Genetic Tests of the Role of Abf1p in Driving Transcription of the Yeast TATA Box Binding Protein-encoding Gene, SPT15*

(Received for publication, March 9, 1998, and in revised form, May 6, 1998)

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In this report we describe studies which utilized yeast strains bearing gain and loss of function alleles of ABF1 in order to attempt to directly implicate Abf1p in modulating transcription of the TBP-encoding gene, SPT15, in vivo. We found that overexpression of Abf1p in a yeast cell increased transcription of the TBP-encoding gene and that this stimulation depended upon the exact sequence of the Abf1p binding site (ABF1) present in the gene. Further, in a yeast strain expressing a temperature sensitive form of Abf1p, occupancy of the chromosomal ABF1 site in the TBP-encoding gene was immediately lost following a temperature shift. Both results suggest that Abf1p drives transcription of the TBP-encoding gene. Surprisingly though we found that continuous ABF1 cis-element occupancy by Abf1p was not acutely required for normal levels of transcription of either the TBP-encoding gene or other "Abf1p-driven" genes tested. We propose a model to explain these results and suggest mechanisms by which Abf1p could activate gene transcription.

We previously described the characterization and purification of a protein which binds with high affinity to the nonconsensus ABF1 site termed PED present in the TBP-encoding gene. A variety of biochemical and immunological tests and finally amino acid sequencing identified this polypeptide as Abf1p. We demonstrated that the integrity of the PED variant ABF1 element was critical for DNA binding both in vitro and in vivo and that the patterns of Abf1p binding to DNA exactly mirrored the ability of the WT and mutant forms of this ABF1 site to drive transcription of the TBP encoding gene in vivo. Finally, we showed that in vitro, the non-consensus TBP gene ABF1 sequence is able to bind Abf1p in a fashion indistinguishable from a consensus ABF1 binding site. Though compelling, this body of evidence does not actually prove that it is Abf1p that drives TBP gene expression inside the yeast cell. In order to more directly implicate Abf1p in controlling TBP gene expression we performed the series of genetic experiments described herein.

* This work was supported by National Institutes of Health Grant GM52461. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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1 The abbreviations used are: ABF1, the DNA binding site for Abf1p; Abf1p, ARS binding factor I protein; TBP, the TATA box binding protein; PED, positive element distal; OFB, open reading frame; UASgal, the enhancer conferring galactose inducibility to transcription; GTF, general transcription factors; TFIIA, B, D, E, F, and H.
2 S. C. Schroeder and P. A. Weil, submitted for publication.

In this study we describe genetic gain and loss of function experiments where we modulated the intracellular levels of (functional) Abf1p in order to assess the impact of these acute changes in activator protein concentration upon the transcription of the TBP-encoding gene. Parallel experiments were performed with a battery of other previously characterized "Abf1p-driven" genes which served as controls for our TBP studies. We utilized genomic footprinting and primer extension to monitor ABF1 occupancy and transcription in vivo in yeast strains carrying temperature sensitive (ts) ABF1 alleles while we used SPT15:lacZ fusion reporter constructs to assess the effects of Abf1p overexpression on transcription of the TBP-encoding gene. Use of these sensitive assay methodologies, coupled with the yeast strains over- and underexpressing Abf1p, allowed us not only to strongly implicate Abf1p in driving TBP gene transcription in vivo but also allowed us to ask whether continuous cis-element occupancy is required for sustained high level gene transcription. Conflicting reports of in vitro studies supporting (1, 2) and refuting (3, 4) this hypothesis have appeared and only a handful of published studies using in vivo methodologies have addressed this question. One of the in vivo studies shows that continuous cis-element occupancy by a "functional" trans-acting factor is absolutely necessary for ongoing activated transcription (5) while three other reports indicate that in fact this may not be the case at least when cis-element occupancy/ function and transcription are monitored acutely (i.e. minute to hours) (6–8).

In our studies, continuous cis-element occupancy by Abf1p, the dominant trans-acting factor for the TBP-encoding gene, was not required for sustained transcription. This behavior was observed not only for the gene encoding TBP but for the entire collection of Abf1p-driven genes which we examined. These results have prompted us to propose a model for Abf1p-mediated gene activation and to speculate on possible molecular mechanisms of Abf1p-driven gene expression based on this paradigm. The possible implications of this model for gene activation events are discussed.

EXPERIMENTAL PROCEDURES

Genomic Footprinting, β-Galactosidase, and Immunoblotting Assays—These assays were performed as detailed previously (9, 10).

Temperature Shift Experiments—To ensure the quickest shift possible from permissive temperature (25 °C) to nonpermissive temperature (37 °C), overnight cultures of JCA30 (ABF1) and JCA31 (abf1-1) were grown at 25 °C in SC-His medium (11) to an absorbance at 600 nm of 0.2, the culture was then split and filtered through separate 500-ml 0.8-μm filter units (Nalgene). Cells from one of the filter units were resuspended in 500 ml of SC-His medium prewarmed to 25 °C while cells from the other were resuspended in 500 ml of the same medium prewarmed to 37 °C. These cultures were then incubated at either 25 or 37 °C, and at intervals (0, 1, 2, and 4 h) cell number was determined and an appropriate volume of cells was harvested for RNA preparation and/or DMS footprinting analysis. For DMS modification at 37 °C, cells were harvested, resuspended in 2 ml of prewarmed media, and incubated for 3 min at 37 °C. 1 μl of DMS (Sigma) was added and the
activation of gene transcription in vivo by abf1p

results

abf1 gain of function experiment—our first set of experiments examined the effect that overexpression of abf1p had on tbp gene expression. we reasoned that if abf1p actually controls transcription of the ebp-encoding gene through its ped variant abf1 element, then by increasing occupancy of this dna element, via mass action through abf1p overexpression, we should effect an increase in gene transcription. complicating this approach was the fact that cells containing just normal amounts of abf1p exhibit full occupancy of abf1 sites. 2 fortunately however, we had a partial loss-of-function mutant allele of the ebp gene abf1 element, termed mutant 3 (fig. 1b). this mutant supports ~50% of wt levels of transcription, and importantly for this experiment, shows only about half of the wt level of ebp1 element occupancy. thus mutant 3 was an ideal strain for testing the effects of abf1p overexpression since abf1p-dependent changes (increases) in transcription should readily be measurable in yeast cells both overexpressing abf1p and carrying this mutant abf1 binding site. conversely overexpression of abf1p in cells carrying the wt ped variant abf1 element should be without effect on transcription.

we generated yeast strains containing a high copy (3µ) expression plasmid where abf1p sequences were driven by the uasgal enhancer (or prs426 plasmid control) as well as either of two spt15::lacZ reporter gene constructs. one reporter plasmid contained ebp gene wt sequences (~1076 to +198) driving lacZ transcription while the second carried ebp gene abf1 mutant 3 sequences in the same ~1076 to +198 backbone, driving lacZ. cells were initially grown in the nonexpressing sugar raffinose then each culture was split and either main-

time point, cells from both 37 °c (i.e. jca30 and jca31) cultures were counted and plated onto ypad plates and incubated at room temperature to measure cell viability after the shift to 37 °c. 2 the cells induced abf1p overexpression—plasmid pmhoefb1 (12) contains a 2µ origin of replication, a ura3 gene, and the abf1p orf under the transcriptional control of the uasgal and gal1 promoter. this plasmid, or as a control the 2µ ura3-marked plasmid prs426 (13), was transformed into yeast strain yph499, matα ura3-52 lys2-801 ade2-101 trp1-901 (14) along with one of two ebp promoter::lacZ fusion plasmids (see fig. 1; ped wt or mutant 3). these cells were grown overnight in selective medium containing 2% raffinose to an absorbance at 600 nm of 0.2. the cultures were then split, this represents zero time or t = 0) and to one-half of the cells, 20% galactose was added to a final concentration (v/v) of 2% while to the other half of the cells was added an identical volume of water. beginning at t = 0 and continuing every 4 h until 20 h, cells were removed from each culture and assayed for β-galactosidase activity and abf1p protein levels. crude protein extracts were prepared from these cells as described previously (10).

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as shown by the abf1p-immunoblot presented in fig. 1a we were able to effect a dramatic (~40 times) overexpression of abf1p in a galactose- and abf1p orf-dependent fashion in these cells. in cells carrying wt ebp gene sequences fused to

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the DNA binding domain of Abf1p (20). Abf1p produced from abf1-1 cells completely fails to bind DNA in vitro indicating that its activity is severely compromised (18) (data not shown). Using the abf1-1 ts strain JCA31 and the WT control strain JCA30, at permissive and nonpermissive temperatures, we performed parallel sets of time course experiments where we simultaneously measured both ABF1 site occupancy and mRNA 5′-end production from the TBP gene and other “Abf1p-driven” genes in the same cells. Upon shifting cells to the nonpermissive temperature, thereby reducing the concentration of functional Abf1p in the abf1-1 cells, we expected to observe dramatic decreases in both ABF1 element occupancy and gene transcription in a strain-specific fashion.

The results of the genomic footprinting analyses to assess cis-element occupancy are presented in Fig. 2. DMS footprints were performed on yeast cells expressing either WT or mutant Abf1p at various times after cultures were shifted from permissive (25 °C) to nonpermissive (37 °C) temperatures (Fig. 2A; ABF1 cells labeled WT and 25 or 37 °C; abf1-1 cells labeled abf1-1 and 25 or 37 °C, respectively). It is important to note that the in vitro half-time for Abf1p dissociation from either a consensus ABF1 element or variant PED element (t1/2) about 30 min at 25 °C and 15 min at 37 °C; (data not shown)2 is long relative to the 1 min of DMS treatment performed during the footprinting procedure therefore allowing an accurate assessment of in vivo occupancy to be obtained using this method.

Isolated naked yeast genomic DNA was also reacted with DMS and served as DMS reactivity control for each gene (lanes marked N). The positive control genes used here either contained consensus (RPL2A (21), TCM1 (22), QCR8 (23)) or a nonconsensus Abf1p binding sites (RPL2B) (21). Abf1p has been implicated as a key transcription factor, one required for high level expression for all of these genes. Simultaneously analyzing all of these genes allowed us to calibrate our assay with bona fide positive and negative signals for comparison to the results we obtained with the TBP-encoding gene. Abf1p produced from the WT ABF1 allele binds the ABF1 cis-elements in all of these genes quite avidly in a temperature independent fashion (occupancy ≥ 90%; Fig. 2A and B). In some of these genes, there are two highly DMS-reactive residues (all marked by arrowheads; Fig. 2A) such as TCM1 and the TBP encoding gene while in other genes, such as RPL2A, RPL2B, QCR8, and PGK, and QCR8 genes to be approximately 15, 2, 10, 20, 6, 30, and 10 min, respectively. It is interesting that the half-lives of mRNA$^{TAF25}$ and mRNA$^{TAF25}$ are so different. The mRNA$^{TAF25}$, at 30 min, is short relative to the 4-h time course of our temperature shift experiment.

Global Measurement of mRNA Synthesis in ABF1 and abf1-1 Strains—To show that a shift of culture temperature per se did not generally alter bulk mRNA metabolism, we prepared total RNA from abf1-1 and ABF1 strains and examined these RNA preparations for total mRNA levels. Using slot blot hybridization of total RNA, and probing these blots with 32P-labeled oligo(dT), we quantitated total mRNA levels at hourly intervals in both WT and abf1-1 cells at permissive and nonpermissive temperatures. The cells used for this analysis were taken from the exact same cultures analyzed for ABF1 element occupancy (cf. Fig. 2). This method has been used extensively by others for the measurement of total mRNA synthesis rates (15, 25, 26). As shown in Fig. 4, there is no dramatic change in mRNA content in either strain regardless of temperature since steady state mRNA levels remain roughly constant over the entire 4-h time period. Clearly for us to observe this result neither mRNA synthesis nor mRNA degradation rates can vary significantly in these cells.

We also performed direct measurements of bulk mRNA half-lives using the slot blot method using RNA prepared from abf1-1 and ABF1 containing cells that had been treated with the antibiotic thiolutin, a potent in vivo and in vitro inhibitor of fungal DNA-dependent RNA polymerases (data not shown). The results of this experiment support the conclusion that bulk mRNA synthesis and degradation rates are constant in both cell types at both temperatures since total mRNA half-lives were not much different between the two cells types (abf1-1

**Measurement of Total and Specific mRNA Metabolism Parameters in ABF1 and abf1-1 Cells**—We next wanted to analyze the kinetics of mRNA 5′-end production from both the TBP-encoding gene and from our positive (RPL2A, RPL2B, TCM1, and QCR8) and negative (TAF25) control genes. We chose TAF25 as a non-Abf1p-regulated gene since there are no ABF1 sites within the 1000 base pairs of DNA either upstream or downstream of the TAF25 ORF. Our goal was to perform these analyses on RNA extracted from the exact same cultures of cells which were probed for cis-element occupancy by DMS footprinting (Fig. 2).

Before these experiments could be performed, however, we first needed to define the parameters of mRNA metabolism in the yeast strains that we were using for our experiments. First we needed to show that the half-lives of the relevant mRNAs were significantly shorter than the time course of our temperature shift experiment. Second, we needed to demonstrate that total mRNA synthesis and degradation was not deranged in abf1-1 cells at either permissive or nonpermissive temperatures. If both of these criteria were met then we could in fact use primer extension quantitation of mRNA 5′-end production as a valid metric of transcription.

Specific mRNA Half-life Determinations—The half-lives of each of the relevant mRNAs were measured using a yeast strain expressing the rpbl1 mutation (24). This mutant gene encodes for a thermosensitive form of the largest subunit of RNAP II, Rpb1p. mRNA synthesis in yeast cells carrying this mutant gene abruptly and specifically ceases when these cells are shifted to nonpermissive temperatures. Utilization of the rpbl1 carrying strain is a facile method for measuring mRNA half-lives in yeast (17). The results of this experiment are presented in Fig. 3. From these data we estimate the half-lives of mRNAs transcribed from the TBP-encoding gene and TAF25, TCM1, RPL2A, RPL2B, PGK, and QCR8 genes to be approximately 15, 2, 10, 20, 6, 30, and 10 min, respectively. It is interesting that the half-lives of mRNA$^{TAF25}$ and mRNA$^{TAF25}$ are so different. The mRNA$^{TAF25}$, at 30 min, is short relative to the 4-h time course of our temperature shift experiment.
ABF1 element occupancy in the

A temperature shift rapidly induces complete loss of strain.

Activation of Gene Transcription in Vivo by Abf1p

RNA Synthesis in abf1-1 and ABF1 Cells at Permissive and Nonpermissive Temperatures—The results of our temperature shift-ABF1 loss of function-specific transcription assays are shown in Fig. 5A and graphically in Fig. 5B. Only the specific extended products are shown in Fig. 5A; however, for all mRNAs measured we were in primer excess (data not shown). In this experiment we monitored mRNATBP 5′-end production in yeast cells expressing WT and ts mutant Abf1p at permissive and non-permissive temperatures. Even after 4 h at the non-permissive temperature there was no significant decrease in mRNA 5′-end production from any of the genes analyzed, except for mRNATCM1. This result is in stark contrast with the observation that ABF1 occupancy by Abf1p was lost within minutes after the temperature shift in these same cells. In the case of TCM1, transcription was affected by the shift to 37 °C, but this decrease was observed in both the wild-type (JCA30) and mutant strain (JCA31). We measured the effect of the temperature shift upon the viability of JCA31 cells. Viability drops to ~75% at 2 h and to ~50% at 4 h after a shift of the abf1-1 cells to the nonpermissive temperature (Fig. 5B, dashed line).

We have also performed these in vivo footprinting/transcriptional analyses with three other temperature-sensitive, loss of function mutant alleles of ABF1, abf1-5 (18), and abf1-102 and of the Abf1p binding sites in various genes as monitored by DMS in vivo footprinting. Yeast strains JCA30 (ABF1) and JCA31 (abf1-1) were grown at permissive (25 °C) and nonpermissive (37 °C) temperatures for the number of hours indicated at the top of the figure. These cells were treated with DMS, and the methylation patterns of isolated genomic DNA was analyzed. The 0° h label for the 37 °C cells (i.e., lanes 6 and 15, top to bottom) indicates the fact that the cells were resuspended in 37 °C prewarmed medium, treated with DMS for 1 min at 37 °C, and then harvested. Therefore, the cells were exposed to 37 °C for approximately 3–4 min. Naked yeast genomic DNA treated with DMS was used as a reactivity control (labeled N). Arrowheads denote the DMS-reactive residues within each ABF1 element. DNA sequence ladders run in parallel (data not shown) allowed assignment of the location of the relevant Abf1p binding sites. Gene names are indicated at the left of each panel. The highly DMS-reactive residues of the ABF1 elements for the various genes are: G-145 and G-138/SPT15; G-220 and G-229/TCM1; G-164/RPL2A; G-173/RPL2B; and G-235/QCR8 (G = guanine). These numbers are counted from the transcription start site for TBP and from the A of ATG for the other four genes. B, quantitation of the in vivo footprinting data. The autoradiograms shown in panel A were densitometrically scanned (naked DNA, solid line; abf1-1 genomic DNA at 25 °C, small dash line; abf1-1 genomic DNA at 37 °C, large dash line), and percent occupancy (% Occ.) determined by quantitation of area under the curves for DMS reactive ABF1 residues (G-145, and G-138/TBP; G-220 and G-229/TCM1; G-164/RPL2A; G-173/RPL2B and G-235/QCR8; numbers are listed above curves) normalized to nonreactive DMS residues (G-164/SPT15; G-220/TCM1; G-164/RPL2A; G-173/RPL2B and G-235/QCR8; numbers are listed above curves) calculated and plotted graphically as a function of time (hours) post temperature shift (analysis of genes from the ABF1 WT strain at 25 °C, open squares; DNA from ABF1 WT strain at 37 °C, filled squares; analysis of genes from abf1-1 strain at 25 °C, open circle; DNA from abf1-1 strain at 37 °C, filled circles). Error bars are included where n was large enough (i.e., ≥4).

Fig. 2. A temperature shift rapidly induces complete loss of ABF1 element occupancy in the abf1-1 strain. A, in vivo occupancy

cells ~10–15 min; ABF1 cells 15–20 min). Such results (i.e. that bulk mRNAs are not globally stabilized in abf1-1 cells at nonpermissive temperatures) is consistent with RNA pulse labeling studies reported by Rhode et al. (18), who showed that total RNA synthesis in abf1-1 cells was lower than WT cells at elevated temperatures.

Taken together then, the in vivo footprinting (Fig. 2), the mRNA half-life (Fig. 3), and the mRNA stability data (Fig. 4) clearly indicate that we can confidently perform and interpret our abf1-1/ABF1 temperature shift loss of function experiment since shifting cells bearing the abf1-1 mutation to high temperatures, although clearly causing dramatic alterations in ABF1 element occupancy, induces no artifacts in total mRNA metabolism.
The implications of this striking result vis-à-vis transcription are discussed below.

**DISCUSSION**

In the present study we attempted to take a direct genetic approach to demonstrate that Abf1p actually drives transcription of the yeast TBP-encoding gene inside the cell. To accomplish this objective we coupled genetic gain and loss of function experiments with in vivo analyses of ABF1 occupancy, SPT15::lacZ expression and assays of TBP gene transcription. The salient features of our approach to analyze the function of Abf1p in TBP gene transcription are worthy of note. First, our two metrics of transcriptional activity, β-galactosidase enzyme levels and mRNA\(_{TBP}\) \(^{-}\)-end production actually measure transcription in two distinct yet complementary ways. One is a “chronic” measure (β-galactosidase) while the other is an “acute” measure of transcription (mRNA\(_{TBP}\) \(^{-}\)-end production) at least in yeast cells. This is due to the fact that the half-lives of the molecules being measured are so dramatically different. β-Galactosidase has a reported half-life of \(\geq 20\) h in yeast cells (27) while, as measured here, mRNA\(_{TBP}\) has a half-life of \(\leq 15\) min. Clearly measurement of β-galactosidase levels can readily be used to chronically assess the requirement(s) for the integrity and/or function of distinct TBP gene cis/trans-elements (9). Although perfectly appropriate for the \(ABF1\) gain of function experiments presented in Fig. 1 where we were expecting to observe an increase in reporter gene expression, analysis of β-galactosidase levels would be wholly inappropriate for our \(ABF1\) loss of function studies because we would be looking for short term rapid (i.e. min) decreases in the level of a very long lived (i.e. hours) reporter. Fortunately both mRNA\(_{TBP}\) \(^{-}\)-end analyses and genomic DMS footprinting represent appropriate analytical tools to acutely measure transcription and ABF1 element occupancy, particularly over the four hour time course of our temperature-shift experiments which utilize the mutant \(abf1-1\)-containing yeast strain. Use of these two biochemical analyses (mRNA \(^{-}\)-end analysis/genomic footprinting) allowed us to take accurate molecular “snapshots” of both TBP gene transcription and PED occupancy as a function of time after inactivation of the ability of Abf1p to bind DNA as induced by shifting cells carrying the \(abf1-1\) allele to the permissive temperature.

Quite surprisingly, as shown in the data of Figs. 2 and 5, Abf1p occupancy of the TBP gene ABF1 element is not required for continuous transcription in vivo. In fact as shown above, ABF1 element occupancy is apparently not required for transcription of other ostensibly “known” Abf1p-driven genes such as RPL2A and RPL2B (21), TCM1 (22) and QCR8 (23, 28). Given the short half-lives of these mRNAs (\(\leq 6\) min, mRNA\(_{RPL2A}\) to \(\leq 20\) min, mRNA\(_{RPL2B}\)) coupled with the facts that simply shifting \(abf1-1\)-containing cells had no artificial effects on bulk mRNA metabolism and that the time course of
First, in order for Abf1p to be irrelevant to the transcription of our collection of “Abf1p-driven” genes (including the gene encoding TBP) a second Abf1p-like DNA-binding transcription factor must exist in yeasts. Second, the plethora of “Abf1p-driven” genes and the involvement of Abf1p in replication and silencing argues that this transcription factor must be a very abundant protein. Third, based upon all of our genetic and biochemical data, this protein must have DNA sequence binding requirements identical to those of Abf1p as defined here.2 The fact that we fail to detect such a protein using sensitive gel shift and UV cross-linking methodologies2 argues strongly against the existence of a hypothetical Abf1p-like factor, although obviously such negative results can not disprove this possibility. Finally, it is also notable that ABF1 element occupancy, and only ABF1 occupancy, is rapidly and specifically lost after a temperature shift only in the abf1-1 cells. Clearly the weight of the data implicates Abf1p as the factor driving transcription inside the cell.

Cis-element Occupancy and Ongoing Transcription—There is a disagreement in the literature over whether continuous cis-element occupancy is absolutely required for sustained high level transcription. This question has been addressed using both in vitro and in vivo approaches. The in vitro data of Hai et al. (3) and Chi and Carey (4) suggest that continuous activator occupancy of cis-elements by their cognate DNA binding factors is not required for activated transcription in vitro. In contrast the in vitro studies of others (1, 2) have shown that activators remain bound to their DNA sites after transcription initiation has occurred and that continuous cis-element occupancy is required to maintain stimulated levels of reinitiation of transcription. Similar results were obtained by Mantovani et al. (29) who observed a requirement for continuous activation domain function for stimulation of reinitiation by the factor NF-Y in vitro. The reasons for the discrepancy between these workers are presently unknown and further experimentation will be required to resolve this. Regardless, extrapolating from these in vitro analyses to the in vivo situation is problematical.

There have been only a few attempts to address the question of obligate cis-element occupancy and transcription using in vivo methods. The major reasons for this are probably the general lack of conditional mutant forms of transcription factors (i.e., ts DNA binding) and the problems attendant with simultaneously measuring transcription and cis-element occupancy via genomic footprinting in complex eukaryotic genomes, particularly in mammalian cells. Despite these difficulties several studies on this problem have appeared. In one, Crabtree, Schreiber and colleagues (5) developed a novel method to conditionally assemble and disassemble a functional transactivator in vivo by utilizing the lipid soluble dimeric ligand FK1012 and separate chimeric DNA binding and activation domains both fused to FKBP12. FKBP12 is a protein which is able to bind the dimeric FK1012 ligand with high affinity. By manipulating the concentration of FK1012 in the culture medium of cells carrying the appropriate chimeric proteins, the authors showed that in vivo, a transcription factor activation domain must be continuously tethered to a cis-linked DNA-bound DNA binding domain in order for activated transcription to occur. These authors observed this phenomenon in both yeast and mammalian cells and thus concluded that it is a universal property of eukaryotic transcription.

In contrast, Darnell and colleagues (6) used run-on transcription assays and genomic footprinting to examine whether in interferon-treated cells all known interferon-specific regulatory cis-elements needed to be continuously occupied to mount and sustain an interferon response. They found that at least one of the multiple upstream cis elements of the guanylate
binding protein gene appeared to only be transiently occupied immediately after interferon application to cells. Thus, they concluded that at least in this system establishment and maintenance of an “activated structure” were distinguishable and separable events. Results in general agreement with this thesis were reported recently. Two laboratories have utilized conditional Flp1p-mediated in vivo recombination to delete either an enhancer (7) or a silencer (8) in order to assess the role that these regulatory cis-elements, and cognate trans factors, played in gene activation and gene repression. Using this elegant approach these groups found that depending upon the system (activation/enhancer or repression/silencer) and the time frame of the analysis (acute versus chronic) maintenance of the prerecognition expression state did not require cis-element occupancy, or, in this case, cis-element presence. Again these results are in contrast to those of Ho et al. (5) and argue strongly for additional investigation of this question. This controversy was one of the reasons we wanted to try and prove that Abf1p functioned in vivo to drive transcription of the TBP-encoding gene. Our results obviously support the hypothesis that continuous cis-element occupancy is not absolutely required for continuous transcription. Several models by which Abf1p could function are presented below.

Models for the Molecular Mechanism of Action of Abf1p—We hypothesize that Abf1p (and perhaps other similar factors) could modulate transcription via a “hit and run” mechanism of activation and in Fig. 6 we present four nonmutually exclusive models by which Abf1p could activate (TBP) gene transcription. An important aspect of all of our models is that we incorporate the fact that once Abf1p has effected the activation event proper (i.e., the “hit”) its continued presence on the gene is not required and it can dissociate from that gene (i.e., the “run”). These properties are at the heart of the hit and run mechanism. Each of the possible mechanisms shown, models I–IV, were formulated with this premise regarding the mode of action of Abf1p on the SPT15 gene in mind.

In models I and II, we suggest that Abf1p binding, either acting alone (model I), perhaps through a DNA bending mechanism (30) or in concert with the transcription machinery to the promoter. Once such repressors have been displaced from the promoter region of the gene other positive activators and the transcription machinery (RNA polymerase II + GTFs) could then stably associate with the TATA-box region of the gene and transcription could commence. Such an activator-GTF-gene complex would, once formed be (relatively) stable and importantly would prevent reformation of the repressive structure. Thus the continued presence of Abf1p at the ABF1 site would no longer be needed. Abf1p is acting as an anti-repressor in both of these models.

In model III, the ability of Abf1p to bend DNA is again considered (30), but in this model, Abf1p binding and thus bending of DNA would not act positively to facilitate contacts between the general transcription machinery and another DNA-bound positive acting factor. Here Abf1p is hypothesized to act as an architectural transcription factor (see Refs. 39–42 for recent reviews on this class of transcription factor) to facilitate the interactions of other factors with GTFs and/or RNAP II. Once an appropriate stereospecific nucleoprotein “enhancerome” complex (43) has formed, the continued presence of Abf1p would not be required to maintain transcription. Alternatively, protein-protein interactions could “hold” Abf1p in place, obviating the requirement for specific contacts between Abf1p and DNA.
In model IV we propose that Abf1p binding would somehow cooperatively stimulate, either directly or by recruiting additional proteins or protein complexes (perhaps proteins with catalytic activity) the formation of additional stable protein-protein contacts between a distinct, non-Abf1p, positive acting factor and components of the general transcription machinery. Again, once the initial interactions between the positive acting transactivator and components of the GTF/RNA Polymerase II complex were established Abf1p would no longer be required for continued gene transcription.

In all of these models of SPT15 gene regulation actively transcribing complexes are stable in the acute absence of Abf1p, consistent with our data. However as we showed previously (9) chronic disruption of binding of Abf1p by mutation of the Abf1p element has dramatic and drastic deleterious effects upon (TBP) gene transcription. Presumably a time dependent, DNA replication-induced disruption of factor-DNA complexes is responsible for the loss of transcription of the TBP encoding gene that we observe in vivo (see Fig. 3) (9). Such a result is consistent with the results of others who examined the requirement for cis-element occupancy/function for gene activation (7) and repression (8, 44). It is important to remember that Abf1p is a multifunctional protein that not only plays important roles in transcription activation but that it is also integrally involved in both DNA replication and transcriptional silencing. Thus yeast cells carrying the abf1-1 allele abruptly halt DNA replication at the nonpermissive temperature (18). We hypothesize that this cessation of DNA replication is the reason that the cells carrying the abf1-1 mutant allele continue to transcribe Abf1p-driven genes at the non-permissive temperature since protein-DNA complexes formed on these genes would not be disrupted by replication. In fact, we believe that this is the reason we were able to make the discovery that Abf1p occupancy is not required for ongoing high level transcription of Abf1p-driven genes.

Conclusions and Perspectives—The data presented here regarding Abf1p and its regulation of SPT15 gene expression could be extended to other systems and other genetic regulatory events. It will be quite interesting to apply the combination of approaches utilized in this study to other eukaryotic gene systems to see whether or not a hit and run mechanism might be used more generally. It will be particularly interesting to perform such experiments in cells or tissues during developmental gene expression programs where gene activation occurs in nonproliferating cells. Finally, it is noteworthy that a hit and run mechanism of gene activation, as proposed here, would potentially allow for a significant level of economy relative to the minimal concentration of a DNA binding transcription factor required to activate a multigene regulatory network. In this scenario the transcription factor(s) could act essentially catalytically. Our future studies will focus upon further defining the molecular mechanisms by which Abf1p controls gene transcription by directly testing which of our models regarding Abf1p action are correct and also testing to see if other transcription factors behave similarly to Abf1p.

Acknowledgments—We thank Drs. Judith Campbell, Jasper Rine, and Shlomo Eisenberg for providing yeast strains, plasmids, and antibodies used for our experiments. We also thank our colleagues in the laboratory for their constructive criticisms, support, and advice throughout the course of this work and Drs. S. Johnston, J. Segall, and M. Groudine for their critical comments on our manuscript.

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