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

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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

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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 (*Mat a, his3Δ1, leu2Δ0, ura3Δ0, met15Δ0, rap1-2 ts::KanMX*) (a generous gift from Charlie Boone, University of Toronto), were grown at 25°C in 50 ml of YPD broth (1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose) to mid-log phase. Cultures were brought rapidly to 37°C by addition of an equal volume of prewarmed YPD broth and incubated 1 h at 37°C. In one of three experiments using the *rap1 ts* and corresponding wild-type (BY4741) strains, cultures were incubated for 2 h at 37°C; results from these cultures were essentially indistinguishable from those incubated for 1 h at 37°C. Cells were then cross-linked by addition of formaldehyde to a final concentration of 2%, incubated 10 min with shaking at 37°C, and the reaction quenched by addition of glycine to a final concentration of 125 mM and incubation for an additional 5 min prior to chromatin preparation. Chromatin was prepared as previously described, with all steps through MNase digestion being carried out at 37°C (31); digestion with MNase was carried out for 8–10 min at 37°C using 100–300 U/ml of MNase. Reactions were stopped by addition of one-sixth volume 5% SDS/5 mg/ml proteinase K, and incubated at 65°C for >2 h prior to cleaning with phenol and chloroform and ethanol precipitation of DNA, which was applied to microarrays without further purification (13). Preparations used for hybridization to tiling arrays were 40–70% mononucleosomal DNA (Supplementary Figure S1).

For indirect end-label analysis, samples were prepared as described above and digested using lower MNase concentrations (2–20 U/ml). For experiments not involving *ts* mutants [WT and *hmo1Δ* strains BY4741 and yDH544 (32) and WT and *ifh1Δ fhl1Δ* strains W303α and DR35 (33)], cultures were grown at 30°C and formaldehyde cross-linking was omitted. Indirect end-label analysis was performed as described previously (27).

Microarray labeling and hybridization

Nucleosomal DNA samples were fragmented with DNase I 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.bpmap (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

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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://fatigo.bioinfo.cnio.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

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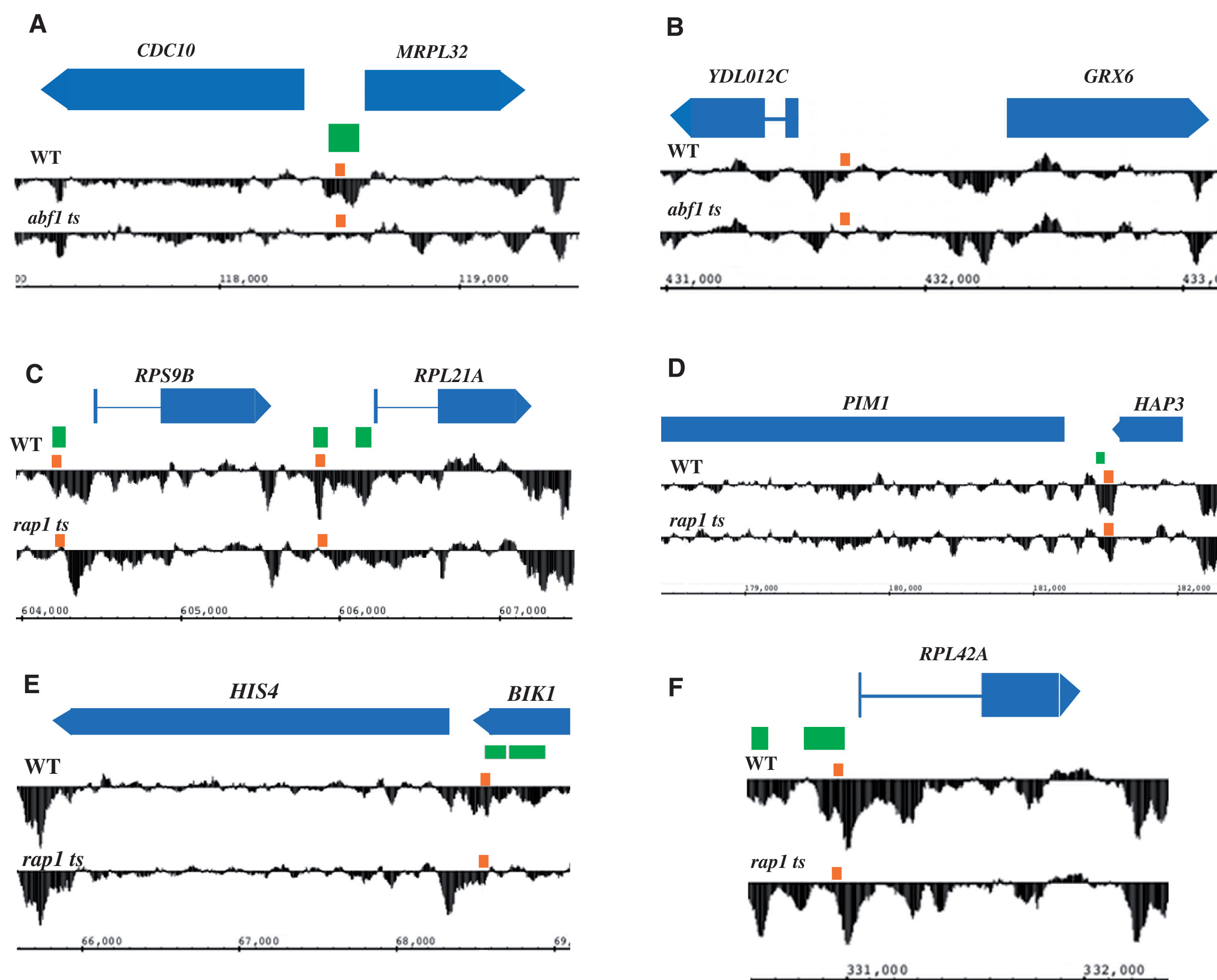

Figure 1. Select genomic regions showing altered nucleosome occupancy profiles in wild-type and *abf1 ts* (**A** and **B**) or *rap1 ts* yeast (**C**–**F**). Relative nucleosome occupancy (black traces) is represented as the $\log_2$ of (nucleosomal DNA/genomic DNA) signal intensity ratio. For each region, blue bars at top show open reading frames, with introns shown as thin lines, pointing to right or left for those on the Watson or Crick strand, respectively (*PIM1* extends beyond the left edge of the region shown); green boxes indicate regions identified as having significantly altered nucleosome occupancy in *ts* mutant yeast; small orange boxes above nucleosome occupancy profiles indicate binding sites for Abf1 or Rap1; and chromosomal coordinates are indicated at bottom. Note that the region in the vicinity of the Abf1-binding site upstream of *YDL102C* (**B**) did not show significantly altered nucleosome occupancy with the parameters used in the Tiling Array Software program; nonetheless, a modest increase in occupancy is seen at this region.

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.

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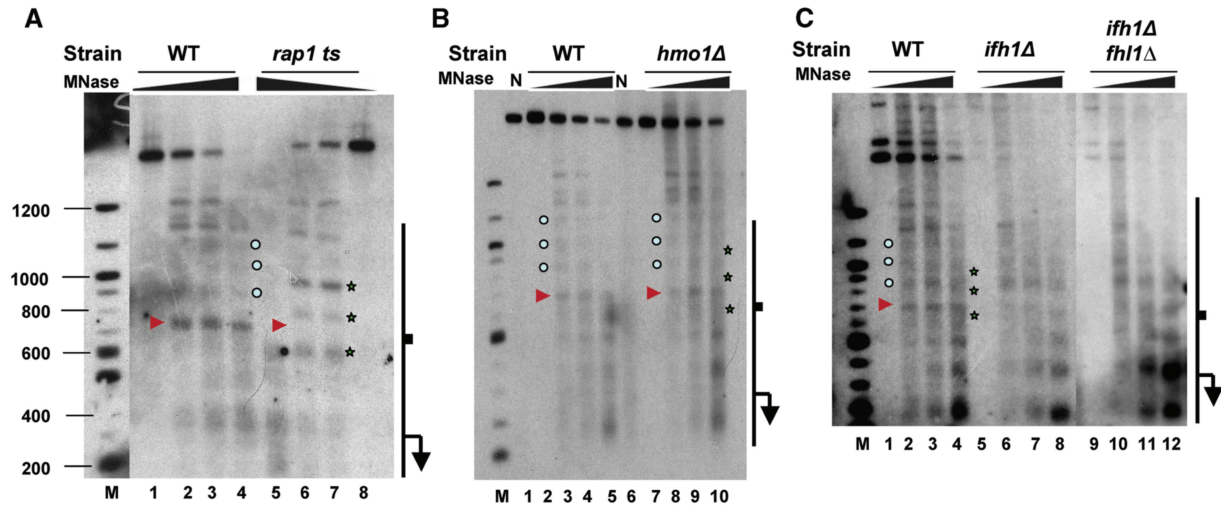

Figure 2. Indirect end-labeling analysis of chromatin structure at the *RPL42A* promoter (**A**) in wild-type and *rap1 ts* yeast, (**B**) in wild-type and *hmo1Δ* yeast, and (**C**) in wild-type, *ifh1Δ*, and *ifh1Δ fhl1Δ* yeast. Increasing amounts of MNase were used, from 0 to 20 U/ml (triangles), to digest chromatin prepared from the indicated strains after 1 h at 37°C (**A**) or grown at 30°C (**B** and **C**), and to digest naked DNA (lanes N). MNase cleavage sites were mapped relative to a BamHI site at +245 bp. Cleavage sites characteristic of chromatin from wild-type cells are indicated by small circles, with a prominent cleavage indicated by the red arrow, while cleavages characteristic of the *ts* mutant are indicated by stars. Note the presence of the former and absence of the latter cleavages in *hmo1Δ*, *ifh1Δ* and *ifh1Δ fhl1Δ* yeast in (**B** and **C**).

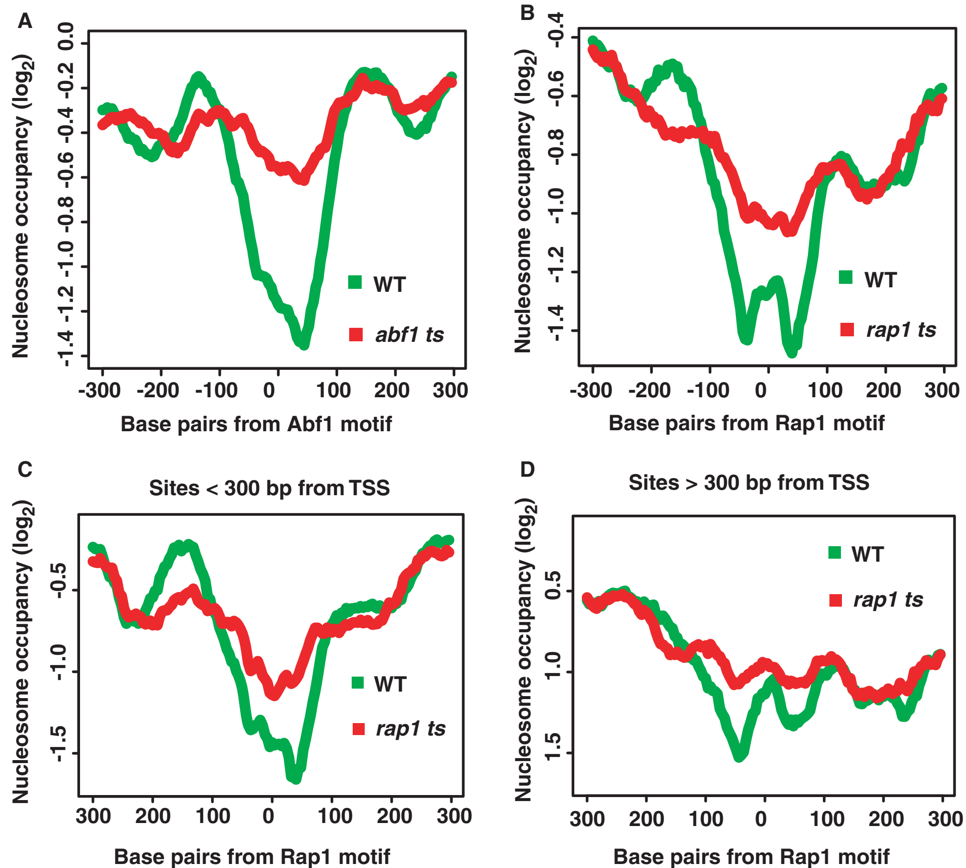

Figure 3. Averaged nucleosome occupancy profiles for (**A**) 67 promoter regions surrounding unique Abf1 sites from genes identified as probable or putative targets of Abf1 (23), in *abf1 ts* and WT yeast; (**B**) 66 promoter regions surrounding unique Rap1 sites from genes identified as probable or 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.

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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%; $P < 10^{-5}$) 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;

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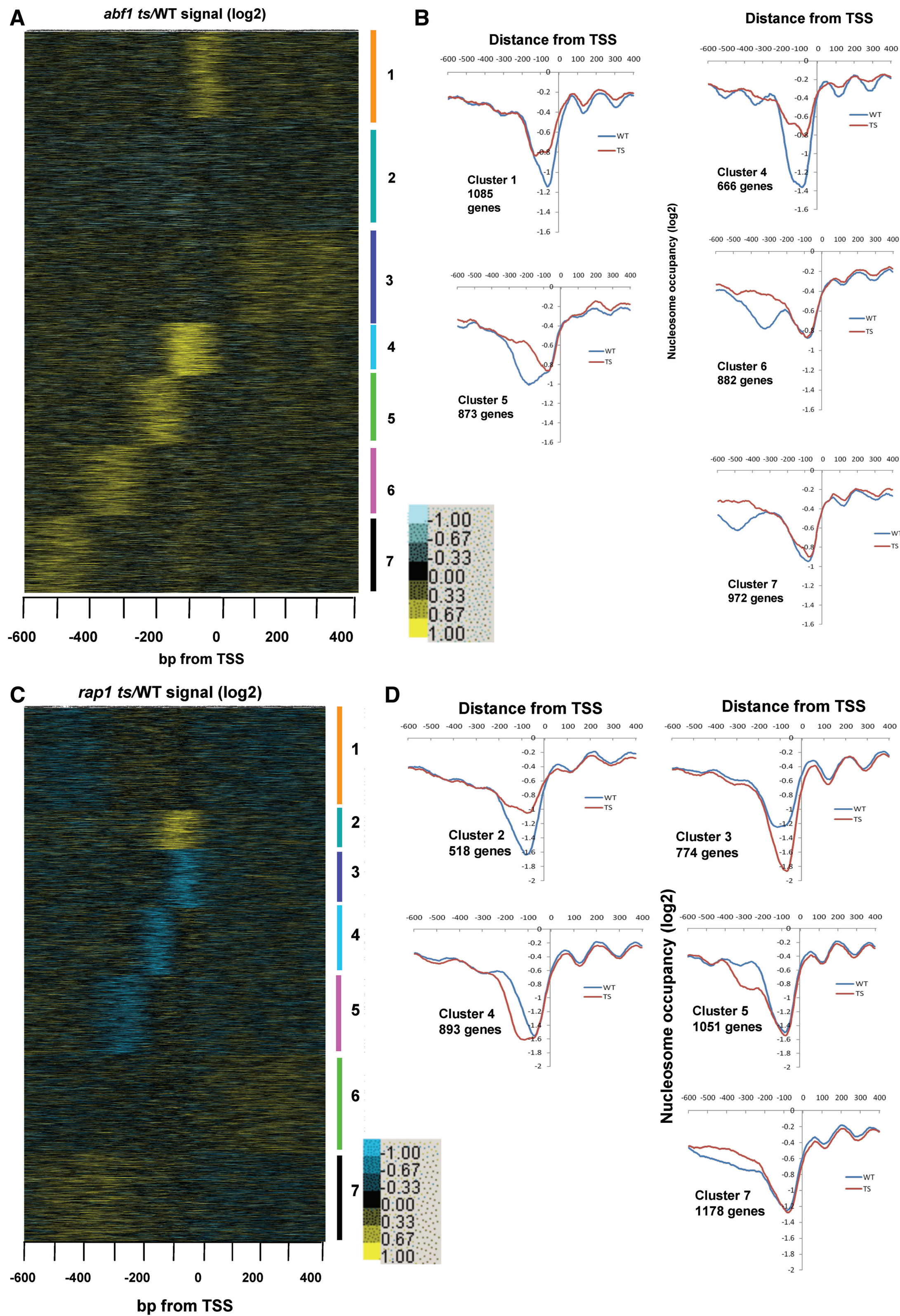

Figure 4. *K*-means clustering ($K = 7$) for $\log_2$ of nucleosome occupancy for (**A**) *abf1 ts* yeast and (**C**) *rap1 ts* over WT for 7052 genes, aligned by TSS; yellow represents increased nucleosome occupancy in the *ts* mutant, while blue represents depletion. Line graphs for average nucleosome 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).

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

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

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.

$P < 10^{-8}$) (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 7×10^{-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 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 TFBSs may exert little effect on transcription in mammalian cells (56), and that nucleosome occupancy diverges much more

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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 ~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 *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 [$\log(P) < -0.5$ for Abf1 and $\log(P) < -0.9$ 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

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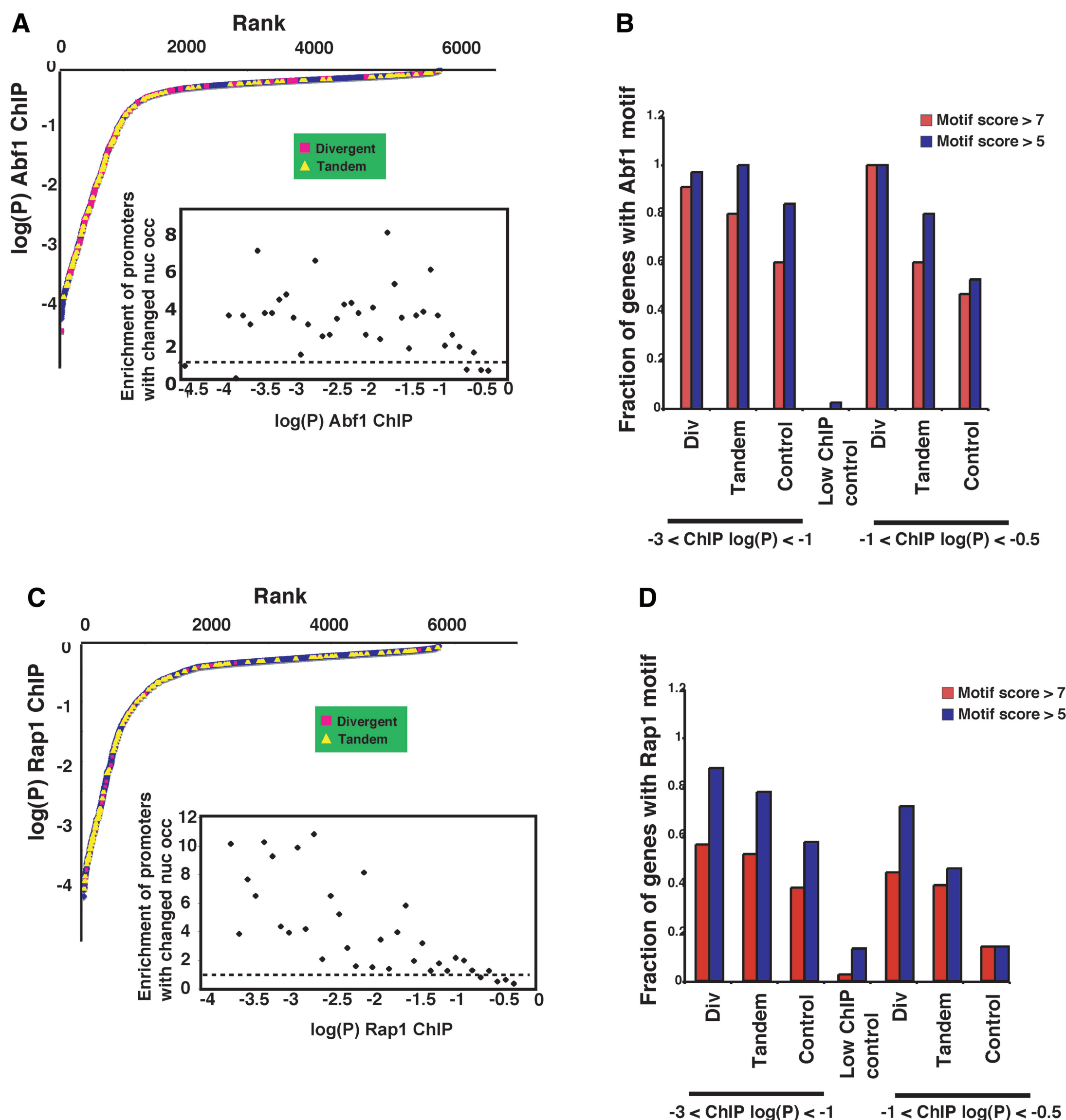

Figure 5. Regions having altered nucleosome occupancy in *abf1 ts* or *rap1 ts* yeast are enriched for both strong and weak Abf1 and Rap1-binding sites. Graphs of genes ranked by log(P) for ChIP against (**A**) Abf1 or (**C**) Rap1 (53) are shown, with promoters containing regions having significantly 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 ChIP 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.

(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* 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

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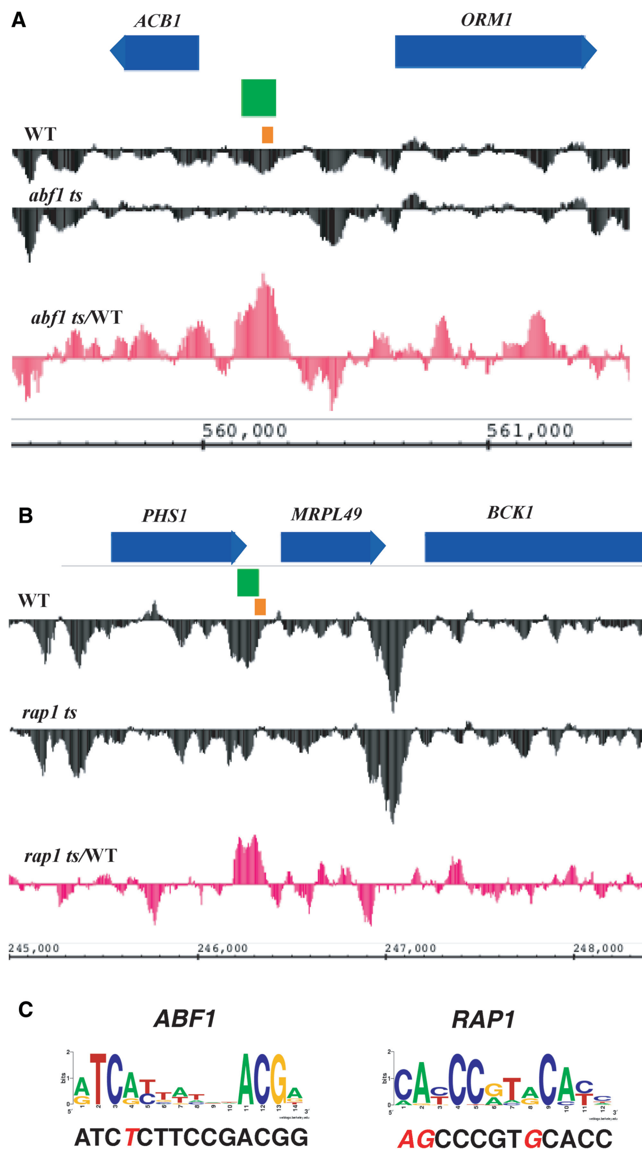

Figure 6. Nucleosome occupancy profiles in the region of the *ORM1* gene in WT and *abf1 ts* yeast (**A**) and in the region of the *MRPL49* gene in WT and *rap1 ts* yeast (**B**); see Figure 1 legend for additional details. (**C**) Motifs found in the regions of altered nucleosome occupancy in (**A** and **B**) [orange boxes in (**A** and **B**)] are compared to logos for the Abf1 and Rap1 consensus motifs (23); differences are highlighted by red italic letters.

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.

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REFERENCES

1. Owen-Hughes,T. and Workman,J.L. (1994) Experimental analysis of chromatin function in transcription control. *Crit. Rev. Eukaryot. Gene Expr.*, **4**, 403–441.

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2. Simpson,R.T. (1991) Nucleosome positioning: occurrence, mechanisms, and functional consequences. *Prog. Nucleic Acid Res. Mol. Biol.*, **40**, 143–184.
3. Thoma,F. (1992) Nucleosome positioning. *Biochim. Biophys. Acta*, **1130**, 1–19.
4. Radman-Livaja,M. and Rando,O.J. (2010) Nucleosome positioning: how is it established, and why does it matter? *Dev. Biol.*, **339**, 258–266.
5. Hirschhorn,J.N., Brown,S.A., Clark,C.D. and Winston,F. (1992) Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev.*, **6**, 2288–2298.
6. Thoma,F. and Simpson,R.T. (1985) Local protein-DNA interactions may determine nucleosome positions on yeast plasmids. *Nature*, **315**, 250–252.
7. Morse,R.H. (1993) Nucleosome disruption by transcription factor binding in yeast. *Science*, **262**, 1563–1566.
8. Satchwell,S.C., Drew,H.R. and Travers,A.A. (1986) Sequence periodicities in chicken nucleosome core DNA. *J. Mol. Biol.*, **191**, 659–675.
9. Tsankov,A.M., Thompson,D.A., Socha,A., Regev,A. and Rando,O.J. (2010) The role of nucleosome positioning in the evolution of gene regulation. *PLoS Biol.*, **8**, e1000414.
10. Ioshikhes,I.P., Albert,I., Zanton,S.J. and Pugh,B.F. (2006) Nucleosome positions predicted through comparative genomics. *Nat. Genet.*, **38**, 1210–1215.
11. Bernstein,B.E., Liu,C.L., Humphrey,E.L., Perlstein,E.O. and Schreiber,S.L. (2004) Global nucleosome occupancy in yeast. *Genome Biol.*, **5**, R62.
12. Kaplan,N., Moore,I.K., Fondufe-Mittendorf,Y., Gossett,A.J., Tillo,D., Field,Y., LeProust,E.M., Hughes,T.R., Lieb,J.D., Widom,J. *et al.* (2009) The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature*, **458**, 362–366.
13. Lee,W., Tillo,D., Bray,N., Morse,R.H., Davis,R.W., Hughes,T.R. and Nislow,C. (2007) A high-resolution atlas of nucleosome occupancy in yeast. *Nat. Genet.*, **39**, 1235–1244.
14. Mavrich,T.N., Ioshikhes,I.P., Venters,B.J., Jiang,C., Tomsho,L.P., Qi,J., Schuster,S.C., Albert,I. and Pugh,B.F. (2008) A barrier nucleosome model for statistical positioning of nucleosomes throughout the yeast genome. *Genome Res.*, **18**, 1073–1083.
15. Whitehouse,I., Rando,O.J., Delrow,J. and Tsukiyama,T. (2007) Chromatin remodelling at promoters suppresses antisense transcription. *Nature*, **450**, 1031–1035.
16. Zhang,Y., Moqtaderi,Z., Rattner,B.P., Euskirchen,G., Snyder,M., Kadonaga,J.T., Liu,X.S. and Struhl,K. (2009) Intrinsic histone-DNA interactions are not the major determinant of nucleosome positions in vivo. *Nat. Struct. Mol. Biol.*, **16**, 847–852.
17. Zhang,Y., Moqtaderi,Z., Rattner,B.P., Euskirchen,G., Snyder,M., Kadonaga,J.T., Liu,X.S. and Struhl,K. (2010) Reply to 'evidence against a genomic code for nucleosome positioning'. *Nat. Struct. Mol. Biol.*, **17**, 920–923.
18. Kaplan,N., Moore,I., Fondufe-Mittendorf,Y., Gossett,A.J., Tillo,D., Field,Y., Hughes,T.R., Lieb,J.D., Widom,J. and Segal,E. (2010) Nucleosome sequence preferences influence in vivo nucleosome organization. *Nat. Struct. Mol. Biol.*, **17**, 918–920.
19. Morse,R.H. (2003) Getting into chromatin: how do transcription factors get past the histones? *Biochem. Cell Biol.*, **81**, 101–112.
20. Morse,R.H. (2007) Transcription factor access to promoter elements. *J. Cell. Biochem.*, **102**, 560–570.
21. Morse,R.H. (2000) RAP, RAP, open up! new wrinkles for RAP1 in yeast. *Trends Genet.*, **16**, 51–53.
22. Shore,D. (1994) RAP1: a protean regulator in yeast. *Trends Genet.*, **10**, 408–412.
23. Yarragudi,A., Parfrey,L.W. and Morse,R.H. (2007) Genome-wide analysis of transcriptional dependence and probable target sites for Abf1 and Rap1 in Saccharomyces cerevisiae. *Nucleic Acids Res.*, **35**, 193–202.
24. Schlecht,U., Erb,I., Demougin,P., Robine,N., Borde,V., Nimwegen,E., Nicolas,A. and Primig,M. (2008) Genome-wide expression profiling, in vivo DNA binding analysis, and probabilistic motif prediction reveal novel Abf1 target genes during fermentation, respiration, and sporulation in yeast. *Mol. Biol. Cell*, **19**, 2193–2207.
25. Lascaris,R.F., Groot,E., Hoen,P.B., Mager,W.H. and Planta,R.J. (2000) Different roles for Abf1p and a T-rich promoter element in nucleosome organization of the yeast RPS28A gene. *Nucleic Acids Res.*, **28**, 1390–1396.
26. Devlin,C., Tice-Baldwin,K., Shore,D. and Arndt,K.T. (1991) RAP1 is required for BAS1/BAS2- and GCN4-dependent transcription of the yeast HIS4 gene. *Mol. Cell. Biol.*, **11**, 3642–3651.
27. Yarragudi,A., Miyake,T., Li,R. and Morse,R.H. (2004) Comparison of ABF1 and RAP1 in chromatin opening and transactivator potentiation in the budding yeast Saccharomyces cerevisiae. *Mol. Cell. Biol.*, **24**, 9152–9164.
28. Yu,L. and Morse,R.H. (1999) Chromatin opening and transactivator potentiation by RAP1 in Saccharomyces cerevisiae. *Mol. Cell. Biol.*, **19**, 5279–5288.
29. Badis,G., Chan,E.T., van Bakel,H., Pena-Castillo,L., Tillo,D., Tsui,K., Carlson,C.D., Gossett,A.J., Hasinoff,M.J., Warren,C.L. *et al.* (2008) A library of yeast transcription factor motifs reveals a widespread function for Rsc3 in targeting nucleosome exclusion at promoters. *Mol. Cell*, **32**, 878–887.
30. Hartley,P.D. and Madhani,H.D. (2009) Mechanisms that specify promoter nucleosome location and identity. *Cell*, **137**, 445–458.
31. Yu,L., Sabet,N., Chambers,A. and Morse,R.H. (2001) The N-terminal and C-terminal domains of RAP1 are dispensable for chromatin opening and GCN4-mediated HIS4 activation in budding yeast. *J. Biol. Chem.*, **276**, 33257–33264.
32. Hall,D.B., Wade,J.T. and Struhl,K. (2006) An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in Saccharomyces cerevisiae. *Mol. Cell. Biol.*, **26**, 3672–3679.
33. Rudra,D., Zhao,Y. and Warner,J.R. (2005) Central role of Ifh1p-Fhl1p interaction in the synthesis of yeast ribosomal proteins. *EMBO J.*, **24**, 533–542.
34. Nagalakshmi,U., Wang,Z., Waern,K., Shou,C., Raha,D., Gerstein,M. and Snyder,M. (2008) The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science*, **320**, 1344–1349.
35. David,L., Huber,W., Granovskaia,M., Toedling,J., Palm,C.J., Bofkin,L., Jones,T., Davis,R.W. and Steinmetz,L.M. (2006) A high-resolution map of transcription in the yeast genome. *Proc. Natl Acad. Sci. USA*, **103**, 5320–5325.
36. Bailey,T.L. and Elkin,C. (1994) *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology*, AAAI Press, Menlo Park, CA, pp. 28–36.
37. van Helden,J. (2003) Regulatory sequence analysis tools. *Nucleic Acids Res.*, **31**, 3593–3596.
38. Teixeira,M.C., Monteiro,P., Jain,P., Tenreiro,S., Fernandes,A.R., Mira,N.P., Alenquer,M., Freitas,A.T., Oliveira,A.L. and Sa-Correia,I. (2006) The YEASTRACT database: a tool for the analysis of transcription regulatory associations in Saccharomyces cerevisiae. *Nucleic Acids Res.*, **34**, D446–D451.
39. Al-Shahrour,F., Diaz-Uriarte,R. and Dopazo,J. (2004) FatiGO: a web tool for finding significant associations of gene ontology terms with groups of genes. *Bioinformatics*, **20**, 578–580.
40. Drazinic,C.M., Smerage,J.B., Lopez,M.C. and Baker,H.V. (1996) Activation mechanism of the multifunctional transcription factor repressor-activator protein 1 (Rap1p). *Mol. Cell. Biol.*, **16**, 3187–3196.
41. Miyake,T., Reese,J., Loch,C.M., Auble,D.T. and Li,R. (2004) Genome-wide analysis of ARS (autonomously replicating sequence) binding factor 1 (Abf1p)-mediated transcriptional regulation in Saccharomyces cerevisiae. *J. Biol. Chem.*, **279**, 34865–34872.
42. Schroeder,S.C. and Weil,P.A. (1998) Genetic tests of the role of Abf1p in driving transcription of the yeast TATA box bindng protein-encoding gene, SPT15. *J. Biol. Chem.*, **273**, 19884–19891.
43. Kuhn,R.M., Karolchik,D., Zweig,A.S., Wang,T., Smith,K.E., Rosenbloom,K.R., Rhead,B., Raney,B.J., Pohl,A., Pheasant,M. *et al.* (2009) The UCSC Genome Browser Database: update 2009. *Nucleic Acids Res.*, **37**, D755–D761.
44. Nedospasov,S.A. and Georgiev,G.P. (1980) Non-random cleavage of SV40 DNA in the compact minichromosome and free in solution by micrococcal nuclease. *Biochem. Biophys. Res. Commun.*, **92**, 532–539.

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45. Wu,C. (1980) The 5′ ends of Drosophila heat shock genes in chromatin are hypersensitive to DNase I. *Nature*, **286**, 854–860.
46. Yuan,G.C., Liu,Y.J., Dion,M.F., Slack,M.D., Wu,L.F., Altschuler,S.J. and Rando,O.J. (2005) Genome-scale identification of nucleosome positions in *S. cerevisiae*. *Science*, **309**, 626–630.
47. Kornberg,R.D. and Stryer,L. (1988) Statistical distributions of nucleosomes: nonrandom locations by a stochastic mechanism. *Nucleic Acids Res.*, **16**, 6677–6690.
48. Garbett,K.A., Tripathi,M.K., Cencki,B., Layer,J.H. and Weil,P.A. (2007) Yeast TFIID serves as a coactivator for Rap1p by direct protein-protein interaction. *Mol. Cell. Biol.*, **27**, 297–311.
49. Schawalder,S.B., Kabani,M., Howald,I., Choudhury,U., Werner,M. and Shore,D. (2004) Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1. *Nature*, **432**, 1058–1061.
50. Wade,J.T., Hall,D.B. and Struhl,K. (2004) The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. *Nature*, **432**, 1054–1058.
51. Kasahara,K., Ohtsuki,K., Ki,S., Aoyama,K., Takahashi,H., Kobayashi,T., Shirahige,K. and Kokubo,T. (2007) Assembly of regulatory factors on rRNA and ribosomal protein genes in Saccharomyces cerevisiae. *Mol. Cell. Biol.*, **27**, 6686–6705.
52. Luscombe,N.M., Babu,M.M., Yu,H., Snyder,M., Teichmann,S.A. and Gerstein,M. (2004) Genomic analysis of regulatory network dynamics reveals large topological changes. *Nature*, **431**, 308–312.
53. Harbison,C.T., Gordon,D.B., Lee,T.I., Rinaldi,N.J., Macisaac,K.D., Danford,T.W., Hannett,N.M., Tagne,J.B., Reynolds,D.B., Yoo,J. *et al*. (2004) Transcriptional regulatory code of a eukaryotic genome. *Nature*, **431**, 99–104.
54. Morris,R.T., O'Connor,T.R. and Wyrick,J.J. (2009) Ceres: software for the integrated analysis of transcription factor binding sites and nucleosome positions in *S. cerevisiae*. *Bioinformatics*, **26**, 168–174.
55. Boorsma,A., Foat,B.C., Vis,D., Klis,F. and Bussemaker,H.J. (2005) T-profiler: scoring the activity of predefined groups of genes using gene expression data. *Nucleic Acids Res.*, **33**, W592–W595.
56. Cao,Y., Yao,Z., Sarkar,D., Lawrence,M., Sanchez,G.J., Parker,M.H., MacQuarrie,K.L., Davison,J., Morgan,M.T., Ruzzo,W.L. *et al*. (2010) Genome-wide MyoD binding in skeletal muscle cells: a potential for broad cellular reprogramming. *Dev. Cell*, **18**, 662–674.
57. Tirosh,I., Sigal,N. and Barkai,N. (2010) Divergence of nucleosome positioning between two closely related yeast species: genetic basis and functional consequences. *Mol. Syst Biol.*, **6**, 365.
58. Grunstein,M. (1997) Molecular model for telomeric heterochromatin in yeast. *Curr. Opin. Cell Biol.*, **9**, 383–387.
59. MacIsaac,K.D., Wang,T., Gordon,D.B., Gifford,D.K., Stormo,G.D. and Fraenkel,E. (2006) An improved map of conserved regulatory sites for Saccharomyces cerevisiae. *BMC Bioinformatics*, **7**, 113.
60. Tanay,A. (2006) Extensive low-affinity transcriptional interactions in the yeast genome. *Genome Res.*, **16**, 962–972.
61. Li,X.Y., MacArthur,S., Bourgon,R., Nix,D., Pollard,D.A., Iyer,V.N., Hechmer,A., Simirenko,L., Stapleton,M., Luengo Hendriks,C.L. *et al*. (2008) Transcription factors bind thousands of active and inactive regions in the Drosophila blastoderm. *PLoS Biol.*, **6**, e27.
62. Goh,W.S., Orlov,Y., Li,J. and Clarke,N.D. (2010) Blurring of high-resolution data shows that the effect of intrinsic nucleosome occupancy on transcription factor binding is mostly regional, not local. *PLoS Comput. Biol.*, **6**, e1000649.
63. van Holde,K. and Zlatanova,J. (2006) Scanning chromatin: a new paradigm? *J. Biol. Chem.*, **281**, 12197–12200.
64. Bruce,A.W., Lopez-Contreras,A.J., Flicek,P., Down,T.A., Dhami,P., Dillon,S.C., Koch,C.M., Langford,C.F., Dunham,I., Andrews,R.M. *et al*. (2009) Functional diversity for REST (NRSF) is defined by in vivo binding affinity hierarchies at the DNA sequence level. *Genome Res.*, **19**, 994–1005.
65. Fu,Y., Sinha,M., Peterson,C.L. and Weng,Z. (2008) The insulator binding protein CTCF positions 20 nucleosomes around its binding sites across the human genome. *PLoS Genet.*, **4**, e1000138.
66. Hertz,G.Z. and Stormo,G.D. (1999) Identifying DNA and protein patterns with statistically significant alignments of multiple sequences. *Bioinformatics*, **15**, 563–577.

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