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# Activation of DNA Replication in Yeast by Recruitment of the RNA Polymerase II Transcription Complex

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**Activators of transcription are known to also play an important and direct role in activating DNA replication. However, the mechanism whereby they stimulate replication has remained elusive. One model suggests that, in the context of replication origins, transcriptional activators work by interacting with replication factors. We show that a defined, single interaction between a DNA-bound derivative of the activator Gal4 and Gal11P, a mutant form of the RNA polymerase II holoenzyme component Gal11, suffices for stimulating DNA replication as it does for transcription. Moreover, recruitment of TBP, which can activate transcription from a gene promoter, also stimulates DNA replication from an origin site. These results strongly argue that transcriptional activators may not necessarily need to contact DNA replication factors directly, but can stimulate replication by recruiting the RNA polymerase II transcription complex to DNA.**

**Key words:** Protein-protein interactions / Regulation of DNA replication / RNA polymerase II holoenzyme / Transcriptional activation by recruitment.

## Introduction

Both DNA replication and transcription are regulated by specific DNA-binding proteins. For example, activation of transcription of protein-coding genes in eukaryotic cells is effected by so-called transcriptional activators. These proteins, which are typically composed of a DNA-binding domain and an activating region, bind regulatory DNA sequences known as enhancers, and interact through their activating regions with components of the RNA polymerase II transcriptional machinery binding the so-called promoter at the transcription start site of a gene (Ptashne, 1992). How do interactions between activators and the transcription complex cause activation of gene transcription? Results of numerous experiments performed in different laboratories over the past few years have indicated that these protein-protein interactions may activate tran-

scription from a gene promoter by simply recruiting the RNA polymerase II (pol II) transcription complex to DNA. In other words, the pol II transcription complex is directed to a promoter by binding cooperatively with enhancer-specific transcription factors to DNA (reviewed by Struhl, 1996; Ptashne and Gann, 1997; Barberis and Gaudreau, 1998).

It has recently become clear that several transcription factors also play an important and direct role in activating DNA replication from viral as well as cellular origins (van der Vliet, 1996). For example, the auxiliary sequences of the SV40 and polyomavirus origins of replication located adjacent to the binding site for the large T antigen contain elements recognized by cellular transcription factors such as Sp1, AP1 and p53 (DePamphilis, 1993). These transcription factors can increase viral origin activity up to 1000-fold (Guo and DePamphilis, 1992). The importance of transcriptional enhancers in regulating viral DNA replication was first demonstrated by de Villiers *et al.* (1984), who showed that substitution of the polyomavirus enhancer with the immunoglobulin gene enhancer conferred tissue-specific replicatory ability to the virus. Metazoan origins of replication also contain binding sites for transcription factors (Heintz, 1996); recognition sequences for octamer-binding and c-Myc proteins, for example, have been reported to contribute to origin function in mammalian cells (Iguchi-Arigo *et al.*, 1993; Taira *et al.*, 1994). The relevance of the interplay between transcription factors and replication in higher eukaryotes is further suggested by the observation that transcriptionally active genes replicate earlier in S phase than do inactive genes (Hatton *et al.*, 1988).

In the yeast *Saccharomyces cerevisiae*, a detailed mutational analysis of a cellular origin, *ARS1* (autonomously replicating sequence 1), has led to the identification of one essential (A) and three important (B1, B2 and B3) elements (Marahrens and Stillman, 1992). The B3 element is a binding site for the transcription factor Abf1p (Diffley and Stillman, 1988). The function of the B3 element of *ARS1* can be replaced by binding sites for other yeast transcription factors such as Gal4 and Rap1p (Marahrens and Stillman, 1992). In addition, activating domains of mammalian transcription factors such as VP16 and p53 can stimulate DNA replication in yeast when tethered to the *ARS1* sequence (Li *et al.*, 1998). How do transcriptional activators stimulate DNA replication from cellular origins? In contrast to the good deal of information that we have recently acquired about the function of transcriptional activators in gene transcription (see above), their mechanism of action in stimulating DNA replication has remained elusive. The following possibilities may be envisioned:

(i) Transcriptional activators might directly contact replication factors to recruit them to origin sites and/or to stimulate their functions in unwinding DNA and initiating DNA synthesis.

(ii) They might remove inhibitory nucleosomal structures from origin sites through direct recruitment of specialized nucleosome remodeling machines such as Swi/Snf and histone acetylases (Workman and Kingston, 1998) to facilitate initiation of replication.

(iii) Transcriptional activators might recruit the RNA polymerase II (pol II) transcription complex near replication origins to synthesize RNA, unwind DNA, and/or displace inhibitory nucleosomes.

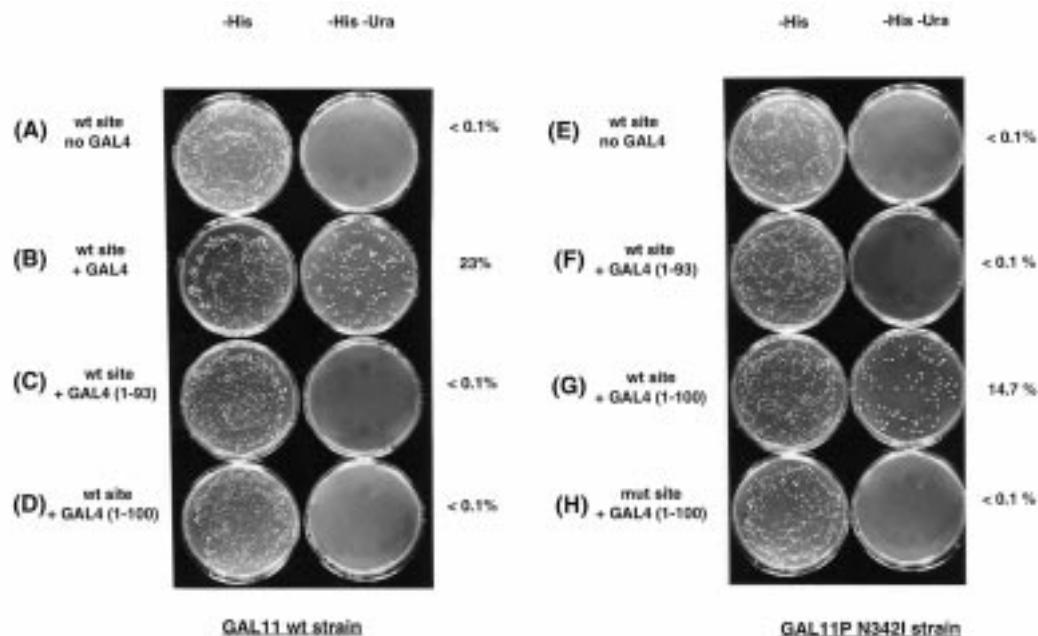
Here we have addressed the following question: what does a sequence-specific DNA-binding protein have to interact with in order to activate replication in the yeast *Saccharomyces cerevisiae*? Some of the transcriptional activators that can stimulate replication from viral as well as yeast origins have been shown to interact with replication protein A (RPA) *in vitro*. It has been suggested that these interactions, in the context of an origin site, are responsible for the stimulation of DNA replication by activators *in vivo* (Li and Botchan, 1993; He *et al.*, 1993; Leiter *et al.*, 1996). Our results show that the same defined protein-protein interactions that cause activation of transcription by recruitment of the pol II transcription complex to DNA can also stimulate DNA replication in yeast cells. Thus, a

DNA-binding protein is not required to directly interact either with replication factors or with specialized nucleosome-remodeling machines to activate replication in yeast.

## Results

### Contact with a Component of the RNA Polymerase II Holoenzyme Activates DNA Replication

It has been shown that interaction between a DNA-bound protein and a single component of the yeast RNA polymerase II holoenzyme can trigger gene activation by recruiting the transcription complex to DNA (Barberis *et al.*, 1995; Farrell *et al.*, 1996; Gaudreau *et al.*, 1998). In this instance of gene activation, a single amino acid change (N342I) in Gal11, a nuclear protein which is almost exclusively found as part of the pol II holoenzyme, creates an interaction between that mutant protein (called Gal11P) and the dimerization region, not the activating region, of the yeast transcriptional activator Gal4. Tethering the relevant portion of the Gal4 dimerization region (residues 58–97) to DNA by fusion with an heterologous DNA-binding domain or as part of Gal4 (1–100), which lacks a classical activating sequence but contains DNA-binding and dimerization domains, leads to strong gene activation specifically in *GAL11P* cells but not in *GAL11* wild-type (wt) cells. *In*



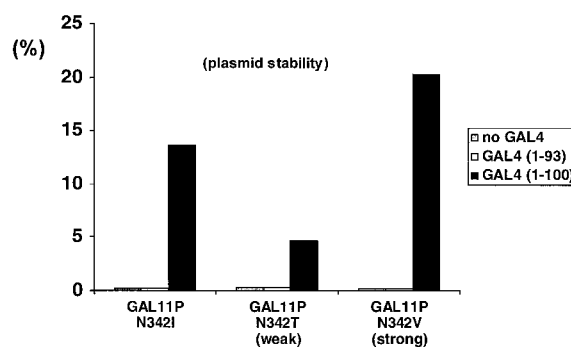
**Fig. 1** Gal4 (1–100) Activates *ARS1* Function in a Site-Dependent Manner through the Interaction with Gal11P, a Component of the RNA Polymerase II Holoenzyme.

*ARS1* plasmids that carry either a wild-type site (wt site; rows A to G) or a mutant site (mut site; row H) for Gal4 protein in place of the Abf1p-binding site were assayed for plasmid stability. The plasmids bearing *HIS3* markers and expressing no Gal4, Gal4 full-length, Gal4 (1–93), or Gal4 (1–100) were introduced into *GAL11* wild-type (wt) strain (SOY23) (left panel) and *GAL11P*N342I strain (SOY28) (right panel) along with the test plasmids. After nonselective growth for 30 h at 30°C, the cells were plated on the control -His (left lanes in each panel) and selective -His -Ura (right lanes in each panel) plates. The ratio of the colony numbers on the two types of plates, expressed as percentage (see Materials and Methods), is indicative of the stability of the test plasmid.

*vitro*, Gal11P, but not Gal11wt, interacts with the Gal4 dimerization domain (Barberis *et al.*, 1995).

Plasmid stability assays (Mahahrens and Stillman, 1992) were performed to determine whether the same, defined interactions that activate transcription by recruitment of the pol II transcription complex to DNA could also stimulate replication from the *ARS1* origin in yeast. As a test plasmid, we used the previously described pARS1/-B23/G24 (Li *et al.*, 1998). This *ARS/CEN* plasmid bears mutated *ARS1* sequences: the B2 element has been substituted with an 8-bp linker, and a 17 base pair Gal4 binding site (17<sup>mer</sup>) has been inserted next to a mutated B3 element which no longer binds the transcription factor Abf1p. As previously shown by Li *et al.* (1998), pARS1/-B23/G24 displays very low stability in the absence of the Gal4 activator (Figure 1, rows A and E). In the presence of full-length Gal4, the stability of the test plasmid increased to 23% (Figure 1, row B). It has been shown that the effect of various activators on the stability of the pARS1/-B23/G24 plasmid precisely mirrors their effect on the efficiency of initiation of DNA replication from the same *ARS1* sequence in a chromosomal location (Li *et al.*, 1998). In our plasmid stability assays we used, in addition to full-length Gal4, its derivatives Gal4 (1–93) and Gal4 (1–100), both of which are capable of dimerizing and binding DNA but unable to activate transcription in *GAL11* wild-type cells because they lack a classical activating region. Gal4 (1–100), but not Gal4 (1–93), interacts with Gal11P, and neither can interact with Gal11wt (Barberis *et al.*, 1995). We first determined stability of the test plasmid pARS1/-B23/G24 by cotransforming *GAL11* wild-type (SOY23) and *GAL11P* (N342I) (SOY28) yeast cells with plasmids expressing either Gal4 (1–93) or Gal4 (1–100). Figure 1 shows that the test plasmid had < 0.1% stability in the presence of Gal4 (1–93) in both *GAL11* wild-type and *GAL11P* cells (rows C and F). In contrast, expression of Gal4 (1–100) significantly increased plasmid stability (14.8% ± 1.9%) in *GAL11P* (N342I) (Figure 1, row G) but not in *GAL11* wild-type cells (Figure 1, row D). No activation of replication was observed with a test plasmid containing a mutated 17<sup>mer</sup> site unable to bind Gal4 proteins (Figure 1, row H). Moreover, no replication was detected with a test plasmid lacking the *ARS1* sequence (data not shown). These results indicated that the same, unique interaction between Gal4 (1–100) and Gal11P, which was shown to cause activation of transcription by recruiting the pol II complex to a promoter, was also sufficient to activate DNA replication from an origin site in yeast.

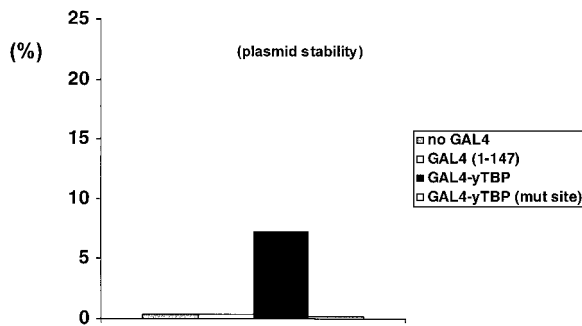
Additional alleles of the original *GAL11P* (N342I) have been characterized, each of which encodes a Gal11 protein that bears a hydrophobic residue in place of the wild-type asparagine at position 342 (Barberis *et al.*, 1995). Farrell *et al.* (1996) have shown that different Gal11P variants have different affinities for the Gal4 dimerization region and that, in agreement with the recruitment model for gene activation, the strength of the Gal4-Gal11P interaction as quantified *in vitro* correlates with the degree of gene activation elicited by Gal4 (1–100) *in vivo*. We compared the



**Fig. 2** The Strength of Interaction between Gal4 (1–100) and Different Gal11P Alleles Correlates with the Degree of Activation of DNA Replication in Yeast Cells.

The plasmids bearing *HIS3* marker and expressing no Gal4, Gal4 (1–93) or Gal4 (1–100) were introduced into yeast cells bearing the original *GAL11P* N342I (SOY28 strain), the weaker *GAL11P* N342T (SOY30 strain), and the stronger *GAL11P* N342V (SOY32 strain) alleles, and tested for their ability to stimulate *ARS1* by measuring the stability of the pARS1/-B23/G24 test plasmid.

degree of stimulation of DNA replication and transcription elicited by Gal4 (1–100) in yeast cells bearing different *GAL11P* alleles. Gal4 (1–100) was expressed in strain SOY28, which contains the original *GAL11P* allele (N342I), as well as in the strains SOY30 and SOY32, which contain the weaker *GAL11P* allele N342T and the stronger *GAL11P* allele N342V, respectively. Figure 2 shows that, in yeast cells bearing the weaker *GAL11P* allele, Gal4 (1–100) stimulated replication of the test plasmid less efficiently than in cells containing the original *GAL11P* allele (4.6% ± 2.4% compared to 13.6% ± 3.1% stability), while in the presence of the stronger *GAL11P* allele, Gal4 (1–100) enhanced replication of the test plasmid more strongly (20.3% ± 3.1% stability). In all three strains, which, with the exception of the point mutation in the *GAL11* gene, are isogenic, expression of Gal4 (1–93) did not increase plasmid stability over the level obtained when not expressing any Gal4 derivatives (Figure 2). Correlation between the strength of the Gal4-Gal11P interaction and the degree of gene activation by Gal4 (1–100) was tested again in these strains by cotransformation with a centromeric plasmid carrying the *lacZ* gene under the control of the Gal4-binding UAS<sub>G</sub> enhancer. The result of this control experiment was very similar to those obtained by Farrell *et al.* (1996) and Barberis *et al.* (1995) with different yeast strains bearing reporter genes integrated into the genome (data not shown). Taken together, these results show that the strength of interaction between Gal4 (1–100) and different Gal11P alleles correlates with the degree of activation of both DNA replication and transcription *in vivo*, thus providing additional support to the idea that a single and specific protein-protein interaction between a DNA-bound and a holoenzyme-bound component can activate DNA replication from an origin site as well as transcription from a pol II promoter.



**Fig. 3** Recruitment of TBP to a Modified *ARS1* through Fusion with Gal4 (1–147) Activates DNA Replication in a Site-Dependent Manner.

The plasmids expressing no Gal4, Gal4 (1–147) and Gal4 (1–147)-yTBP were introduced into cells of the *S. cerevisiae* SOY23 strain along with *ARS1* test plasmids that carry either a wild-type or a mutant (mut) Gal4 site, and stability of the *ARS1* plasmids was tested.

### Recruitment of TBP near an Origin Site Activates DNA Replication

It has been shown that the TATA-binding protein (TBP), a subunit of the TFIID complex which is not an integral part of the yeast pol II holoenzyme (Kim *et al.*, 1994; Koleske and Young, 1994), can activate transcription when tethered to promoters as a fusion with the Gal4 DNA-binding domain (Xiao *et al.*, 1995). Similar results have been obtained by tethering TBP through fusion or interaction with other DNA-binding proteins (Chatterjee and Struhl, 1995; Klages and Strubin, 1995). These results, taken together with those of Barberis *et al.* (1995), Farrell *et al.* (1996), and Gaudreau *et al.* (1998), have led to the suggestion that interactions between transcriptional activators and either the TFIID or the holoenzyme complexes (or both) activate transcription by recruiting the complete pol II machinery to DNA (Barberis *et al.*, 1995; Barberis and Gaudreau, 1998). To determine whether recruitment of TBP to the replication origin is sufficient to activate *ARS1* function in yeast, we expressed a hybrid protein containing yeast TBP (yTBP) fused to the DNA-binding Gal4 (1–147) protein (Xiao *et al.*, 1995) in the yeast strain SOY23, and performed plasmid stability assays. While the Gal4-yTBP hybrid protein had no effect on the stability of an *ARS1* plasmid containing a mutant Gal4-binding site, it enhanced the stability of the test plasmid carrying the wild-type Gal4 site to  $7.2\% \pm 2.5\%$  (Figure 3). Thus, artificial recruitment of TBP close to the *ARS1* origin of replication also suffices for activation of DNA replication *in vivo*.

### Discussion

Results of experiments performed with animal viruses and with yeast have demonstrated that transcription factors play a major and direct role in stimulating DNA replication (Heintz, 1992; DePamphilis, 1993; van der Vliet, 1996). It has been postulated that the involvement of the same

transcription factors in the activation of DNA replication from origin sites as well as transcription from gene promoters requires that these proteins have either two different biochemical activities or one single activity that stimulates two distinct processes (Marahrens and Stillman, 1992). That some transcriptional activators might indeed be endowed with different biochemical activities has been indicated by results of *in vitro* experiments which showed that these proteins can interact not only with components of the RNA pol II transcription complex (see Introduction) but also with replication factors. Thus, these *in vitro* experiments have led to the suggestion that, in the context of origin sites, transcriptional activators might directly contact replication factors to stimulate DNA replication, while, in the context of gene promoters, they would function by interacting with components of the RNA pol II transcription complex (Li and Botchan, 1993; He *et al.*, 1993; Leiter *et al.*, 1996). The results of our *in vivo* experiments show that the same biochemical activity (protein-protein interaction) of a DNA-binding protein can activate transcription from a gene promoter as well as replication from an origin site in yeast. More specifically, defined molecular interactions that tether components of either the pol II holoenzyme complex or the TFIID complex to DNA are shown to restore DNA replication from a yeast origin of replication that lacks the natural binding site for the transcription factor Abf1p. Since the same molecular interactions can activate transcription from a pol II promoter by recruiting the pol II transcription complex to DNA (Ptashne and Gann, 1997; Barberis and Gaudreau, 1998), these results strongly suggest that the same mechanism, *i.e.* recruitment of the pol II transcription complex, applies to this instance of activation of DNA replication. In agreement with the recruitment mechanism, a fusion protein bearing the DNA-binding domain of Gal4 (residues 1–100) linked to the holoenzyme-bound 282 carboxyl-terminal residues of Gal11, which has been shown to strongly activate gene transcription in yeast (Barberis *et al.*, 1995; Farrell *et al.*, 1996), also efficiently stimulated replication of the test plasmid (not shown). Thus, a DNA-binding protein does not need to directly interact with replication factors in order to activate replication from *ARS1*. Moreover, in analogy with the implications of the Gal4-Gal11P interaction in gene activation already discussed by Barberis *et al.* (1995), stimulation of replication in our experiments does not require direct interaction of the DNA-binding protein with machinery that helps remove inhibitory chromatin structures. Our results more broadly raise the possibility that natural transcriptional activators binding near replication origins also activate replication by recruitment of the pol II transcription complex through direct interactions with one or more of its components.

We note that in the case of the yeast origin of replication *ARS121*, the Abf1p binding sites that stimulate the activity of this origin could not be functionally replaced by binding sites for other transcriptional activators (Wiltshire *et al.*, 1997). It is possible that Abf1p has distinct biochemical activities at different origins, only one of which corre-

sponds to its transcriptional activation function and can be replaced by other activators, as it is the case at the *ARS1* locus tested here. Alternatively, while at the *ARS1* origin a number of interactions between different DNA-bound proteins (including Abf1p) and the pol II transcription complex may cause stimulation of replication, at *ARS121* only the particular interaction between Abf1p and the transcription complex may be able to stimulate this origin function. In analogy with this scenario, it has been reported that there are promoters that can only be activated by some enhancer-binding proteins and not by others, suggesting the existence of specificity in the enhancer/promoter relationship (Li and Noll, 1994; Das *et al.*, 1995; Barberis and Gaudreau, 1998). It is conceivable that there is a similar kind of specificity in the enhancer/origin relationship.

What is the function of the recruited pol II transcription complex in the enhancement of DNA replication from an origin site? Three mechanisms may be considered for further investigation:

(i) recruitment of the pol II complex might lead to initiation of RNA synthesis by RNA polymerase II near or at the origin site; this mechanism would be reminiscent of those described for mitochondrial DNA replication, in which the RNA product serves as a primer for DNA synthesis (Clayton, 1991), and for DNA replication from the origins of the *E. coli* chromosome and phage  $\lambda$ , in which transcription appears to activate replication by altering the local DNA structure, thus facilitating formation of the replication complex on DNA (Baker and Kornberg, 1988; Learn *et al.*, 1993).

(ii) Separation of DNA strands, which is required for initiation of both replication and transcription, may be carried out by helicase enzymes that are part of TFIIH, a component of the pol II holoenzyme (Koleske and Young, 1994; Guzder *et al.*, 1994; Sung *et al.*, 1996).

(iii) Recruitment of the pol II transcription complex to DNA might remodel chromatin structures which act as general repressors of promoter and origin functions. Such a chromatin remodeling might be a consequence of the activities of the pol II transcription complex, or it might be carried out by specialized machines, e.g. Swi/Snf and histone acetylases, which can associate with the recruited transcription complex (Barberis and Gaudreau, 1998). It has been shown that recruitment of the pol II holoenzyme to the *PHO5* promoter suffices to remodel positioned nucleosomes, even in the absence of transcription (Gaudreau *et al.*, 1997).

## Materials and Methods

### Yeast strains and media

The following yeast strains were used: SOY23 (*MAT $\alpha$  ura3-52 his3 $\Delta$ 200 leu2 $\Delta$ 1 trp1 $\Delta$ 63 lys2 $\Delta$ 385 GAL11*), SOY28 (*MAT $\alpha$  ura3-52 his3 $\Delta$ 200 leu2 $\Delta$ 1 trp1 $\Delta$ 63 lys2 $\Delta$ 385 GAL11P-N342*), SOY30 (*MAT $\alpha$  ura3-52 his3 $\Delta$ 200 leu2 $\Delta$ 1 trp1 $\Delta$ 63 lys2 $\Delta$ 385 GAL11P-N342T*), SOY32 (*MAT $\alpha$  ura3-52 his3 $\Delta$ 200 leu2 $\Delta$ 1 trp1 $\Delta$ 63*

*lys2 $\Delta$ 385 GAL11P-N342V*). These strains did not show significant differences in the rate of growth on media containing glucose (data not shown). Media and solutions were prepared according to standard methods (Rose *et al.*, 1990).

### Plasmids

All plasmids, as well as the protein products of yeast expression vectors, were previously described (see below). pRS313 is a *HIS3*-marked, CEN/ARS-based vector (Sikorski and Hieter, 1989). pNS43 is a *HIS3*-marked, CEN/ARS-based plasmid that expresses Gal4 (1–93) (Barberis *et al.*, 1995). pRJR217 is a *HIS3*-marked, CEN/ARS-based plasmid that expresses Gal4 (1–100) (Barberis *et al.*, 1995). pRJR191 is a *HIS3*-marked, CEN/ARS-based plasmid that expresses full-length Gal4 (Reece and Ptashne, 1993). pGAL4-yTBP is a *HIS3*-marked, CEN/ARS-based plasmid that expresses a yeast TBP derivative fused to the DNA-binding Gal4 (1–147) protein (Xiao *et al.*, 1995). The test plasmid pARS1/-B23/G24 as well as its derivative carrying the mutated Gal4-binding site used for the stability assays described in this work were kindly provided by Drs. Rong Li and Bruce Stillman, and described by Li *et al.* (1998).

### Plasmid Stability Assay

Plasmid stability assays were performed as detailed by Marahrens and Stillman (1992) and Li *et al.* (1998). Vectors expressing Gal4 and its derivatives (or not expressing any Gal4 protein) and the test plasmid pARS1/-B23/G24 (Li *et al.*, 1998) were transformed simultaneously into yeast strains SOY23, SOY28, SOY30 and SOY32 by the standard lithium acetate method (Rose *et al.*, 1990). Single transformants were inoculated in 2 ml of synthetic complete medium (SCM) -His -Ura and grown overnight at 30°C. Overnight cultures were diluted to an OD<sub>546</sub> of 0.0003 in 3 ml of YPD medium and grown for 30 hours (approximately 14 generations) at 30°C. Cultures were diluted, and equal numbers of cells (~ 1000 cells) were plated on selective SCM -His -Ura and control SCM -His plates. Plasmid stability is reported as: [(number of colonies on SCM -His -Ura plates) / (number of colonies on SCM -His plates)] × 100. The stability assay for each Gal4 derivative is an average of data from at least three independent experiments, each using colonies from separate transformation.

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