Extensive role of the general regulatory factors, Abf1 and Rap1, in determining genome-wide chromatin structure in budding yeast

Mythily Ganapathi¹, Michael J. Palumbo¹, Suraiya A. Ansari¹, Qiye He¹,², Kyle Tsui³, Corey Nislow⁴ and Randall H. Morse¹,²,*

¹Laboratory of Molecular Genetics, Wadsworth Center, New York State Department of Health, ²Department of Biomedical Sciences, State University of New York at Albany School of Public Health, Albany, NY 12201-0509, USA, ³Department of Pharmaceutical Sciences and ⁴Banting and Best Department of Medical Research and Department of Molecular Genetics, University of Toronto, Toronto, ON M5S 3E1, Canada

Received August 14, 2010; Accepted October 28, 2010

ABSTRACT
The packaging of eukaryotic DNA into chromatin has profound consequences for gene regulation, as well as for other DNA transactions such as recombination, replication and repair. Understanding how this packaging is determined is consequently a pressing problem in molecular genetics. DNA sequence, chromatin remodelers and transcription factors affect chromatin structure, but the scope of these influences on genome-wide nucleosome occupancy patterns remains uncertain. Here, we use high resolution tiling arrays to examine the contributions of two general regulatory factors, Abf1 and Rap1, to nucleosome occupancy in Saccharomyces cerevisiae. These factors have each been shown to bind to a few hundred promoters, but we find here that thousands of loci show localized regions of altered nucleosome occupancy within 1 h of loss of Abf1 or Rap1 binding, and that altered chromatin structure can occur via binding sites having a wide range of affinities. These results indicate that DNA-binding transcription factors affect chromatin structure, and probably dynamics, throughout the genome to a much greater extent than previously appreciated.

INTRODUCTION
The packaging of DNA into chromatin has a major impact on eukaryotic gene regulation. One critical facet of chromatin-mediated gene regulation is the precise placement of nucleosomes with respect to DNA sequence, along with their relative density, or occupancy, as incorporation into nucleosomes of DNA sequences that are binding sites for transcription factors or the general transcription machinery can inhibit transcription (1). Recognition of this potential regulatory role has generated great interest in understanding the determinants of nucleosome positioning and occupancy (2–4). Numerous studies have documented effects of DNA sequence, chromatin remodeling proteins and DNA-binding transcription factors on nucleosome occupancy and positioning for specific loci in vivo and in vitro (4–8). Correspondingly, recent studies employing new technologies to elucidate nucleosome occupancy genome-wide have begun to confirm these influences on genome-wide chromatin architecture (4,9–15). However, the extent to which these different variables contribute to both specific and stereotypical patterns of nucleosome positioning is currently unclear and, in fact, controversial (12,16–18).

DNA-binding transcription factors can be inhibited from binding nucleosomal sites in some cases, but in other circumstances can out-compete histones for their binding sites, thus creating regions of open chromatin (19,20). Factors in the latter category have the potential to dictate chromatin structure at a significant portion of the genome if their binding sites are widespread. In yeast, a small group of multifunctional, DNA-binding proteins termed General Regulatory Factors (GRFs), including Abf1, Rap1 and Reb1, have this potential; two of these factors, Abf1 and Rap1, are the subject of this study. Abf1 and Rap1 are abundant, essential DNA-binding proteins that function in transcriptional activation at hundreds of promoters in Saccharomyces cerevisiae, as well as playing roles in replication, silencing and DNA repair (21–24). Previous investigations have shown that Rap1 and Abf1 can influence local chromatin structure (25,26), and in fact

*To whom correspondence should be addressed. Tel: +1 518 486 3116; Fax: +1 518 474 3181; Email: randall.morse@wadsworth.org

© The Author(s) 2010. Published by Oxford University Press. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/2.5), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
can out-compete nucleosomal histones for their binding sites (27,28). Studies on genome-wide chromatin structure have shown that Rap1 and Abf1-binding motifs are enriched at promoters having low nucleosome occupancy (11,13). Furthermore, comparison of native yeast chromatin to yeast genomic DNA packaged into nucleosomes in vitro reveals lower nucleosome occupancy at GRF-binding sites in vivo, suggesting a direct influence on nucleosome occupancy (12). Two other recent studies have documented the influence of several GRFs on nucleosome occupancy, one for yeast Chromosome III and one genome-wide, and report effects at up to several hundred (or ~10% of) yeast promoters for individual GRFs, mostly at NDRs (29,30). However, these studies focused on regions showing most pronounced changes in nucleosome occupancy, and did not examine closely effects of binding site strength or at promoter locations away from the NDR. Here we report on the influence of Rap1 and Abf1 binding on genome-wide chromatin structure at high resolution, by using tiling arrays to compare nucleosome occupancy in yeast harboring abf1-1 or rap1-2 ts alleles with the corresponding wild-type strains. Our work shows that both Abf1 and Rap1 contribute to local regions of chromatin structure by acting at both strong and weak binding sites, at proximal promoter regions and at sites farther upstream, over a very large fraction of the yeast genome. These results indicate that transcription factors are likely to play a much larger role in determining genome-wide nucleosome occupancy and dynamics in both yeast and higher eukaryotes than previously appreciated.

**MATERIALS AND METHODS**

**Nucleosomal DNA isolation**

Yeast strain TMY86 lacking the chromosomal copy of ABF1 and harboring ABF1 or the abf1-1 ts allele on pRS415 (23), and strains BY4741 and CBY10037 (Toronto), were grown at 25°C (a generous gift from Charlie Boone, University of Toronto). Cells were then cross-linked by addition of formaldehyde to an average size of ~50–70 bp, followed by labeling with biotinylated ddATP as previously described (13). Labeled DNA samples were hybridized to Affymetrix tiling arrays (P/N 520055) and processed as described (13).

**Data analysis**

Raw data from Affymetrix GCOS software were analyzed using Affymetrix Tiling Analysis Software (TAS) v1.1.02 (http://www.affymetrix.com/support/developer/downloads/TilingArrayTools/index.affx), and the BPMAP file 2006Feb_S288c_All_BothStrands_7G.bmap (http://www-sequence.stanford.edu/S288c/bpmap.html). A two-sample analysis was conducted using three nucleosomal DNA samples as the ‘treatment’ group and three whole genome fragmented DNA samples (13) as the ‘control’ group for each wild-type and ts mutant strain. Data were normalized using built-in quantile normalization and probe-level analysis with both perfect match and mismatch (PM/MM) probes and run with a bandwidth of 30. Nucleosome occupancy profiles were visualized with Affymetrix Integrated Genome Browser (IGB) (http://www.affymetrix.com/support/developer/tools/download_igb.affx).

For identification of regions showing altered nucleosome occupancy, we used TAS to generate .bar files using three wild-type nucleosomal DNA samples as treatment group and three ts samples as control (anticipating increased nucleosome occupancy would be most typical of the ts mutant samples) using parameters as above and two-sided P-value selection, and then employed the Interval Analysis feature of TAS with a minimum run of 50 and maximum gap of 20 probes, and P-value cutoff of 0.05. PERL scripts were written to associate chromosomal regions identified in the resulting .bed files with gene promoters or coding regions, using the January 2005 yeast genome build. Comparison between data sets (e.g. ChIP-chip data and genes associated with altered nucleosome occupancy) were made using Microsoft Excel. K-means clustering was performed using Cluster 3.0 (http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/), using the ‘organize genes’ option and default options of ‘Euclidean distance’ and 100 runs, and
visualized using Java Treeview (http://jtreeview.sourceforge.net/). For alignment by TSS, we used published data (34) after removing redundancies by choosing only the most 5′-transcript corresponding to genes having multiple exons. Clustering using TSSs identified in an earlier study (35) yielded similar results (data not shown). Motif analysis was performed using MEME (http://meme.sdsc.edu/meme/), RSA Tools (http://rsat.ccb.sickkids.ca/) (36,37) and YEASTRACT (38). We used position weight matrices (PWMs) from Yarragudi et al. (23); use of independently derived motifs (29) yielded similar results. Motif enrichment in regions showing altered nucleosome occupancy (Supplementary Figure S8) was compared to a control set of sequences equal in total length to tested regions selected randomly from the yeast genome. Functional classification was done using FatiGo (39) (http://fatiigo.bioinfo.cnb.es/). P-values for enrichments (or depletions) were calculated using the hypergeometric distribution (Fisher’s exact test) (http://www.alewand.de/stattab/tabdiske.htm), and corrected for multiple category testing for functional classifications.

Microarray data are available at the Gene Expression Omnibus under accession number GSE22514, and processed data can be downloaded from www.wadsworth.org/resnres/bios/morse.

RESULTS

Abf1 and Rap1 influence promoter chromatin structure near strong binding sites

To examine the influence of Rap1 and Abf1 binding on chromatin structure genome-wide and at high resolution, we used tiling arrays to compare nucleosome occupancy in yeast harboring abf1-1 or rap1-2 ts alleles and the corresponding wild-type strains. Both of these ts mutants have been shown by DMS footprinting and chromatin IP to vacate their binding sites rapidly after being shifted to restrictive conditions (27,40–42), and growth for 1 h at the restrictive temperature suffices for altered expression of many Rap1- and Abf1-dependent genes (23). Wild-type and corresponding ts mutant yeast were grown at 25°C to mid-log phase, rapidly shifted to 37°C by addition of pre-warmed media, and grown for 1 h at 37°C before preparing mononucleosomal DNA. For each condition, three independent samples of primarily mononucleosomal DNA were hybridized to Affymetrix tiling arrays and nucleosome occupancy determined by comparison to fragmented naked genomic DNA (13).

Comparison of nucleosome occupancy profiles at individual gene loci for wild-type and ts samples, visualized with the Interactive Genome Browser (IGB) (http://genome.ucsc.edu) (43), reveals that many Rap1- and Abf1-dependent promoters exhibit distinct alterations in nucleosome occupancy (Figure 1). To identify regions having altered chromatin structure between wild-type and ts mutant yeast in an unbiased manner, we used the Interval Analysis feature of the Affymetrix Tiling Array Software (TAS), which identifies regions that differ according to specified P-values, number of consecutive probes, and allowed gaps (‘Materials and Methods’ section); such regions are denoted by the green rectangles in Figure 1. Notably, these regions frequently are found close to or overlapping binding sites for Abf1 or Rap1 (orange boxes, Figure 1A and C–F), and typically show increased nucleosome occupancy in the relevant ts mutant, as expected. In some cases, regions of altered nucleosome occupancy near binding sites for Abf1 or Rap1 can be discerned in spite of not being diagnosed by TAS (Figure 1B). Regions of altered chromatin structure are most typically localized within 50–100 bp of Abf1 or Rap1-binding sites, but can sometimes extend several hundred base pairs from the binding site (Figure 1C, E and F). We confirmed a more extended change in chromatin structure in rap1 ts compared to WT yeast for one example, the RPL42A promoter (Figure 1F) by mapping MNase cleavage sites in chromatin by indirect end-labeling (44,45) (Figure 2A). Thus, Abf1 and Rap1 contribute to chromatin structure close to their binding sites at many yeast promoters, and in some cases affect occupancy and positioning over more extended regions.

To examine the effect of Abf1 and Rap1 on nucleosome occupancy at strong binding sites on a more general basis, we compiled profiles of nucleosome occupancy surrounding sites identified as functional targets of Abf1 and Rap1, using only promoters having unique strong binding sites (23). These sites were identified on the basis of belonging to promoters controlling genes showing altered expression in the corresponding abf1 or rap1 ts mutant, scoring positive for binding of Abf1 or Rap1 in ChIP data, and showing a strong match to the relevant binding motif (23). The results reveal prominent valleys of low nucleosome occupancy centered on the binding sites in the wild-type strains (Figure 3A and B), in agreement with previous work (12). In the corresponding ts strains, nucleosome occupancy increases at the Rap1 and Abf1-binding sites, with this change being mostly localized to within 50–100 bp on either side of the binding site. Control plots, in which nucleosome occupancy from wild-type and abf1 ts yeast was plotted centered on Rap1-binding sites and conversely, showed almost no change, as expected, although a slight decrease surrounding Abf1-binding sites is seen in the rap1 ts mutant (see below) (Supplementary Figure S2A and B). Notably, the Rap1-binding sites examined are broadly distributed from 100- to 600-bp upstream of the transcription start site (TSS) (23), indicating that Rap1 is a potent organizer of local chromatin structure independently of the nucleosome-depleted region (NDR) upstream of the TSS (13,46); this can also be seen by examining only Rap1-binding sites that are >300 bp from the corresponding ATG (Figure 3D). Interestingly, a maximum in nucleosome occupancy that occurs about 120-bp upstream of the Abf1-binding site and about 150-bp upstream of the Rap1-binding site is substantially reduced in magnitude and shifted toward the WT minimum in the ts mutants. A similar shift in nucleosome occupancy at a fraction of proximal promoter regions on yeast chromosome III upon loss of Abf1 or Reb1 has been recently reported (30). These observations suggest that Abf1 and Rap1 act as
boundaries for an upstream positioned nucleosome at many sites (14,47). In contrast, the downstream occupancy profiles, which also suggest a positioned nucleosome flanking the Abf1 and Rap1-binding sites, are nearly unperturbed in the $ts$ mutant. This could indicate that specific sequences, or other factors that are not perturbed in the $ts$ mutants, contribute to nucleosome positioning proximal to the promoter relative to Abf1 or Rap1-binding sites.

We also noted a distinct bimodal minimum in nucleosome occupancy surrounding the Rap1-binding sites (Figure 3B). This was not due to the asymmetric nature of the Rap1-binding site (data not shown), nor to differences in profiles for ribosomal protein (RP) and non-RP genes (Supplementary Figure S2C). However, plotting nucleosome occupancy surrounding Rap1 sites found at >300 bp from the corresponding start sites separately from those found at <300 bp from the start sites revealed distinct patterns (Figure 3C and D). A single minimum was found for the more promoter-proximal sites, with a slight decrease in nucleosome occupancy on the promoter side (Figure 3C). This is likely to reflect the contribution of the NDR to this pattern for these sites. The nucleosome occupancy pattern surrounding Rap1 sites found at >300 bp from the start site is strikingly different, both in wild-type and the $rap1$ $ts$ mutant yeast (Figure 3D). Notably, in $rap1$ $ts$ yeast, nucleosome occupancy appears to be restored throughout this region. Thus, the odd ‘double minimum’ pattern seen surrounding Rap1-binding sites (Figure 3B) may arise from the contribution of sites relatively distant from the promoter, although its precise origin remains unclear.
Figure 3. Averaged nucleosome occupancy profiles for (A) 67 promoter regions surrounding unique Abf1 sites from genes identified as probable or putative targets of Abf1 (23), in *abf1 ts* and WT yeast; (B) 66 promoter regions surrounding unique Rap1 sites from genes identified as probable or putative targets of Rap1 (23), in *rap1 ts* and WT yeast; (C and D) 30 promoter regions having unique Rap1-binding sites located <300 bp or >300 bp from the TSS, respectively, centered on Rap1-binding sites, in *rap1 ts* and WT yeast.
Rap1 can alter chromatin structure independently of Ifh1, Fhl1 and Hmo1

Partners for Abf1 in transcriptional activation are not known, but Rap1 is known to collaborate with other DNA-associated proteins, particularly at RP gene promoters (32,33,48–51). One such protein, Hmo1, associates with many promoters of both RP and non-RP genes, and at many RP genes Hmo1 is required for association of Ifh1 and Fhl1, which are important for RP gene activation (32,51). We tested the dependence on Hmo1 of chromatin structure of two Hmo1-associated promoters, RPL42A and ENO1, by MNase digestion followed by indirect end-labeling. In both cases the MNase cleavage pattern was identical in hmo1Δ and wt yeast, and differed substantially in rap1 ts mutant yeast (Figure 2A, B and Supplementary Figure S3). Similarly, ifh1Δ and ifh1Δ fhl1Δ yeast do not show altered chromatin structure at RPL42A compared to wild-type yeast (Figure 2C). These results indicate that Rap1 can affect chromatin structure independently of Ifh1, Fhl1 and Hmo1. In previously published work, we showed that rap1 mutants lacking portions of the C-terminus, including the activation domain and other protein-interacting domains, could perturb nucleosome positioning at Rap1-binding sites as well as intact Rap1 could (31); thus, it seems likely that many sites at which nucleosome occupancy is altered in rap1 ts yeast are affected directly by Rap1. Given the number of sites at which Abf1 affects chromatin structure (see below) and earlier results showing that protein-interacting domains of Abf1 are not required to perturb local chromatin structure (27), it seems likely that Abf1 also directly influences chromatin structure by binding to its cognate sites.

K-means clustering reveals extensive roles for Abf1 and Rap1 in determining genome-wide nucleosome occupancy

To examine the effect of Abf1 and Rap1 on genome-wide nucleosome occupancy, we used K-means clustering to group nucleosome occupancy profiles relative to transcription start sites (TSS) for wild-type and ts mutant yeast. Distinct clusters observed for K = 4 were consistent with earlier observations for wild-type BY4741 yeast grown at 30°C (13), and indicated similar nucleosome occupancy patterns between wild-type and mutant yeast (Supplementary Figure S4A). However, clustering profiles of nucleosome occupancy ratios between ts mutant and wild-type yeast, which allows changes in nucleosome occupancy against this background to be readily discerned, revealed a surprisingly large number of genes showing altered nucleosome occupancy in abf1 and rap1 ts mutants (Figure 4A–D).

K-means clustering revealed five clusters showing localized changes in nucleosome occupancy for abf1 ts/WT nucleosome occupancy profiles, containing regions upstream of approximately 4500 defined ORFs (4478/7052, or 63%) (Figure 4A and B). These clusters all showed modest but significant, localized increases in nucleosome occupancy in the ts mutant. Remarkably, the five clusters exhibiting strongest signals all occurred in promoter regions; in addition, one cluster (Cluster 3 in Figure 4A) showed more weakly increased nucleosome occupancy over a less localized region within ORFs. The five strong clusters differed mainly in being localized to the NDR just upstream of the TSS or to similarly restricted regions farther upstream (Figure 4A and B). As a control, we clustered ratios of nucleosome occupancy between the two wild-type yeast strains used here, and did not observe any clusters showing localized changes upstream of the NDR as seen for the abf1 ts/WT nucleosome occupancy ratios (Supplementary Figure S5). Thus, the localized changes seen in Clusters 5–7 of Figure 4A are unlikely to have arisen adventitiously during clustering.

Most Abf1-binding sites are located between 100- and 200-bp upstream of the TSS, but sites are also found farther upstream and may account at least in part for Clusters 5–7 in Figure 4 (23). The five clusters exhibiting increased nucleosome occupancy at localized promoter regions in abf1 ts yeast all show enrichment for the Abf1-binding motif in those regions compared to the same region in a control cluster (Cluster 3), suggesting that many of the genes showing increased occupancy in abf1 ts mutant yeast are direct targets of Abf1 (Table 1). A modest GO enrichment was found for RP genes (P < 0.015); failure to discover other GO enrichments is not surprising, given the wide range of functional categories containing genes regulated by Abf1 (24,52). Consistent with the observed motif enrichment, gene promoters found to bind Abf1 in a genome-wide localization experiment (53) were enriched in clusters exhibiting increased nucleosome occupancy: 718/904, or 79% of genes binding Abf1 with P < 0.1 were contained within these clusters (P < 10−28) (Supplementary Figure S6A). Enrichment was observed both for high affinity sites (ChIP-chip P < 0.001; 200/267, or 75%; P < 10−25) as well as for low affinity sites (ChIP-chip 0.001 < P < 0.1; 518/637 or 81%; P < 10−24). Thus, Abf1 contributes to localized regions of decreased nucleosome occupancy near binding sites at a substantial fraction of promoter regions in the yeast genome, and does so at many sites at which ChIP results suggest relatively poor binding. These findings are consistent with the presence of a large number of Abf1-bound sites in yeast, but suggest that previous estimates err on the low side with respect to the number of gene promoters at which Abf1 binds and affects nucleosome occupancy (24,29).

Similar analysis of profiles of altered nucleosome occupancy between rap1 ts and WT yeast revealed two clusters exhibiting increased occupancy in rap1 ts yeast, as well as three clusters showing decreased nucleosome occupancy in the ts mutant (Figure 4C–D). Cluster 2, containing 518 genes (7.3%), shows increased nucleosome occupancy in the NDR and is strongly enriched for RP genes (P < 10−14) and for the Rap1 motif (P < 10−16; Table 1). Cluster 7, which contains 1178 genes (17%), shows increased occupancy farther upstream that is broader and more diffuse, and weaker in magnitude than the increased occupancy seen for Cluster 2. Nonetheless, this cluster is enriched for the Rap1-binding motif (P < 10−10; Table 1). Furthermore, genes binding Rap1 in a ChIP-chip experiment (P < 0.1) (53) were highly enriched in both Clusters 2 (161/518; P < 10−27) and 7 (222/1178;
Figure 4. K-means clustering ($K = 7$) for log$_2$ of nucleosome occupancy for (A) abf1 ts yeast and (C) rap1 ts over WT for 7052 genes, aligned by TSS; yellow represents increased nucleosome occupancy in the ts mutant, while blue represents depletion. Line graphs for average nucleosome occupancy of indicated clusters are depicted in (B) and (D); line graphs for Abf1 cluster 3 and Rap1 cluster 6, used as controls for enrichment, are shown in Supplementary Figure S4B and C. Clustering for $K = 6$ or $K = 8$ yielded similar results, with some clusters being grouped together or sub-divided further (Supplementary Figure S10).
For each cluster analyzed, the same region from control cluster 3 for Abf1 or cluster 6 for Rap1, which did not show significant change in these Rap1-binding motifs, yeast therefore seems likely to be caused by rap1 ts ChIP-chip data. Decreased nucleosome occupancy in (Table 1), and are depleted for genes binding Rap1 in nucleosome occupancy in genome. Interestingly, all three clusters showing decreased occupancy over a considerable fraction of the yeast presumably indirect effects, Rap1 exerts an effect on nucleosome occupancy is that loss of Rap1 binding allows nucleosome positions to shift, presumably by an indirect mechanism, in a way that amplifies the exclusionary effect of A/T rich motifs on nucleosome occupancy [possibly in cooperation with Abf1 in some cases (25)]; additional investigations will be needed to test this possibility.

We also examined the distribution of genes showing altered expression in abf1-1 or rap1-2 ts mutants among the clusters identified as having, or not having, changes in nucleosome occupancy from Figure 4 (Supplementary Table S1). Genes showing altered expression (FDR < 0.1) in abf1-1 ts yeast at 37°C were highly enriched in Cluster 4 and slightly enriched in Cluster 1. These are the two clusters showing increased occupancy at the NDR, with Cluster 4 showing the larger effect; this finding is consistent with the observation that the majority of genes whose expression is directly controlled by Abf1 have Abf1-binding sites between 100- and 200-bp upstream of the TSS (13,23). In contrast, genes controlled by Rap1 have widely dispersed binding sites from 100- to 600-bp upstream of the TSS; correspondingly, only mild enrichment is seen for genes showing altered expression in rap1 ts yeast in Cluster 2 (Supplementary Table S1), which shows increased occupancy in the NDR (Figure 4). Notably, only a small fraction of those promoters showing altered nucleosome occupancy in abf1 or rap1 ts yeast correspond to genes whose expression is affected in these mutants. This observation is consistent with reports showing that a large fraction of occupied TFBs may exert little effect on transcription in mammalian cells (56), and that nucleosome occupancy diverges much more

\[ P < 10^{-8} \] (Supplementary Figure S6B). Clustering of RP genes also reveals regions of increased nucleosome occupancy varying from 50 to 500 bp upstream of the TSS in a large fraction (Supplementary Figure S6C), consistent with Rap1 motifs being located over a more extended region upstream of the TSS compared to Abf1 sites (13,14,23,54). Thus, like Abf1, Rap1 appears to contribute to low nucleosome occupancy at a substantial fraction of yeast promoters at upstream regions as well as at the NDR.

Clusters 3–5 exhibit decreased nucleosome occupancy at or just upstream of the NDR, and are thus unlike any clusters seen for abf1 ts yeast compared to wild-type. These clusters show modest depletion for RP genes (\( P < 0.012 \), are not enriched for Rap1-binding sites (Table 1), and are depleted for genes binding Rap1 in ChIP-chip data. Decreased nucleosome occupancy in rap1 ts yeast therefore seems likely to be caused by indirect effects. Nonetheless, these effects appear specific to loss of Rap1 binding, as similar changes were not seen upon loss of Abf1 binding; thus, between direct and apparently indirect effects, Rap1 exerts an effect on nucleosome occupancy over a considerable fraction of the yeast genome. Interestingly, all three clusters showing decreased nucleosome occupancy in rap1 ts yeast are enriched for A/T rich motifs, consistent with these motifs contributing to nucleosome exclusion (Supplementary Figure S7); in addition, Cluster 4 was enriched for Abf1 binding in ChIP-chip data (\( E \)-value of \( 7 \times 10^{-4} \) in T-profiler (55)) and was correspondingly enriched for the Abf1 motif [Supplementary Figure S7 and data not shown]. One speculative explanation for this observed decreased

### Table 1. Enrichment of Abf1 and Rap1-binding motifs (23) was identified using default values for Patser (66) in clusters from Figure 4

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Region relative to TSS taken for scoring motifs</th>
<th>No. of sequences</th>
<th>No. of motifs</th>
<th>No. of sequences with motif (%)</th>
<th>Fisher’s exact test 2 tail P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abf1-binding motifs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cluster 1</td>
<td>50 to –150 bp</td>
<td>1622</td>
<td>156</td>
<td>152 (9.4)</td>
<td>1.144E-05</td>
</tr>
<tr>
<td>Control cluster 3</td>
<td>50 to –150 bp</td>
<td>1165</td>
<td>55</td>
<td>58 (5.0)</td>
<td>2.413E-69</td>
</tr>
<tr>
<td>cluster 4</td>
<td>0 to –250 bp</td>
<td>666</td>
<td>285</td>
<td>269 (40.4)</td>
<td>1.942E-05</td>
</tr>
<tr>
<td>Control cluster 3</td>
<td>0 to –250 bp</td>
<td>1165</td>
<td>71</td>
<td>76 (6.5)</td>
<td>5.027E-11</td>
</tr>
<tr>
<td>cluster 5</td>
<td>–150 to –350 bp</td>
<td>873</td>
<td>84</td>
<td>88 (10.1)</td>
<td>6.213E-11</td>
</tr>
<tr>
<td>Control cluster 3</td>
<td>–150 to –350 bp</td>
<td>1165</td>
<td>56</td>
<td>59 (5.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>cluster 6</td>
<td>–250 to –500 bp</td>
<td>882</td>
<td>95</td>
<td>107 (12.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>Control cluster 3</td>
<td>–250 to –500 bp</td>
<td>1165</td>
<td>47</td>
<td>50 (4.3)</td>
<td>0.012</td>
</tr>
<tr>
<td>cluster 7</td>
<td>–350 to –650 bp</td>
<td>972</td>
<td>96</td>
<td>110 (11.3)</td>
<td>1.144E-05</td>
</tr>
<tr>
<td>Control cluster 3</td>
<td>–350 to –650 bp</td>
<td>1165</td>
<td>40</td>
<td>46 (3.9)</td>
<td>5.72E-17</td>
</tr>
<tr>
<td><strong>Rap1-binding motifs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cluster 2</td>
<td>50 to –150 bp</td>
<td>518</td>
<td>67</td>
<td>64 (12.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>control cluster 6</td>
<td>50 to –150 bp</td>
<td>1290</td>
<td>28</td>
<td>28 (2.2)</td>
<td>0.31</td>
</tr>
<tr>
<td>cluster 3</td>
<td>50 to –150 bp</td>
<td>774</td>
<td>10</td>
<td>10 (1.3)</td>
<td>3.66E-11</td>
</tr>
<tr>
<td>control cluster 6</td>
<td>50 to –150 bp</td>
<td>1290</td>
<td>37</td>
<td>35 (2.7)</td>
<td>0.012</td>
</tr>
<tr>
<td>cluster 4</td>
<td>–50 to –250 bp</td>
<td>893</td>
<td>34</td>
<td>31 (3.5)</td>
<td>5.027E-11</td>
</tr>
<tr>
<td>control cluster 6</td>
<td>–50 to –250 bp</td>
<td>1290</td>
<td>37</td>
<td>35 (2.7)</td>
<td>6.213E-11</td>
</tr>
<tr>
<td>cluster 5</td>
<td>–150 to –350 bp</td>
<td>1051</td>
<td>39</td>
<td>31 (2.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>control cluster 6</td>
<td>–150 to –350 bp</td>
<td>1290</td>
<td>32</td>
<td>30 (2.3)</td>
<td>0.36</td>
</tr>
<tr>
<td>cluster 6</td>
<td>–150 to –550 bp</td>
<td>1178</td>
<td>210</td>
<td>169 (13.1)</td>
<td>5.72E-17</td>
</tr>
<tr>
<td>control cluster 6</td>
<td>–150 to –550 bp</td>
<td>1290</td>
<td>84</td>
<td>64 (5.4)</td>
<td>3.66E-11</td>
</tr>
</tbody>
</table>

For each cluster analyzed, the same region from control cluster 3 for Abf1 or cluster 6 for Rap1, which did not show significant change in these regions (Supplementary Figure S4B and C) were used as controls, and P-values for enrichment of indicated clusters were calculated using Fisher’s exact test.
than gene expression in hybrid diploids from related yeast species (57). Thus, TFBSs can contribute to local patterns of chromatin structure at many genes with little direct impact on gene expression.

**Regions exhibiting changed nucleosome occupancy are enriched in both strong and weak binding sites for Abf1 and Rap1**

As a second approach to examining genome-wide effects of Abf1 and Rap1 on nucleosome occupancy, we identified 444 genomic regions showing significantly \( (P < 0.05) \) altered nucleosome occupancy in \( abf1 \) \( ts \) compared to wild-type yeast, and 552 such regions for \( rap1 \) \( ts \) yeast (‘Materials and Methods’ section). In both cases promoter regions were enriched relative to coding sequences and regions between convergently transcribed genes, consistent with the clustering results discussed above (data not shown). K-means clustering showed that \( \sim 80\% \) of the regions affected by loss of Abf1 binding showed increased occupancy, whereas about half of the regions affected by loss of Rap1 binding exhibited increased nucleosome occupancy and half decreased occupancy, consistent with clustering results discussed above (Supplementary Figure S8). Interestingly, regions having decreased occupancy showed lower but still significant enrichment for Rap1 motifs (6.8% compared to 0.8% in a control dataset) (Supplementary Figure S8C), in contrast to the lack of enrichment for Rap1 motifs in clusters showing decreased nucleosome occupancy in \( rap1 \) \( ts \) yeast in Figure 4C. These results suggest that an effect of Rap1 in increasing nucleosome occupancy can be seen in those promoter regions showing the most significant effects upon loss of Rap1 binding, but is masked by indirect effects when all promoters are clustered. Interestingly, many of the sequences identified as Rap1-binding motifs in regions showing nucleosome depletion in the \( ts \) mutant correspond to CACACCCACAC ACC repeats, and 20/23 genes flanking these regions were identified as telomeric or subtelomeric. This observation is consistent with Rap1 contributing to stable nucleosome formation in telomeric heterochromatin (58).

Among regions having altered nucleosome occupancy in \( abf1 \) \( ts \) compared to WT yeast, substantial enrichment for Abf1 motifs was found in both the cluster showing stronger (68%) and weaker (35%) increased nucleosome occupancy (Supplementary Figure S8C). Searching \textit{de novo} for enriched motifs (data not shown) yielded readily identifiable Abf1 and Rap1-binding motifs from the respective regions; Abf1-associated regions were also enriched for A-rich tracts, consistent with such tracts cooperating with Abf1 to generate regions of open chromatin (25).

To investigate further the extent to which Abf1 and Rap1 contribute to genome-wide chromatin structure, for each promoter showing changed nucleosome occupancy we determined the \( P \)-values for Abf1 or Rap1 binding from a genome-wide ChIP-chip analysis (53). Figure 5A and C show all gene promoters ranked according to the \( \log(P) \) for ChIP of Abf1 or Rap1, respectively, with those promoters that show altered nucleosome occupancy indicated by pink squares and yellow diamonds, according to whether they are divergent or tandem. Marked enrichment is seen for gene promoters with altered nucleosome occupancy in the \( ts \) mutants among those binding Abf1 and Rap1 (i.e. for promoters with low \( P \)-values) in ChIP-chip experiments; seventy percent of promoters showing altered nucleosome occupancy in \( rap1 \) \( ts \) yeast were in the top quartile for ChIP enrichment, while 67% of promoters showed similar enrichment for Abf1 (Supplementary Figure S9). We also plotted the enrichment for promoters having altered nucleosome occupancy compared to total promoters against the \( \log(P) \) for ChIP of Abf1 or Rap1, in 0.1 increments of \( \log(P) \) (Figure 5A and C, insets). These plots clearly show enrichment for promoters having altered nucleosome occupancy in \( abf1 \) and \( rap1 \) \( ts \) yeast even among promoters showing relatively weak enrichment in ChIP experiments \( [\log(P) < -0.5 \text{ for } Abf1 \text{ and } \log(P) < -0.9 \text{ for } Rap1] \) (Figure 5A and C, insets), consistent with Abf1 and Rap1 contributing to chromatin structure at a very large number of promoters. In further support of this notion, promoters showing poorer Rap1 or Abf1 binding in ChIP experiments (with \( P > 0.001 \)) and having altered nucleosome occupancy in the \( ts \) mutants were also enriched for Rap1 or Abf1-binding sites relative to control promoters having similar ChIP \( P \)-values but not showing significantly altered nucleosome occupancy (Figure 5B and D and Supplementary Figure S9C). This enrichment was observed both for high and low affinity binding sites, underscoring the large number of locations at which Abf1 and Rap1 are able to affect nucleosome occupancy. To test further the idea that weak sites can contribute to decreased nucleosome occupancy caused by Abf1 or Rap1 binding, we scrutinized promoters identified as having significantly altered nucleosome occupancy for the presence of weak motifs, based both on occupancy determined in ChIP-chip experiments and on PWM score (37,53). We then examined those having identifiable but weak binding sites for altered nucleosome occupancy, and in several cases found altered occupancy in the immediate vicinity of the identified weak binding motif (Figure 6). This further supports the notion that even weakly binding Abf1 and Rap1 can influence local nucleosome occupancy. Taken together, these results indicate that Abf1 and Rap1 contribute to chromatin structure at thousands of sites, including relatively weak binding sites, throughout the yeast genome.

**DISCUSSION**

In this work, we show that Abf1 and Rap1 influence chromatin structure at thousands of sites genome-wide in yeast, and can affect nucleosome occupancy even at binding sites of relatively low affinity. Several lines of evidence support these conclusions. First, because we compare nucleosome occupancy between wild-type and \( ts \) mutant yeast after both are incubated at 37°C for 1 h, it is unlikely that effects due to heat stress underlie observed changes in nucleosome occupancy. Furthermore, the patterns of altered nucleosome occupancy are quite different between \( abf1 \) and \( rap1 \) \( ts \) yeast.
(Figure 4), whereas effects caused by heat stress would be expected to be in common between these two data sets. Second, regions showing increased nucleosome occupancy in abf1 ts or rap1 ts yeast are highly biased towards promoter sequences and reflect highly localized changes in nucleosome occupancy at sites enriched for Abf1 or Rap1-binding motifs and for promoters identified as binding Abf1 or Rap1 in genome-wide localization experiments (Figures 1, 3 and 4; Table 1), suggesting that at many promoters binding of Abf1 or Rap1 results in decreased occupancy at a specific nucleosomal site. Third, we show that regions showing significantly altered nucleosome occupancy are enriched for sites binding Abf1 or Rap1 with relatively low affinity in ChIP-chip data (Figure 5), and for sites having low affinity by motif stringency (Figures 5 and 6). As hundreds of relatively stringent binding sites for both Abf1 and Rap1 have been identified in the yeast genome (59), it is thus not surprising

**Figure 5.** Regions having altered nucleosome occupancy in abf1 ts or rap1 ts yeast are enriched for both strong and weak Abf1 and Rap1-binding sites. Graphs of genes ranked by log(P) for ChIP against (A) Abf1 or (C) Rap1 (53) are shown, with promoters containing regions having significantly changed nucleosome occupancy, and associated with divergent or tandem promoters, indicated by pink squares and yellow triangles, respectively. Regions found to lie in divergent promoters were mapped to only one of the two divergently transcribed genes. Insets show the relative enrichment of promoters having altered nucleosome occupancy in ts mutant yeast against the log(P) for Abf1 or Rap1, for increments of log(P) of 1/10. This was calculated by determining the fraction of promoters having altered nucleosome occupancy for each increment of log(P) and dividing by the fraction of all promoters having log(P) in the same increment. The horizontal dashed lines correspond to a value of 1, or no enrichment. In (B) and (D) are shown the fraction of genes showing altered nucleosome occupancy in abf1 or rap1 ts yeast, respectively, that contain Abf1 or Rap1 motifs defined stringently [motif score >7 in Patser (66), corresponding approximately to ln(P) <–9.5] or loosely [motif score >5, corresponding approximately to ln(P) <–8], as indicated, and having ChIP log(P) values as indicated at bottom. Control sets were closely matched for ChIP P-values but did not show altered nucleosome occupancy according to the criteria applied (‘Materials and Methods’ section), while the ‘low ChIP control’ was a group of genes at about 75th percentile for ChIP P-value.
that a large fraction of promoters would contain motifs with more relaxed specificity.

Two recent studies have similarly reported a role for Abf1, Reb1 and Rap1 in creating local regions of nucleosome depletion at a few hundred proximal promoter sites (29,30). However, the findings in these studies were restricted to showing a correlation of increased nucleosome occupancy upon loss or inactivation of these GRFs with the presence of appropriate binding motifs, principally at proximal promoter regions. Here, by using tiling arrays that allow nucleosome occupancy to be determined at high resolution over the entire yeast genome (13), employing biological replicates and monitoring chromatin structure just 1 h after factor inactivation, we have been able to go considerably beyond the analyses presented in previous work.

The observation that even low affinity binding sites for Abf1 and Rap1 play a role in nucleosome occupancy is consistent with previous studies reporting extensive, low-affinity interactions of transcription factors with the yeast and Drosophila genomes (60,61). In a related analysis, Goh et al. (62) have shown reduced nucleosome occupancy surrounding Abf1 motifs even for cohorts having modest P-value in ChIP-chip data (e.g. for P-value between 0.1 and 0.5 compared to P > 0.5). However, this approach does not distinguish whether increased binding is caused by reduced nucleosome occupancy or the converse, whereas we have shown directly that regions showing altered nucleosome occupancy in abf1 ts yeast are enriched even among Abf1 bound regions with modest P-value for binding, as determined by ChIP-chip (Figure 5A). Such interactions may influence chromatin dynamics as well, thereby playing a role in the transient exposure of DNA sequences that are incorporated into nucleosomes (20,63).

It seems likely that transcription factors that bind to large numbers of sites in metazoans play a similar role in influencing genome-wide chromatin structure and dynamics. Factors such as CTCF, NRSF and MyoD in mammalian cells are candidates for this role (29,56,64,65). Combining genome-wide observations with specific genetic perturbations will be essential to revealing these relationships, and future studies will undoubtedly benefit by adopting this approach.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS
The authors thank Charlie Boone, Kevin Struhl and Jon Warner for providing yeast strains, Hope Parker and Stefanie DeVito for contributing to MNase and ChIP analyses and Luisa Battistella for technical assistance. The authors also acknowledge the Wadsworth Center Applied Genomic Technologies and Bioinformatics Cores, and Joe Wade for a critical reading of the article.

FUNDING
National Science Foundation (grant MCB0641776 to R.H.M.); Canadian Institutes of Health Research (MOP-86705; for work in C.N.’s laboratory). Funding for open access charge: National Science Foundation (grant MCB0641776).

Conflict of interest statement. None declared.

REFERENCES


