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Three classes of mammalian transcription activation domain stimulate transcription in *Schizosaccharomyces pombe*

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Representatives of three distinct classes of mammalian protein domain activating RNA polymerase II were fused to the yeast GAL4p DNA-binding domain. The resulting fusion proteins were tested in the fission yeast *Schizosaccharomyces pombe* for their ability to activate transcription of different reporter constructs containing GAL4-binding sites in positions close to or far from the TATA box. The acidic-rich activation domain of VP16 stimulates transcription in *S.pombe* from proximal and distal positions, suggesting that the mechanism of activation is conserved from man to budding and fission yeasts. Unlike in *Saccharomyces cerevisiae*, the glutamine-rich activation domains of Sp1, Oct1 and Oct2 activate transcription in *S.pombe* when tested in a proximal TATA box context. Similarly to mammalian cells, these domains are inactive or weakly active when tested in a distal position. Moreover, the proline-rich activation domains of AP-2 and CTF/NF1 display strong transcriptional activities from a TATA box-proximal position, and weak activities when tested in a remote position. Consequently, proline-rich and glutamine-rich activation domains act differently in *S.cerevisiae* and mammalian cells, but similarly in *S.pombe* and mammalian cells.

Keywords: GAL4p fusion proteins/mammalian transcription activation domains/promoter organization/*Schizosaccharomyces pombe*

Introduction

Initiation of transcription in eukaryotes occurs through a complex set of DNA–protein and protein–protein interactions involving RNA polymerase II (which is presumably part of a complex holoenzyme), promoter DNA, a set of general transcription factors (GTFs) and adaptors, and promoter-specific regulatory proteins (Zawel and Reinberg, 1995; Künzler *et al.*, 1996). The latter are sequence-specific DNA-binding proteins that in many cases have been shown to function as transcriptional activators. Typically, these activators are composed of distinct domains including separable DNA-binding and activation domains (Hope and Struhl, 1986; Keegan *et al.*, 1986; for a review, see Triezenberg, 1995). The activation domains have been classified somewhat arbitrarily depending on whether they are rich in acidic amino acids, glutamine or proline. However, for the acidic-rich activation domains, recent observations revealed that specific patterns of hydrophobic and aromatic amino acids were equally or even more critical than the acidic ones (Cress and Triezenberg, 1991; Regier *et al.*, 1993; Drysdale *et al.*, 1995). These classes of activation domain differ significantly in their ability to activate transcription from distinct promoter positions in mammalian cells (Seipel *et al.*, 1992). 'Proximal' activation domains, exemplified by glutamine-rich domains of Sp1, Oct1 and Oct2, stimulate transcription only from a position close to the TATA box, usually in response to a remote enhancer. Acidic-type activation domains, represented by yeast GAL4p and herpes simplex virus VP16, stimulate transcription from remote (e.g. enhancer) as well as proximal promoter positions. Finally, the proline-rich activation domains of AP-2 and CTF/NF1 display considerable proximal and low but significant remote promoter activity.

Several mammalian proteins containing acidic-rich and/or proline-rich activation domains have been shown to stimulate transcription in the budding yeast (Lech *et al.*, 1988; Metzger *et al.*, 1988; Sadowski *et al.*, 1988; Schena and Yamamoto, 1988; Struhl, 1988). Notably, the yeast transcription factor GAL4p, which has an acidic-type activation domain, also activates transcription in plant and mammalian cells, suggesting that its mechanism of activation is conserved (Ma *et al.*, 1988; Webster *et al.*, 1988). In contrast, the human Sp1 transcription factor, which contains well characterized glutamine-rich activation domains, fails to stimulate transcription in *Saccharomyces cerevisiae* even in concert with human TATA-binding protein (TBP) or human–yeast TBP hybrids (Ponticelli *et al.*, 1995). The glutamine-rich activation domains of Oct1, Oct2 and Sp1, when fused to the DNA-binding domain of GAL4p, cannot stimulate transcription in *S.cerevisiae* from promoters containing GAL4-binding sites (Künzler *et al.*, 1994). Consequently, in contrast to the acidic activation domains, the function of glutamine-rich domains in transcription differs between humans and *S.cerevisiae*. One explanation for this may be that glutamine-rich activation domains require for their function in mammals additional factors which are absent or have other functions in *S.cerevisiae*.

For several aspects, such as chromosome size, centromere structure, intron splicing mechanism, cell cycle control and heat shock response, the fission yeast seems to be more closely related to mammalian cells than to budding yeast (Glick, 1996). In addition, as in mammalian cells, initiation of transcription from *Schizosaccharomyces pombe* promoters occurs ~30 bp downstream of the TATA

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Table I. Summary of the transactivation results

GAL4 fusion constructs	Prevalent amino acid	Net charge	Activation in *S.pombe* (reporter constructs: Figure 1)		
			TATA-proximal (A)	87 bp spacing (B)	Remote (C)
GAL4(1–93)			–	–	–
GAL4(1–93)–Sp1(132–243)$_1$	Q	0	+	+/–	–
GAL4(1–93)–Sp1(340–485)$_2$	Q	+1	+	+/–	–
GAL4(1–93)–Oct1(175–269)	Q	+1	+	+/–	–
GAL4(1–93)–Oct2(99–161)	Q	+2	++	+	+/–
GAL4(1–93)–AP2(31–117)$_1$	P>S + T	+5	+++	+++	+/–
GAL4(1–93)–AP2(31–76)$_2$	P>S + T	0	++	+	+/–
GAL4(1–93)–CTF(399–499)	P>S + T	+3	++	++	+/–
GAL4(1–93)–VP16(c)(413–454)	D + E	−7	++	++	++
GAL4(1–93)–VP16(413–490)	D + E	−18	+++	+++	+++

Amino acid positions are given in parentheses. The exact amino acid sequences of the fusion proteins are described elsewhere (Seipel *et al.*, 1992).
+++ indicates very strong, ++ strong, + medium, +/– weak and – no activation.
$_1$ and $_2$ denote different TADs in the factor (Sp1) or different forms of the same factor (AP-2).

box, whereas in *S.cerevisiae* distances can vary between 40 and 120 bp (Li *et al.*, 1994). Moreover, initiation from several mammalian promoters introduced into *S.pombe* occurs at the same site(s) as in mammalian cells (Toyama and Okayama, 1990). Since TFIIB and RNA polymerase II are mainly responsible for selecting the start site of transcription, this suggests a higher degree of conservation of the functions of these proteins between *S.pombe* and mammalian cells, when compared with *S.cerevisiae*. Several transcription factors have been cloned from *S.pombe* by other groups (Sugimoto *et al.*, 1991; Tang *et al.*, 1994; Wu and McLeod, 1995; Wilkinson *et al.*, 1996). However, the characterization of their potential transcriptional activation domains (TADs) has not been performed yet. Therefore, there are hardly any data on the function of such domains at various promoter positions in *S.pombe*. Consequently, it is interesting to document whether mammalian transcriptional activators work in *S.pombe* as they do in mammalian cells or as they do in *S.cerevisiae*.

We tested, in a systematic approach, representatives of the three classes of mammalian TAD in *S.pombe*. For this study, we used exactly the same $GAL4_{DBD}$–TAD fusions that were tested in mammals and in *S.cerevisiae* (Seipel *et al.*, 1992; Künzler *et al.*, 1994). These hybrid proteins are based on the GAL4p DNA-binding domain ($GAL4_{DBD}$; amino acids 1–93) fused to the activation domains from well-characterized mammalian transcription factors (Seipel *et al.*, 1992; Künzler *et al.*, 1994). These different $GAL4_{DBD}$–TAD fusions were tested for transactivation of several *S.pombe* reporter constructs containing GAL4p-binding sites located at different positions from the TATA box. Acidic-type activation domains stimulate transcription strongly from positions both close to and remote from the TATA box. As in mammalian cells, proline-rich domains stimulate transcription strongly from a position close to the TATA box but only weakly from a remote position. Most importantly, glutamine-rich activation domains stimulate transcription in *S.pombe,* but only from a position close to the TATA box, just as is observed in mammals. These results indicate that, in contrast to *S.cerevisiae*, *S.pombe* is able to use glutamine-rich activation domains. These observations on the use of different types of activation domains in *S.pombe* significantly support the emerging picture that *S.pombe*, in many respects and in particular with regard to transcription, is much more related to and comparable with mammalian cells than *S.cerevisiae*.

Results

Overexpression of mammalian activation domains is toxic in the fission yeast

TADs of different well-characterized mammalian transcription factors fused to the DNA-binding domain of GAL4p ($GAL4_{DBD}$) have been analysed previously for transactivation in the budding yeast *S.cerevisiae* and in mammalian cells (Seipel *et al.*, 1992; Künzler *et al.*, 1994). Therefore, to allow comparison of the transcriptional activity of the different types of activation domains in *S.pombe*, *S.cerevisiae* and mammals, we tested in *S.pombe* exactly the same $GAL4_{DBD}$–TADs fusions that were studied both in *S.cerevisiae* and in mammalian cells. We selected representatives of all three classes of activation domain from this collection of chimeric activators and used these in different *S.pombe* reporter strains (Table I and Figure 1). Acidic-rich activation domains are exemplified by different portions of the VP16 activation domain. Different portions of the AP-2 activation domain and the activation domain of CTF/NF1 represent the proline-rich class of activation domain. Finally, the class of glutamine-rich activation domains is represented by Oct1 and Oct2, and by different portions of the Sp1 activation domain. As a negative control, $GAL4_{DBD}$ was used without an activation domain. Chimeric activators were cloned into an episomal plasmid under the control of the thiamine-repressible *S.pombe PHO4* promoter (Silvestre and Jacobs, 1997). Transcription from this promoter is induced on medium lacking thiamine.

All the different $GAL4_{DBD}$–TAD expression plasmids were transformed into the *S.pombe* P2 strain. For each plasmid, one half of the transformation mixture was spread on selective medium containing thiamine (MMRT) and, therefore, expression of chimeric activators was repressed; the other half was plated on selective medium lacking thiamine (MMR), which enables synthesis of chimeric activator polypeptides. No differences in number and size of the colonies on both selection agar plates were detected

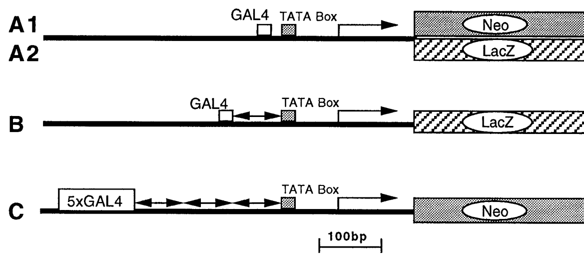


Fig. 1. The arrangement of the GAL4p-binding sites in the different ADH_{min} reporter genes. The $GAL4_{DBD}$–TAD fusion proteins were tested for transactivation in *S.pombe* by introducing the different expression plasmids in four *S.pombe* reporter strains: A1, A2, B and C. The construction of these strains is described in Materials and methods. The strain C (*neo* reporter gene) enables us to test the different $GAL4_{DBD}$–TAD in a position remote ('235 bp') from the TATA box of the ADH_{min} promoter. For this, 5GAL4-binding sites were separated from the TATA box by three copies of a transcriptionally silent 71 bp DNA fragment (symbolized by the double arrow) of the firefly luciferase gene. For transactivation in a proximal position from the TATA box, we used strains A1 and A2, which contain a 17mer GAL4-binding site positioned immediately upstream of the ADH TATA box. The *neo* gene (strain A1) and the *lacZ* gene (strain A2) were used as reporter genes. The strain B (*lacZ* reporter gene) allows transactivation to be tested for in an intermediate position ('87 bp') upstream of the TATA box. To construct this strain, one copy of the same 71 bp luciferase DNA fragment was inserted between one GAL4-binding site and the ADH TATA box.

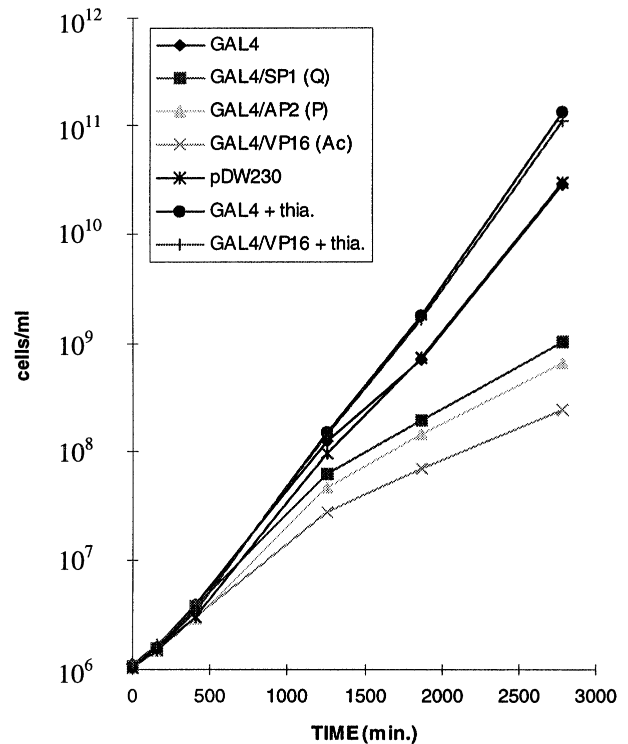


Fig. 2. Growth curve of *S.pombe* strain P2 expressing different $GAL4_{DBD}$–TAD fusions. To induce the expression of the $GAL4_{DBD}$–TAD fusions by the activation of the thiamine-repressible *PHO4* promoter, cells were washed three times and put into culture in the absence of thiamine. In control samples (+ thia), the cells were grown in the presence of thiamine. At different times points, samples were taken and the cell number determined. The growth rate was estimated 35 h after induction.

with the $GAL4_{DBD}$ control plasmid (data not shown). When the $GAL4_{DBD}$–TAD fusion genes were repressed, the URA4 colonies obtained on MMRT plates displayed the same size as the $GAL4_{DBD}$ control. However, when the fusion gene expressions were induced on medium lacking thiamine (MMR), no colony was obtained for the GAL4 hybrids with the proline-rich and acidic-rich activation domains. With the glutamine-rich activation domains, the size of these colonies was significantly smaller than the size of the colonies obtained with the $GAL4_{DBD}$ plasmid.

To investigate this growth phenotype further, we measured the growth rate of yeast strains containing expression plasmids for the different types of activation domain in liquid culture. The cultures were cultivated in thiamine-containing medium (MMRT) and shifted to medium lacking thiamine (MMR). The growth rate (measured 35 h after the medium shift) of the strain expressing the $GAL4_{DBD}$ was 2.8 h per division, which is identical to the growth rate of the strain containing the pDW230 control plasmid. This suggests that no toxicity was observed for the overexpression of the $GAL4_{DBD}$ (Figure 2). The growth rates of the strains expressing $GAL4_{DBD}/SP1_{(1)}$ (glutamine-rich), $GAL4_{DBD}$/CTF (proline-rich) and $GAL4_{DBD}$/VP16 (acidic-rich) were only 6.3, 6.8 and 8.3 h per division, respectively. In MMRT, no difference in growth rate was observed between the yeast containing the $GAL4_{DBD}$ expression plasmid and the yeast containing the $GAL4_{DBD}$/VP16 expression plasmid. Therefore, the reduction in growth rate is due to the overexpression of the different $GAL4_{DBD}$–TAD fusion proteins. These observations indicate that the tested $GAL4_{DBD}$–TAD fusions are toxic when overexpressed in *S.pombe*. This toxicity may result from the squelching of components of the yeast transcriptional machinery by the overexpressed activation domains. Overexpression of acidic domains was also shown to be toxic in *S.cerevisiae* (Gill and Ptashne, 1988), and the screening for extragenic suppressors of this toxicity has allowed the isolation of transcriptional adaptors for acidic activation domains (Berger *et al.*, 1992).

All three types of mammalian activation domains stimulate transcription in S.pombe in a position close to the TATA box

The different $GAL4_{DBD}$–TAD fusions were tested for transactivation in a proximal position in two reporter yeast strains, A1 and A2 (Figure 1). In both, one GAL4p 17mer recognition sequence ($GAL4_{UAS}$) was inserted immediately upstream of the TATA box from the *S.pombe ADH* minimal (ADH_{min}) promoter.

In reporter strain A1, this chimeric $GAL4_{UAS}$–ADH_{min} promoter was cloned upstream of the *neo* gene, and the reporter construct was stably integrated into the *ARG3* locus of the *S.pombe* genome. Transactivation in that reporter strain was determined by the relative G418 resistance level of the yeast transformants. Therefore, the yeast colonies were grown on minimal medium (MM) containing a limiting concentration of thiamine (5 μM) to repress the expression of the chimeric activators and replica-plated on YPD medium lacking thiamine and containing different concentrations of G418.

In strain A2, the $GAL4_{UAS}$–ADH_{min} chimeric promoter was cloned upstream of *lacZ*, and this fusion reporter gene was integrated into *ADE6* locus. Transactivation of that reporter gene was determined by measuring

β-galactosidase activity from yeast cells grown in liquid culture. β-Galactosidase activities were measured 8 h after shifting the culture from MM containing thiamine to MM lacking thiamine. Under these conditions, the different fusion proteins were produced in similar amounts, at least as determined by Western blot using GAL4 antibody (data not shown), and were not toxic for the cells (see Figure 2).

The basal level of resistance for the A1 yeast is 100 mg of G418/l, which is equivalent to the resistance level observed for strain P2 that does not contain a *neo* reporter cassette (data not shown). The transformation of the $GAL4_{DBD}$ expression plasmid and the empty vector pDW230 as controls does not increase the resistance level of the A1 strain, indicating that no transactivation was observed (Figure 3A). In parallel, this was confirmed with the other test system by measurement of the β-galactosidase activity in the A2 strain (Figure 3B).

The acidic-type $GAL4_{DBD}/VP16_{TAD}$ activators yield high G418 resistance levels in strain A1 and high β-galactosidase levels in strain A2 (Figure 3A and B, respectively). These activations are comparable with the results obtained for the same fusion proteins expressed in *S.cerevisiae* and in mammalian cells (Seipel *et al.*, 1992; Künzler *et al.*, 1994). In addition, and like in mammalian cells (Seipel *et al.*, 1992), we observed that the truncated version of VP16 TAD (amino acids 454–490) activates only half as potently as compared with the intact VP16 TAD (amino acids 413–490).

The proline-rich activation domains of AP-2 and CTF also strongly stimulate transcription in *S.pombe* from close to the TATA box. In contrast to *S.cerevisiae*, the level of stimulation for the proline-rich and acidic-rich activation domains in *S.pombe* is equivalent, similar to what was found previously in mammalian cells. We also observed that the truncated version of AP-2 TAD (amino acids 31–76) is half as active as the intact AP-2 TAD (amino acids 31–117). This shorter AP-2 TAD was also less active in *S.cerevisiae*.

Finally, the glutamine-rich activation domains of Sp1, Oct1 and Oct2 increase both the G-418 resistance levels of the A1 strain and the β-galactosidase activity of the A2 strain, indicating that these domains are functional in *S.pombe*. Therefore, unlike *S.cerevisiae*, the glutamine-rich activation domains stimulate transcription in *S.pombe*.

We conclude from these results that *S.pombe* resembles, more so than *S.cerevisiae*, a mammalian cell with regard to the functionality of the different activation domains, at least when the latter operate close to the TATA-box.

The three types of activation domains stimulate S.pombe transcription 87 bp upstream of the TATA box

A 71 bp fragment of the luciferase gene was inserted between the $GAL4_{UAS}$ and the *ADH* minimal promoter, resulting in a final spacing of 87 bp. This chimeric promoter was cloned upstream of the *lacZ* gene and allowed the different TA domains to be tested in a more upstream context. As for the A2 strain, this reporter gene was also integrated at the *ADE6* locus in the *S.pombe* genome, and this yeast reporter has been named strain B. β-Galactosidase activity was determined as for strain A2. The selected luciferase fragment does not contain any known binding site for yeast transcription factors, and the reporter strain B displays the same low β-galactosidase activity as the A2 strain (data not shown).

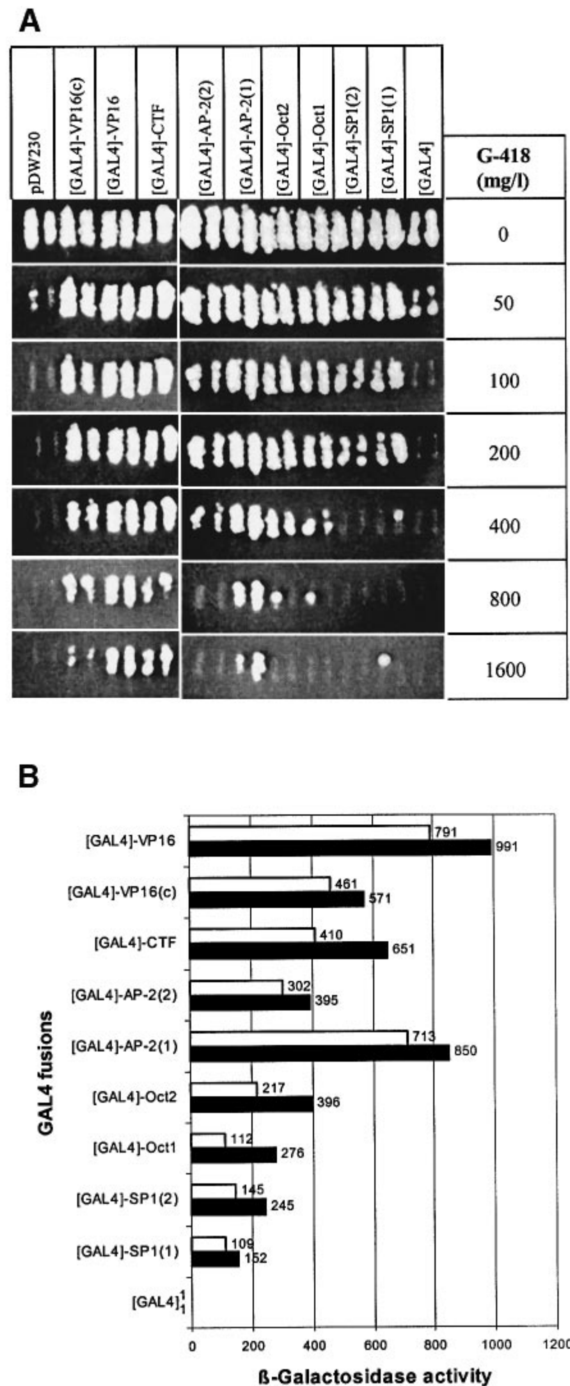


Fig. 3. Activation of transcription by the different $GAL4_{DBD}$–TADs close to the TATA box and 87 bp upstream from the TATA box. Transcriptional activation of the reporter genes by the $GAL4_{DBD}$–TADs placed close to the TATA box was estimated by the relative G418 resistance levels (**A**) in strain A1. Transcriptional activation by the $GAL4_{DBD}$–TADs in a proximal context (solid bar) and 87 bp upstream of the TATA box (open bar) was determined by the relative β-galactosidase activities (**B**) in strains A2 and B, respectively. The values listed in (B) represent the β-galactosidase activities obtained with the different $GAL4_{DBD}$–TADs after subtraction of the β-galactosidase background activity of strain A2 and B, respectively.

The levels of transactivation observed for the acidic-rich TA domains in strain B are between 15 and 20%

lower than the levels obtained in strain A2 (Figure 3B), indicating that a 87 bp upstream shift between the $GAL4_{UAS}$ and the TATA box had a limited impact on the level of transactivation by these domains in *S.pombe*. Similar observations were made for the proline-rich activation domains, with the exception of the CTF domain for which the level of activation drops by 40% in reporter B. Stimulation of transcription by the glutamine-rich domains seems to be more efficient in close proximity to the TATA box, because the insertion of an extra 87 bp between the TATA box and the $GAL4_{UAS}$ leads to a decrease in transcription activity of up to 50%. Therefore, when tested 87 bp usptream of the TATA box, all three types of activation domains still stimulate transcription in *S.pombe*. However, glutamine-rich TA domains seem to be more affected by the introduced 87 bp spacing than do the proline-rich and the acidic-rich TA domains.

Only acidic-rich and proline-rich TA domains stimulate S.pombe transcription in a remote position 235 bp upstream of the TATA box

To investigate the transactivation by these different TA domains in an enhancer-like position even more remote from the TATA box, we inserted 235 bp of spacer DNA derived from the luciferase open reading frame between the five copies of $GAL4_{UAS}$ and the ADH_{min} promoter. This $5GAL4_{UAS}$–ADH_{min} chimeric promoter was cloned upstream of the *neo* gene and, as in strain A1, this reporter was integrated at the *ARG3* locus in the *S.pombe* genome, generating reporter strain C. Transactivation is documented by G418 resistance levels and Neo antigen levels (Figure 4A and B, respectively).

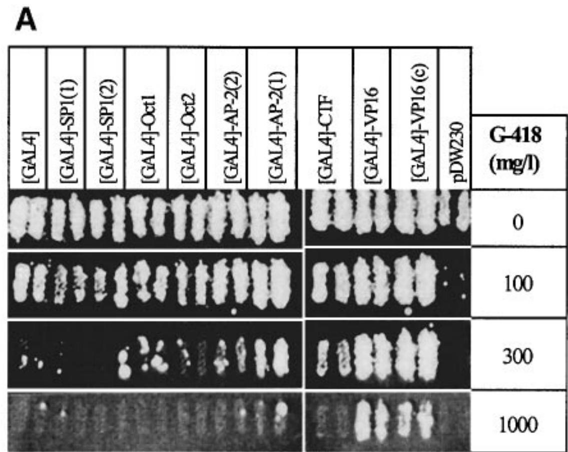
We observed a strong stimulation by the acidic TADs acting from the remote promoter position. As in the proximal context, we observed that the truncated TAD of VP16 is only half as potent as its intact counterpart. The proline-rich TADs can still stimulate transcription in *S.pombe* from a remote position. However, in contrast to the results which were observed at the proximal TATA box position, these domains are 12- to 25-fold less potent than the VP16 activation domain for activation of transcription from a '235 bp' remote position. This differs from the results obtained in *S.cerevisiae* where the same proline-rich domains were inactive when tested in a similar '226 bp' remote position (Künzler *et al.*, 1994). Glutamine-rich activation domains fail to transactivate from a '235 bp' remote position in *S.pombe*, except for the Oct2 domain, for which we observed a weak activation equivalent to the level obtained with the proline-rich activation domains. Therefore, as in mammalian cells, the glutamine-rich activation domains display very low transcriptional activity when tested in a context distal from the TATA box in *S.pombe*.

In summary, all types of mammalian transactivation domain function in the fission yeast *S.pombe* similarly to how they do in mammalian cells but differently from how they do in the budding yeast *S.cerevisiae*.

Discussion

Striking similarity between mammalian cells and S.pombe with respect to stimulation of transcription by three classes of mammalian TAD

In this study, we tested the three different types of TADs for their capacity to stimulate transcription in *S.pombe* from proximal and remote positions upstream of the TATA box. We have shown that acidic activation domains strongly stimulate transcription from both proximal or remote positions. These domains thus function similarly in *S.pombe*, in mammalian cells and in *S.cerevisiae* (Seipel *et al.*, 1992; Künzler *et al.*, 1994), indicating a conserved mechanism between mammalian cells and both yeast species. The proline-rich activation domains display strong activities when tested in a close proximal position to the TATA box in *S.pombe*. The levels of induction observed for these domains are comparable with the levels obtained with the acidic-rich domains, at least in the same promoter context. However, in contrast to the acidic-rich domains, the proline-rich domains stimulate transcription in a position remote (235 bp upstream) from the TATA box only weakly. Although these domains were shown to have considerable promoter activity and weak enhancer activity in mammalian cells (Seipel *et al.*, 1992), they stimulate



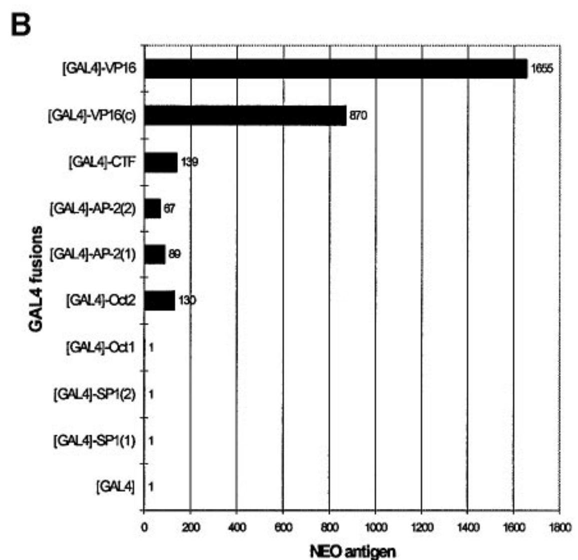


Fig. 4. Transcriptional activation by the different $GAL4_{DBD}$–TADs remote ('235 bp' away) from the TATA box. Transcription activation by the $GAL4_{DBD}$–TADs in strain C is documented in terms of G418 resistance levels (**A**) and Neo antigen levels (**B**). The values listed in (B) represent the Neo antigen levels obtained with the different $GAL4_{DBD}$–TADs after subtraction of the Neo antigen background level of strain C.

transcription only weakly from a position close to the TATA box in *S.cerevisiae* (Künzler *et al.*, 1994). Finally, the glutamine-rich activation domains were shown to have strong promoter activities and no enhancer activity in mammalian cells (Seipel *et al.*, 1992). In *S.pombe*, we also showed that these domains stimulate transcription only from a TATA box-proximal context, with the exception of the Oct2 TAD which is able to activate transcription weakly from a remote position. These glutamine-rich activation domains do not function in *S.cerevisiae* in any promoter context (Künzler *et al.*, 1994), suggesting a divergence between the budding and the fission yeast for the utilization of glutamine-rich activation domains. Therefore, with respect to the use of the different types of activation domains, we conclude that *S.pombe* resembles mammalian cells more than *S.cerevisiae* does.

Possible targets for the glutamine-rich activation domains in S.pombe

All activation domains probably induce transcription by accelerating, during the assembly of the pre-initiation complex, the recruitment of GTFs such as TBP, TBP-associated factors (TAFs) and TFIIB (Zawel and Reinberg, 1995; Pugh, 1996), or by contacting the already partially pre-assembled RNA polymerase II holoenzyme (Koleske and Young, 1994). For example, it was shown previously that the glutamine-rich activation domains of Sp1 and *Antennapedia* (*Antp*) bind directly and specifically to the C-terminal domain of TBP, which is evolutionarily conserved from *Drosophila* to man (Emili *et al.*, 1994). In addition, the ability of Sp1 activation domains to interact directly with the TBPs of various species correlates well with their ability to activate transcription in extracts derived from these species. In contrast, these activation domains interact weakly with the *S.cerevisiae* TBP (Emili *et al.*, 1994). This weak interaction between TBP and the glutamine-rich domains may explain why these domains failed to stimulate transcription in *S.cerevisiae*. However, glutamine-rich activation domains still failed to activate transcription in *S.cerevisiae* strains in which the yeast TBP was substituted by a hybrid human–yeast TBP or by the human TBP R231K variant. In contrast to wild-type human TBP, this TBP variant was shown to support cell viability of a yeast strain carrying a deletion of the TBP-encoding gene. These observations indicate that the divergence between human and *S.cerevisiae* TBP is not the underlying cause of the inability of glutamine-rich domains to activate in *S.cerevisiae* (Ponticelli *et al.*, 1995). Here, we showed that mammalian glutamine-rich domains can stimulate transcription in *S.pombe*. However, the C-terminal domain of TBP that mediates the interaction with Sp1 is highly conserved between *S.cerevisiae* and *S.pombe* (Hoffmann *et al.*, 1990), suggesting that the divergence observed between *S.pombe* and *S.cerevisiae* for the transactivation by glutamine-rich domains does not result from differences in TBP recruitment. It is more likely that activation by Sp1 requires additional factors which are present in *S.pombe* but absent in *S.cerevisiae*, or whose function is well conserved between *S.pombe* and humans only.

It is well documented that purified human TBP cannot support Sp1-mediated transcription in a reconstituted *in vitro* assay, whereas crude TFIID preparations support this activation (Pugh and Tjian, 1990). Several TAFs have been isolated, and the glutamine-rich Sp1 activation domain was shown to interact specifically with one of them (TAF110; Hoey *et al.*, 1993). Mutations that interfere with this interaction also decreased the transactivation by Sp1. Moreover, the combination of TAF250, TAF150, TAF110 and TBP can support robust Sp1 activation (Chen *et al.*, 1994). Interestingly, a *S.cerevisiae* homologue of this TAF110 has not been identified, suggesting that the absence of this TAF may be the reason why Sp1 activation domains are not active in this budding yeast (Tansey and Herr, 1997). The fact that the Sp1 activation domains are active in *S.pombe* suggests that the fission yeast may contain a functional homologue of mammalian TAF110.

In addition, a mutation in TFIIB was shown previously to inhibit *in vivo* the activation mediated by the strong glutamine-rich activation domain of the *fushi tarazu (ftz)* transcription factor in *Drosophila* (Colgan *et al.*, 1995). A direct and specific interaction between this activation domain and TFIIB was demonstrated *in vitro*. The domains of TFIIB and of *ftz* that are required for the interaction *in vitro* are also necessary for *in vivo* function. These results support the idea that a direct interaction between glutamine-rich activation domains and TFIIB may be important for transcriptional activation *in vivo*. It is well documented that the mechanism controlling the selection of the transcriptional initiation site(s) is more similar between *S.pombe* and mammalian cells than between budding yeast and mammalian cells (Li *et al.*, 1994). Together with RNA polymerase II, TFIIB also plays an important role in this mechanism, suggesting a conserved function of TFIIB between *S.pombe* and mammalian cells. Therefore, as in mammalian cells, glutamine-rich domains may stimulate transcription in *S.pombe* by interacting with TFIIB (which remains to be cloned), and by accelerating its recruitment to the assembling pre-initiation complex.

Genetic approaches in S.pombe for studying activation by proline-rich and glutamine-rich activation domains

The results from our systematic study also provide new possibilities for investigating fundamental aspects of trans-activation in general, in particular with regards to the proline-rich and glutamine-rich TADs. It is known that transcriptional activation by these domains in higher eukaroytes is mediated through several interactions with several GTFs and/or TAFs. In addition, it cannot be excluded that as yet unknown factors may also be involved in their activation. These activation domains also stimulate strongly transcription in *S.pombe*, and it is likely that the GTFs involved in this are the functional homologues of the human factors. Therefore, *S.pombe* opens up the possibility of using genetic approaches to investigate the interactions between these two classes of activation domain and the general factors in their natural chromosomal context under conditions where all proteins are present at physiological concentrations. Furthermore, we showed that these domains are toxic when overexpressed in *S.pombe*, possibly as a result of the squelching of general factors important for *S.pombe* gene transcription. Isolation of mutations that suppress this toxicity, an approach which is not possible in mammalian cells, may enable the identification of the target of these domains, which in

some cases may represent new functions. Similar screening in *S.cerevisiae* for suppressors of the toxic effect mediated by the overexpression of $GAL4_{DBD}$/$VP16_{TAD}$ has allowed the isolation of transcriptional adaptors for acidic-rich activation domains (Berger *et al.*, 1992). We therefore would like to emphasize that the fission yeast may represent an attractive organism to investigate in the future the molecular mechanisms leading to the control of the transcriptional activation by proline-rich and glutamine-rich activation domains.

Materials and methods

Plasmids, yeast strains and media

For analysis in *S.pombe*, various *GAL4* fusion constructs (see Table I; Künzler *et al.,* 1994) were cloned downstream of the *PHO4 S.pombe* promoter into vector pTG5702 (from E.Jacobs, Transgène, Strasbourg). The *URA4*-containing plasmid pDW230 was used as a control for yeast transformation (Weilguny *et al.*, 1991).

The 17mer binding site for the GAL4p protein ($GAL4_{UAS}$) (Giniger and Ptashne, 1988) was inserted upstream of an *Nco*I–*Eco*RI fragment carrying the minimal promoter including the TATA box of the *S.pombe ADH1* gene (ADH_{min} promoter; Russell and Hall, 1983). This chimeric $GAL4_{UAS}$–ADH_{min} promoter was inserted upstream of the *Escherichia coli lacZ* gene into the integrative vector pPIADE6βGal to generate the reporter plasmid pPIADE6GAL4/ADHβGal. In this vector, the *lacZ* gene is followed by the *LEU2* gene of *S.cerevisiae*, which can complement the *leu1 S.pombe* mutation. The *lacZ* reporter/*LEU2* gene sequences are flanked by 5′ and 3′ fragments of the *ADE6* gene of *S.pombe*. Upon digestion of this reporter plasmid with *Xho*I, stable integration of the *lacZ* reporter is obtained by replacement of the chromosomal *ADE6* gene. The yeasts carrying the resulting *ADE6* disruption lack the *ADE6*-encoded enzyme and therefore accumulate a red pigment, hence they are easy to recognize. The A2 *lacZ*-reporter strain was obtained by transforming *S.pombe* strain P3 (*h^-, ura4Δ18, leu1)* with the pPIADE6G-AL4/ADHβGal vector linearized by *Xho*I.

In addition, the $GAL4_{UAS}$–ADH_{min} chimeric promoter was inserted upstream of the *neo* gene in pTG4726 (E.Jacobs, Transgène, Strasbourg) to generate pPIARG3GAL4/ADHNEO. This vector contains the *ARG3* gene for stable integration at the *ARG3* locus. The A1 *neo* reporter strain was obtained after transforming the P2 *S.pombe* strain (*h^{90}, ura4Δ18, arg3*) with linearized pPIARG3GAL4/ADHNEO vector.

The reporter gene with five copies of the $GAL4_{UAS}$ in a remote position from the TATA box contained, upstream of the *ADH* minimal promoter, three copies of the 71 bp *Sau*3A fragment (+598, +669, with +1 for the ATG) obtained from the firefly luciferase gene (De Wet *et al.*, 1987). The insertion of these fragments resulted in an overall spacing of 235 bp between the (proximal) copy of the $GAL4_{UAS}$ and the TATA box. This chimeric promoter was inserted upstream of the *neo* gene in pTG4726. The *neo* reporter strain C was generated by the integration of this *neo* reporter upon transformation of the P2 yeast, as described for the A1 yeast reporter strain.

Finally, a single copy of the same *Sau*3A luciferase fragment was also inserted between the $GAL4_{UAS}$ and the *ADH* minimal promoter in vector pPIADE6GAL4/ADHβGal to generate the *lacZ*-reporter strain B. In this strain, the TATA box and the GAL4-binding site are 87 bp apart.

Complex medium (YPD) contains 10 g of yeast extract (Difco), 20 g of glucose and 20 g of bactopeptone (Difco) per liter, and was supplemented with different concentrations of G418 (see Figures 3A and 5A). Minimal medium (MM) was prepared as described previously (Alfa *et al.*, 1993). MMR corresponds to MM supplemented with 75 mg of arginine/l. MMRT corresponds to MMR supplemented with 50 μg thiamine/l. For the selection of the yeast integrants, adenine and/or uracil were added to the MM medium at 75 mg/l. All solid media contain 20 g of agar (Gibco) per litre.

Schizosacharomyces pombe was transformed using the alkali cation method (Okazaki *et al.*, 1990).

Western blotting

$GAL4_{DBD}$ antibody used for detection of $GAL4_{DBD}$–TAD in total yeast extract was purchased from Clontech. Total yeast protein extract, polyacrylamide gel electrophoresis, blotting and immunodetection were performed according to the $GAL4_{DBD}$ antibody manufacturer's protocol. Protein extracts were prepared from yeast harvested 8 h after shifting the culture from MMRT to MMR.

Neomycin phosphotransferase (NPTII) ELISA

To quantify NPTII protein in crude extracts of yeast cells, we used an NPTII enzyme-linked immunosorbent assay (ELISA) kit (5′→3′ Inc.). The yeast cells were grown in MMRT medium then shifted to MMR medium and cultured for an additional 8 h until the final density was 1×10^7 cells/ml. Approximatively 5×10^8 cells/ml were harvested by slow spin centrifugation, resuspended in 1.5 ml of TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA) containing 1 mM phenylmethylsulfonyl fluoride (PMSF), and transferred in 15 ml round bottom plastic tubes containing 2.5 g of glass beads (0.3–0.5 mm diameter; Sigma). The yeast cell wall was broken by vigorous vortexing for 3 min. The suspension was centrifuged and the protein concentration of the supernatant was determined (Bio-Rad). Samples of it were diluted to 400 μg protein/ml and tested in the ELISA. NPTII levels were expressed as picograms of NPTII protein per milligram of total protein. Each experiment was performed at least twice with independent transformants, and all assays were done in duplicate. Data from experiments done in parallel always varied $<15\%$.

β-Galactosidase assay

The yeast crude extract was prepared as for the NPTII ELISA. β-Galactosidase activity was measured using a Galacto-light kit (Tropix, Bedford, USA). The samples were measured using a TopCount Scintillation counter (Canberra, Packard) and normalized to their protein concentration. The results were expressed in relative light units (c.p.m.) per microgram of total protein. Each experiment was performed twice with three independent yeast transformants. Data from experiments done in parallel always varied $<15\%$.

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References

Alfa,C., Fantes,P., Hyams,J., McLeod,M. and Warbrick,E. (1993) *Experiments with Fission Yeast*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Berger,S.L., Pina,B., Silverman,N., Marcus,G.A., Agapite,J., Regier,J.L., Triezenberg,S.J. and Guarente,L. (1992) Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. *Cell*, **70**, 251–265.

Chen,J.L., Attardi,L.D., Verrijzer,C.P., Yokomori,K. and Tjian,R. (1994) Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell*, **79**, 93–105.

Colgan,J., Ashali,H. and Manley,J.L. (1995) A direct interaction between a glutamine-rich activator and the N-terminus of TFIIB can mediate transcriptional activation *in vivo*. *Mol. Cell. Biol.*, **15**, 2311–2320.

Cress,W.D. and Triezenberg,S.J. (1991) Critical structural elements of the VP16 transcriptional activation domain. *Science*, **251**, 87–90.

DeWet,J.R., Wood,K.V., DeLuca,M., Helinski,D.R. and Subramani,S. (1987) Firefly luciferase gene: structure and expression in mammalian cells. *Mol. Cell. Biol.*, **7**, 725–737.

Drysdale,C.M., Duenas,E., Jackson,B.M., Reusser,U., Braus,G.H. and Hinnebush,A.G. (1995) The transcriptional activator GCN4 contains multiple activation domains that are critically dependent on hydrophobic amino acids. *Mol. Cell. Biol.*, **15**, 1220–1233.

Emili,A., Greenblatt,J. and Ingles,C.J. (1994) Species-specific interaction of the glutamine-rich activation domains of Sp1 with TATA box-binding protein. *Mol. Cell. Biol.*, **14**, 1582–1593.

Gill,G. and Ptashne,M. (1988) Negative effect of the transcriptional activator GAL4. *Nature*, **334**, 721–724.

Giniger,E. and Ptashne,M. (1988) Cooperative DNA binding of the yeast transcriptional activator GAL4. *Proc. Natl Acad. Sci. USA*, **85**, 382–386.

Glick,B.S. (1996) Alternatives to baker's yeast. *Curr. Biol.*, **12**, 1570–1572.

Hoey,T., Weinzierl,R.O.J., Gill,G., Chen,J.L., Dynlacht,B.D. and Tjian,R. (1993) Molecular cloning and functional analysis of *Drosophila* TAF110 reveal properties expected of coactivators. *Cell*, **72**, 247–260.

Hoffmann,A., Horikoshi,M., Wang,C.K., Schroeder,S., Weil,P.A. and Roeder,R.G. (1990) Cloning of the *Schizosaccharomyces pombe* TFIID gene reveals a strong conservation of functional domains present in *Saccharomyces cerevisiae*. *Genes Dev.*, **4**, 1141–1148.

Hope,I.A. and Struhl,K. (1986) Functional dissection of a eukaryotic transcriptional activator protein, GCN4 of yeast. *Cell*, **48**, 885–894.

Keegan,L., Gill,G. and Ptashne,M. (1986) Separation of DNA binding from the transcription activation function of a eukaryotic regulatory protein. *Science*, **231**, 699–704.

Koleske,A.J. and Young,R.A. (1994) An RNA polymerase II holoenzyme responsive to activators. *Nature*, **368**, 466–469.

Künzler,M., Braus,G.H., Georgiev,O., Seipel,K. and Schaffner,W. (1994) Functional differences between mammalian transcription activation domains at the yeast *GAL1* promoter. *EMBO J.*, **13**, 641–645.

Künzler,M., Springer,C. and Braus,G.H. (1996) The transcriptional apparatus required for mRNA encoding genes in the yeast *Saccharomyces cerevisiae* emerges from a jigsaw puzzle of transcription factors. *FEMS Microbiol. Rev.*, **19**, 117–136.

Lech,K., Anderson,K. and Brent,R. (1988) DNA-bound Fos proteins activate transcription in yeast. *Cell*, **52**, 179–184.

Li,Y., Flanagan,P.M., Tschochner,H. and Kornberg,R.D. (1994) RNA polymerase II initiation factor interactions and transcription start site selection. *Science*, **263**, 805–807.

Ma,J., Przibilla,E., Hu,J., Bogorad,L. and Ptashne,M. (1988) Yeast activators stimulate plant gene expression. *Nature*, **334**, 631–633.

Metzger,D., White,J.H. and Chambon,P. (1988) The human oestrogen receptor functions in yeast. *Nature*, **334**, 31–36.

Okazaki,K., Okazaki,N., Kume,K., Jinno,S., Tanaka,K. and Okayama,H. (1990) High-frequency transformation method and library transducing vectors for cloning mammalian cDNAs by trans-complementation of *Schizosaccharomyces pombe*. *Nucleic Acids Res.*, **18**, 6485–6489.

Ponticelli,A.S., Pardee,T.S. and Struhl,K. (1995) The glutamine-rich activation domains of human Sp1 do not stimulate transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **15**, 983–988.

Pugh,B.F. (1996) Mechanisms of transcription complex assembly. *Curr. Opin. Cell Biol.*, **8**, 303–311.

Pugh,B.F. and Tjian,R. (1990) Mechanism of transcriptional activation by Sp1: evidence for coactivators. *Cell*, **61**, 1187–1197.

Regier,J.L., Shen,F. and Triezenberg,S.J. (1993) Pattern of aromatic and hydrophobic amino acids critical for one of two subdomains of the VP16 transcriptional activator. *Proc. Natl Acad. Sci. USA*, **90**, 883–887.

Russell,P.R. and Hall,B.D. (1983) The primary structure of the alcohol dehydrogenase gene from the fission yeast *Schizosaccharomyces pombe*. *J. Biol. Chem.*, **258**, 143–149.

Sadowski,I., Ma,J., Triezenberg,S. and Ptashne,M. (1988) GAL4-VP16 is an unusually potent transcriptional activator. *Nature*, **335**, 563–564.

Schena,M. and Yamamoto,K.R. (1988) Mammalian glucocorticoid receptor derivatives enhance transcription in yeast. *Science*, **241**, 965–967.

Seipel,K., Georgiev,O. and Schaffner,W. (1992) Different activation domains stimulate transcription from remote ('enhancer') and proximal ('promoter') positions. *EMBO J.*, **11**, 4961–4968.

Silvestre,N. and Jacobs,E. (1997) Characterization of upstream activating sequences involved in activation and regulation of PHO4 expression in *Schizosaccharomyces pombe*. *Mol. Gen. Genet.*, **253**, 428–438.

Struhl,K. (1988) The JUN oncoprotein, a vertebrate transcription factor, activates transcription in yeast. *Nature*, **332**, 649–650.

Sugimoto,A., Iino,Y., Maeda,T., Watanabe,Y. and Yamamoto,M. (1991) *Schizosaccharomyces pombe ste11*$^{+}$ encodes a transcription factor with an HMG motif that is critical regulator of sexual development. *Genes Dev.*, **5**, 1990–1999.

Tang,C.S., Bueno,A. and Russel,P. (1994) *ntf1*$^{+}$ encodes a 6-cysteine zinc finger-containing transcription factor that regulates the *nmt1* promoter in fission yeast. *J. Biol. Chem.*, **269**, 11921–11926.

Tansey,W.P. and Herr,W. (1997) TAFs: guilt by association. *Cell*, **88**, 729–732.

Toyama,R. and Okayama,H. (1990) Human chorionic gonadotropin alpha and human cytomegalovirus promoters are extremely active in the fission yeast *Schizosaccharomyces pombe*. *FEBS Lett.*, **268**, 217–221.

Triezenberg,S.J. (1995) Structure and function of transcriptional activation domains. *Curr. Opin. Genet. Dev.*, **5**, 190–196.

Webster,N., Jin,J.R., Green,S., Hollis,M. and Chambon,P. (1988) The yeast UASG is a transcriptional enhancer in human HeLa cells in the presence of the GAL4 trans-activator. *Cell*, **52**, 169–178.

Weilguny,D., Praetorius,M., Carr,A., Egel,R. and Nielsen,O. (1991) New vectors in fission yeast: application for cloning the *HIS2* gene. *Gene*, **99**, 47–54.

Wilkinson,M.G., Samuels,M., Takeda,T., Toone,W.M., Shieh,J.C., Toda,T., Millar,J.B. and Jones,N. (1996) The Atf1 transcription factor is a target for the Sty1 stress-activated MAP kinase pathway in fission yeast. *Genes Dev.*, **10**, 2289–2301.

Wu,S.Y. and McLeod,M. (1995) The *sak1*$^{+}$ gene of *Schizosaccharomyces pombe* encodes an RFX family DNA-binding protein that positively regulates cyclic AMP-dependent protein kinase-mediated exit from mitotic cell cycle. *Mol. Cell. Biol.*, **15**, 1479–1488.

Zawel,L. and Reinberg,D. (1995) Common themes in assembly and function of eukaryotic transcription complexes. *Annu. Rev. Biochem.*, **64**, 533–561.

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