INVESTIGATION

Chromatin Mediation of a Transcriptional Memory Effect in Yeast

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ABSTRACT Previous studies have described a transcriptional “memory effect,” whereby transcript levels of many Abf1-regulated genes in the budding yeast Saccharomyces cerevisiae are undiminished even after Abf1 has dissociated from its regulatory sites. Here we provide additional support for this effect and investigate its molecular basis. We show that the effect is observed in a distinct abf1 ts mutant from that used in earlier studies, demonstrating that it is robust, and use chromatin immunoprecipitation to show that Abf1 association is decreased similarly from memory effect and transcriptionally responsive genes at the restrictive temperature. We also demonstrate that the association of TATA-binding protein and Pol II decreases after the loss of Abf1 binding for transcriptionally responsive genes but not for memory effect genes. Examination of genome-wide nucleosome occupancy data reveals that although transcriptionally responsive genes exhibit increased nucleosome occupancy in abf1 ts yeast, the promoter regions of memory effect targets show no change in abf1 ts mutants, maintaining an open chromatin conformation even after Abf1 eviction. This contrasting behavior reflects different inherent propensity for nucleosome formation between the two classes, driven by the presence of A/T-rich sequences upstream of the Abf1 site in memory effect gene promoters. These sequence-based differences show conservation in closely related fungi and also correlate with different gene expression noise, suggesting a physiological basis for greater access to “memory effect” promoter regions. Thus, our results establish a conserved mechanism underlying a transcriptional memory effect whereby sequences surrounding Abf1 binding sequences affect local nucleosome occupancy following loss of Abf1 binding. Furthermore, these findings demonstrate that sequence-based differences in the propensity for nucleosome occupancy can influence the transcriptional response of genes to an altered regulatory signal.

KEYWORDS transcription yeast Abf1 chromatin nucleosome ChIP-seq

Abf1 is an essential, abundant general regulatory factor found in Saccharomyces cerevisiae and conserved in other fungi (Wapinski et al. 2007). Abf1 is involved in transcriptional activation, DNA replication, DNA repair, and gene silencing (Hardy et al. 1992; Miyake et al. 2007). Abf1 regulates approximately 200—300 targets in yeast, including the ABF1 gene itself, which it negatively regulates (Miyake et al. 2004; Yarragudi et al. 2007). Regulatory responsibilities include transcriptional activation/repression of various gene families involved in amino acid transport, carbon source regulation, sporulation, meiosis, and mitochondrial function (de Boer et al. 2000; de Winde and Grivell 1992; Loo et al. 1995; Schlecht et al. 2008; Silve et al. 1992). Previous work demonstrated that Abf1 is able to outcompete histones for occupancy of its binding site at natural and synthetic locations (Venditti et al. 1994; Yarragudi et al. 2004). Additionally, increased nucleosome occupancy was observed at numerous promoter sites throughout the yeast genome upon Abf1 depletion, indicating that Abf1 maintains an open chromatin conformation at its binding sites (Badis et al. 2008; Ganapathi et al. 2011; Hartley and Madhani 2009).
Experiments performed previously in \textit{abf1-1 ts} yeast demonstrated a transcriptional memory effect occurred at select \textit{Abf1}-regulated targets at the restrictive temperature (Schroeder and Weil 1998). Continuous binding of transcriptional activators to their cognate promoter binding sites is generally viewed as being necessary for ongoing transcription (Ho et al. 1996). However, Schroeder and Weil (1998) showed that in an \textit{abf1-1 ts} mutant, mRNA levels of several genes known to depend on \textit{Abf1} binding sites for activation were undiminished after 1 hr at the restrictive temperature (Schroeder and Weil 1998). DMS footprinting showed loss of \textit{Abf1} binding under these same conditions, suggesting that these target genes retained a molecular memory of \textit{Abf1} binding that allowed their continued transcription at the restrictive temperature. Later examination of genome-wide expression in \textit{abf1-1 ts} yeast revealed at least 80 \textit{Abf1} probable regulatory targets that did not show significantly reduced transcription at 37°C, whereas 37 stringently defined \textit{Abf1}-regulated target genes showed reduced mRNA levels by at least 1.5-fold (Yarragudi et al. 2007). We refer to these two classes of \textit{Abf1} targets, which in fact represent two ends of a continuum, as “memory effect” and “transcriptionally responsive” genes, respectively.

The molecular mechanisms responsible for the transcriptional memory effect exerted at \textit{Abf1}-regulated genes are unknown. We examined previously the possibility that memory effect genes use different \textit{Abf1} binding motifs than do promoters of transcriptionally responsive targets but found identical \textit{Abf1} consensus motifs for the two classes (Yarragudi et al. 2007). Here we examine other possible causes of this memory effect and identify an evolutionarily conserved, sequence-directed propensity for nucleosome formation as a distinguishing feature and likely contributing factor to this phenomenon.

### MATERIALS AND METHODS

**Yeast strains and growth**

Yeast strains are listed in Table 1. Binding site mutants were constructed using the delitto perfetto technique to replace the 14-bp \textit{Abf1} binding site with the sequence CTACTAGTTA or its complement depending on the orientation of the \textit{Abf1} binding site (Storici and Resnick 2006). Yeast cells were grown in rich medium, Yeast Peptone Dextrose [1% bacto-yeast extract (10 g/L), 2% bacto-peptone extract (20g/L), 2% glucose (20g/L) in dH2O]. For temperature shift experiments, cultures were grown to mid-log phase (OD\(_{600}\) = 0.6–1.2), diluted to OD\(_{600}\) = 0.3–0.5 and allowed to grow for 2 hr at the permissive temperature (25°C). Culture density was measured to ensure doubling, and cultures were shifted to the restrictive temperature (37°C) by adding prewarmed media and incubated at 37°C for 60 min. All other cultures were grown to mid-log phase (OD\(_{600}\) = 0.6–1.2), diluted to OD\(_{600}\) = 0.3–0.5 and allowed to grow for 2 hr at 30°C.

**Chromatin immunoprecipitation (ChIP) and library preparation and amplification**

Whole-cell extracts were prepared from 50 mL of yeast cells as described previously (Ansari et al. 2012). Conventional ChIP was performed as described previously, using 180 μL of whole-cell extracts with the following antibodies: α-\textit{Abf1} (5 μg; Santa Cruz Biotechnology; Santa Cruz, CA), α-TBP (2.5 μg; gift from PA Weil, Vanderbilt University, TN), and α-Rpb3 (1 μg; Neoclon; Madison, WI; www.neoclon.com). Bar-coded libraries for ChIP-seq were prepared as recently described (Paul et al. 2015) and were sequenced at the University of Buffalo Next-Generation Sequencing and Expression Analysis Core (University at Buffalo, State University of New York; Buffalo, New York).

**RNA isolation and cDNA synthesis**

Total RNA isolation was carried out from 10-mL cultures using the Hot Phenol protocol adapted from Schmitt et al. (1990). RNA levels were quantified by quantitative real-time polymerase chain reaction of cDNA generated using First-Strand cDNA Synthesis Kit for Real-Time PCR (USB/Affymetrix, Cleveland, OH). For cDNA synthesis, either a mix of oligo dT and random hexamers (proprietary concentrations) or a mix of appropriate gene-specific oligonucleotides was used for reverse transcriptase primers. Reactions were run at 44°C for 1 hr and 92°C for 10 min, as per manufacturer’s recommendation.

**Quantitative Real-Time Polymerase Chain Reaction**

Quantitative real-time polymerase chain reaction was conducted as described previously (Ansari et al. 2012) using a StepOnePlus Real Time PCR System (Applied Biosystems/Life Technologies, Green Island, NY). Technical replicates of individual 12.5-μL reactions were averaged prior to averaging biological replicates and calculating standard deviations. Reactions were run on Fast protocol (20 sec at 95°C, followed by 40 cycles of 1 sec at 95°C and 20 sec at 60°C; data capture was at end of extension period). Relative enrichment for (ChIP) or expression levels (for RNA, following cDNA synthesis) were obtained by determining the differences in Ct values compared with a reference; the values obtained this way were rescaled for presentation by addition of a constant. Primers are for coding regions or \textit{Abf1} binding sites in gene promoters and are listed in Table 2.

### Computational analysis

Average profiles for memory effect and transcriptionally responsive genes were generated using data for 69 transcriptionally responsive targets and 64 memory effect targets (Yarragudi et al. 2007) containing unique \textit{Abf1} sites. ChIP-seq data were analyzed with the ArchiTEx program (Lai et al. 2012) and Z-scored to normalize for variance between experiments after calculating the log\(_2\) ratio. To obtain Z-scores, sequence tags were first extended to a total length of 120 bp to account for the gap between forward and reverse reads and the total number of tags were summed at each base pair in the genome. Counts were divided at each base pair by the genomic average (120 bp × total sequence tags / genome size) and log\(_2\) values were generated for each ratio. The mean and variance of log\(_2\) ratios were calculated across the genome and normalized to N(0,1) for every base pair to yield Z-scores. Peaks were identified using MACS (P < 10\(^{−5}\)) (Zhang et al. 2008); peaks identified using this relatively low stringency still generally showed decreased magnitude in \textit{abf1-101 ts} yeast.

Predicted and in vitro nucleosome occupancy profiles were generated using the algorithm and data from Kaplan et al. (Kaplan et al. 2009). dA/dT motifs that disfavor nucleosome occupancy were defined as any 7bp sequence that contains at least 6 As or 6 Ts. The frequency of such sequences was examined in 150-bp windows surrounding each \textit{Abf1} motif, and these frequencies were averaged over the responsive vs. the memory-effect genes (Figure 4C). This analysis was performed for \textit{S. cerevisiae} as well as for four closely related species, using aligned promoter sequences defined previously (Clifton et al. 2003; Kellis et al. 2003). Average expression noise was calculated using data from Newmann et al. which was centered at zero (Newman et al. 2006), and error bars were defined by bootstrapping: genes having “open” (DPN, depleted proximal nucleosome) and “closed” (OPN, occupied promoter nucleosome) promoter nucleosome configurations are taken from Tsui et al. (2011).
Table 1 Yeast strains used in this study

| Strain            | Genotype                              | Reference                           |
|-------------------|---------------------------------------|-------------------------------------|
| TMY86             | Mata ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 abf1Δ::HIS3MX6 [pRS416-ABF1] | (Miyake et al. 2002)               |
| abf1-1            | TMY86-1/pPM629; pRS415-abf1-1         | (Miyake et al. 2002)               |
| BY4741            |                                       | Yeast Deletion Library              |
| CYBY11502         | Mat a, his3Δ1, leu2Δ0, ura3Δ0, met15Δ0, abf1-101 ts::KanMX | Gift from C. Boone                   |
| Z579              | Mat a, his3Δ200, leu2-3,122, ura3-52, srb4Δ2::HIS3 [pCT181/RY2882/SRB4 LEU2 CEN] | (Thompson and Young 1995)           |
| Z111              | Mat alpha, ura3-52, his3Δ200, leu2-3,112, rpb1-1, ade2 | (Nonet et al. 1987)                |
| EPY10             | BY4741/mutant Abf1 binding site (10mer) @ pRPS28A | This study                           |
| EPY26             | BY4741/mutant Abf1 binding site (10mer) @ pRPL3 | This study                           |
| EPY66             | BY4741/mutant Abf1 binding site (10mer) @ pCNY1 | This study                           |
| EPY67             | BY4741/mutant Abf1 binding site (10mer) @ pAR03 | This study                           |

Accession number
ChIP-seq data have been deposited at ArrayExpress under accession number E-MTAB-3208.

RESULTS

Transcription continues at a subset of Abf1-regulated genes in two abf1 ts yeast mutants

The categorization of putative Abf1 targets as “memory effect” or “responsive” genes was based on microarray analysis of gene expression using the abf1-1 ts mutant in a W303 background (Rhode et al. 1992; Yarragudi et al. 2007). Comparison of changes in gene expression from published microarray analysis of a distinct ts mutant, abf1-101 (Loo et al. 1995), in a BY4741 background, revealed that this categorization is robust: changes in the two ts mutants strongly correlate (Figure 1A; R = 0.755; P = 2.977e-36) and the two classes of targets are for the most part well demarcated. Memory effect targets showed little change in expression in both the abf1-1 ts and abf1-101 ts strains, whereas the transcriptionally responsive targets showed on average a twofold decrease in expression in both backgrounds. We further validated the differing behavior of memory effect and transcriptionally responsive genes by comparing transcript levels for two memory effect genes in wild type and abf1-101 yeast (Figure 1B). The two transcriptionally responsive targets are for the most part well demarcated. Memory effect targets showed little change in expression in both the abf1-1 ts and abf1-101 ts strains, whereas the transcriptionally responsive targets showed on average a twofold decrease in expression in both backgrounds.

Table 2 Primers used in this study

| Name        | Sequence                                      |
|-------------|-----------------------------------------------|
| YKT6-A      | TATCCGTGACGACGATACACAC                        |
| YKT6-B      | TGGACAACCGCAGCTAACCTAGCC                     |
| YKT6-1Fe    | CCA AAA TTC GGC TCC TTT TCC CTT              |
| YKT6-1Re    | GAG CGA AAT ACA CCG ATG TAG ATG             |
| VID27-1E    | CTA CAC CAT TGG TGA TTG GTG TTT             |
| VID27-1R    | GAC TAC TTT GCT TTT ATG GCT GTC             |
| TCM1-3      | GACACGTTGCAGAACTTACGCG                       |
| TCM1-1      | GCCCTCCATCAAGCTAGCAGCTAGTAAAG               |
| SPT15-A     | CCC CTG TGA TAG CTG AGA CCG GTG CTT CC       |
| SPT15-C     | CCAAAGTTCCTTGACCGAGAATTTTTTTTGG             |
| Rps28a-P1   | CAAGACATCAATACCCCTTTAGCAGCATATC             |
| Rps28a-P2   | CGTAGTGGCAGTGGTTTTTTTGC                     |
| Rps28a-1    | GGATACACCCCAAGCGCTACGCTAGTACG               |
| Rps28a-2    | GAC GAG CCT CAT GGT CAT ATT CCA TTA G        |
| SNR6-1Fcr   | TTC GGC AAG TAA CCC TTC GTG GA              |
| SNR6-1Rcr   | GTC AAA CGG TTC ATC ATC ATG AGC             |
| RPN8-1Fcr   | TAC GTG AAG TAG CAC AGC CTG                 |
| RPN8-1Rcr   | GGT TGG TGA TCC AAG TGA TGG TGG            |
| RPL3-1F     | CTC AGC AAG ACT GTA AAT GGC                 |
| RPL3-1R     | ATA TCG ACG AAG CAA CAA AGA GAC            |
| RAD23-1F    | CTA GGC TGG GTT TTT TAT CTC GGG             |
| RAD23-1R    | AAA TTT CAA TTT CCC CAC CGA GCC             |
| QCR8-1F     | GCT GAT GTC TTA ATG CCG GTG TGG            |
| QCR8-1R     | CCG TAA TTT CCC ATG ACC ATG TCC             |
| PIK1-1Fcr   | TGA TTCCA ACT CTA GTG AAT TCA CCC           |
| PIK1-1R     | GGT AGG GTT CTT CTT TCG GTG TGC            |
| PIK1-1Rcr   | ATG GTG AGG ACC GTT ACC CTG TGC             |
| IPP1-1F     | GTC ATC GCA GAC GCT AAG GTT GTT            |
| IPP1-1Rcr   | TAC ACTACC AGA CAA ATT GGT GCC             |
| IPP1-1R     | GCC TCG TGT TAT AAT CTA ACT TGT CTG         |
| CNB1-1F     | GGA TTT GAT CGC GAA GAC GTT AGT            |
| CNB1-1R     | TAA GAT GAT ACC CGG CCT TCC AGC            |
| CNb1-1Fcr   | GCTGCCCTTTCCAAATTTGGGAT                    |
| CNb1-1Rcr   | AAACCTCCATTATACGCTCACAGAG                 |
| Chv-down    | CACCCCGAAGACTTCTTACAAATAC                  |
| Chv-up      | GGCTGTCAAGAATGTTGCGCCCTGTA                 |
| ARO3-1F     | GAA GCA GCT GCG TAT CTT CTC AAA            |
| ARO3-1R     | ATG CAG CAA GCA TAC TTT CCG ATG            |

The distinct behavior of memory effect and transcriptionally responsive genes could be compared by differential transcript abundance. To examine this possibility, we compared mRNA half-lives, as measured in a recent study that measured mRNA half-lives by a different method (Geisberg et al. 2014) (data not shown). As an independent test, we measured transcript levels for two memory effect genes in abf1-101 and rpb1-1 ts yeast along with the corresponding wild type strains after 1 hr at 37°C (Figure 1D). Transcription by RNA polymerase II ceases at 37°C in the rpb1-1 mutant, providing a measurement of mRNA stability (Nonet et al. 1987). Strains were grown and analyzed in parallel. Two "memory effect" genes, CNB1 and RPN8, which show slightly increased transcript levels in abf1 ts yeast in both microarray experiments [log2 increase of 0.2 in both abf1-1 and abf1-101 ts yeast (Badis et al. 2008; Yarragudi et al. 2007)] and by quantitative polymerase chain reaction
Figure 1D), exhibited more than fourfold decreased transcript levels in rpb1-1 ts yeast while showing no decrease in abf1-101 ts yeast (Figure 1D). A third putative “memory effect” gene, RPL3 (also known as TCM1) behaved similarly; however, we show below that RPL3 transcription does not depend on its Abf1 binding site. Transcript levels of IPP1 decreased about threefold in the abf1-101 ts strain in this experiment, and ARO3 transcript levels decreased approximately 1.6-fold. Although these decreases are somewhat less than seen in the experiment of Figure 1B, likely because of the variability in the temperature shift, they are consistent with the magnitude of the effects seen in microarray experiments and show that the temperature shift was effective (as do the decreased transcript levels seen in rpb1-1 yeast). These results indicate that the continued expression of these Abf1 targets is not due to transcript stability.

Memory effect genes are not distinguished by other contributing transcription factors or by lack of dependence on Abf1
Many yeast promoters bind multiple transcription factors. This could lead to redundancy in transcriptional activation, such that putative “memory effect” genes do not depend on Abf1 because of contributions from other activators. We compared transcription factor (TF) binding to memory effect and transcriptionally responsive genes using the CERES web tool (Morris et al. 2009) and found neither class to be enriched for binding sites for any specific TF (including Hsf1, which could conceivably be responsible for activating memory effect genes after the temperature shift to 37°C). Furthermore, the average number of non-Abf1 TF binding sites within 500 bp of the starting ATG was very similar for the two classes, with memory effect genes averaging 1.5 TF binding sites/promoter and transcriptionally responsive genes averaging 1.24 TF binding sites/promoter. It therefore seems unlikely that other TFs make an important contribution to the distinction between memory effect and transcriptionally responsive promoters.

A more direct test of Abf1 dependence would be to mutate promoter binding sites and examine the effect on transcript levels. Previous work has demonstrated the need for the Abf1 binding site at the promoter region of selected memory effect targets. Mutation of Abf1 binding sites at the RPL3, QCR8, RPS28A, HIS7, and ADE5,7 promoters in plasmid-borne reporter constructs resulted in strongly decreased expression, whereas chromosomal mutations at the Abf1 binding site in the promoter of the memory effect gene TOM6 resulted in a decrease in TOM6 expression (Della Seta et al. 1990; Hornung et al. 2012; Lascaris et al. 2000; Yarragudi et al. 2004; Yarragudi et al. 2007). To test further the importance of the Abf1 binding site for
transcription initiation at select memory effect genes. Abf1 binding sites were mutated in promoters of three memory effect targets, RPL3, RPS28A, and CNB1, and one transcriptionally responsive gene, ARO3, in their native chromosomal context by replacing the recognition sequence with a sequence to which Abf1 cannot bind. Expression analysis reveals that mutating the Abf1 binding site decreased transcription of RPS28A by about threefold, CNB1 and ARO3 by eightfold, and RPL3 surprisingly not at all (Figure 2). These results indicate that although some “memory effect” genes may in fact not depend on Abf1 binding for their transcription, Abf1 binding is needed for full transcription initiation of at least a fraction of memory effect genes.

As an additional test for the functional importance of Abf1 sites in memory effect compared with transcriptionally responsive genes, we examined data on site conservation from previous work of Bulyk and colleagues (Mukherjee et al. 2004). Of 64 memory effect promoters having unique Abf1 binding sites, 41 showed conservation of the site in four related yeast species (Saccharomyces mikatae, Saccharomyces kudriavzevi, Saccharomyces bayanus, and Saccharomyces paradoxus) using a criterion of motif conservation within two standard deviations of the motif average, whereas 29 of 69 transcriptionally responsive promoters with unique Abf1 binding sites showed equivalent conservation. Abf1 sites in memory effect genes therefore do not appear to be less functionally important, based on evolutionary conservation, than those sites in transcriptionally responsive genes. Taken together, data from previous site mutation experiments and those shown here, together with information on evolutionary conservation, indicate that the memory effect is not likely explained by Abf1 being unimportant for transcriptional activity at these promoters.

**Abf1 dissociates from its binding site in abf1-ts yeast**

Another possible explanation for the distinct behavior of memory effect and transcriptionally responsive Abf1 targets is that loss of Abf1 binding occurs differentially in these two classes in abf1 ts yeast. Abf1 binding motifs in the promoters of these two classes were indistinguishable, making this seem less likely (Yarragudi et al. 2007). To further examine this possibility, individual loci were examined for Abf1 enrichment through ChIP in wild type and abf1-101 ts yeast. Abf1-regulated targets showed a clear decrease in binding of Abf1 in abf1-101 ts yeast at both transcriptionally responsive and memory effect genes after 1 hr at 37°C (Figure 3A). There was a 4.5-fold average decrease in enrichment for four transcriptionally responsive targets and a 2.6-fold average decrease in Abf1 binding at seven memory effect targets. This difference in average fold decrease did not meet standard criteria for significance ($P = 0.07$), and the decrease in Abf1 enrichment at memory effect targets such as RPN8 and CNB1 confirms that transcript levels remain high in spite of Abf1 dissociating from its binding site at genes (at least some) that depend on their Abf1 binding site for normal levels of transcription (Figure 2). Nonetheless, these results left open the possibility that Abf1 may not be dissociating from memory effect targets as well as it does at transcriptionally responsive targets.

To expand this analysis, we used ChIP-Seq to examine Abf1 binding genome-wide in wild type and abf1-101 ts yeast after 1 hr at 37°C. We identified a large number of Abf1 peaks in wild-type yeast, nearly all promoter-associated, in reasonable agreement with the number identified in a previous large-scale genome-wide association study (Harbison et al. 2004) and roughly comparable to the ~1200 identified in another recent study (Kasinathan et al. 2014) (Figure 3B). Only a fraction of the genes associated with these sites show decreased transcription in abf1 ts yeast (Badis et al. 2008; Yarragudi et al. 2007). Average Abf1 binding profiles were generated and centered over the Abf1 binding motif in the promoter regions of transcriptionally responsive and memory effect targets. Both classes of genes show a decrease in Abf1 enrichment levels when comparing the levels in wild-type yeast with those in abf1-101 ts yeast (Figure 3, C and D). The decrease was slightly greater at the transcriptionally responsive targets with log2 enrichment values centered on the Abf1 binding site decreasing from 2.0 in the wild type strain to 0.8 in the abf1 ts strain while enrichment at the memory effect targets decreased from 2.2 in the wild-type strain to 1.2 in abf1-101 ts yeast. The enrichment levels above background were essentially equivalent for both gene classes in wild-type yeast, indicating that Abf1 binds equally well to both memory effect and transcriptionally responsive targets, consistent with the two classes being associated with indistinguishable motifs (Yarragudi et al. 2007). Thus, results of both ChIP-seq and conventional ChIP followed by quantitative polymerase chain reaction at select loci indicate substantial loss of Abf1 binding from both memory effect and transcriptionally responsive targets, with the loss being slightly greater from responsive gene promoters.

To determine whether association of the general transcription machinery differs at the two classes of Abf1 targets, enrichment of pre-initiation complex components, TATA-binding protein (TBP) and RNA Polymerase II (Rpb3 subunit), was examined by ChIP-Seq in wild type and abf1-ts yeast after 1 hr at 37°C. As expected, TBP association was observed at promoters of both memory effect and transcriptionally responsive promoters (Figure 3D). However, association of TBP at transcriptionally responsive targets decreased approximately 25% in the abf1-101 ts strain, whereas no decrease at all was observed at memory effect genes (Figure 3D). Similarly, Pol II enrichment downstream of Abf1 binding sites (i.e., over coding regions) of Abf1-regulated targets decreased in abf1-101 ts yeast, while virtually no change was seen at memory effect genes (Figure 3D). These results are consistent with the distinct effects on mRNA expression levels for memory effect and transcriptionally responsive Abf1 targets being a direct result of altered response of the transcriptional machinery to decreased Abf1 binding at these two classes of genes.
Memory effect and transcriptionally responsive promoters differ in nucleosome-forming propensity

Chromatin structure of promoter regions can affect transcriptional activity, as a more open chromatin structure can allow increased access to activators and general TFs, thus facilitating transcription (Zaret and Carroll 2011). To test whether chromatin structure contributes to the memory effect phenomenon, we used previously generated data to compare nucleosome occupancy for Abf1-regulated transcriptionally responsive and memory effect targets after 1 hr at 37°C in wild-type and abf1ts yeast (Badis et al. 2008; Ganapathi et al. 2011). As expected,
promoters for both classes of Abf1-regulated genes have an open chromatin structure over the Abf1 binding site in wild type yeast (Figure 4A) (Badis et al. 2008; Ganapathi et al. 2011; Hartley and Madhani 2009; Kaplan et al. 2009). This open chromatin structure was mostly lost in transcriptionally responsive gene promoters in \textit{abf1-1 ts} yeast after 1 hr at 37°C, indicating that dissociation of Abf1 from its binding site in these promoters is accompanied by generation of a closed chromatin conformation (Figure 4A). This finding is in accord with previous studies indicating that local domains of low nucleosome occupancy in the vicinity of Abf1 binding sites generally depend on Abf1 (Badis et al. 2008; Ganapathi et al. 2011). Remarkably, however, memory effect targets showed very little change in nucleosome occupancy over the Abf1 binding site in \textit{abf1-1} yeast, instead exhibiting low nucleosome occupancy similar to that seen in wild-type yeast (Figure 4A). Essentially identical results were obtained in comparing nucleosome occupancy of transcriptionally responsive and memory effect genes in \textit{abf1-101 ts} yeast (Supporting Information, Figure S1). These results suggest that open chromatin structure that persists in the promoter regions of the Abf1 memory effect genes after dissociation of Abf1 may allow the general transcription machinery continued access to the transcription start site, resulting in persistent expression.

We next asked what might cause the maintenance of low nucleosome occupancy observed at memory effect genes in \textit{abf1 ts} yeast. One potential mechanism that could mark memory effect promoters would

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Figure 4 Transcriptionally responsive and memory effect targets differ in properties related to nucleosome occupancy. (A) In vivo average nucleosome occupancy in wild-type (WT, W303; green line) and \textit{abf1-1}ts (red line) yeast after 1 hr at 37°C centered over the Abf1 binding site shows an open chromatin structure at memory effect targets in \textit{abf1 ts} yeast. Top panel shows the average in vivo occupancy for 69 Abf1 transcriptionally responsive targets and bottom panel shows average occupancy for 64 memory effect targets (both limited to those containing only a single Abf1 binding site) as defined by Yarragudi et al. (2007). (B) (Kaplan et al. 2009; top panel) and in vitro (bottom panel) average nucleosome occupancy profiles, centered over the Abf1 binding site, for 69 transcriptionally responsive targets (black line) and 64 memory effect targets (red line). (C) Conservation of T-rich regions, based on frequency of 7-mers containing at least 6 A or T residues (see the section Materials and Methods), upstream of the Abf1 binding sites in memory effect and responsive promoters (blue) and transcriptionally responsive targets (black) in \textit{S. cerevisiae} and four closely related yeast species. Averaged plots are centered over the Abf1 binding site. (D) Average gene expression noise (Newman et al. 2006) for genes having “Open” or “Closed” nucleosome occupancy configurations at their promoters (Tsui et al. 2011), and for transcriptionally responsive and memory effect genes as defined in the text.
be recruitment of a particular accessory transcriptional activator, which might require Abf1 for its initial recruitment but not for continued binding and maintenance of an open chromatin structure. However, as mentioned previously, we observed no differential enrichment in type or number of TF binding sites between the promoters of memory effect and transcriptionally responsive Abf1 targets, including other general regulatory factors (Cbf1, Rap1, Reb1).

We then asked whether the propensity to form nucleosomes might differ in the vicinity of the Abf1 binding site of memory effect and transcriptionally responsive promoters; this could result in a greater tendency for one class than the other (presumably transcriptionally responsive over memory effect promoters) to be occupied by a nucleosome after loss of Abf1 binding. Previous work from the Widom and Segal labs measured nucleosome occupancy obtained upon packaging yeast genomic DNA into nucleosomes in vitro, and then developed an algorithm based on the results to predict relative nucleosome-forming propensity based on DNA sequence (Kaplan et al. 2009). We examined the in vitro nucleosome occupancy and predicted occupancy for memory effect and transcriptionally responsive promoter sequences, and found that memory effect promoter sequences showed considerably lower values in the vicinity of Abf1 binding sites (Figure 4B). Memory effect promoters showed enrichment for A/T-rich elements (see the section Materials and Methods), which are relatively unfavorable for nucleosome formation and can affect transcription of the associated gene, and which also have been shown to cooperate with Abf1 in transcriptional activation (Figure S2) (Lascaris et al. 2000; Segal and Widom 2009). This effect is confined to the upstream-flanking region of the motif and is almost completely absent in the downstream flanking region. The enrichment of A/T-rich elements at the upstream region of memory effect promoters is mostly for T-tracts and less evident for A-tracts (reading 5′ to 3′ on the upper strand), which is consistent with the observation that nucleosome-depleted regions are typically defined by T-tracts followed by A-tracts (Figure S2). These results strongly suggest that the differential response of memory effect and transcriptionally responsive promoters to loss of Abf1 binding is due at least in part to memory effect promoters showing a reduced propensity, based on DNA sequence, to form a closed chromatin structure upon loss of Abf1 binding in abf1 ts yeast.

The experimental conditions that reveal distinct behavior of memory effect and transcriptionally responsive Abf1 target genes are non-physiological, in that they are observed using abf1 ts yeast. Therefore, to determine whether the observed differences in promoter type are physiologically significant, we examined DNA sequences of promoters of genes homologous to these two classes for nucleosome-forming propensity in four additional yeast species, S. paradoxus, S. mikatae, S. kudriavzevi, and S. bayamas. Similar to the results observed in S. cerevisiae, three of the four yeast species have increased levels of da/dT upstream of the Abf1 binding site in the promoters of memory effect genes as compared to the promoters of transcriptionally responsive genes (Figure 4C). This conservation of the da/dT tracts, and consequently the predicted lower nucleosome occupancy, suggests that this memory effect reflects an underlying and physiologically relevant property. Previous investigation of gene properties related to nucleosome occupancy has revealed two broad categories of yeast promoters; these have depleted proximal nucleosome, or more open, structure, and those having an occupied proximal nucleosome, or more closed, structure (Figure 4D) (Tirosh and Barkai 2008; Tirosh et al. 2009a). Promoters belonging to these two classifications differ on average in a number of properties, including TATA element, expression divergence, and expression noise (Tirosh and Barkai 2008; Tirosh et al. 2009a). Transcriptionally responsive and memory effect promoters are similar in their possession of consensus TATA elements and corresponding dependence on SAGA and TFIIID (Huisings and Pugh 2004). However, examination of the transcriptional noise associated with these two categories revealed a substantial difference. Memory effect promoters are associated with extremely low transcriptional noise, even lower than the average for promoters having depleted promoter nucleosome structure, while transcriptionally responsive promoters, although tending to have a nucleosome depleted region, have average noise (i.e., similar to the genome-wide average) which is higher than other depleted proximal nucleosome promoters (Figure 4D). Thus, the memory effect that is characterized by continued transcription at Abf1-dependent promoters upon loss of Abf1 appears to reflect an evolved property that results in lower “on-off” switching, or noise, thus ensuring robust continuation of transcription of this class of genes.

**DISCUSSION**

Previous work by us and others has indicated the existence of two classes of genes whose transcription is regulated by Abf1: memory effect genes and transcriptionally responsive targets (Schroeder and Weil 1998; Yarragudi et al. 2007). These two categories were first defined based on analysis of genome-wide expression and ChIP-chip results in abf1-1 ts yeast (Yarragudi et al. 2007), and actually represent the two ends of a continuum. Abf1 targets were defined as genes whose promoters bind Abf1 in vivo, based on ChIP-chip (Harbison et al. 2004), and have an Abf1 binding motif or bind Abf1 in vitro (Yarragudi et al. 2007). Transcriptionally responsive targets were then defined as those whose transcription decreased at least 1.5-fold after 1 hr at 37° in abf1-1 ts yeast, whereas memory effect genes were defined as having log2 of transcriptional change less than 0.2. This categorization omits a substantial number of Abf1 targets with intermediate response, but is useful for exploring the varied behavior of Abf1-controlled genes.

Here we show that this same categorization holds for a different abf1 ts mutant, abf1-101, thus demonstrating that the effect is robust and not an artifact of one mutant. We also address a potentially trivial explanation for the difference between transcriptionally responsive and memory effect genes: that the former depend on Abf1 binding sites for their transcription and the latter do not. Previous work had indicated, in the context of plasmid reporter genes, that loss of Abf1 binding sites at several memory effect genes resulted in strongly diminished transcription (Della Seta et al. 1990; Hornung et al. 2012; Lascaris et al. 2000; Yarragudi et al. 2004; Yarragudi et al. 2007). Here we tested the effect of mutation of Abf1 binding sites in the native chromosomal context of three memory effect genes and one transcriptionally responsive gene (Figure 2). Two of the three memory effect genes showed four- to eightfold reduction in transcription, as did the transcriptionally responsive gene, whereas one, RPL3, showed no effect. Another study found mutation of the Abf1 binding site in the TOM6 promoter in the chromosomal context resulted in about a threefold reduction in transcription [Figure 2E of (Hornung et al. 2012)]. Furthermore, conservation across Saccharomyces species of Abf1 binding sites is actually somewhat stronger among memory effect than transcriptionally responsive genes. Thus, although some “memory effect” genes may in fact not depend on Abf1 at all, this seems likely to represent a minor fraction of this category.

Consistent with the differential transcription of memory effect and transcriptionally responsive targets, ChIP-seq results show that although Abf1 binding is decreased in both classes, they differ in retention of the general transcription machinery in abf1 ts yeast. Finally, we show that memory effect and transcriptionally responsive genes display a major difference in their promoter chromatin structure, with memory effect gene promoters retaining low nucleosome occupancy in abf1 ts yeast.
while Abf1 binding sites of transcriptionally responsive promoters become occupied by nucleosomes. Importantly, transcriptionally responsive and memory effect genes defined in abf1-ts yeast show the same difference in nucleosome occupancy properties assessed in both abf1-ts and abf1-101(ts) yeast, showing that this functional distinction according to an independent criterion also holds for distinct abf1-ts mutations. This distinguishing behavior is reflected by differential, sequence-directed propensity for nucleosome occupancy between the two classes, which displays evolutionary conservation and may be important for governing differential expression noise between the two classes.

Our ChiP-seq results for Abf1 complement previous work identifying Abf1 binding sites (Badis et al. 2008; Ganapathi et al. 2011; Harbison et al. 2004; Kasinathan et al. 2014). Early ChiP-chip studies indicated Abf1 binding to approximately 200 targets although expression data suggested there were additional loci affected by the loss of Abf1 (Harbison et al. 2004). A more recent study from our lab revealed that Abf1 contributes to low nucleosome occupancy at many of these additional loci (Ganapathi et al. 2011), while recent work from the Henikoff lab used a modified ChiP-seq protocol to identify 1258 binding sites for Abf1 (Kasinathan et al. 2014).

Although our ChiP-seq results showed that Abf1 dissociates from both classes of targets in abf1-ts yeast, transcriptionally responsive promoters showed a slightly more efficient loss of Abf1 than did memory effect genes. We believe this is more likely to reflect underlying, chromatin-mediated differences in these two classes of promoters than it is to be the cause of the difference. First, the Abf1 motif identified using MEME is identical for memory effect and transcriptionally responsive promoters (Bailey and Elkan 1994; Yarragudi et al. 2007). Second, although the average Abf1 occupancy at memory effect promoters is slightly higher than at transcriptionally responsive genes in abf1-ts yeast (Figure 3D), there is substantial overlap in the range of occupancies observed at the two promoter types. Rather, we suggest that the slightly more efficient eviction of Abf1 at the promoter region of the transcriptionally responsive targets in the ts mutant could be due to differential competition with the histone proteins. Upon shift to the restrictive temperature, the weak binding of the abf1-ts protein may not be strong enough to retain Abf1 at its binding site at transcriptionally responsive promoters because of their stronger propensity to assemble into nucleosomes, whereas the decreased likelihood of the nucleosome assembly due to nucleosome-disfavoring tracts (principally dT tracts) at the promoters of Abf1 memory effect targets could contribute to modest retention of Abf1 in the ts mutant at these loci. With weaker competition between Abf1 and the histone proteins, Abf1 is not evicted as efficiently.

The details of how the chromatin-mediated mechanism that we have uncovered here contributes to the memory effect seen at select Abf1-dependent genes remain unclear. It is difficult to ascertain the temporal limits of the memory effect, as yeast cells deficient in Abf1 function cannot progress through G1 into S phase (Rhode et al. 1992). The precise nature of the sequences that distinguish transcriptionally responsive and memory effect genes is not clear at present. Our initial efforts to interconvert responsive and memory effect Abf1 target genes by swapping sequences upstream of the Abf1 binding site did not succeed in clearly changing transcriptional response to loss of Abf1 binding in abf1-ts yeast. Thus, although sequence-directed differences in propensity for nucleosome occupancy appear important for distinguishing transcriptionally responsive from memory effect genes, other factors, including sequences downstream of Abf1 binding sites, may also contribute to this effect. An alternative and likely more efficient means to dissecting the sequences that distinguish transcriptionally responsive and memory effect promoters would be to measure this effect using engineered abf1-ts mutant strains of Saccharomyces species closely related to S. cerevisiae, as this would simultaneously monitor the effect of sequence changes in hundreds of promoters (Tirosh et al. 2009b). Future studies should lead to a more detailed understanding of the transcriptional memory effect studied here, and will likely provide insights into transcriptional responsiveness and the mechanisms underlying gene expression noise as well.

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