Review Article

Chromatin Opening and Potentiation of Transcriptional Activation by the General Regulatory Factors, ABF1 and RAP1, in the Budding Yeast *Saccharomyces cerevisiae*

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ABF1 (autonomously replicating sequence binding factor) and RAP1 (repressor activator protein) are well known transcription factors from the budding yeast *Saccharomyces cerevisiae*. Both are multifunctional, site specific DNA binding proteins, with roles in replication, gene transcription and repression. A property of RAP1 likely to be important for these various functions is its ability, supported by experiments by ourselves and others, to create a local region of "open" chromatin. More recently we have found that ABF1 also is able to create a local region of open chromatin that contributes to its ability to potentiate transcription. In this review we discuss the evidence for chromatin opening by RAP1 and ABF1, and the similarities and differences in their mechanisms of action.

Key Words: Chromatin, yeast, transcription, RAP1, ABF1, nucleosomes, epigenetic

Introduction

ABF1 and RAP1 are abundant, multifunctional, site specific DNA binding proteins found in the budding yeast *Saccharomyces cerevisiae* (Miyake et al. 2002, Morse 2000, Shore 1994). RAP1 and ABF1 are essential for cell viability and have roles in gene activation and gene silencing, telomere structure and recombination; their multifunctional character has caused them to be termed General Regulatory Factors, or GRFs.. Binding sites for ABF1 and RAP1 are found in a large number of promoters, and have been shown to be important for activation of many of the corresponding genes. Genome-wide localization, or "ChIP-on-chip" experiments indicate that RAP1 and ABF1 bind to about 300 promoters each, and RAP1 has been found to bind to 122 of 137 ribosomal protein genes (Lee et al. 2002, Lieb et al. 2001). Genes containing RAP1 or ABF1 sites principally comprise ribosomal protein genes and genes encoding proteins involved in amino acid biosynthesis, regulation of carbon source, and sporulation. Many of these genes are among those most highly expressed in the yeast genome.

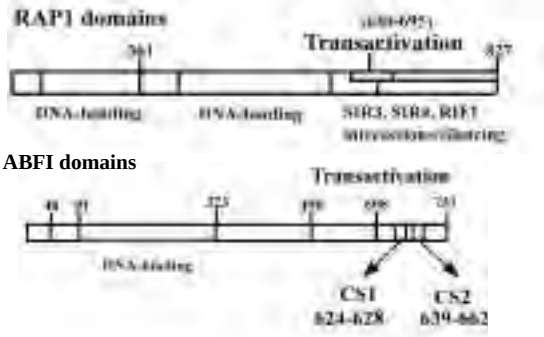

Figure 1: *Schematic diagram of domains of RAP1 and ABF1*

Though ABF1 and RAP1 are similar in some functional aspects, they share only limited homology (Fig. 1). Both proteins possess central regions required for DNA-binding. The DNA-binding domain of RAP1 has been crystallized in complex with telomeric DNA, and shows structural similarity to the homeodomain and the proto-oncogene protein Myb, but also shows novel features distinct from these (Konig et al. 1996). ABF1, in contrast, possesses a bipartite DNA-bindng domain, consisting of a zinc finger region near the N-terminus, and a second more central DNA-binding motif (Cho et al. 1995). Both proteins also possess essential C-terminal regions. The C-terminus of RAP1 interacts with proteins involved in silencing and telomere function, and also possesses a putative trans-activation domain that can activate reporter genes in the context of fusions with the GAL4 DNA-binding domain (Hardy et al. 1992). Specific interaction of other proteins with the C-terminal region of ABF1 has not been demonstrated, but two small clusters of amino acids, CS1 and CS2, have been show to be important for transcriptional activation as well as chromatin remodeling. Correspondingly, deletion of these regions results in slow growth while, perhaps surprisingly, mutation of either CS1 or CS2 is lethal (Miyake et al. 2002). Interestingly, the small area of homology between RAP1 and ABF1 encompasses the CS1 and CS2 domains (Diffley and Stillman 1989, Miyake et al. 2002), and deletion of the portion of RAP1 containing this domain is much more deleterious to cell growth than deletion of the more C-terminal region that is responsible for silencing and telomere structure. No mutational analysis of these regions has been performed for RAP1. The homology in the C-terminal regions of RAP1 and ABF1 appears to reflect functional similarity,

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as fusing the C-terminal region of ABF1 or RAP1 to the remainder of the other suppresses the lethality of deletion of the RAP1 or ABF1, respectively (Goncalves et al. 1996).

Transcriptional roles of ABF1 and RAP1

By themselves, ABF1 and RAP1 only weakly activate transcription (Buchman and Kornberg 1990, Chasman et al. 1990, Goncalves et al. 1995), but both participate in transcription at different promoters with the help of cooperating activators. RAP1 is largely dedicated to facilitating activating of ribosomal protein genes, and typically functions at these in cooperation with T-rich elements (Lascaris et al. 2000, Lascaris et al. 1999). ABF1 also functions in cooperation with T-rich elements, and both RAP1 and ABF1 can also facilitate activation by GCN4 or BAS1/BAS2 (Devlin et al. 1991, Lascaris et al. 2000, Martens and Brandl 1994, Rolfes et al. 1997, Yarragudi et al. 2004). RAP1 can also facilitate activation of genes encoding glycolytic enzymes, such as *TPI* and *ENO2*, in conjunction with the activator GCR1 (Scott and Baker 1993, Willett et al. 1993). In this case, RAP1 aids GCR1 binding by protein-protein interaction (Drazinic et al. 1996, Tornow et al. 1993). However, evidence for such classic cooperativity at other genes for which ABF1 or RAP1 contribute to activation is lacking. In fact, at the *HIS4* promoter (Fig. 2), where RAP1 can activate transcription synergistically with GCN4 or BAS1/BAS2, altering the separation between GCN4 and RAP1 sites by 5 bp or 10 bp (half or a whole helical turn of the DNA helix) does not alter transcriptional activation, indicating that protein-protein interactions are not likely to contribute to this synergy (Yu and Morse 1999). How then do RAP1 and ABF1 collaborate with various other activators to turn on expression of a large variety of genes?

Chromatin opening by ABF1 and RAP1

One mechanism by which RAP1 and ABF1 could contribute to transcriptional activation of a variety of genes, and that has been suggested by various researchers, is by opening chromatin to facilitate binding of other activators (Devlin et al. 1991, Rolfes et al. 1997, Schroeder and Weil 1998). We tested this idea by performing experiments based on activation of the *HIS4* promoter, at which RAP1 and GCN4 synergistically activate transcription. GCN4 similarly requires assistance from a poly(dA-dT) sequence at the *HIS3* promoter, again suggesting that chromatin opening might be needed to facilitate activation by GCN4 (Iyer and Struhl, 1995). To test the idea that GCN4 is not able to efficiently access its binding site in chromatin, we used a yeast plasmid based on the *TRP1ARS1* plasmid, which is packaged into positioned nucleosomes in yeast (Thoma et al. 1984). We engineered a GCN4 binding site into a sequence that is incorporated into a positioned nucleosome, and found that the nucleosome remained at the same region regardless of the expression of GCN4.(Yu and Morse, 1999). In contrast, introduction of a RAP1 binding site completely abolished nucleosome positioning in its vicinity. These results are consistent with the idea that RAP1 binding could assist GCN4 binding by opening chromatin. More recently, we have used chromatin IP to show directly that GCN4 binding at the *HIS4* promoter is reduced by mutation of the nearby RAP1 binding site (A. Y., L. Yu, C. Yu and R.H.M., unpublished results).

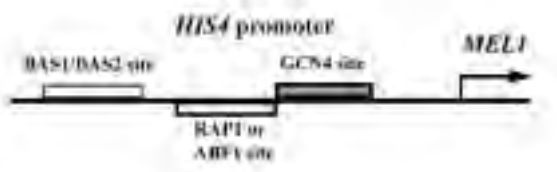

Figure 2: *Schematic diagram of the* HIS4-MEL1 *reporter gene. Binding sites for BAS1/BAS2, RAP1, and GCN4 are depicted; the RAP1 binding site was replaced by a binding site for ABF1 in some experiments. The yeast* MEL1 *gene encodes α-galactosidase. Binding sites are not to scale.*

Interestingly, a recent study of global nucleosome occupancy in yeast showed a correlation of RAP1 binding sites with sites of reduced nucleosome occupancy (Bernstein et al. 2004). The preponderance of RAP1 binding sites are in the promoters of ribosomal protein genes, as mentioned earlier. It remains to be seen whether activators associated with these and other genes to which RAP1 binds are helped in their binding to sites in chromatin by the presence of binding sites for RAP1.

Given the considerable functional overlap between RAP1 and ABF1, it seemed possible that ABF1 might possess an ability to open chromatin and thereby assist binding of other transcriptional activators similarly to RAP1. To compare the abilities of ABF1 and RAP1 to open chromatin, we examined chromatin structure in yeast of four episomes, again based on the *TRP1ARS1* plasmid, that contained either a RAP1 or ABF1 binding site, or the corresponding mutant binding sites, placed into a sequence normally packaged into a positioned nucleosome in yeast. As already mentioned, RAP1 abolished nucleosome in the vicinity of its bindng site (Fig. 3). ABF1 was similarly able to perturb nucleosome positioning. However, ABF1 appeared to exert an effect on chromatin structure that was somewhat more localized than RAP1, with the region immediately surrounding the binding site being cleared of nucleosomes and nucleosomes adopting altered positions at a slight distance from the ABF1 binding site (Yarragudi et al. 2004). This difference may account for the finding that lower nucleosome occupancy in yeast correlates with RAP1 binding sites but was not found to correlate with binding sites for ABF1 (Bernstein et al. 2004).

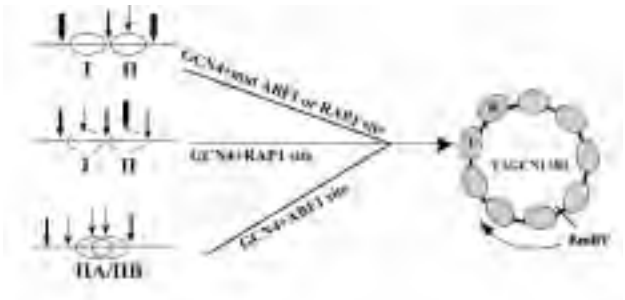

Figure 3: *Chromatin structure is affected differently by RAP1 and ABF1 binding. A yeast episome derived from the* TRP1ARS1 *(shown at right) was used to create similar episomes having binding sites for RAP1 or ABF1, or corresponding mutated sites, placed in a region that is positioned into a nucleosome in the parent plasmid, TAGCN1Δ80, in yeast. Characterization of chromatin structure of the resulting minichromosomes was performed by micrococcal nuclease digestion followed by indirect end-labeling, and the results are depicted schematically at the left (only the region near nucleosome I in the parent TAGCN1Δ80 plasmid is shown). Plasmids containing mutant ABF1 or RAP1 binding sites retained positioning of nucleosomes I and II (solid circles), while the presence of ABF1 or RAP1 binding sites perturbed these nucleosomes. However, while RAP1 essentially abolished nucleosome positioning (dashed circles represent loss of positioning), ABF1 binding prevented nucleosome formation in its immediate vicinity and caused altered nucleosome positioning nearby the overlapping circles in the construct having an ABF1 binding site (left, bottom) indicate alternate nucleosome positions in this plasmid episome.*

Interchangeability of ABF1 and RAP1 for transcriptional activation

If chromatin opening by RAP1 and ABF1 contributes to their ability to facilitate activation of numerous gene promoters, one might expect these GRFs to be fairly interchangeable. RAP1 and ABF1 can both synergize with T-rich elements in the rp*S33* and rp*L45* promoters, and have also been reported to be interchangeable at the *TRP3* promoter (Goncalves et al. 1995, Martens and Brandl 1994). We recently found that at the *HIS4* promoter, which normally has a RAP1 binding site, and at the *HIS7* and *ADE5,7* promoters, which have ABF1 sites, RAP1 and ABF1 sites function nearly indistinguishably to facilitate transcriptional activation (Yarragudi et al. 2004).

Which domains contribute to chromatin opening and transactivation potentiation by ABF1 and RAP1?

As discussed earlier, ABF1 and RAP1 possess DNA-binding domains and putative transactivation domains in their C-terminal regions (Fig. 1). Although deletion of the C-terminal regions that include the putative activation domains of ABF1 and RAP1 strongly affect cell growth, we were surprised to find that these regions are dispensable for chromatin perturbation via a nucleosomal binding site (Yarragudi et al. 2004, Yu et al. 2001). Furthermore, using either the native *HIS4* promoter (which contains a RAP1 binding site), or a modified *HIS4* promoter that contains an ABF1 binding site (Fig. 2), we found that the C-termini of RAP1 and ABF1 show at most a modest contribution to transcriptional activation (Yarragudi et al. 2004, Yu et al. 2001). Importantly, we found that ABF1 lacking its activation domain was completely unable to activate *HIS4* on its own, and yet could contribute to activation mediated by GCN4. This finding, taken together with the ability of ABF1 to open chromatin via a nucleosomal binding site even when lacking its C-terminal activation domain, strongly supports the idea that chromatin opening contributes to activation mediated by ABF1. Furthermore, we have more recently used chromatin IP to show directly that the presence of an ABF1 site near the GCN4 binding site in the *HIS4* promoter enhances GCN4 binding, similar to findings with RAP1 mentioned earlier (AY and RHM, unpublished results). However, it is also worth noting that the C-terminal region of ABF1 that contains the CS1 and CS2 domains is needed to remodel chromatin from sites near positioned nucleosomes, as opposed to within a positioned nucleosome, where the competition with histones is direct. It may be that chromatin is remodeled by more than one mechanism in vivo by ABF1 (and RAP1), and that this results in mechanistic differences in the way they contribute to gene activation at specific promoters. This remains an area for future investigation.

ABF1 and RAP1 differ in their requirements for continuous binding to maintain transcriptional activation

It seemed possible that RAP1 might be required at the *HIS4* promoter only until GCN4 is stably bound, or it might be required continuously to prevent reformation of a repressive chromatin structure that would inhibit GCN4 access. We tested this in a yeast strain harboring a *rap1* mutation that is *ts* for DNA binding. Shifting this strain to the restrictive temperature caused loss of GCN4-mediated *HIS4* transcription within 45 minutes, indicating a continuous requirement for RAP1 (Yu et al. 2001). Previous work had shown that loss of ABF1 binding (using a *ts* mutant for binding) from several ABF1-driven promoters, including *SPT15, RPL2A, RPL2B, TCM1,* and *QCR8,* surprisingly did not result in decreased transcription (Schroeder and Weil 1998). To test whether this contrasting behavior of ABF1 and RAP1 reflected different properties of the relevant promoters or of the factors themselves, we tested whether ABF1 binding is continuously required to enhance transcription from the modified *HIS4* promoter having a binding site for ABF1 in place of that for RAP1. Using yeast carrying the *abf1-1* mutation, which is *ts* for ABF1 binding, we found, in contrast to our results for RAP1, that no decrease in transcription is seen from the modified *HIS4* promoter after shifting to the restrictive temperature, in

spite of a clear dependence on the ABF1 site for transcription (Yarragudi et al. 2004). Previous work had shown by DMS footprinting that ABF1 binding is lost after 30 minutes in the *abf1-1 ts* mutant, and we observed loss of ABF1 binding to the *SPT15* and the modified *HIS4* promoters at 37°C in the *abf1-1 ts* strain by ChIP. Thus, at the same promoter, RAP1 binding is continuously required for ongoing transcription, whereas ABF1 binding is not. These results suggest that ABF1 may leave a "mark" on promoters that it regulates, in contrast to RAP1. Intriguingly, a recent microarray study identified only 50 genes that showed significantly decreased transcription at 37 °C in *abf1-1* compared to wild type yeast (Miyake et al. 2004), in spite of about 300 genes being identified as binding ABF1 in a genome-wide ChIP analysis (Lee et al. 2002). This result is consistent with our own preliminary microarray results, in which only 66 genes show two-fold or greater reduction in expression after 1 hr at 37 °C in *abf1-1* compared to wild type yeast (A Yarragudi and RHM unpublished). Taken together, these results suggest that ABF1 confers an epigenetic mark, so that continual binding is not required for continued activation of ABF1-stimulated transcription. Current investigations in our lab are addressed at understanding the mechanistic basis for this unusual "memory effect".

Outstanding questions

It now seems well established that ABF1 and RAP1 are both well able to open chromatin, and this property contributes substantially to their ability to contribute to transcriptional activation and likely to other properties as well (Morse 2000). However, they show subtle differences in their effects on a positioned nucleosome containing their respective binding sites (Fig. 3), and the mechanistic basis for this difference remains obscure. Furthermore, RAP1 binding was found to correlate with reduced nucleosome occupancy in yeast, whereas ABF1 binding was not (Bernstein et al. 2004), whether this is connected with the difference in their effects on local chromatin structure is not yet known.

A major difference between RAP1 and ABF1 was found in examining their requirements for ongoing transcription: loss of RAP1 binding resulted in a rapid loss of transcription, whereas transcription continued unabated at a number of promoters even after ABF1 binding was lost. The mechanistic basis for this "memory effect" exerted by ABF1 is unknown and of clear interest. This effect may involve histone modifications or preserved changes in local chromatin structure. Epigenetic memory contributes to important biological phenomena such as pattern formation in developing *Drosophila* embryos and X-chromosome inactivation in mammals, suggesting that understanding the basis for the ABF1 memory effect may reveal broader insights. Future investigations of RAP1, ABF1, and other GRFs may well yield additional surprises and novel insights.

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