD3*. Mutations in the SAS complex were previously shown to increase the silencing efficiency of a defective *hmra-e*** locus (EHRENHOFER-MURRAY *et al.* 1997; XU *et al.* 1999), and *sas2* mutations were first identified as leading to increased silencing in *sir1* mutants (REIFSNYDER *et al.* 1996). Since *hmra-e*** lacks the boundary element, it is likely that mutations in the SAS complex globally affect the robustness of silencing by affecting cellular levels of histone H4 lysine 16 acetylation (MEIJSING and EHRENHOFER-MURRAY 2001; KIMURA *et al.* 2002; SUKA *et al.* 2002; SHIA *et al.* 2005), as opposed to specifically affecting the integrity of the boundary element. Mutations in *RPD3* are also

well documented as leading to a global increase of silencing at all three yeast heterochromatin loci (mating loci, rDNA, and telomeres) by an unknown mechanism (RUNDLETT *et al.* 1996; VANNIER *et al.* 1996; KIM *et al.* 1999; SMITH *et al.* 1999; SUN and HAMPSEY 1999).

Interestingly, eight independent isolates containing transposon insertions in *YTA7*, representing five different insertion sites, were isolated in the screen. It is not clear why *yta7* mutants would occur so frequently in the library compared to other genes. It should be noted that the screen, while calculated to be saturating, was not, as mutations in genes previously known to induce a mating phenotype in this reporter system, such as the acetyltransferase subunit of SAS, *SAS2*, and the bromodomain-acetyltransferase *GCN5*, were not recovered (DONZE and KAMAKAKA 2001). Additionally, insertions into other genes known to be required for restriction of silencing at *HMR*, *BDF1* (LADURNER *et al.* 2003), and *HTZ1* (MENEHINI *et al.* 2003) were also not recovered. However, as discussed below, direct deletion of *BDF1* or *HTZ1* does lead to a spread of silencing in this reporter assay (Figure 3). The Sir protein dependence of the observed spread of silencing is shown for a *yta7* mutant (DDY2078, Figure 1) and was also confirmed for *rpd3* and *SAS* mutants (our unpublished data).

YTA7 contains AAA family ATPase domains and a single bromodomain module, schematically depicted in Figure 2A. Since bromodomains have been demonstrated to be acetyllysine-binding modules (DHALLUIN *et al.* 1999; HUDSON *et al.* 2000; JACOBSON *et al.* 2000; OWEN *et al.* 2000) and have been suggested to mediate their functions by binding to acetylated histones and other chromatin-associated proteins (ZENG and ZHOU 2002; YANG 2004; DE LA CRUZ *et al.* 2005), it seemed likely that Yta7p may be functioning through histone recognition. The bromodomain protein Bdf1p is also known to have an antisilencing function at *HMR*, as *bdf1* mutants show decreased gene expression and increased Sir3p association in regions adjacent to yeast silenced loci (LADURNER *et al.* 2003). Alignment of the *YTA7* bromodomain with other yeast bromodomains (Figure 2B), however, reveals that *YTA7* lacks a critical conserved tyrosine residue required for acetyllysine recognition by P/CAF, Gcn5p, and Bdf1p via a key hydrogen bond formation within the binding pocket (DHALLUIN *et al.* 1999; OWEN *et al.* 2000; LADURNER *et al.* 2003). Yta7p does contain a serine and threonine at the same location.

Due to the absence of this key tyrosine residue, we wanted to test whether the Yta7p bromodomain module was able to bind to histones. We cloned the coding sequence of the *YTA7* bromodomain into a GST fusion vector and purified the GST-Yta7p fusion protein from *Escherichia coli* cells. This fusion protein was used in a pull-down assay with purified chicken erythrocyte core histones. Figure 2C shows that GST-Yta7p efficiently binds to histones H3 and H4, compared to no binding

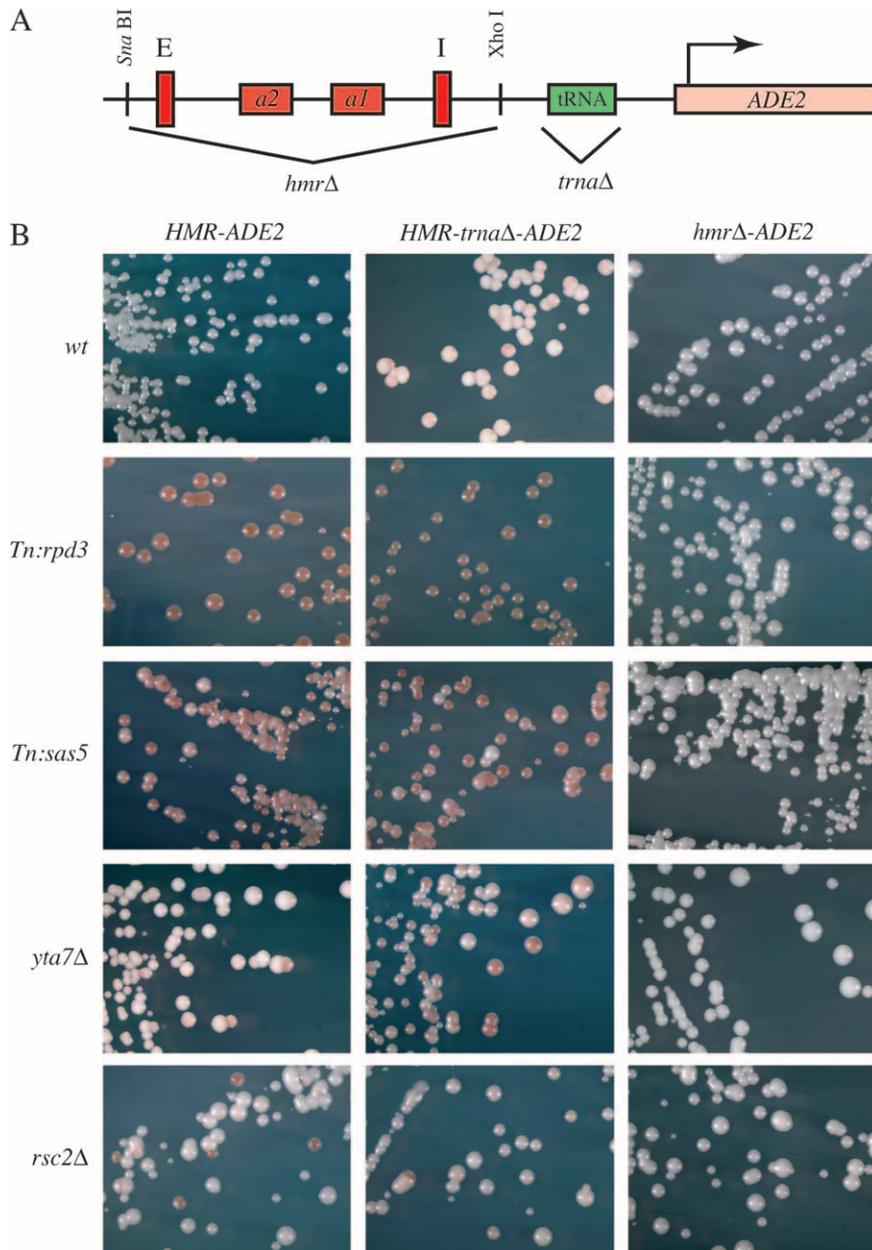


FIGURE 4.—*YTA7* and the *HMR*-tRNA function through different pathways to restrict the spread of silencing at *HMR*. (A) The *ADE2* gene was inserted into chromosome III downstream of *HMR* in *S. cerevisiae* (*HMR-ADE2*). Isogenic strains lacking the *HMR*-tRNA (*HMR-trnaΔ-ADE2*) or lacking *HMR* (*hmrΔ-ADE2*) were also constructed. (B) Each strain was crossed into *rpd3*, *sas5*, *yta7*, or *rsc2* mutant backgrounds, and silencing was assessed by the degree of pigmentation exhibited by the presence or absence of *ADE2* expression. The combination of *yta7Δ trnaΔ* leads to a more silenced phenotype (more and darker red colonies), suggesting that each functions independently. No difference in phenotype is seen between strains containing *rsc2Δ* and *rsc2Δ trnaΔ*. Strains depicted are: first row [wild type (*wt*)], DDY814, DDY811, and DDY2114; second row (*rpd3*), DDY2093, DDY2143, and DDY2128; third row (*sas5*), DDY2106, DDY2156, and DDY2142; fourth row (*yta7*), DDY2205, DDY2200, and DDY2198; and fifth row (*rsc2*), DDY2496, DDY2489, and DDY2450.

near-complete mating phenotype in the mating assay, we developed a second system for assessing the effects of tRNA deletion on heterochromatin spread at *HMR*. The *S. cerevisiae* *ADE2* gene was integrated downstream of *HMR* (referred to here as *HMR-ADE2*), and isogenic strains that were *HMR-trnaΔ-ADE2* or *hmrΔ-ADE2* were created. Repression of *ADE2* expression leads to the accumulation of a red pigment derived from the Ade2p substrate (FISHER 1969). Phenotypes of these parent strains are shown in the top row of Figure 4B; strains containing a normal *HMR* locus or a deletion of *HMR* (including both silencers) give rise to all-white colonies, demonstrating expression of *ADE2* at this integration site; deletion of only the *HMR*-tRNA gives rise to a variegated phenotype with some sectorial pink colonies arising due to epigenetic silencing of *ADE2*.

These *ADE2* reporter strains were crossed to strains containing mutations that resulted in spreading of silencing in the mating assay. Mutation of *RPD3* resulted in completely red colonies with or without the tRNA present. This complete penetrance of the *rpd3* phenotype prevented any epistatic analysis using this assay. Mutation of *SAS5* resulted in a variegated but mostly pink-red colony phenotype, which was unchanged when the *HMR*-tRNA gene was deleted. Again, on the basis of the qualitative nature of this assay, it was impossible to determine epistatic effects of *sas5Δ trnaΔ* mutations.

Deletion of *YTA7* in *HMR-ADE2* strains yielded a variegated pink-white phenotype, similar in magnitude to the *HMR-trnaΔ-ADE2* strain; however, in *yta7Δ trnaΔ* strains, a darker red color was observed in a greater percentage of colonies, including some completely red

colonies, demonstrating a more robust spread of silencing when both mutations are present (Figure 4B, fourth row, center). This additive effect suggests that *YTA7* functions independently of the *HMR*-tRNA in restricting the spread of silencing. *RSC2* deletion shows a weakly variegated phenotype in the *HMR-ADE2* background, with rare silenced colonies that were somewhat darker red. Deletion of the *HMR*-tRNA in the *rsc2Δ* background did not increase the severity of the phenotype, suggesting that *RSC2* and the *HMR*-tRNA may function through the same pathway in restricting the spread of silencing. We attempted to determine epistatic relationships among bromodomain-containing genes, but we were unable to obtain *yta7-rsc2* or *yta7-bdf1* double mutants by crosses of single-mutant strains or by homologous recombination, suggesting a synthetic lethality of these mutant combinations in our W303 background (our unpublished results).

Importantly, when *HMR* was deleted in each of these mutant backgrounds (Figure 4B, right column), all-white colonies were observed. This confirms that phenotypes observed for each of these mutations are due to the spread of silencing from *HMR* and not due to global histone acetylation effects or spreading of silencing from the telomere of chromosome III. Deletion of *SIR2* or *SIR4* in these backgrounds also results in completely white colonies, confirming the Sir dependence of the phenotype (our unpublished data). Also, it should be noted that the spreading of silencing observed in all experiments described here is due to normal levels of Sir proteins and that no overexpression of Sir3p was employed to obtain the observed phenotypes.

DISCUSSION

***YTA7* encodes a histone-binding chromatin boundary protein:** The mutational analysis of boundary function described here revealed that the AAA family ATPase/bromodomain protein Yta7p plays a role in the integrity of the euchromatin/heterochromatin boundary at the *S. cerevisiae* *HMR* locus. *YTA7* contains a region of bromodomain homology from amino acids 1003 to 1091 and two separate AAA family ATPase domains from amino acids 449 to 580 and 771 to 957 (Figure 2A). Comparison of the homology of the *YTA7* bromodomain to other yeast bromodomains (Figure 2B) revealed that *YTA7* lacks a key tyrosine residue (conserved in most bromodomains), which makes a critical water-mediated hydrogen bond to acetyllysine in Gcn5p and is required for the interaction of acetylated histones with Bdf1p (DHALLUIN *et al.* 1999; OWEN *et al.* 2000; LADURNER *et al.* 2003). However, the results shown in Figure 2C demonstrate that the *YTA7* bromodomain is a functional histone-binding module. The *YTA7* sequence contains tandem serine and threonine residues at the position of the conserved tyrosine, which may contribute

the necessary hydrogen-bonding potential within the bromodomain-binding pocket. Bioinformatic analysis of *YTA7* orthologs in the related yeast species *S. paradoxus*, *S. mikatae*, and *S. bayanus* and the less-related *Debaryomyces hansenii* and *Yarrowia lipolytica* revealed that these *YTA7* orthologs have the same serine-threonine substitutions in their respective bromodomains (CHRISTIE *et al.* 2004), and the same substitution is seen in both human and mouse Tif1β (ZENG and ZHOU 2002). Yta7p is also a confirmed nuclear protein, as determined by a genome-wide analysis of yeast protein localization (HUH *et al.* 2003).

While this work was in progress, TACKETT *et al.* (2005) published a proteomic study that also implicated Yta7p in boundary function. They found that Yta7p associated with complexes containing Dpb4p, a component of the DNA polymerase ε and ISW2/CHRAC chromatin complexes, and that both of these complexes are involved in regulating telomeric silencing (IDA and ARAKI 2004). TACKETT *et al.* (2005) also showed that deletion of *YTA7* led to spreading of silencing at *HMR*, and most significantly, they localized Dpb4p-Yta7p complexes to sites near *HMR*, *HML*, chromosome ends, and the conditionally expressed and epigenetically regulated *FLO* genes, suggesting a specific targeted role for Yta7p in restricting repression from multiple loci. The results presented in this article confirm the role of Yta7p in preventing the spread of silencing and extend these results to demonstrate independent functions of Yta7p and the *HMR*-tRNA with an epistasis analysis (discussed below).

Additionally, the study by TACKETT *et al.* (2005) identified roles for DNA polymerase ε, Isw2, and Sas3-Spt16 chromatin-remodeling complexes in boundary function. We previously tested mutations in *SAS3* (DONZE and KAMAKAKA 2001) and *SPT16* (our unpublished results) and saw no spreading of silencing in our mating assay. TACKETT *et al.*'s (2005) analysis of the effect of *SAS3* deletion showed a reduction in transcript levels of genes adjacent to *HMR* (*GIT1* and *YCR095C*), but it is unknown if this reduction is Sir protein dependent, as expression of these genes was not reported in a *sirΔ* or *hmrΔ* background.

Our genetic analysis also uncovered a role for remodels the structure of chromatin (RSC) complex proteins in boundary function. RSC is a multisubunit, highly abundant, and essential chromatin-remodeling complex first purified from *S. cerevisiae* cells (CAIRNS *et al.* 1996). Genome-wide analysis of the location of the RSC complex has demonstrated that RSC is targeted to many tRNA genes *in vivo* (NG *et al.* 2002), suggesting a potentially direct connection between the function of the *HMR*-tRNA and the RSC complex in heterochromatin boundary function. Our epistasis analysis demonstrating no difference in phenotype between *rsc2Δ* and *rsc2Δ trnaΔ* strains shown in Figure 4 is consistent with this proposed connection.

Yta7p appears to function independently of the *HMR*-tRNA, as deletion of both the *cis*-element and the *trans*-factor leads to a more silenced phenotype (Figure 4). We could not determine the epistatic relationships between the *HMR*-tRNA and *BDF1*, *BDF2*, *RSC1*, or *GCN5* in this assay, as strains containing deletions of these genes grew as all-white colonies in the *HMR-ADE2* background and had no apparent effect on colony color when in the *HMR-trnaΔ-ADE2* background (our unpublished results). Mutations in *BDF2*, *RSC1*, and *GCN5* all exhibited a weaker phenotype in the mating assay, an effect that may be too weak to affect the *ADE2* promoter. However, we observed a strong increase in silencing upon deletion of *BDF1* in the mating assay, but this mutation did not give rise to pigmented colonies in the *HMR-ADE2* background. It should be noted that *bdf1* strains are very slow growing, and it is uncertain how growth rate might affect the accumulation of pigment in *ADE2* silenced strains.

The role of multiple chromatin-associated proteins in maintaining euchromatin-heterochromatin boundaries: Since the discovery and characterization of a discrete heterochromatin boundary downstream of the *HMR* locus (LOO and RINE 1994; DONZE *et al.* 1999; DONZE and KAMAKAKA 2001), many mutations have been uncovered that alter the integrity of the convergence point of heterochromatin with downstream euchromatin at this locus. As described here and in other studies, in addition to mutation of the *HMR*-tRNA or its associated RNA polymerase III complex proteins, loss of function of chromatin-associated complexes containing Yta7p, Rsc2p, Rpd3p, Sas2, -4, or -5 proteins, Htz1p, or Bdf1p leads to spreading of silencing through the *HMR* boundary element. These effects either appear to be targeted to the boundary region in the case of the RNA polymerase III complex, Yta7p, and Bdf1p (DONZE and KAMAKAKA 2001; LADURNER *et al.* 2003; TACKETT *et al.* 2005) or may act more globally to affect histone acetylation levels, as has been suggested for the SAS complex (KIMURA *et al.* 2002; SUKA *et al.* 2002).

Several recent studies have demonstrated that direct targeting of transcription factors or components of chromatin-modifying complexes can act as a barrier, preventing the spread of SIR-mediated heterochromatin in yeast (DONZE and KAMAKAKA 2001; FOUREL *et al.* 2001; CHIU *et al.* 2003; JACOBSON and PILLUS 2004; OKI *et al.* 2004). The barrier effect created by acetyltransferase targeting was not necessarily due to the enzymatic activity of acetyltransferase fusions, but did require the presence of other components of the respective complexes (CHIU *et al.* 2003; JACOBSON and PILLUS 2004; OKI *et al.* 2004), suggesting that targeting of a specific factor results in the recruitment of multiple proteins. Global models of heterochromatin barrier function suggest that acetyltransferases and deacetylases establish “negotiable borders” or “fuzzy boundaries” by convergence of the effects of their activities, without the

need for a specific barrier element or localized targeting (FOUREL *et al.* 2004; KIMURA and HORIKOSHI 2004). However, while both targeted and negotiable models are consistent with current data, the effects of global genome alteration (such as in a *sas2* background) on the normal targeting of chromatin-associated proteins is not well defined. Any natural targeting of proteins occurs within the context of normal cellular chromatin acetylation. Therefore, while domains of global histone acetylation may be negotiable in mutant strains, such genome-wide effects may lead to specific alterations in acetylation-state-dependent localization of proteins, such as the targeting of bromodomains.

Taken together, the current evidence suggests that euchromatin-heterochromatin boundaries are determined by both site-specific interactions and global influences. Sir-protein-dependent silencing in yeast appears to propagate by successive Sir2p deacetylation, Sir3p binding to deacetylated nucleosomes, and recruitment of the Sir4p-Sir2p complex, allowing spreading of the heterochromatic chromatin along a nucleosomal array after initial targeting of the SIR complex at silencers or telomeres (HOPPE *et al.* 2002; RUSCHE *et al.* 2002). In certain cases, simply excluding nucleosomes by binding of LexA to multimerized sites can block the spread of Sir-protein-mediated heterochromatin propagation in *S. cerevisiae* (BI *et al.* 2004), perhaps by simply distancing the next nucleosome from the previous Sir2-3-4-nucleosome complex. Part of the function of the *HMR*-tRNA may involve creating such a nucleosomal gap, as fully assembled RNA polymerase III complexes footprint close to 150 bp (CHEDIN *et al.* 1998; KASSAVETIS *et al.* 1998), approximately the length of the DNA occupied by a nucleosome. However, since the *HMR*-tRNA alone cannot provide full boundary function in a chromosomal context (our unpublished results), other chromatin-associated factors are clearly involved.

Additional studies will be required to identify the roles of bromodomains and other histone-binding proteins like CTF-1 (FERRARI *et al.* 2004) in establishing heterochromatin-euchromatin boundaries. Bdf1p has been shown to protect histone H4 from Sir2p deacetylation *in vitro* (LADURNER *et al.* 2003), suggesting one possible mechanism of bromodomain action in restricting silencing. However, bromodomains are known to bind to acetylated proteins other than histones (YANG 2004; DE LA CRUZ *et al.* 2005); therefore the effects on boundary establishment reported in this study may involve more than bromodomain-histone interactions. The results presented here suggest that the RSC complex may be acting through the *HMR*-tRNA, which is supported by the fact that RSC preferentially associates with tRNA genes *in vivo* (NG *et al.* 2002). However, it is currently unknown whether RSC is targeted to assembled RNA polymerase III genes or if prior RSC binding promotes RNA polymerase III complex assembly. Yta7p appears to be targeted to a limited set of chromosomal

sites (TACKETT *et al.* 2005), and while the mechanism of this targeting is unknown, it may involve the histone acetylation state near the boundary. The variability of bromodomain mutant phenotypes on boundary function may reflect a varied array of target proteins for bromodomain binding or could be a function of varied effects on the different multisubunit complexes in which most bromodomain factors have been identified. It will be of interest to determine the acetylation requirements for Yta7p binding at heterochromatin boundaries and how mutations that cause specific or global alterations in the acetylation states of histones, or possibly other chromatin-associated proteins, affect the targeting and function of boundary-associated bromodomain factors.

We thank David Stillman (University of Utah Health Sciences Center) for the *gcn5* allele, Rohinton Kamakaka (National Institute of Child Health and Human Development, Bethesda, MD) for the *snf2* and *htz1* alleles, and Andreas Ladurner (European Molecular Biology Laboratory, Heidelberg, Germany) for the *bdf1* and *bdf2* alleles. Research in the Donze laboratory is generously supported by grants from the Human Frontier Science Program (RGY11/2002) and the National Science Foundation (MCB-0342113).

LITERATURE CITED

- BI, X., and J. R. BROACH, 1999 UASrpg can function as a heterochromatin boundary element in yeast. *Genes Dev.* **13**: 1089–1101.
- BI, X., Q. YU, J. J. SANDMEIER and Y. ZOU, 2004 Formation of boundaries of transcriptionally silent chromatin by nucleosome-excluding structures. *Mol. Cell Biol.* **24**: 2118–2131.
- BURNS, N., B. GRIMWADE, P. B. ROSS-MACDONALD, E. Y. CHOI, K. FINBERG *et al.*, 1994 Large-scale analysis of gene expression, protein localization, and gene disruption in *Saccharomyces cerevisiae*. *Genes Dev.* **8**: 1087–1105.
- CAIRNS, B. R., Y. LORCH, Y. LI, M. ZHANG, L. LACOMIS *et al.*, 1996 RSC, an essential, abundant chromatin-remodeling complex. *Cell* **87**: 1249–1260.
- CARMEN, A. A., L. MILNE and M. GRUNSTEIN, 2002 Acetylation of the yeast histone H4 N terminus regulates its binding to heterochromatin protein SIR3. *J. Biol. Chem.* **277**: 4778–4781.
- CHEDIN, S., M. L. FERRI, G. PEYROCHE, J. C. ANDRAU, S. JOURDAIN *et al.*, 1998 The yeast RNA polymerase III transcription machinery: a paradigm for eukaryotic gene activation. *Cold Spring Harbor Symp. Quant. Biol.* **63**: 381–389.
- CHENNA, R., H. SUGAWARA, T. KOIKE, R. LOPEZ, T. J. GIBSON *et al.*, 2003 Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res.* **31**: 3497–3500.
- CHIU, Y. H., Q. YU, J. J. SANDMEIER and X. BI, 2003 A targeted histone acetyltransferase can create a sizable region of hyperacetylated chromatin and counteract the propagation of transcriptionally silent chromatin. *Genetics* **165**: 115–125.
- CHRISTIE, K. R., S. WENG, R. BALAKRISHNAN, M. C. COSTANZO, K. DOLINSKI *et al.*, 2004 *Saccharomyces Genome Database (SGD)* provides tools to identify and analyze sequences from *Saccharomyces cerevisiae* and related sequences from other organisms. *Nucleic Acids Res.* **32**: D311–D314.
- DE LA CRUZ, X., S. LOIS, S. SANCHEZ-MOLINA and M. A. MARTINEZ-BALBAS, 2005 Do protein motifs read the histone code? *BioEssays* **27**: 164–175.
- DHALLUIN, C., J. E. CARLSON, L. ZENG, C. HE, A. K. AGGARWAL *et al.*, 1999 Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **399**: 491–496.
- DION, M. F., S. J. ALTSCHULER, L. F. WU and O. J. RANDO, 2005 Genomic characterization reveals a simple histone H4 acetylation code. *Proc. Natl. Acad. Sci. USA* **102**: 5501–5506.
- DONZE, D., 2004 Breaking the histone code of silence: the propagation and blocking of heterochromatin. *Curr. Org. Chem.* **8**: 211–221.
- DONZE, D., and R. T. KAMAKAKA, 2001 RNA polymerase III and RNA polymerase II promoter complexes are heterochromatin barriers in *Saccharomyces cerevisiae*. *EMBO J.* **20**: 520–531.
- DONZE, D., and R. T. KAMAKAKA, 2002 Braking the silence: how heterochromatic gene repression is stopped in its tracks. *BioEssays* **24**: 344–349.
- DONZE, D., C. R. ADAMS, J. RINE and R. T. KAMAKAKA, 1999 The boundaries of the silenced HMR domain in *Saccharomyces cerevisiae*. *Genes Dev.* **13**: 698–708.
- EHRENHOFER-MURRAY, A. E., D. H. RIVIER and J. RINE, 1997 The role of Sas2, an acetyltransferase homologue of *Saccharomyces cerevisiae*, in silencing and ORC function. *Genetics* **145**: 923–934.
- FERRARI, S., K. C. SIMMEN, Y. DUSSERRE, K. MULLER, G. FOUREL *et al.*, 2004 Chromatin domain boundaries delimited by a histone-binding protein in yeast. *J. Biol. Chem.* **279**: 55520–55530.
- FISHER, C. R., 1969 Enzymology of the pigmented adenine-requiring mutants of *Saccharomyces* and *Schizosaccharomyces*. *Biochem. Biophys. Res. Commun.* **34**: 306–310.
- FOUREL, G., E. REVARDEL, C. E. KOERING and E. GILSON, 1999 Cohabitation of insulators and silencing elements in yeast subtelomeric regions. *EMBO J.* **18**: 2522–2537.
- FOUREL, G., C. BOSCHERON, E. REVARDEL, E. LEBRUN, Y. F. HU *et al.*, 2001 An activation-independent role of transcription factors in insulator function. *EMBO Rep.* **2**: 124–132.
- FOUREL, G., F. MAGDINIER and E. GILSON, 2004 Insulator dynamics and the setting of chromatin domains. *BioEssays* **26**: 523–532.
- HOPPE, G. J., J. C. TANNY, A. D. RUDNER, S. A. GERBER, S. DANAIE *et al.*, 2002 Steps in assembly of silent chromatin in yeast: Sir3-independent binding of a Sir2/Sir4 complex to silencers and role for Sir2-dependent deacetylation. *Mol. Cell Biol.* **22**: 4167–4180.
- HUDSON, B. P., M. A. MARTINEZ-YAMOUT, H. J. DYSON and P. E. WRIGHT, 2000 Solution structure and acetyl-lysine binding activity of the GCN5 bromodomain. *J. Mol. Biol.* **304**: 355–370.
- HUH, W. K., J. V. FALVO, L. C. GERKE, A. S. CARROLL, R. W. HOWSON *et al.*, 2003 Global analysis of protein localization in budding yeast. *Nature* **425**: 686–691.
- IIDA, T., and H. ARAKI, 2004 Noncompetitive counteractions of DNA polymerase epsilon and ISW2/ γ CHRAC for epigenetic inheritance of telomere position effect in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **24**: 217–227.
- JACOBSON, S., and L. PILLUS, 2004 Molecular requirements for gene expression mediated by targeted histone acetyltransferases. *Mol. Cell Biol.* **24**: 6029–6039.
- JACOBSON, R. H., A. G. LADURNER, D. S. KING and R. TJIAN, 2000 Structure and function of a human TAFII250 double bromodomain module. *Science* **288**: 1422–1425.
- JENUWEIN, T., and C. D. ALLIS, 2001 Translating the histone code. *Science* **293**: 1074–1080.
- KASSAVETIS, G. A., A. KUMAR, E. RAMIREZ and E. P. GEIDUSCHEK, 1998 Functional and structural organization of Brf, the TFIIB-related component of the RNA polymerase III transcription initiation complex. *Mol. Cell Biol.* **18**: 5587–5599.
- KIM, S., A. BENGURIA, C. Y. LAI and S. M. JAZWINSKI, 1999 Modulation of life-span by histone deacetylase genes in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **10**: 3125–3136.
- KIMURA, A., and M. HORIKOSHI, 2004 Partition of distinct chromosomal regions: negotiable border and fixed border. *Genes Cells* **9**: 499–508.
- KIMURA, A., T. UMEHARA and M. HORIKOSHI, 2002 Chromosomal gradient of histone acetylation established by Sas2p and Sir2p functions as a shield against gene silencing. *Nat. Genet.* **32**: 370–377.
- KURDISTANI, S. K., S. TAVAZOIE and M. GRUNSTEIN, 2004 Mapping global histone acetylation patterns to gene expression. *Cell* **117**: 721–733.
- LADURNER, A. G., C. INOUE, R. JAIN and R. TJIAN, 2003 Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. *Mol. Cell* **11**: 365–376.
- LOO, S., and J. RINE, 1994 Silencers and domains of generalized repression. *Science* **264**: 1768–1771.
- MARCHLER-BAUER, A., J. B. ANDERSON, P. F. CHERUKURI, C. DEWEESE-SCOTT, L. Y. GEER *et al.*, 2005 CDD: a conserved domain database for protein classification. *Nucleic Acids Res.* **33**: D192–D196.

- MEIJSING, S. H., and A. E. EHRENHOFER-MURRAY, 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., M. WU and H. D. MADHANI, 2003 Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. *Cell* **112**: 725–736.
- NG, H. H., F. ROBERT, R. A. YOUNG and K. STRUHL, 2002 Genome-wide location and regulated recruitment of the RSC nucleosome-remodeling complex. *Genes Dev.* **16**: 806–819.
- OKI, M., L. VALENZUELA, T. CHIBA, T. ITO and R. T. KAMAKAKA, 2004 Barrier proteins remodel and modify chromatin to restrict silenced domains. *Mol. Cell. Biol.* **24**: 1956–1967.
- OWEN, D. J., P. ORNAGHI, J. C. YANG, N. LOWE, P. R. EVANS *et al.*, 2000 The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. *EMBO J.* **19**: 6141–6149.
- REIFSNYDER, C., J. LOWELL, A. CLARKE and L. PILLUS, 1996 Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. *Nat. Genet.* **14**: 42–49.
- ROSS-MACDONALD, P., A. SHEEHAN, G. S. ROEDER and M. SNYDER, 1997 A multipurpose transposon system for analyzing protein production, localization, and function in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **94**: 190–195.
- ROSS-MACDONALD, P., A. SHEEHAN, C. FRIDDLE, G. S. ROEDER and M. SNYDER, 1999 Transposon mutagenesis for the analysis of protein production, function, and localization. *Methods Enzymol.* **303**: 512–532.
- RUNDLETT, S. E., A. A. CARMEN, R. KOBAYASHI, S. BAVYKIN, B. M. TURNER *et al.*, 1996 HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. *Proc. Natl. Acad. Sci. USA* **93**: 14503–14508.
- RUSCHE, L. N., A. L. KIRCHMAIER and J. RINE, 2002 Ordered nucleation and spreading of silenced chromatin in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **13**: 2207–2222.
- RUSCHE, L. N., A. L. KIRCHMAIER and J. RINE, 2003 The establishment, inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae*. *Annu. Rev. Biochem.* **72**: 481–516.
- SHIA, W. J., S. OSADA, L. FLORENS, S. K. SWANSON, M. P. WASHBURN *et al.*, 2005 Characterization of the yeast trimeric-SAS acetyltransferase complex. *J. Biol. Chem.* **280**: 11987–11994.
- SMITH, J. S., E. CAPUTO and J. D. BOEKE, 1999 A genetic screen for ribosomal DNA silencing defects identifies multiple DNA replication and chromatin-modulating factors. *Mol. Cell. Biol.* **19**: 3184–3197.
- STOTZ, A., and P. LINDER, 1990 The ADE2 gene from *Saccharomyces cerevisiae*: sequence and new vectors. *Gene* **95**: 91–98.
- SUKA, N., K. LUO and M. GRUNSTEIN, 2002 Sir2p and Sas2p oppositely regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat. Genet.* **32**: 378–383.
- SUN, Z. W., and M. HAMPSEY, 1999 A general requirement for the Sin3-Rpd3 histone deacetylase complex in regulating silencing in *Saccharomyces cerevisiae*. *Genetics* **152**: 921–932.
- TACKETT, A. J., D. J. DILWORTH, M. J. DAVEY, M. O'DONNELL, J. D. AITCHISON *et al.*, 2005 Proteomic and genomic characterization of chromatin complexes at a boundary. *J. Cell Biol.* **169**: 35–47.
- VANNIER, D., D. BALDERES and D. SHORE, 1996 Evidence that the transcriptional regulators SIN3 and RPD3, and a novel gene (SDS3) with similar functions, are involved in transcriptional silencing in *S. cerevisiae*. *Genetics* **144**: 1343–1353.
- WEST, A. G., M. GASZNER and G. FELSENFELD, 2002 Insulators: many functions, many mechanisms. *Genes Dev.* **16**: 271–288.
- XU, E. Y., S. KIM, K. REPLOGLE, J. RINE and D. H. RIVIER, 1999 Identification of SAS4 and SAS5, two genes that regulate silencing in *Saccharomyces cerevisiae*. *Genetics* **153**: 13–23.
- YANG, X. J., 2004 Lysine acetylation and the bromodomain: a new partnership for signaling. *BioEssays* **26**: 1076–1087.
- ZENG, L., and M. M. ZHOU, 2002 Bromodomain: an acetyl-lysine binding domain. *FEBS Lett.* **513**: 124–128.

Communicating editor: A. P. MITCHELL