Repressor activator protein 1 (RAP1) assists GCN4-mediated HIS4 activation by overcoming some repressive aspect of chromatin structure to facilitate GCN4 binding. RAP1 also participates in other nuclear processes, and discrete domains of RAP1 have been shown to have specific properties including DNA binding, DNA bending, transcriptional activation, and silencing and telomere functions. To investigate whether specific domains of RAP1 are required to "open" chromatin and help GCN4 to activate the HIS4 gene, we examined the abilities of different truncated RAP1 proteins to perturb positioned nucleosomes via a nucleosomal RAP1 site in a yeast episome in vivo, and we tested HIS4 activation in yeast strains harboring truncated RAP1 mutants. We found that neither the DNA bending domain nor the putative activation domain of RAP1 is required for its ability to perturb the chromatin structure of a plasmid containing a RAP1 site. Similarly, neither the putative activation domain nor the N-terminal DNA-bending domain was required for GCN4-mediated activation of HIS4. We also used a rap1ts mutant to show that continuous occupancy of the HIS4 promoter by RAP1 is required for GCN4-mediated gene activation.

Repressor activator protein 1 (RAP1) is an essential protein in yeast. Binding sites for RAP1 have been found in promoters, silencers, and telomeres, and correspondingly, RAP1 participates in gene transcription, silencing, and telomere maintenance (1). RAP1 has been implicated in transcriptional activation of many genes including the mating-type genes Matα1 and Matα2, ribosomal protein genes, and glycolytic genes. RAP1 also contributes to a meiotic recombination at the HIS4 locus (2), and a cluster of RAP1 binding sites from the TEF1 promoter has recently been shown to function as a boundary element that can prevent the spread of silent chromatin (3).

The ability of RAP1 to play such disparate roles in yeast depends in part on its ability to interact with a variety of other proteins. In many cases, the sites of interaction have been mapped, and a domain structure for RAP1 has been constructed based on these and other findings (Fig. 1). The central part of this 827-amino acid protein contains the DNA-binding domain, and the C terminus contains domains that interact with the SIR and RIF proteins, which are important for silencing and telomeric length control (4). Also, within this C-terminal part, amino acids 630–695 function as an activation domain in a hybrid GAL4-RAP1 fusion protein (5). The N terminus of RAP1 is a large region that is not essential for cell viability, although it may be involved in regulating the activity of RAP1 through a putative BRCT domain (6). It has also been shown to potentiate DNA bending by RAP1 in vitro (7). RAP1 also contributes to the transcription of glycolytic enzyme genes via cooperative interactions with the activator GCR1, and in this case, in vitro experiments have shown that either the N-terminal or the C-terminal moiety of RAP1 together with the DNA-binding domain suffices to assist GCR1 binding, although the DNA-binding domain alone does not (8).

A property of RAP1 that is likely to contribute to its function in disparate processes is a potent ability to perturb chromatin structure in a way that increases access by DNA-binding proteins and nucleases, a property we refer to as "chromatin opening" (9, 10). This property most clearly contributes to RAP1 function at the HIS4 promoter where a RAP1 binding site is required to overcome the repressive effect of chromatin structure and allow GCN4 to bind and activate transcription in response to conditions of amino acid starvation (10, 11). When HIS4 activation is made to depend on other activators with a better ability to outcompete histones for binding to DNA, the RAP1 site becomes dispensable, indicating that the RAP1 requirement is a consequence of the poor ability of GCN4 to bind to chromatin under physiological conditions (10). More recently, we have used chromatin immunoprecipitation to show directly that GCN4 binding to the HIS4 promoter is reduced when the RAP1 binding site is mutated.2 The ability of RAP1 to open chromatin can also be assessed by placing a RAP1 site into a stable episome that serves as a chromatin reporter in yeast cells. An introduction of a mutant RAP1 site allows a positioned nucleosome to form that incorporates the mutant site, whereas a wild-type RAP1 binding site (the same one present in the HIS4 promoter) prevents nucleosome positioning in its near vicinity (10).

Nucleosome perturbation via a GAL4 site near the center of a positioned nucleosome or via a PHO4 binding site just outside a positioned nucleosome requires the binding protein to have a functional activation domain (12–14). Therefore, it was natural

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This paper is dedicated to the memory of a good friend, Alan Wolffe.

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1 The abbreviations used are: RAP, repressor activator protein; HIS, histidine; MNase, micrococcal nuclease; CSM, complete synthetic medium.

2 L. Yu, J. P. Madigan, and R. H. Morse, unpublished results.
to imagine that the ability of RAP1 to open chromatin and to contribute to HIS4 activation and perhaps the activation of other genes might likewise require its putative activation domain. To determine whether the putative activation domain or other domains of RAP1 are required for it to open chromatin, we used a series of yeast strains expressing truncated derivatives of RAP1 to examine the abilities of these derivatives to perturb positioned nucleosomes containing RAP1 binding sites and to contribute to the expression of HIS4 mediated by GCN4. We also used a strain expressing a RAP1 mutant that is conditional for DNA binding to examine whether RAP1 binding is required for continuous expression of HIS4 or only for the initial chromatin opening event that allows GCN4 to bind.

RESULTS

Both the N-terminal and the C-terminal Domains of RAP1, Including the Putative Activation Domain, Are Dispensable for Its Ability to Perturb a Positioned Nucleosome in Vivo—Full-length RAP1 has a strong ability to perturb nucleosome positioning (10). In the assay used to demonstrate this property of RAP1, the chromatin structures of two yeast epims, TAR/GCN1Δ80 and TARmut/GCN1Δ80, are compared (Fig. 2A). These two epims were constructed by placing a wild-type or mutant RAP1 binding site, respectively, into the parent plasmid TAR/GCN1Δ80. This parent plasmid has a GCN4 binding site in a strongly positioned nucleosome, and GCN4 perturbs this nucleosome very little at normal physiological levels under either uninduced or induced conditions. The introduction of the mutant RAP1 binding site results in a mild perturbation of the chromatin structure in yeast, but a positioned nucleosome that incorporates the mutant RAP1 binding site remains clearly evident. In contrast, an introduction of the wild-type RAP1 binding site abolishes any trace of nucleosome positioning in its vicinity with the pattern of micrococcal nuclease cleavage sites being identical in TAR/GCN1Δ80 for chromatin and naked DNA (10).

To determine which domains of RAP1 are needed for its ability to perturb positioned nucleosomes in vivo, we tested the abilities of different truncated derivatives of RAP1 to perturb the chromatin structure of TAR/GCN1Δ80 relative to TARmut/ GCN1Δ80 in yeast. The chromatin structure of TAR/GCN1Δ80 and TARmut/GCN1Δ80 was examined by MNase cleavage followed by indirect-end labeling (18, 19). Fig. 2 shows the results for rap1Δ622 cells in which the silencing domain of RAP1 and a part of the putative transactivation domain are absent. The MNase digestion pattern of TAR/GCN1Δ80 chromatin is very similar to that of the naked DNA (Fig. 2B, lanes 3–7 compared with lane 1) as reported previously in cells expressing full-length RAP1 (10) with the positioning of nucleosomes I and II essentially abolished. In contrast, the cleavage pattern characteristic of positioned nucleosomes I and II is largely retained in TARmut/GCN1Δ80 (Fig. 2C), note the two cleavages indicated by stars that are present in naked DNA and are reduced or absent in the chromatin samples as well as the enhanced cleavage at the “upper” border of nucleosome II. This finding suggests that the C-terminal domain of RAP1 that is important for its functions at silencers and telomeres is not required for its strong ability to perturb chromatin structure. Consistent with this result, an absence of SIR4, which is part of the telomeric complex that includes RAP1 (20, 21), does not impair the ability of RAP1 to perturb nucleosome positioning (Fig. 2D).

In rap1Δ628 cells in which the entire putative transactivation domain of RAP1 is missing, the cleavage pattern of TAR/ GCN1Δ80 chromatin was again similar to that of naked DNA (Fig. 3A). RAP1 lacking the N-terminal domain also retained
its ability to perturb TAR/GCN1Δ80 chromatin (Fig. 3B). Changes in MNase cleavages, characteristic of positioned nucleosomes I and II, were still observed in TARmut/GCN1Δ80 in these mutant rap1 backgrounds (data not shown). Thus, the DNA-bending domain, the silencing domain, and the putative activation domain of RAP1 are dispensable for its ability to perturb a positioned nucleosome in vivo.

Both the N-terminal and C-terminal Domains of RAP1 Are Dispensable for GCN4-mediated HIS4 Activation—RAP1 opens up the chromatin structure at the HIS4 promoter to facilitate GCN4-mediated activation (10, 11). If perturbing chromatin structure for other activators is the only function of RAP1 at the HIS4 promoter, we would predict that if a particular truncated RAP1 maintains its ability to perturb chromatin structure in vivo, its ability to help GCN4 to activate HIS4 should also be maintained. To test this hypothesis, we examined mRNA levels of HIS4 in the same rap1 mutant strains used to analyze the perturbation of nucleosome positioning in the preceding section.

We examined levels of HIS4 mRNA in wild-type yeast and in yeast harboring the rap1Δ662, rap1Δ628, and rap1ΔN mutations treated with 5-methyltryptophan. This approach necessitated using TRP+ cells, so we first transformed strains with TRP1-containing plasmids. The uninduced levels of HIS4 mRNA in these strains were fairly high, most probably because all four strains are his− (22). As shown in Fig. 4, the observed levels of HIS4 expression normalized to ACT1 mRNA levels were essentially identical in wild-type cells and the three mutant strains. We conclude that GCN4-mediated HIS4 expression is not substantially affected by the deletion of the N-terminal DNA-bending domain or the C-terminal silencing domain or putative activation domain. We also found that HIS4 expression was not affected by the deletion of SIR4, consistent with the finding that the silencing domain of RAP1 is unimportant for HIS4 regulation (data not shown).

Continuous RAP1 Binding to DNA Is Not Required for Perturbation of Nucleosome Positioning in TAR/GCN1Δ80—RAP1 is needed at the HIS4 promoter to facilitate activator binding by overcoming the repressive effects of the chromatin structure. We were interested in determining whether RAP1 was required only for the establishment of an open chromatin structure or for its maintenance even after transcriptional activation had begun. In the former case, RAP1 should be dispensable after the induction of transcription. In the latter case, RAP1 would be required for continued transcription after induction.

We first examined whether the effects on chromatin structure caused by the binding of RAP1 would persist after a loss of

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**TABLE I**

Yeast strains used in this study

| Strain         | Genotype                  | Reference |
|----------------|---------------------------|-----------|
| rap1Δ662       | ade2–1, trp1–1, leu2 his3–11, rap1Δ662 | 42        |
| rap1Δ628       | ade2–1, trp1–1, leu2 his3–11, rap1Δ628 | 42        |
| JLG1–45D       | MATα, ade2–1, ade3::KnMX, can1–100, his3–11,15, leu2–3,112, trp1–1, uro3–1 | 20        |
| JLG1–25A       | MATα, ade2–1, ade3::KnMX, can1–100, his3–11,15, leu2–3,112, trp1–1, uro3–1, sir4Δ::HIS3 | 20        |
| LYY275         | MATα, ade2–1, can1–100, his3–11,15, leu2–3,112, trp1–1, uro3–1 | 4         |
| YDS2           | MATα, ade2–1, can1–100, his3–11,15, leu2–3,112, trp1–1, uro3–1, rap1ΔΔ2–2 | 4         |

This study
RAP1. To address this question, we used the rap1–2ts mutant that is temperature sensitive for binding to DNA both in vitro and in vivo (23, 24). We introduced TAR/GCN1Δ80 and TARmut/GCN1Δ80 into yeast harboring the rap1–2ts allele and monitored the chromatin structure at the permissive temperature and at various times after a shift to nonpermissive temperatures. The results of such an experiment are shown in Fig. 5. When cells were grown at 25 °C, the permissive temperature, the chromatin structure of TAR/GCN1Δ80 was affected by RAP1 binding as we have seen before (Fig. 5A, lanes 1–3). At 2 and 4 h after the cells were shifted to 37 °C, no significant change of MNase accessibility was seen in this plasmid (Fig. 5, lanes 4 and 5). Only after 6 h after the shift to nonpermissive temperature was a significant chromatin structure change detected in the vicinity of nucleosomes I and II (Fig. 5A, lane 6; note the protection against the cleavage in the region of nucleosome I is most easily visualized in the densitometric scan of Fig. 5C). As a control, we monitored the chromatin structure of TARmut/GCN1Δ80 in a similar experiment and found no change in its chromatin structure during 6 h at 37 °C (Fig. 5, B and C).

Because dimethyl sulfate footprinting experiments have shown that the RAP1–2ts protein is lost from the TPI promoter within 30 min of the shift to a nonpermissive temperature (24), these results suggest that the chromatin structure of TAR/GCN1Δ80 resulting from RAP1 binding is maintained long after RAP1 is lost from the plasmid. Furthermore, although RAP1 is an essential protein, the survival rate of the rap1–2ts strain 8 h after being shifted to a nonpermissive temperature is about 70% of its wild-type counterpart (23). This finding implies that the majority of the cells are still viable even 6 h after the temperature shift, so that the failure to recover nucleosome positioning in TAR/GCN1Δ80 after a loss of RAP1 is not because of cell death. The alterations seen 6 h after the temperature shift provide strong evidence that the chromatin structure of TAR/GCN1Δ80 is indeed dictated by RAP1 binding, that RAP1 is lost upon temperature shift, and that chromatin remodeling is not prevented by the failure of basic cellular processes at the nonpermissive temperature. These results indicate that the chromatin structure dictated by RAP1 binding to TAR/GCN1Δ80 persists for considerable time after RAP1 is lost from the DNA.

Continuous RAP1 Binding Is Required for GCN4-mediated HIS4 Activation—To test whether continuous binding of RAP1 at the HIS4 promoter is required to maintain GCN4-mediated HIS4 activation, we examined the mRNA level of HIS4 in a rap1–2ts yeast strain before and after shifting the cells from permissive temperature to a nonpermissive temperature. To simplify the experiment, GCN4 was constitutively expressed from the DED1 promoter in a single copy plasmid. Either the DED1-GCN4 plasmid or an empty vector was transformed into the rap1–2ts strain and its wild-type counterpart. Cells were grown in media having suboptimal levels of histidine for strains containing the DED1-GCN4 plasmid or in media containing histidine for cells containing the empty vector at 25 °C overnight until they reached the log phase, and then they were inoculated into fresh medium preequilibrated to the appropriate temperature and grown at either 25 °C or 37 °C. We then harvested mRNA from the cells at different time points. Because the half-life of HIS4 mRNA is approximately 17 min (25), we chose our first time point for mRNA sampling at 1.5 h after the temperature shift to allow ample time for the decay of preexisting HIS4 mRNA.

As shown in Fig. 6A, constitutive expression of GCN4 from the DED1 promoter in RAP1 wild-type cells resulted in an elevated HIS4 expression at both 25 °C and 37 °C. The increase in HIS4 mRNA levels at later time points is probably because of the gradual depletion of exogenous histidine in the medium. Thus, the temperature shift per se does not have a significant effect on HIS4 transcription mediated by GCN4. In contrast to cells expressing wild-type RAP1, the levels of HIS4 mRNA in rap1–2ts cells containing the GCN4 expression plasmid dropped significantly after the cells were shifted from 25 °C to 37 °C (Fig. 6B). As in cells expressing wild-type RAP1, HIS4...
expression depended on the expression of GCN4. As an additional control we measured in an independent experiment levels of HIS3, HIS4, and PYK1 mRNA; HIS3 does not contain binding sites for RAP1 but does depend on GCN4 for its expression. The results showed that HIS4 levels declined at 37 °C only in the rap1-2ts strain, whereas HIS3 levels were unaffected (Fig. 6C, and data not shown). These experiments also confirmed that PYK1 expression was not affected by the temperature shift despite its promoter having a binding site for RAP1 (26, 27). Thus, RAP1 binding is required for the maintenance of ongoing transactivation of HIS4 that is mediated by GCN4.

Because of the relatively long half-life (17 min) of HIS4 mRNA (25), as stated above, we first sampled mRNA 1.5 h after the temperature shift to 37 °C. Although we did not examine the replication status of the rap1-2ts cells after the shift to 37 °C, it seemed possible that many cells would have undergone replication in the time before first sampling the mRNA. If the HIS4 locus replicated after a loss of RAP1 in a high proportion of the cells, it could reassemble into a chromatin structure that was refractory to GCN4 binding and fail to be transcribed. To examine this possibility, we first arrested rap1-2ts cells with α-factor at the permissive temperature and then shifted cells to the non-permissive temperature (or kept them at the permissive temperature as a control) and monitored HIS4 expression 45 and 90 min later, respectively. The results of two such experiments are averaged in Fig. 7 and show that after 90 min, rap1ts cells show clearly diminished amounts of HIS4 mRNA at 37 °C but not at 25 °C. Similar results were obtained in a separate experiment in which HIS4 mRNA levels were normalized to HIS3 mRNA (data not shown). We conclude that RAP1 is required for ongoing HIS4 transcription mediated by GCN4, even in nonreplicating cells.

**DISCUSSION**

**Dispensability of the C-terminal and N-terminal Domains of RAP1 for Chromatin Perturbation and HIS4 Activation**—We have examined the ability of truncated versions of RAP1, lacking different of its known functional domains, to perturb chromatin structure via a nucleosomal RAP1 binding site, and we find that neither the C-terminal part containing the silencing domain and the putative activation domain nor the N-terminal portion that is required for DNA bending by RAP1 is needed for RAP1-mediated chromatin perturbation. Chromatin opening by RAP1 is essential to GCN4-mediated HIS4 activation, and consistent with the results using the artificially engineered RAP1 site in a positioned nucleosome, we also find that HIS4...
can be activated to wild-type levels in yeast lacking either the C-terminal or N-terminal domains of RAP1. Although we have not been able to test derivatives of RAP1 lacking both C-terminal and N-terminal domains, the simplest interpretation of our findings is that the ability of RAP1 to perturb chromatin and to participate in \textit{HIS4} activation resides in its DNA-binding domain alone.

In our \textit{in vivo} experiments, the RAP1 binding site in TAR/GCN1\textit{Δ}80 is located at a DNA sequence that is near the center of a positioned nucleosome in TAR\textit{mut}/GCN1\textit{Δ}80 in which the RAP1 binding site is mutated. Interestingly, a recent \textit{in vitro} study shows that although RAP1 can bind to a site located near the edge of a nucleosome and somewhat less well to a site located approximately 40 base pairs from the edge, binding to a site near the dyad (or center) was essentially inhibited completely (28). Furthermore, binding to the less centrally located

**FIG. 6. Continuous RAP1 binding is required for efficient \textit{HIS4} activation.** Total RNA was harvested from wild-type (A) and \textit{rap1-2ts} (B) cells. Cells were grown in media containing histidine for the cells containing only an empty vector or in media containing half the optimal concentration of histidine for the cells containing a GCN4 expression plasmid. The cells were grown at either 25°C or 37°C, and RNA was collected at different time points. The RNA blot was hybridized with probes specific for \textit{HIS4} and \textit{PYK1} mRNA, and the signals were quantitated by PhosphorImager analysis. C, the results from an independent experiment in which \textit{HIS4} and \textit{HIS3} mRNAs were quantitated as described above. \textit{PYK1} mRNA was also measured in this experiment, and \textit{HIS4}/\textit{PYK1} ratios were consistent with the experiments of A and B.
We are somewhat surprised that neither the partial loss of the putative activation domain (in the Δ662 mutant) nor a complete loss (in the Δ628 mutant) affected the ability of RAP1 to open chromatin in the nucleosome-positioning assay (Fig. 3) or to contribute to HIS4 activation (Fig. 4). Previous work by us and others has shown that the GAL4 and PHO4 activators perturb chromatin in vivo in an activation domain-dependent manner (12–14, 33). In particular, the perturbation of nucleosomes I and II of TA17A80, which is identical to the chromatin reporter plasmid TAR/GCN1Δ80 used in this work save for the presence of a single 17-base pair GAL4 binding site in place of the RAP1 and GCN4 binding sites, requires activating GAL4 or related derivatives (12). Similar results have been observed with the related chromatin reporter plasmid TALS (14, 34). We have recently found that the perturbation of nucleosome positioning in TALS by GAL4-ER-VP16 is greatly reduced in gcn5 yeast, consistent with the idea that the activation domains may recruit other activities that contribute to chromatin perturbation via nucleosomal binding sites in vivo (35). Thus, it seemed reasonable that RAP1 might also require an activation domain to recruit chromatin remodeling or modification enzymes to open chromatin, but as stated above, it apparently does not. However, RAP1 has recently been shown to recruit the histone acetyltransferase Esa1p to the promoters of some genes in yeast (36). Perhaps this recruitment occurs through some domain of RAP1 other than its putative transactivation domain and contributes to the ability of RAP1 to remodel chromatin and/or activate HIS4. Future experiments will be aimed at testing this possibility.

We also did not see any effect of loss of the putative activation domain of RAP1 on induced levels of HIS4 mRNA. This observation is in contrast to a report in which the abilities of different domains of RAP1 fused to the GAL4 DNA-binding domain to activate HIS4 transcription were assessed using a modified HIS4 promoter containing two GAL4 binding sites (37). In this work, a GAL4 fusion protein containing the activation domain of RAP1 allowed the growth of cells on plates lacking histidine, whereas the fusion of the GAL4 DNA-binding domain to the silencing domain of RAP1 alone or together with a substantial part of the DNA-binding domain did not. The modified HIS4 promoter used in these experiments lacked a RAP1 binding site.

We suggest that the high affinity of the RAP1 DNA-binding domain for its cognate site (38) makes the contribution made by activation domains of more weakly binding proteins, such as GAL4, unnecessary for RAP1 to bind to sites in chromatin. Although the affinity of GAL4 for its binding site is fairly high, it is nonetheless approximately 50-fold lower than that of RAP1 (38, 39). Furthermore, Western analysis of hemagglutinin-tagged GAL4(1–147) expressed from the ADH1 promoter in yeast, which perturbs TA17A80 chromatin only slightly (12), indicates that it is present at over 100,000 molecules/cell (data not shown), which is much more abundant than RAP1 (40). Hence, the requirement for an activation domain for nucleosome perturbation by GAL4 in contrast to our results for RAP1 cannot be explained by the relative abundance of these proteins.

Our model suggests that the function of RAP1 at the HIS4 promoter is strictly to open the chromatin to allow access of the weakly binding activator GCN4 to the promoter. RAP1 alone does not suffice for activating HIS4. A true activator such as GCN4 is needed in addition to recruit the transcriptional machinery (41). At the modified HIS4 promoter used in the experiments of Kirkpatrick et al. (37), no additional activation domain is brought to the promoter, because the normal upstream-activating sequences have been removed. Thus, an activation domain must be fused to the GAL4 DNA-binding domain to allow activation. Previous work has shown that the RAP1 putative activation domain is important at some promoters at which RAP1 functions but not at other promoters, consistent with the results presented here (42). Certainly, this domain of RAP1 contributes to some of its important functions, as yeast bearing the Δ628 mutation of RAP1 are much sicker than those bearing the Δ662 mutation (42); however, more work will be required to determine where the RAP1 putative activation domain exerts its critical function.

Continuous Binding of RAP1 Required for HIS4 Activation—We used a RAP1 mutant that is temperature-sensitive for DNA binding to show that RAP1 binding is needed for ongoing transcriptional activation of HIS4. Interestingly, chromatin remodeling by the SWI-SNF complex at the SUC2 promoter is needed similarly for ongoing transcriptional activation and not just for the establishment of the competent state (43, 44). In contrast, the binding of the RAP1-related protein ABF1 to its site in the SPT15 promoter is not needed to maintain transcription of that gene (45). It may be a general property of chromatin in living cells that a reversion to a nonpermissive transcriptional state is fairly rapid in the absence of proteins specialized for perturbing the chromatin structure and/or in the presence of repressor proteins (46), but this issue has not received sufficient attention to draw conclusions at present.

Curiously, in contrast to the relatively rapid inactivation of HIS4 transcription after the shift to restrictive temperature, the perturbed chromatin structure of TAR/GCN1Δ80 persists for up to 4 h following this shift. One possible explanation for this result is that transient RAP1 binding may still occur at the restrictive temperature, and that this transient binding, although not sufficient to maintain HIS4 activation, does prevent nucleosome positioning in TAR/GCN1Δ80. Alternatively, it may be that the repressive chromatin structure at HIS4 that forms in the absence of RAP1 is of a different type than the positioned nucleosomes of our chromatin reporters and therefore differs in its kinetic properties. Either explanation sug-
suggests that the plasmid chromatin reporters, which we have used to examine RAP1 binding to chromatin directly, are not in some respects accurate models for the HIS4 promoter. This finding seems probable despite the use of these chromatin reporters in assessing the ability of various transcription factors to bind to nucleosomal sites (10, 12, 14, 47, 48), as we and others have not found the HIS4 promoter to be packaged into strongly positioned nucleosomes (49).3

Chromatin is folded into higher order structures beyond the level of the nucleosome in vivo. It may be that the HIS4 promoter in the absence of RAP1 binding is repressed by a higher order folding that inhibits binding by GCN4. The transition to such a repressed configuration could be relatively rapid after a loss of RAP1 binding, which is consistent with the results presented here. Furthermore, we have found that the loss of the histone H3 N terminus suppresses the requirement for RAP1 at the HIS4 promoter without substantially affecting nucleosome positioning.4 The histone H3 N termini and particularly that of histone H3 have been reported to contribute to a higher order folding of chromatin in vitro (50–52). Future studies will be aimed at examining more closely the mechanism by which chromatin represses HIS4 expression in the absence of RAP1 binding.

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