A Noncanonical Bromodomain in the AAA ATPase Protein Yta7 Directs Chromosomal Positioning and Barrier Chromatin Activity[▽]

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Received 4 February 2009/Returned for modification 7 March 2009/Accepted 21 June 2009

Saccharomyces cerevisiae Yta7 is a barrier active protein that modulates transcriptional states at the silent mating locus, *HMR*. Additionally, Yta7 regulates histone gene transcription and has overlapping functions with known histone chaperones. This study focused on deciphering the functional role of the noncanonical Yta7 bromodomain. By use of genetic and epistasis analyses, the Yta7 bromodomain was shown to be necessary for barrier activity at *HMR* and to have overlapping functions with histone regulators (Asf1 and Spt16). Canonical bromodomains can bind to acetylated lysines on histones; however, the Yta7 bromodomain showed an association with histones that was independent of posttranslational modification. Further investigation showed that regions of Yta7 other than the bromodomain conferred histone association. Chromatin immunoprecipitation-chip analyses revealed that the Yta7 bromodomain was not solely responsible for histone association but was also necessary for proper chromosomal positioning of Yta7. This work demonstrates that the Yta7 bromodomain engages histones for certain cellular functions like barrier chromatin maintenance and particular Spt16/Asf1 cellular pathways of chromatin regulation.

Cellular processes such as gene transcription are governed on one level by epigenetics. Chemical modifications occur on chromosomal DNA and proteins to establish a given epigenetic state (e.g., CpG DNA methylation and histone acetylation/methylation/phosphorylation). Histones are rich with modifiable amino acids and thus can be found with particular sets of posttranslational modifications (PTMs) (16). The particular mark or set of marks establishes regions of active (euchromatin) and silent (heterochromatin) gene transcription. Chromatin binding proteins can utilize a variety of domains to recognize these modifications. One highly specialized domain is the bromodomain, which binds specifically to acetylated lysines and serves as a protein-protein interaction domain (10).

The bromodomain has been found in proteins ranging from histone acetyltransferases to transcriptional activators. The reported three-dimensional structures of bromodomains are highly conserved, consisting of a four-helix bundle (α_Z , α_A , α_B , and α_C) that has interhelical ZA and BC loops (10). The loops form a hydrophobic pocket that interacts with the acetylated lysine, with the ZA loop likely determining the binding dynamics. ZA and BC loops show high sequence diversity, but three amino acids are conserved for acetyllysine recognition (in *Saccharomyces cerevisiae* histone acetyltransferase Gcn5, these are Y364, Y406, and N407) (10, 11). Interestingly, the conserved Asn is not found in human TIF1 β —suggesting that this tran-

scription intermediate factor may not have acetyllysine specificity but may still serve as a non-PTM-dependent protein-protein interaction domain (10).

The yeast tat-binding analog 7 (Yta7) protein falls into the aforementioned category of proteins with potential noncanonical bromodomains, which may utilize the bromodomain as a protein-engaging module in a PTM-independent manner. Within the ZA loop, Yta7 has only two of five conserved amino acids necessary for Gcn5 binding to acetylated lysine but does contain two of three conserved amino acids needed for Gcn5 binding to the histone peptide backbone (11). This simple homology analysis led us to hypothesize that Yta7 contains a noncanonical bromodomain that modulates protein-protein interactions in an acetyllysine-independent manner. We postulated that the protein-protein association mediated by the Yta7 bromodomain (Yta7BD) directs a histone interaction because affinity purification of Yta7 yields a strong interaction with core histones (14). We also proposed that the Yta7BD is noncanonical, as in vitro binding studies revealed an association with the unmodified N-terminal tail of histone H3 (6). To test these hypotheses, we performed a functional analysis of the Yta7BD and further explored histone PTMs that may enhance bromodomain binding.

Yta7 is a barrier active protein modulating transcriptional states at the silent mating locus, *HMR* (8, 14). Deletion of the *YTA7* gene leads to silencing of genes near the *HMR* region, likely due to a misregulation of barrier chromatin maintenance (14). Further studies found that double deletion of the *YTA7* gene with histone chaperones or chromatin remodeling proteins impairs cell growth under stress conditions, suggesting that Yta7 plays a role in the maintenance of chromatin structure and transcriptional regulation (6). We recently showed that Yta7 binds histone proteins, associates with histone loci,

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[▽] Published ahead of print on 6 July 2009.

TABLE 1. *S. cerevisiae* strains used in this study

Genotype	Background	Source or reference
<i>MATα his3Δ1 leu2Δ0 lys2Δ ura3Δ0</i>	BY4742 WT ^a	Open Biosystems
<i>MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	BY4741 WT	Open Biosystems
<i>MATα ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	W303a WT	Frederick Cross, Rockefeller University
<i>MATα ade2 LYS URA3-HMR ppr1::HIS3</i>	W303 ROY508	3
<i>MATα ade2 LYS HMR::URA3 ppr1::HIS3</i>	W303 ROY648	3
<i>MATα yta7::KAN ade2 LYS URA3-HMR ppr1::HIS3</i>	W303 ROY508	This study
<i>MATα YTA7bd::MYC ade2 LYS URA3-HMR ppr1::HIS3</i>	W303 ROY508	This study
<i>MATα yta7::KAN ade2 LYS HMR::URA3 ppr1::HIS3</i>	W303 ROY648	This study
<i>MATα YTA7bd::MYC ade2 LYS HMR::URA3 ppr1::HIS3</i>	W303 ROY648	This study
<i>MATα yta7::KAN his3Δ1 leu2Δ0 lys2Δ ura3Δ0</i>	BY4742	Open Biosystems
<i>MATα hir2::KAN his3Δ1 leu2Δ0 lys2Δ ura3Δ0</i>	BY4742	Open Biosystems
<i>MATα hir2::KAN yta7::LEU2 his3Δ1 leu2Δ0 lys2Δ ura3Δ0</i>	BY4742	6
<i>MATα hir2::KAN YTA7bd::PrA his3Δ1 leu2Δ0 lys2Δ ura3Δ0</i>	BY4742	This study
<i>MATα YTA7bd::PrA his3Δ1 leu2Δ0 lys2Δ ura3Δ0</i>	BY4742	This study
<i>MATα yta7::KAN ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	W303	6
<i>MATα spt16-11 trp1 leu2 ura3 his7</i>	W303 8515-8-1	4
<i>MATα spt16-11 yta7::KAN ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	W303 8515-8-1	6
<i>MATα spt16-11 YTA7bd::PrA ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	W303 8515-8-1	This study
<i>MATα asf1::KAN his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	BY4741	Open Biosystems
<i>MATα asf1::KAN yta7::LEU2 his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	BY4741	6
<i>MATα asf1::KAN YTA7bd::PrA his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	BY4741	This study
<i>MATα YTA7bd::PrA his3Δ1 leu2Δ0 lys2Δ ura3Δ0</i>	W303	This study
<i>MATα yta7-1200-MYC_o ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	W303	This study
<i>MATα yta7bd-MYC_o ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100</i>	W303	This study
<i>MATα YTA7-1200-PrA his3Δ1 leu2Δ0 lys2Δ ura3Δ0</i>	BY4742	This study

^a WT, wild type.

and acts as a repressor of histone gene transcription (6). In addition to the bromodomain region, Yta7 contains one well-conserved and a second putative AAA ATPase domain (8). AAA ATPase domains contain Walker motifs for ATP hydrolysis as well as regions for protein-protein interactions (7). Since Yta7 is implicated in barrier chromatin maintenance, genetically overlaps with histone chaperone activities, and represses histone transcription, we wanted to better understand how the noncanonical bromodomain affects these cellular functions. Our results not only better define how the bromodomain affects Yta7 activity but also shed light onto the role of noncanonical bromodomains in modulating chromatin structure and function.

MATERIALS AND METHODS

Yeast strains. Yeast strains are listed in Table 1. Genomic tagging and domain deletions with PrA or Myc_o were done as reported previously (5, 6). To check for expression of Yta7 and Yta7BD in deletion strains, equal amounts of cell lysates from the strains indicated in Fig. 2B were subjected to Western blotting first for the PrA tag (Dako P0450; 1:100 dilution) on Yta7 (or the internal PrA tag used to delete the bromodomain) and second for general histone H3 (Abcam 1791; 1:5,000 dilution) as a loading control.

Epistasis analysis. For temperature sensitivity assays, cells were grown to saturation at 25°C, 10-fold serially diluted, spotted on yeast extract-peptone-dextrose plates, and incubated at increasing temperatures (25°C and 34°C). For damage assays, cells were grown to saturation at 25°C, 10-fold serially diluted, and spotted on yeast extract-peptone-dextrose plates containing hydroxyurea (HU; 0.1 and 0.2 M).

Barrier activity assay. The YTA7 gene or the YTA7 bromodomain was deleted in cells harboring a URA3 reporter in the transcriptionally expressed barrier (ROY508) and repressed (ROY648) areas of HMR (3). Cells were 10-fold serially diluted on plates with or without 5-fluoroacetic acid (5-FOA; 1 g/liter) and incubated at 30°C for 3 days. Expression of the URA3 gene in the presence of 5-FOA leads to cell death.

High-resolution ChIP-chip. Chromatin immunoprecipitation (ChIP)-chip analyses of YTA7-MYC and YTA7bdΔ-MYC cells were performed in quadruplicate

with dye swapping (13, 15). Full-genome *S. cerevisiae* microarrays were utilized (4 × 44K slides; Agilent). The ratio of immunopurified DNA signal to whole-cell extract was considered significant beyond 2 standard deviations from the mean array signal. Standard ChIP with real-time PCR readout was used to validate selected ChIP-chip results as previously described (14).

Transcription assay. ROY508, ROY508 yta7::KAN, and ROY508 YTA7bdΔ::MYC_o cells were grown to mid-log phase, and pellets were frozen in liquid nitrogen after being washed with cold water. Hot acidic phenol extraction of RNA was performed, and reverse transcription was conducted with the Invitrogen Superscript first-strand kit (14). Transcription levels of the *HTB1* and *ACT1* genes were determined by real-time PCR (6).

Affinity purification of YTA7 truncations. Strains shown in Fig. 5A were made by replacing segments of the YTA7 gene with a PrA (or Myc) tag or by inserting the tag at the indicated positions (5). Affinity purifications were done as previously reported (14). The amount of cell lysate used in the truncation analysis was normalized by Western blotting for the PrA tag. Histone- and Yta7-containing gel bands were identified by tandem mass spectrometry (MS).

Yta7BD binding to histones. For histone binding studies, 30 μg of recombinant Yta7BD-glutathione *S*-transferase (GST) (or GST control) was incubated with *S. cerevisiae* core histones (gift from Ines Pinto, University of Arkansas at Fayetteville). Core histones were purified in the presence of sodium butyrate to prevent histone deacetylase activity (12, 14). Histones and recombinant proteins were incubated in 500 μl of binding buffer (20 mM HEPES, pH 7.9, 25% glycerol, 100 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 1% Triton X-100, 0.2 μg/μl bovine serum albumin, 1:100 Sigma yeast protease inhibitor cocktail) for 2 h at room temperature. Yta7BD-GST or GST alone was collected for 2 h at room temperature with glutathione Sepharose 4B (Amersham Biosciences) and washed four times with low-salt buffer (4 mM HEPES, pH 7.9, 10 mM NaCl). Associated histones were eluted after the mixture was heated to 95°C for 10 min in sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer, resolved with a 4 to 20% Tris-glycine gel, and visualized by Coomassie blue staining. Histone-containing gel bands were chemically treated with *d*6-acetic anhydride and subjected to trypsin digestion (2, 14).

Histone peptide binding. Recombinant fusions of GST with the PHD finger of Yng1 and with the bromodomain of CBP were purified as reported previously and confirmed with MS (15). Histone peptide binding studies with GST-Yng1 (PHD finger) and GST-CBP (bromodomain) were performed as reported previously with buffers (and washing) as described above for Yta7BD binding methodology (6).

FIG. 1. The bromodomain of Yta7 is required for barrier activity. The *YTA7* gene or the bromodomain region of the gene was deleted in strains harboring a *URA3* reporter gene at the transcriptionally active barrier (*ROY508*) or silent region (*ROY648*) of *HMR*. Cells were 10-fold serially diluted on medium with or without 5-FOA. WT, wild type.

MS. Mass spectra of the histone peptides were collected with a matrix-assisted laser desorption ionization (MALDI)-prOTOF MS (PerkinElmerSciex). Peptides were confirmed by tandem MS with a vMALDI-LTQ ion trap MS (Thermo). m-over-z software was used to extract the monoisotopic peak areas for the heavy and light versions of each histone peptide. Quantitative measurements of acetylation were calculated from peak areas of fragment ions of samples treated with *d6*-acetic anhydride (14). The *d6*-acetic anhydride treatment converts unmodified lysines to triply deuterated acetyllysines (14). This allows one to differentiate chemically acetylated lysines (+45 Da) from in vivo-acetylated lysines (+42 Da) (14). Measurements of methylation were semiquantitative, as the isotopic label was an acetyl group and therefore may have had slightly different ionization properties. The percentage of methylation was obtained by calculating the light peptide area relative to total light- plus heavy-peptide area. Enrichment of acetylated or methylated peptides was evaluated by comparison to levels of the same modification in the input sample of histones.

RESULTS

The bromodomain of Yta7 is required for barrier activity.

Genomic deletion of *YTA7* results in the loss of barrier maintenance at the right- and left-hand barriers of *HMR* (8, 14). In accordance, we investigated the requirement for the Yta7BD in *HMR* barrier maintenance by using a *URA3* gene silencing assay (3, 6, 14). Two different cellular backgrounds lacking the Yta7BD were used: *ROY508*, which contains a *URA3* gene in the transcriptionally active left-hand barrier of *HMR*, and *ROY648*, which contains a *URA3* gene in the silenced *HMR* region (Fig. 1). *ROY508* cells died in the presence of 5-FOA, while *ROY648* cells that carry the *URA3* gene in the silenced region grew normally (Fig. 1). In contrast, *YTA7* gene deletion or Yta7BD deletion conferred survival of *ROY508* cells on 5-FOA plates. Deletions of *YTA7* in the *ROY648* background did not affect growth, thus demonstrating that the silent region

of *HMR* was not perturbed. These results show that the Yta7BD is necessary for maintenance of the left-hand barrier around *HMR*, as bromodomain deletion resulted in spreading of the silent transcriptional state and silencing of the *ROY508 URA3* gene reporter.

The functional overlap of Yta7 and certain histone regulators is bromodomain dependent. Previous epistasis analyses revealed that double deletion of the *YTA7* gene and certain histone regulators leads to growth defects under stress conditions. For example, cells harboring both a mutation in the yFACT (facilitates chromatin transcription) component *SPT16* (*spt16-11*) and a deletion of the *YTA7* gene exhibit temperature sensitivity and a mild HU sensitivity (6). Double deletion of known nucleosome assembly proteins (*HIR1*, *HIR2*, or *ASF1* gene products) in *yta7Δ* cells leads to temperature, methyl methanesulfonate, and/or HU sensitivity (6). We used the same epistasis analysis to investigate the importance of the Yta7BD.

The bromodomain of Yta7 was genomically replaced with a PrA tag (*YTA7bdΔ*). Cells were serially diluted and grown at elevated temperature (34°C) or under HU (0.1 M and 0.2 M) stress conditions. *YTA7bdΔ* cells with *hir2Δ*, *asf1Δ*, or *spt16-11* were subjected to the different treatments. The *spt16-11* point mutant of Spt16 was used because the gene deletion mutant is inviable and this mutant shows growth defects under the conditions utilized (4, 6). In *hir2Δ* cells exposed to high-temperature or HU treatment, deletion of the bromodomain had no effect on cell growth relative to controls (not shown). In contrast, deletion of the Yta7BD with *spt16-11* or *asf1Δ* showed an intermediate growth defect in comparison with the single (*YTA7bdΔ*, *spt16-11*, or *asf1Δ*) and double (*spt16-11 yta7Δ* or *asf1Δ yta7Δ*) gene deletions. The synthetic defect with *asf1Δ YTA7bdΔ* was mild but reproducible. Western blotting for the PrA tag on Yta7 or Yta7BD showed that the observed effects were not due to altered protein expression (Fig. 2B). Our results show that the Yta7BD contributes to certain Yta7 activities and that the bromodomain confers overlapping function with Spt16 and Asf1.

Deletion of the bromodomain genomically redistributes Yta7. We have shown that the bromodomain of Yta7 associates with histones via the N-terminal tail of histone H3 (6). Our data presented above suggest that the bromodomain impacts the function of Yta7 in barrier activity and exhibits overlapping functions with histone chaperones (Fig. 1 and 2). Therefore, we sought to better understand whether the bromodomain of Yta7 localized the activity of Yta7 to certain regions of chromosomes.

To determine the importance of the bromodomain for chromosomal positioning of Yta7, genome binding of Yta7 with or without the bromodomain was assessed using ChIP-chip analyses (Fig. 3A). Deletion of the Yta7BD resulted in a significant redistribution of Yta7 binding to more chromosomal sites (Fig. 3A). Only 104 binding sites (<15%) were conserved between *YTA7bdΔ-MYC* and *YTA7-MYC* cells. Bromodomain deletion led to a loss of binding in some sites, such as *GLN4* (Fig. 3B), or to a gain of binding such as at YCR095C, located next to the *HMR* region (Fig. 3C). Gaining and losing binding suggest that the bromodomain is not solely responsible for chromatin association but may actually fine-tune Yta7 chromosomal positioning. Our previous work shows that Yta7 binds to histone

FIG. 2. The functional overlap of Yta7 and certain histone regulators is bromodomain dependent. (A) To assay temperature sensitivity, indicated cells were 10-fold serially diluted and incubated at 25°C or 34°C for the indicated number of days (d). To assay HU sensitivity, cells were 10-fold serially diluted and incubated at 25°C for the indicated number of days. (B) Deletion/mutation of the Yta7BD, Asf1, or Spt16 does not affect protein levels of Yta7 or Yta7 with a bromodomain deletion. Western blotting was used to detect the PrA tag on Yta7 or the internal PrA tag used to delete the Yta7BD in the indicated strains. Western blotting for histone H3 served as a loading control.

loci to regulate S-phase transcriptional activation (6). In the present ChIP-chip analyses, histone locus binding was not lost upon deletion of the Yta7BD (Fig. 3D). We observed significant association of both Yta7 and Yta7BDΔ at the *HHT1/HHF1* and *HTB1/HTA1* loci. Binding distributions shown in Fig. 3B, C, and D were confirmed by conventional ChIP (Fig. 3E). To functionally verify Yta7BDΔ binding at the *HTB1/HTA1* locus, we used real-time PCR to measure the transcrip-

tion level of *HTB1* relative to that of the control gene *ACT1* (Fig. 3F). We observed that *YTA7* gene deletion caused an increase in *HTB1* transcription, while deletion of the bromodomain mimicked wild-type transcription. These results suggest that the Yta7BD is not required for physical retention or activity at histone loci.

The Yta7BD binds to histones in a PTM-independent manner. Functional and localization data suggest that the bromodomain of Yta7 is necessary for particular cellular functions (Fig. 1 to 3). To evaluate whether binding of the Yta7BD to histones is dependent on a particular set of histone PTMs, recombinant Yta7BD-GST fusion protein or GST alone was incubated with yeast core histones and affinity isolated on glutathione resin (Fig. 4A). Histones copurifying with Yta7BD-GST or GST alone were resolved on a denaturing gel and visualized with Coomassie blue staining. Compared to the GST control, all four histones were strongly enriched with Yta7BD (Fig. 4A). Under the conditions of the assay, histone octamers are likely dissociating; thus, the Yta7BD may have affinity for H3-H4 pairs, H2A-H2B pairs, and/or individual histones. In previous work, we observed a Yta7BD enrichment of *Tetrahymena thermophila* histone H3 and H2B (6). The conditions used in the experiment shown in Fig. 4A have been optimized relative to our initial work, which is evident in our now-minimal binding of histones to the GST control (and use of histones from *S. cerevisiae*). Thus, under the conditions used, Yta7BD was found to purify the core histones.

To determine if the buffer composition and resin washing procedure used in this approach would preserve protein-histone interactions, we performed histone peptide binding studies with two protein domains that bind histone PTMs (Fig. 4B). Recombinant GST fusions were made with the PHD finger from the yeast Yng1 protein and the bromodomain of human CBP. The Yng1 PHD finger shows a relatively strong and specific interaction with H3K4me3 (K_D [equilibrium dissociation constant] = 9 μM) (15). Canonical bromodomains show a weaker binding to the acetyllysine moieties on histones (K_D ≈ 100 to 400 μM) (1, 17). To test binding, the biotinylated histone peptides listed in Fig. 4B were coupled to streptavidin Dynabeads and incubated with the respective protein (6). Under the binding and washing conditions used in this work for Yta7BD, both the higher-affinity interaction of the Yng1 PHD finger with H3K4me3 and the lower-affinity interaction of the CBP bromodomain with a tetra-acetylated version of the H4 N-terminal tail were detected (Fig. 4B). These results indicate that any histone PTM enrichment with the Yta7BD pulldown should be detectable, since the relative association of histones with Yta7BD is greater than that with the CBP canonical bromodomain (Fig. 4A) (6).

Gel bands corresponding to histones in Fig. 4A were excised, treated with *d*6-acetic anhydride for quantitative analysis of histone acetylation, and subjected to in-gel trypsin digestion (6, 14). This *d*6-acetic anhydride treatment will heavily acetylate (45-Da) unmodified lysines while not affecting in vivo acetylations (42 Da), thereby providing an isotopic label for quantitation. Resulting tryptic histone peptides were subjected to quantitative MALDI-MS and MALDI-tandem MS analysis (6, 14). MS analysis identified all four histones, but posttranslationally modified peptides were identified only for H3/H2B/H4. As an example, the MALDI mass spectra of the histone

FIG. 3. Deletion of the bromodomain genomically redistributes Yta7. (A) ChIP-chip analysis of Yta7-Myc and Yta7BDΔ-Myc. A Venn diagram shows a redistribution of Yta7 binding with deletion of the bromodomain. Binding sites from the microarray are reported at the 95% confidence level. (B) Yta7 binding at *GLN4* was lost with bromodomain deletion. Binding is reported as the $\log_2$ of the immunopurified signal to whole-cell lysate array signals. The asterisk indicates significant binding (95% confidence level). (C) Bromodomain deletion resulted in detectable Yta7 binding at YCR095C. The asterisk indicates significant binding (95% confidence level). (D) Deletion of the bromodomain did not affect Yta7 association at the *HTB1-HTA1* histone locus. The asterisks indicate significant binding (95% confidence level). (E) Conventional ChIP for the Myc tag on Yta7 and Yta7BDΔ confirms the redistribution shown in panels B, C, and D. Error bars are the standard errors from triplicate analyses. (F) Bromodomain deletion does not affect *HTB1* transcription. Transcript levels of *HTB1* relative to *ACT1* in asynchronous cells harboring full-length Yta7 or the bromodomain deletion. Error bars are the standard deviations. Asterisks show significant differences determined by one-tailed testing ($P < 0.001$). WT, wild type.

H4 4-17 peptide is shown for the input and affinity-purified histone (Fig. 4C). The MS analysis of Yta7BD-associated histones relative to input histones did not reveal any enriched acetylation or methylation (Table 2), suggesting that histone association was PTM independent for the detected histone peptides.

Yta7 contains histone binding regions distinct from the bromodomain. The ChIP-chip studies in Fig. 3 suggest that Yta7 can maintain chromatin association without the bromodomain. To determine other regions of Yta7 that facilitate histone association, we constructed a series of truncations of or insertions in the *YTA7* gene (Fig. 5A). Each of these Yta7 proteins was affinity purified via the PrA tag, and copurifying histones were identified by MS (Fig. 5B). Interestingly, the two smallest constructs (1 to 400 and 1 to 650) copurified with the largest

amount of histones (Fig. 5B). Each construct longer than 650 amino acids exhibited histone binding comparable to that of full-length Yta7. In concurrence with those results, insertion of the PrA tag within the first 400 amino acids of the gene (at position 106) greatly impaired histone purification, suggesting that there is at least one additional histone binding region in the N-terminal 400 amino acids of the protein. The largely unstructured first 200 amino acids of Yta7 are 35% acidic amino acids with a pI of 4.3, while the remaining portion of the protein is 15% acidic amino acids with a pI of 5.7. This suggests that the acidic N-terminal region of Yta7 may have electrostatic interactions with charged, unmodified lysine and arginine residues on histones. One possibility is that the N-terminal region and the bromodomain function synergistically to provide histone binding and proper in vivo localization.

FIG. 4. The Yta7BD binds to histones in a PTM-independent manner. (A) Recombinant Yta7BD-GST or GST alone was incubated with purified histones and isolated on glutathione resin. Associated histones were resolved on Tris-glycine gels (4 to 20%) and visualized by Coomassie blue staining. (B) Yng1 PHD finger and the CBP bromodomain show specific binding under the Yta7BD binding assay conditions. Recombinant GST, GST-Yng1 PHD finger, or GST-CBP bromodomain was incubated with the indicated biotinylated histone peptides immobilized on streptavidin-coated Dynabeads. Bound protein was resolved by denaturing gel electrophoresis and visualized by Coomassie blue staining. (C) Quantitative analysis of histone acetylation copurifying with Yta7BD-GST. Copurifying and input histones shown in panel A were treated with *d6*-acetic anhydride and digested with trypsin. Shown are overlaid MALDI spectra of the N-terminal tail of histone H4 corresponding to peptide 4–17 containing K5, K8, K12, and K16 that have been chemically (45 Da) or in vivo (42 Da) acetylated. No significant difference in histone acetylation was observed. The complete set of results is in Table 2.

DISCUSSION

The Yta7 protein has reported activities in (i) the maintenance of barrier chromatin surrounding *HMR* and (ii) pathways involving histone-regulating proteins (like Spt16, Hir1/2, and Asf1) (6, 8). The functionality of the Yta7BD was tested for these various activities. Deletion of the bromodomain mimicked deletion of the entire *YTA7* gene for barrier maintenance activity at *HMR* (Fig. 1). Since an in vivo effect was observed upon deletion of a histone binding module, one would anticipate that Yta7 association at *HMR* had been perturbed. In contrast, upon removal of the bromodomain, Yta7 showed significant association with the barrier relative to that of wild-type Yta7 (Fig. 3C). This suggests that the barrier maintenance activity of Yta7 may be more of a transient association that is attenuated by the bromodomain. In support, removal of the bromodomain leaves the N-terminal acidic region of Yta7 to modulate the histone association, which showed increased histone binding relative to that observed with the bromodomain (Fig. 5). In accordance with these data, removal of the Yta7BD favors Yta7 association at the *HMR* barrier, which in turn

TABLE 2. Yta7BD does not enrich for specific histone PTMs

Histone	Residue(s)	PTM	Bromodomain enrichment ^a
H2B	K12, K15	ac1	0.96
		ac2	NR ^b
H3	K36	me	0.86
		me2	0.97
		me3/ac	1.00
	K56	ac	0.93
	K79	me	1.28
		me2	NR
		me3	0.85
H4	K5, K8, K12, K16	ac1	1.04
		ac2	NR
		ac3	NR
		ac4	NR

^a Ratio of Yta7BD enriched to input histone PTMs.
^b NR, very low to nonrecordable levels in both input and enriched.

FIG. 5. Yta7 contains histone binding regions distinct from the bromodomain. (A) Schematic of Yta7 constructs used to determine histone binding. The shapes represent: PrA tag (circles), AAA ATPase domain (filled squares), putative AAA ATPase domain (striped squares), and bromodomain (open squares). Constructs were made genomically. (B) Affinity purification of the constructs in panel A. Yta7 constructs were purified on immunoglobulin G-coated Dynabeads under conditions that preserve *in vivo* protein associations. PrA-tagged Yta7 and associated proteins were resolved by denaturing gel electrophoresis and visualized by Coomassie blue staining. Yta7 constructs and histones were identified by MS.

perturbs the maintenance of the barrier (Fig. 1 and 3C). One explanation of these observations is that Yta7 functions as a chromatin-organizing protein that serves a transient function in establishing or maintaining the barrier chromatin. In the case of a bromodomain deletion, the prolonged Yta7 activity serves as a bottleneck along the pathway for barrier maintenance.

In a similar situation of transient association, Yta7 shows binding at the *HTB1-HTA1* histone locus prior to gene transcription but dissociates during S-phase transcription of *HBT1* (A. Gradolatto and A. J. Tackett, unpublished observations). Thus, Yta7 binding and activity can be transient during the cell cycle. The transient association of Yta7 at the histone genes does affect gene transcription (Fig. 3F); thus, the normal dosage of histones is altered in the S phase and could have implications for the repackaging of chromatin during replication. Additional links of Yta7 to chromatin packaging were found with our epistasis analyses (Fig. 2). Deletion of the Yta7BD with *spt16-11* or *asf1Δ* showed an intermediate effect relative to that of full deletion of the *YTA7* gene and controls. These synthetic effects link the Yta7BD to pathways of chromatin assembly and disassembly for gene transcription.

To test whether the bromodomain targets Yta7 to particular chromatin sites, a ChIP-chip analysis was performed. Removal of the bromodomain did perturb wild-type binding, as Yta7BDΔ was largely redistributed across the chromosomes (Fig. 3A). We previously reported the avid association of Yta7 with histone loci (6). The histone locus association was preserved upon deletion of the bromodomain, and the bound Yta7BDΔ protein was functionally active for the regulation of histone gene transcription (Fig. 3D and F). The redistribution supports the finding that histone association is not solely conferred through the Yta7BD but additionally via the N-terminal acidic region (Fig. 5). Chromatin association through an acidic (~50%) C-terminal half of a protein has also been seen for the histone regulator Asf1 (9). Furthermore, the redistribution ef-

fect correlates with the mixed phenotypes observed with our epistasis analyses. These data support the idea that redistribution of Yta7 upon deletion of the bromodomain can alter particular activities of Yta7; however, retention of Yta7 through bromodomain-independent mechanisms occurs.

In previous work, we were unable to uncover a histone PTM that would enhance Yta7BD association with histones by using an *in vitro* system with synthetic histone peptides but rather found that additional PTMs perturbed the Yta7BD-histone H3 interaction (6). These analyses were expanded upon by utilizing purified yeast histones (Fig. 4A and Table 2). These studies also did not reveal a histone acetylation that was enriched with Yta7. It is possible that a given acetylation precluded our MS analysis; however, the femtomole-level sensitivity of the MS combined with the avid enrichment of histones with Yta7BD would be expected to identify any observable acetylated peptide. These results support the concept that the Yta7BD serves as a protein-protein interaction domain for PTM-independent histone association.

The data presented provide evidence that the Yta7 protein contains a noncanonical bromodomain that serves primarily as a histone recognition domain. The function of the bromodomain appears to be for chromatin localization in some cases and attenuation of binding in others. Specifically, the ability of Yta7 to engage histones via the bromodomain is needed for certain cellular functions like barrier maintenance at *HMR* and particular Spt16/Asf1 cellular pathways of chromatin regulation. Future work will focus on understanding the molecular details of how the bromodomain and the AAA ATPase domain confer *in vivo* activity.

ACKNOWLEDGMENTS

The following National Institutes of Health grants supported this work: R01DA025755, P20RR015569, P20RR016460, R01GM075152, and U54RR022220.

We acknowledge support from the Arkansas Biosciences Institute, David Terrano for critical reading, Ana Raman for technical assistance, Haitao Li and Dinshaw Patel for the GST-Yng1 (PHD) expression construct, and Phillip A. Cole and Blair Dancy for *Escherichia coli* containing induced GST-CBP (bromodomain).

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