

# Association of Taf14 with acetylated histone H3 directs gene transcription and the DNA damage response

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**The YEATS domain, found in a number of chromatin-associated proteins, has recently been shown to have the capacity to bind histone lysine acetylation. Here, we show that the YEATS domain of Taf14, a member of key transcriptional and chromatin-modifying complexes in yeast, is a selective reader of histone H3 Lys9 acetylation (H3K9ac). Structural analysis reveals that acetylated Lys9 is sandwiched in an aromatic cage formed by F62 and W81. Disruption of this binding in cells impairs gene transcription and the DNA damage response. Our findings establish a highly conserved acetyllysine reader function for the YEATS domain protein family and highlight the significance of this interaction for Taf14.**

Supplemental material is available for this article.

Received February 3, 2015; revised version accepted August 11, 2015.

Histone post-translational modifications (PTMs) play a fundamental role in the control of DNA-templated processes in the cell. While many PTMs mediate their effects through histone–histone or histone–DNA interaction, a large fraction of these PTMs function through the recruitment of chromatin-associated proteins that harbor conserved “reader” domains (Musselman et al. 2012; Rothbart and Strahl 2014). Histone lysine acetylation is a dynamic PTM that is typically associated with 5′ pro-

moter regions and transcriptionally active chromatin but also plays a role in nucleosome assembly and the DNA damage response (Shahbazian and Grunstein 2007; Bannister and Kouzarides 2011). Acetylation neutralizes the positively charged ε-amino group of lysine residues, which reduces electrostatic interactions that can intrinsically loosen chromatin structure, and serves to recruit bromodomain-containing effector proteins to facilitate the biological functions of these proteins (Grunstein 1997; Rothbart and Strahl 2014).

Until recently, bromodomains were considered the primary readers of histone acetylation (Dhalluin et al. 1999), and, consequently, the structures and biological significance of these domains have been studied extensively (Zeng and Zhou 2002; Filippakopoulos et al. 2012). However, a new acetyllysine reader domain was recently described in human AF9, a chromatin-associated protein that is a common fusion partner of the mixed-lineage leukemia (MLL) protein (Li et al. 2014). This study found that the YEATS domain of AF9 selectively recognized acetylated Lys9 on histone H3 (H3K9ac) to facilitate the recruitment of additional histone modifiers, thus highlighting the potential for other domains outside of bromodomains to interpret the acetyllysine mark (Li et al. 2014). However, it is unclear whether other YEATS domain-containing proteins act as acetyllysine readers to facilitate the DNA-templated process within chromatin, similar to AF9.

The budding yeast *Saccharomyces cerevisiae* has three YEATS domain-containing proteins: Sas5, Yaf9, and Taf14 (Fig. 1A). Together, these proteins are in nine chromatin- and transcription-associated complexes that mediate histone acetylation, chromatin remodeling, and transcription (Schulze et al. 2009). Structural studies of the Yaf9 (Wang et al. 2009) and Taf14 (Wen et al. 2011) YEATS domains show that they both adopt an immunoglobulin (Ig)-like elongated β-sheet sandwich similar to that described for AF9 (Li et al. 2014). Yaf9 is a member of the NuA4 histone acetyltransferase complex and SWR1 chromatin remodeling complex and was shown to have the capacity to bind to H3K27ac in vitro (Li et al. 2014). However, it is unclear whether the YEATS domains of Taf14 and Sas5 also recognize histone acetylation and, more importantly, how the YEATS domain contributes to the functional roles of these proteins.

In our efforts to uncover the role of the YEATS domain in these chromatin regulators, we found that the YEATS domain of Taf14 interacts with acetylated histone H3 with high preference for H3K9ac. The 1.9-Å-resolution crystal structure of the Taf14 YEATS domain in complex with the H3K9ac peptide reveals an elongated binding site with H3K9ac sandwiched in a threonine-lined aromatic cage formed by F62 and W81 of the protein. Importantly, disruption of the Taf14–H3Kac interaction in vivo impairs transcription and sensitizes cells to DNA damage. Our results define a critical role for this YEATS domain in mediating the functions of Taf14 and broadly establish the role of the YEATS domain as an acetyllysine reader.

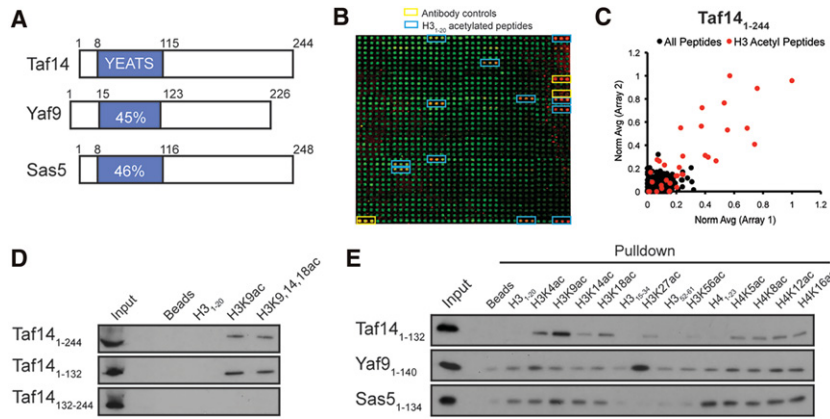
[*Keywords*: Taf14; YEATS domain; histone acetylation; DNA damage; gene transcription]

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Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.269977.115>.

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**Figure 1.** The YEATS domain of the transcriptional regulator Taf14 binds acetylated histone peptides. (A) Schematic of the YEATS proteins in *S. cerevisiae*. The YEATS domains in Yaf9 and Sas5 have 45% and 46% shared identity with the Taf14 YEATS domain. (B) Representative images of histone peptide microarrays of full-length Taf14. (C) A scatter plot of the relative binding intensity normalized to the most intense signal, highlighting binding of Taf14 to histone H3 acetylated peptides on duplicate arrays. (D) Western blot analyses of peptide pull-down assays with the corresponding GST-tagged Taf14 proteins and histone peptides. (E) Western blot analyses of peptide pull-down assays with the corresponding GST-tagged YEATS domains and histone peptides.

## Results and Discussion

### The YEATS domain of Taf14 selectively binds acetylated H3K9

To determine whether the YEATS-containing proteins in *S. cerevisiae* bind histone PTMs, we interrogated the ability of recombinant full-length Sas5, Yaf9, and Taf14 to associate with histone peptides on our microarray platform, which consists of core histone peptides (H2A, H2B, H3, and H4) harboring >250 unique single or multiple PTMs (Supplemental Table S1; Fuchs et al. 2011; Rothbart et al. 2012b). Of the three proteins examined, Taf14 showed a strong interaction with a variety of acetylated H3 peptides (Fig. 1B,C; Supplemental S1A), and this selective binding was mediated through the N-terminal YEATS domain as determined by in-solution peptide pull-down assays (Fig. 1D; Supplemental S1B,C). Further analysis of the exogenously expressed YEATS domain of Taf14 in both wild-type and histone deacetylase (HDAC)-deleted strains containing increased levels of histone acetylation further confirmed the ability of this domain to associate with acetylated histones (Supplemental Fig. S1D). As Taf14 is a member of the preinitiation complex of transcription (TFIID and TFIIF) and several chromatin-modifying complexes, including INO80, Swi/Snf, RSC, and NuA3 (see the schematic in Fig. 4A, below), these data suggest an important role for histone acetylation binding by Taf14 in one or more of these complexes' function.

Although peptide microarrays can interrogate a factor's association to histone peptides with individual or combinatorial PTMs, this approach is most effectively used to detect relatively high-affinity interactions. To explore the possibility that the YEATS domain of Taf14 as well as those found in Sas5 and Yaf9 might bind other acetylated histone peptides below the detection limit of the array platform, we next tested the PTM-binding specificity of these proteins using in-solution peptide pull-down assays (Fig. 1E). Analysis of the YEATS domain of Sas5 showed that it interacted with both unmodified H3 and

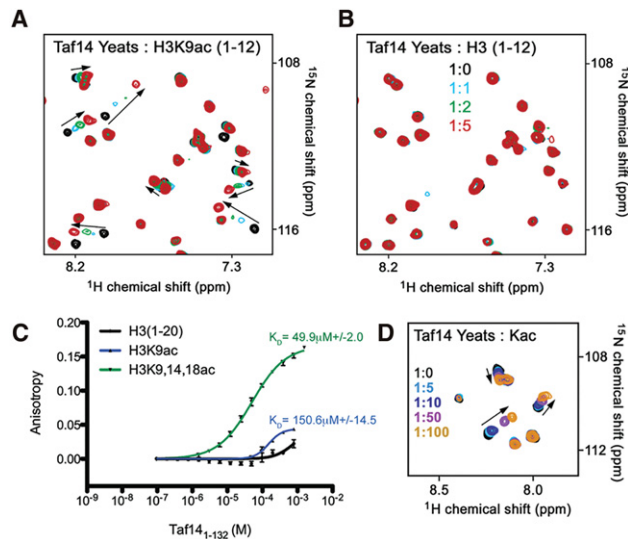
H4 N-terminal peptides, but the Yaf9 YEATS domain showed a preference instead for H3K27ac as previously described (Li et al. 2014). Notably, we found that the YEATS domain of Taf14 had no detectable interaction with unmodified histones and showed a strong preference for H3K9ac. A weak interaction was also observed for the H3K4ac, H3K14ac, H3K18ac, and H3K27ac peptides along with individual acetylation sites on the H4 N terminus (H4K5ac, H4K8ac, H4K12ac, and H4K16ac). However, the Taf14 YEATS domain exhibited a marked selectivity for acetyl-H3, as determined by fluorescence polarization (Supplemental Fig. S1C).

We next examined the known human YEATS domains found in AF9, ENL, and GAS41 to determine the extent to which they associate with acetylated histone H3. In agreement with a recent report, we found that the YEATS domain of AF9 showed a strong preference for H3K9ac (Supplemental Fig. S1E). Strikingly, we also found that the YEATS domains of ENL and GAS41 also have a preference for both H3K9ac and H3K27ac. Collectively, these results demonstrate that the YEATS domain is a highly conserved reader module of histone acetylation.

### The Taf14 YEATS domain forms an aromatic cage around H3K9ac

Given that Taf14 showed the highest specificity for binding acetylated peptides, we focused on this protein further to understand the structural basis and biological significance of the association with H3K9ac. To identify key residues involved in this interaction, nuclear magnetic resonance (NMR) chemical shift perturbation (CSP) experiments were carried out on uniformly  $^{15}\text{N}$ -labeled Taf14 YEATS (residues 1–132) (Fig. 2A). The  $^1\text{H}$ ,  $^{15}\text{N}$  heteronuclear single quantum coherence (HSQC) spectra of Taf14 YEATS revealed good dispersion of amide resonances, indicative of a well-folded protein. Upon addition of the H3K9ac<sub>1–12</sub> peptide, substantial changes in amide resonances were observed globally, confirming direct interaction between the protein and the peptide (Fig. 2A). Increasing the peptide concentration resulted in progressive shifting, broadening, and disappearance of cross-peaks. This pattern of CSPs indicated a strong interaction. Contrary to the H3K9ac peptide, no resonance changes were observed upon addition of an unmodified H3<sub>1–12</sub> peptide (Fig. 2B). Based on NMR CSP and fluorescence polarization experiments (Fig. 2A,C, respectively), the  $K_d$  for the interaction between the Taf14 YEATS and acetylated H3K9 was estimated to be 150  $\mu\text{M}$ . We observed a stronger interaction in the context of polyacetylated H3 (Fig. 2C). Interestingly, while this binding affinity for H3K9ac is ~50-fold higher than that of the human AF9 YEATS for H3K9ac, it is comparable with the binding affinity of the yeast Yaf9 YEATS for H3K27ac (Li et al. 2014). This suggests that tighter regulation of these marks may be necessary in more complex multicellular organisms.

The acetyl-dependent resonance changes observed in NMR CSP signify that acetylation of K9 is required for



**Figure 2.** Taf14 YEATS selectively binds H3K9ac. (A,B) Superimposed  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of the Taf14 YEATS domain (residues 1–132) collected upon titration with the H3K9ac<sub>1–12</sub> peptide (A) and unmodified H3<sub>1–12</sub> peptide (B). Spectra are color-coded according to the protein:peptide molar ratio. (C) Fluorescence polarization binding assays of the Taf14 YEATS domain with fluorescently labeled H3<sub>1–20</sub> (black), H3<sub>1–20</sub>K9ac (blue), and H3<sub>1–20</sub>K9,14,18ac (green) peptides. (D) Superimposed  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of the Taf14 YEATS domain collected upon titration with acetylated lysine (free amino acid).

the H3<sub>1–12</sub> peptide to be recognized by Taf14 YEATS. To determine what residues of the histone tail are involved in the interaction, the H3K9ac<sub>5–13</sub> peptide was tested. The H3K9ac<sub>5–13</sub> peptide induced a nearly identical resonance perturbation pattern in the Taf14 YEATS domain, although binding was slightly decreased as compared with binding of the H3K9ac<sub>1–12</sub> peptide (Supplemental Fig. S2A). Binding of a free acetylated lysine amino acid to the Taf14 YEATS domain led to CSPs primarily in an acetyllysine-binding pocket; however, an  $\sim 10$ -mM binding affinity suggested that the surrounding K9ac residues provide additional favorable interactions that enhance the affinity of Taf14 YEATS (Fig. 2D).

Of the acetylated lysine residues found in histones, H3K9 and H3K27 are surrounded by strikingly similar landscape (i.e., ARKS). Both the H3K9 and H3K27 sequences possess an A<sub>–2</sub>R<sub>–1</sub> and S<sub>–1</sub> relative to acetylated lysine. Therefore, we examined whether Taf14 could also recognize H3K27ac (Supplemental Fig. S2B). The CSPs observed in the Taf14 YEATS domain upon addition of the H3K27ac<sub>21–31</sub> peptide were similar in direction to CSPs seen upon addition of H3K9ac, indicating that the binding mode is conserved. However, the CSPs were smaller in magnitude, inferring that the binding is weaker. These results further corroborate pull-down experiments showing that the Taf14 YEATS domain selects for the H3K9ac mark.

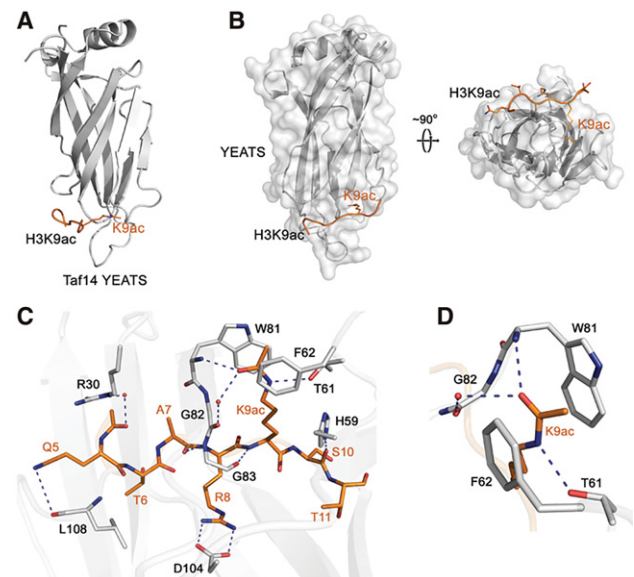
To establish the molecular mechanism for recognition of H3K9ac by the YEATS domain of Taf14, we determined the crystal structure of the Taf14–H3K9ac<sub>5–13</sub> complex to a resolution of 1.9 Å. In the complex, the Taf14 YEATS domain adopts a characteristic Ig scaffold (Fig. 3A). It folds into an elongated  $\beta$  sandwich composed of eight anti-parallel  $\beta$  strands, with two short N-terminal and C-terminal  $\alpha$  helices positioned at one of the open ends of the  $\beta$  sandwich. The opposite end of the  $\beta$  sandwich is capped by four

loops: the longest loop ( $\beta 1$ – $\beta 2$ ) and the three short loops ( $\beta 3$ – $\beta 4$ ,  $\beta 5$ – $\beta 6$ , and  $\beta 7$ – $\beta 8$ ) that create a binding site for the H3K9ac peptide (Fig. 3B).

Analysis of the Taf14:H3K9ac interface revealed a set of polar interactions between the main chain of H3K9ac and Taf14 (Fig. 3C). The N-terminal acetyl group, which mimics the backbone carbonyl of H3K4, forms a bridging water H bond with the guanidinium group of R30. The carbonyl group of H3A7 interacts with the backbone amide of G83. The main chain amide of K9ac is hydrogen-bonded to the carbonyl oxygen of G83. The imidazole group of H59 provides further stabilization of the H3 peptide via a H bond with the main chain carbonyl group of H3S10.

A number of intermolecular hydrogen bonds involve the side chains of the H3 peptide (Fig. 3C). The side chain amide of H3Q5 forms a hydrogen bond with the carbonyl oxygen of L108, and the guanidinium group of H3R8 forms a salt bridge with the carboxylate group of D104. The carbonyl oxygen of the K9ac side chain makes two H bonds: one via bridging water to the carbonyl group of G82 and the other with the main chain nitrogen amide of W81. The  $\epsilon$  nitrogen atom of K9ac is in an apparent H bond with the hydroxyl group of T61.

In addition to the polar interactions, the acetyl group of H3K9ac is sandwiched between two aromatic residues, F62 and W81, located in the  $\beta 3$ – $\beta 4$  and  $\beta 5$ – $\beta 6$  loops, respectively (Fig. 3D). This mode of K9ac recognition is reminiscent of that seen in the AF9:H3K9ac complex (Li et al. 2014), although the Taf14 YEATS domain has a threonine-lined aromatic cage as opposed to a serine-lined aromatic cage in AF9. Additionally, the AF9 YEATS domain contains a tyrosine residue in the positional equivalent of W81 (Fig. 3D).



**Figure 3.** Structural basis for the interaction between Taf14 YEATS and H3K9ac. (A) The overall structure of the Taf14 YEATS domain (gray) in complex with the H3K9ac peptide (orange) depicted as cartoon. (B) Surface representation of the Taf14 YEATS domain reveals a deep binding pocket for acetylated lysine. (C) Hydrogen-bonding network (blue dashes) between the H3K9ac peptide and the Taf14 YEATS domain. Water molecules are shown as red spheres. (D) Close-up view of the K9ac-binding pocket, with the hydrogen-bonding interaction represented by blue dashes.



To determine the importance of the aromatic cage for H3K9ac binding, a W81A mutant in the Taf14 YEATS domain was generated and examined by NMR. The lack of CSPs in the HSQC spectra of the Taf14 W81A mutant upon addition of the H3K9ac peptide implied that there is no binding (Supplemental Fig. S2C). This was further confirmed by in-solution peptide pull-down assays and chromatin association assays *in vivo* (Fig. 4B,C). These results demonstrate that the polar and electrostatic interactions observed between the peptide and protein are not sufficient to stabilize the complex and that the aromatic cage of Taf14 is essential for binding of H3K9ac.

#### *Disruption of the YEATS domain impairs transcription and the DNA damage response*

Taf14 is a member of at least three chromatin remodeling complexes, including INO80 (Shen et al. 2004), SWI/SNF, and RSC (Cairns et al. 1996; Kabani et al. 2005), as well as the NuA3 histone acetyltransferase complex (John et al. 2000) and the TFIID and TFIIF general transcription factor complexes (Fig. 4A; Henry et al. 1994). Given the observed interaction between H3K9ac and TAF14 YEATS, we postulated that the YEATS domain might be a critical determinant for the function of one or more of these complexes. We therefore screened for genetic interactions between the Taf14 YEATS domain and several genes involved in transcription and histone acetylation by generating *taf14Δ* cells that exogenously express full-length HA-tagged *TAF14* wild type or a YEATS domain point mutant (W81A), which are expressed at equivalent levels (Supplemental Fig. S3A). Under optimal growth conditions, the sickness of the *taf14Δ* strain was rescued to a similar degree by both wild-type *TAF14* and the *taf14<sub>W81A</sub>* mutant. In contrast, the YEATS point mutant exhibited increased heat and cold sensitivity (Fig. 4D). Further genetic analyses did not reveal any genetic interactions with the catalytic subunits of INO80, SWI/SNF, and NuA3 (Supplemental Fig. S3B), possibly due to redundant roles of other complex members in mediating histone interactions.

We next asked whether loss of Gcn5, a transcriptional coactivator and H3 acetyltransferase that shows synthetic genetic interactions with many of the Taf14-containing complexes (Grant et al. 1999; Howe et al. 2001; Barbaric et al. 2007; Roberts and Winston 1997), would result in a synthetic growth defect when Taf14 was deleted and, in particular, when the Taf14 YEATS–H3K9ac interaction is disrupted. Using a *GCN5* shuffle strain harboring a wild-type exogenous copy of *GCN5* covering the endogenous deletion of this gene (Gilbert et al. 2014), we found that loss of *GCN5* is lethal when combined with a deletion of *TAF14*, a result not previously reported (Supplemental Fig. S3C). As expected, expression of the wild-type version of *TAF14* was able to rescue lethality (Fig. 4E). In contrast, the expression of the *taf14<sub>W81A</sub>* mutant was unable to fully rescue the lethality caused by the double deletion (Fig. 4E). These data imply an important role for Taf14–H3ac interaction in coordinating transcriptional regulation with Gcn5.

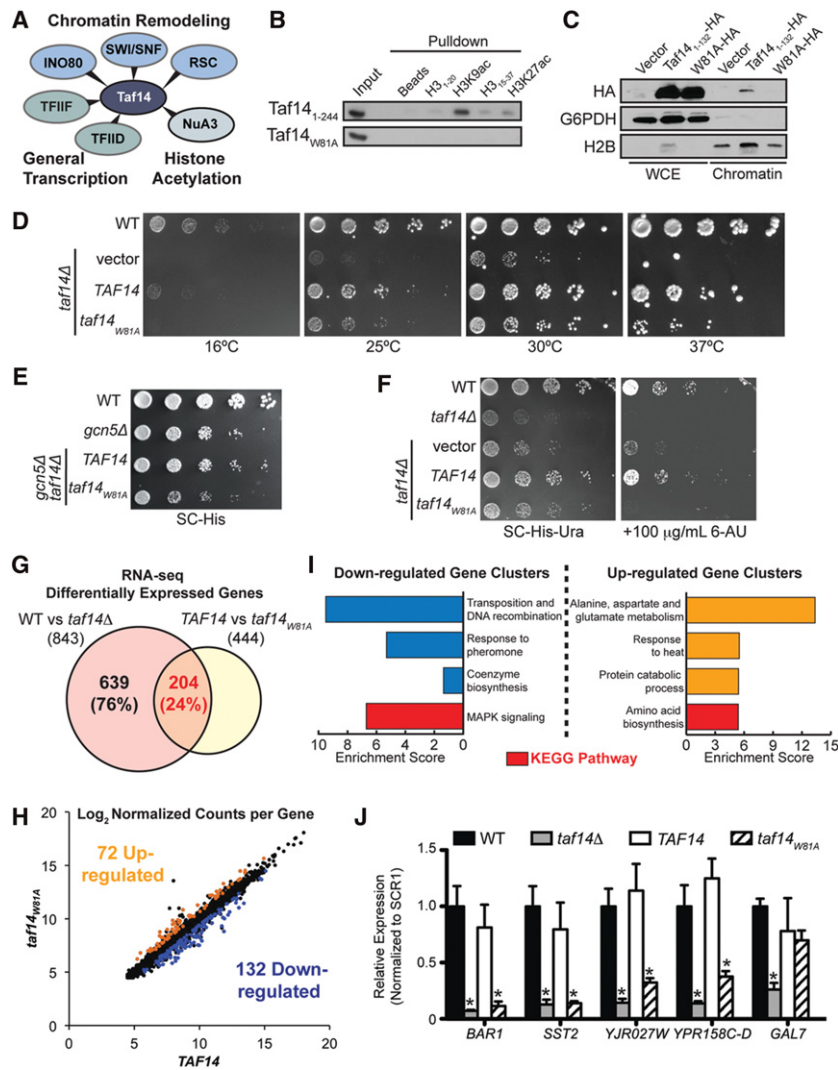
Consistent with a role of Taf14 in transcriptional regulation, we reconfirmed the previously shown sickness of *taf14Δ* in the presence of the transcriptional elongation inhibitor 6-azauracil (6-AU) (Fig. 4F; Schulze et al. 2010). Significantly, while exogenously expressed *TAF14* could rescue the 6-AU sickness, the *taf14<sub>W81A</sub>* mu-

tant was unable to rescue this growth. These results suggest that Taf14 functions in transcription at least in part through acetylated H3 interaction. In order to determine whether the YEATS domain globally impacted transcription, we performed high-throughput sequencing of transcripts expressed in wild-type and *taf14Δ* cells and compared them with transcripts expressed in *taf14Δ* cells expressing either full-length wild-type *TAF14*-HA or a *taf14<sub>W81A</sub>*-HA mutant. Of the 843 Taf14-dependent genes that were differentially expressed between wild-type and *taf14Δ* cells ( $P < 0.01$ , fold change  $> 1.5$ ), 204 genes (24%) were differentially regulated in cells expressing the YEATS point mutant compared with wild-type Taf14 (Fig. 4G). Of these YEATS-dependent Taf14 target genes, ~65% (132 genes) were down-regulated in *taf14<sub>W81A</sub>* (Fig. 4H). These genes were involved in transposition, DNA replication, pheromone response, coenzyme biosynthesis, and MAPK signaling (Fig. 4I). Genes involved in amino acid metabolism and heat response were enriched in the genes up-regulated in *taf14<sub>W81A</sub>* (Fig. 4I). Further validation by quantitative real-time PCR confirmed the YEATS-dependent expression of several of these genes, while other Taf14-dependent genes did not require a functional YEATS domain for proper expression (Fig. 4J). Collectively, these results demonstrate an important role for the Taf14 YEATS domain in transcriptional regulation.

It had been shown previously that Taf14 plays a role in the DNA damage response, and *taf14Δ* cells are sensitive to DNA-damaging agents such as UV light, methane methylsulfonate (MMS), and hydroxyurea (HU) (Zhang et al. 2004). Taf14 also genetically interacts with both Rad5 and Rad6, which are involved in the post-replication repair process (Erlich et al. 2008). Given the connection of Taf14 to DNA repair, we postulated that the YEATS domain of Taf14 and its ability to bind to H3 acetylation might also be important for the proper function of Taf14 in the response to genotoxic stress. To examine this, we screened *taf14Δ* cells expressing wild-type or *taf14<sub>W81A</sub>* for sensitivity to UV, MMS, and HU. We found that disruption of the Taf14 YEATS domain significantly impaired the response to MMS (Supplemental Fig. S3D). However, sensitivity to other DNA-damaging agents (UV, HU, and phleomycin) observed in the *taf14Δ* strain was rescued with both the wild type and the *taf14<sub>W81A</sub>* mutants (Supplemental Fig. S3D). These results reveal that H3 acetyl binding by Taf14 is required for some, but not all, forms of DNA repair, a result that may be explained through its association with one or more complexes that are also sensitive to MMS, such as SWI/SNF, INO80, RSC, or the Mediator complex (Erlich et al. 2008).

#### *Concluding remarks*

The YEATS domain is a conserved domain found in >100 proteins from at least 50 different species (Schulze et al. 2009). Many YEATS-containing proteins are associated with transcription and chromatin, including the three YEATS proteins found in budding yeast (Fig. 1A). The YEATS domain of the human protein AF9 was recently found to be an acetyllysine reader domain (Li et al. 2014), and we here established this conserved function of the YEATS domain in budding yeast. Our structural analysis of the Taf14 YEATS domain in complex with H3K9ac peptide reveals that the YEATS domain forms an aromatic cage around the acetyllysine residue.



**Figure 4.** The Taf14 YEATS domain regulates transcription and DNA damage response. (A) Schematic of the Taf14-containing complexes in *S. cerevisiae* with the known interacting members listed. (B) Western blot analysis of peptide pull-down assays performed with full-length Taf14 showing disruption of H3K9ac binding when the aromatic cage residue W81 is mutated. (C) Chromatin association assays with wild-type or W81A mutant Taf14 YEATS-HA expressed in wild-type BY4741 showing loss of chromatin association in the point mutant. (D) Spotting assays with BY4741 or *taf14Δ* strains transformed with empty vector or plasmids expressing HA-tagged full-length wild-type or *taf14*<sub>W81A</sub> at the indicated temperatures. (E) Spotting assays with wild-type (BY4741) or *gcn5Δ* strains demonstrating that Taf14 YEATS acetyl binding becomes necessary for normal cell growth in the context of depleted histone acetylation levels. (F) Spotting assays with wild-type (BY4741) or *taf14Δ* strains transformed with the indicated plasmids and grown on control (SC-Ura-His) and 6-azauracil (6-AU) medium. (G) Venn diagram of differentially expressed (DE) genes identified by RNA sequencing ( $P < 0.01$ , fold change  $> 1.5$ ). Of the 843 DE genes identified in *taf14Δ* (red), 204 genes (24%) were also identified as DE between TAF14 and *taf14*<sub>W81A</sub> (yellow). (H) Scatter plot of log<sub>2</sub>-normalized gene counts in TAF14 and *taf14*<sub>W81A</sub>. Of the 204 genes identified in G, 72 genes were up-regulated (orange), and 132 genes were down-regulated (blue) in *taf14*<sub>W81A</sub>. (I) Gene ontology analysis of the 204 genes identified in G, showing that up-regulated genes and down-regulated genes were enriched in several molecular functions (enrichment scores shown in orange and blue, respectively) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (red). (J) Quantitative RT-PCR showing reduced transcription of several genes in both *taf14Δ* and *taf14*<sub>W81A</sub>. In contrast, *GAL7* transcript levels do not depend on Taf14 YEATS. Error bars represent standard deviations of three biological replicates. (\*)  $P < 0.05$  compared with wild type.

We further demonstrate a critical role for the Taf14 YEATS domain in mediating transcription and the DNA damage response. Because Taf14 is a member of at least

six key chromatin-associated complexes, defining the function of Taf14 and, in particular, its YEATS domain in each complex will be a challenge for future studies. Our study focused on determining whether there would be a function for the Taf14 YEATS domain in two key chromatin-templated processes: transcription and DNA repair. We provide evidence that the YEATS domain of Taf14 regulates transcription in a YEATS–acetyl-binding manner. With respect to DNA damage, Taf14 has been shown previously to play a role in post-replication repair induced by the alkylating agent MMS, probably through several of its associated complexes, including SWI/SNF, INO80, RSC, and the Mediator complex (Erich et al. 2008). We further established this role and determined that the YEATS domain of Taf14 is a critical determinant in the response to DNA damage induced by MMS. While the studies presented here further our understanding of Taf14 and YEATS domain function, it will be interesting to understand how the YEATS domains of Sas5 and Yaf9 as well as the other uncharacterized human YEATS domains contribute to chromatin regulation.

## Materials and methods

### Peptide microarray

The peptide microarray platform was generated as described previously (Fuchs et al. 2011; Rothbart et al. 2012a) with the exception that the array contained four triplicate spots of each peptide listed in Supplemental Table S1.

### In-solution peptide pull-down assays

In-solution peptide pull-down assays were performed as described previously (Rothbart et al. 2012b) with the exception that 500 pmol of biotinylated peptide was immobilized on streptavidin-coated magnetic beads (Pierce) prior to incubation with 40 pmol of protein for 1 h at 4°C with rotation. Bound protein was eluted from the beads with 1× SDS buffer and analyzed by Western blot detection with GST antibody (Sigma).

### X-ray crystallography

Taf14 (residues 1–138) was purified as described in the Supplemental Material and incubated for 10 min at room temperature with the H3K9ac<sub>5–13</sub> peptide (acetylated at the N terminus) in a 1:5 molar ratio prior to crystallization. Crystals of the complex were grown using the sitting-drop diffusion method at 25°C by mixing 800 nL of protein–peptide solution with 800 nL of well solution composed of 0.2 M sodium citrate (pH 5.5) and 48% (v/v) PEG600. X-ray diffraction data were collected from a single crystal on beamline 4.2.2

at the Advance Light Source administered by the Molecular Biology Consortium. Processing of raw diffraction data and further structural refinement are described in the Supplemental Material. Data and refinement

statistics are summarized in Supplemental Table S2. Structural data have been deposited to Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank with accession number 5D7E.

### NMR spectroscopy and fluorescence polarization

NMR experiments and fluorescence polarization were performed as described in the Supplemental Material.

### Spotting assays

Spotting assays were performed with serial dilutions of saturated overnight cultures of the indicated yeast strains, and growth was assessed after 2–3 d. All yeast strains used in this study are described in Supplemental Table S3, and plasmids used in this study are presented in Supplemental Table S4. Chromatin association assays are described in the Supplemental Material.

### RNA sequencing and quantitative PCR

Total RNA was extracted from triplicate log-phase cultures of BY4741 (wild type) transformed with pRS313-HA3-SSN6 and *taf14Δ* transformed with pRS313-HA3-SSN6 (empty vector), full-length pRS313-*TAF14*-HA3-SSN6, or pRS313-*taf14<sup>WR1A</sup>*-HA3-SSN6 cultured in synthetic complete medium lacking histidine. Library preparation and sequencing were performed at the University of North Carolina High-Throughput Sequencing Facility. Details of the methods used and downstream analyses are described in the Supplemental Material. All data have been made publicly available through Gene Expression Omnibus (accession no. GSE71768). Quantitative PCR was performed as described in the Supplemental Material.

### Acknowledgments

We thank Mark Bedford for his contribution of the human YEATS proteins, and Michael Keogh for the pRS313-HA3-SSN6 vector. This work was supported by National Institutes of Health grants R01 GM110058 to B.D.S. and GM100907 and GM101664 to T.G.K. E.K.S. was supported by National Institute of General Medical Sciences Institutional Research and Academic Career Development Award grant K12-GM000678, and F.H.A. was supported by National Institutes of Health grant T32AA007464. S.L.M. and J.V.D. were supported in part by a grant from the National Institute of General Medical Sciences under award 5T32 GM007092.

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