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# Functional differences between mammalian transcription activation domains at the yeast *GAL1* promoter

**Markus Künzler[1], Gerhard H.Braus[1,2,3], Oleg Georgiev[4], Katja Seipel[4] and Walter Schaffner[4]**

[1]Mikrobiologisches Institut, Eidgenössische Technische Hochschule (ETH), ETH-Zentrum, CH-8092 Zürich, Switzerland, [2]Institut für Mikrobiologie and Biochemie, Friedrich-Alexander-Universität, Staudtstrasse 5, D-91058 Erlangen, Germany and [4]Institut für Molekularbiologie II der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland
[3]Corresponding author

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**We have fused representatives of three structurally and functionally distinct classes of mammalian transcription activation domains for RNA polymerase II to the yeast GAL4 DNA binding domain. All fusion proteins were stable when expressed in yeast and were tested for their ability to activate transcription from various positions in the yeast *GAL1* promoter. Activation domains functional from remote as well as TATA-proximal positions in mammalian cells, e.g. the acidic-type domain of VP16, also stimulate transcription in yeast from various promoter positions. Proline-rich domains, as e.g. in AP-2 and CTF/NF1, with considerable promoter activity and low enhancer activity in mammalian cells stimulate transcription in yeast only from a position close to the TATA box. The glutamine-rich domains of Oct1, Oct2 and Sp1, which activate transcription in mammalian cells from close to the TATA box in response to a remote enhancer, are inactive in the yeast *GAL1* promoter. This finding might reflect some basic difference between the organization of yeast and mammalian promoters.**

*Key words:* GAL4-fusion proteins/mammalian transcription activation domains/promoter organization/transactivation/yeast *GAL1* promoter

## Introduction

Many fundamental mechanisms involved in initiation of mRNA synthesis are conserved from yeast to mammals (for reviews see Ptashne, 1988; Mitchell and Tjian, 1989; Guarente and Bermingham-McDonough, 1992). Transcriptional activation by proteins binding to specific *cis*-regulatory sequences is a prominent example. Most sequence specific DNA binding transcriptional activators are composed of separable DNA binding and activation domains (Ptashne, 1988). Analysis of these two types of domains in mammalian cells revealed a limited number of structural motifs (Mitchell and Tjian, 1989). Activation domains can be divided into three classes with respect to their amino acid composition. They are either rich in acidic amino acid residues, in glutamine residues or in proline residues. The three classes of activation domains differ in their ability to activate transcription from different promoter positions in mammalian cells (Seipel *et al.*, 1992). Acidic-type activation domains, as represented by the yeast GAL4 and the mammalian herpesvirus VP16 activation domain, are able to stimulate transcription from remote enhancer as well as from proximal promoter positions. In contrast, the glutamine-rich activation domains of Oct1, Oct2 and Sp1 mediate transcriptional activation only from a position close to the TATA box, usually in response to a remote enhancer. The proline-rich activation domains of AP-2 and CTF/NF1 reveal considerable promoter activity and low enhancer activity.

Several mammalian transcriptional activators containing proline-rich and/or acidic-type activation domains, or at least the activation domains thereof, such as the estrogen receptor (Metzger *et al.*, 1988), the glucocorticoid receptor (Schena and Yamamoto, 1988), the herpesvirus VP16 activation domain (Sadowski *et al.*, 1988), c-Jun (Struhl, 1988) and c-Fos (Lech *et al.*, 1988), are able to stimulate transcription in yeast. So far, there is no example of a mammalian transcription factor containing a glutamine-rich activation domain such as Sp1 (Courey and Tjian, 1988) or the octamer factors (Müller-Immerglück *et al.*, 1990), where single or very short (two to four residues) stretches of glutamine residues are interspersed with hydrophobic and other amino acid residues, stimulating transcription in yeast. There are a few yeast transcription factors, such as HAP1 (Pfeifer *et al.*, 1989), HAP2 (Pinkham *et al.*, 1987), MCM1 (Passmore *et al.*, 1988), OPI1 (White *et al.*, 1991), PHO2 (Sengstag and Hinnen, 1987) and GAL11 (Suzuki *et al.*, 1988) which contain homopolyglutamine stretches (e.g. 23 residues in GAL11), however, no activation function has been so far demonstrated for these. It remains to be seen whether they are related to homopolyglutamine stretches present in some mammalian transcription factors which can, at least in certain configurations, modulate transcriptional activity (H.P.Gerber and W.Schaffner, unpublished data).

Here we show that the three classes of activation domains identified in mammalian cells also differ in their transcription activation behavior in the yeast *Saccharomyces cerevisiae*. For analysis in yeast we used the same fusion proteins as used in mammalian cells (Seipel *et al.*, 1992), based on the GAL4 DNA binding domain (amino acid residues 1–93) fused to the activation domains from well-characterized mammalian transcription factors. We tested representatives of all three classes of mammalian transcription activation domains for transactivation from different positions in the yeast *GAL1* promoter. While acidic-type activation domains stimulated transcription from all reporter constructs, and proline-rich domains were capable of slight activation from a TATA-proximal position, glutamine-rich domains did not show any transactivation under these conditions. The inactivity of glutamine-rich activation domains in yeast might reflect some basic difference in the organization of *cis*-regulatory sequences between yeast and mammals.

**Table I.** Summary of transactivation results

| GAL4 fusion construct | Prevalent amino acid | Net charge | Activation in yeast (reporter construct according to Figure 1) | | |
|---|---|---|---|---|---|
| | | | Remote (A) | TATA-proximal (B) | GCN4-responsive (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)–AP-2(31–117)$_1$ | P>S+T | +5 | – | ± | ± |
| GAL4(1–93)–AP-2(31–76)$_2$ | P>S+T | 0 | – | ± | ± |
| GAL4(1–93)–CTF(399–499) | P>S+T | +3 | – | ± | ± |
| GAL4(1–93)–VP16(n)(413–454) | D+E | −11 | + | + | + |
| GAL4(1–93)–VP16(c)(454–490) | D+E | −7 | + | + | + |
| GAL4(1–93)–VP16(413–490) | D+E | −18 | ++ | + | + |

Amino acid residues are given in brackets. The exact amino acid sequences of the fusion proteins are described elsewhere (Seipel *et al.*, 1992). ++ indicates very strong, + strong, ± weak, – no activation and – – repression of transcription by the corresponding fusion protein.

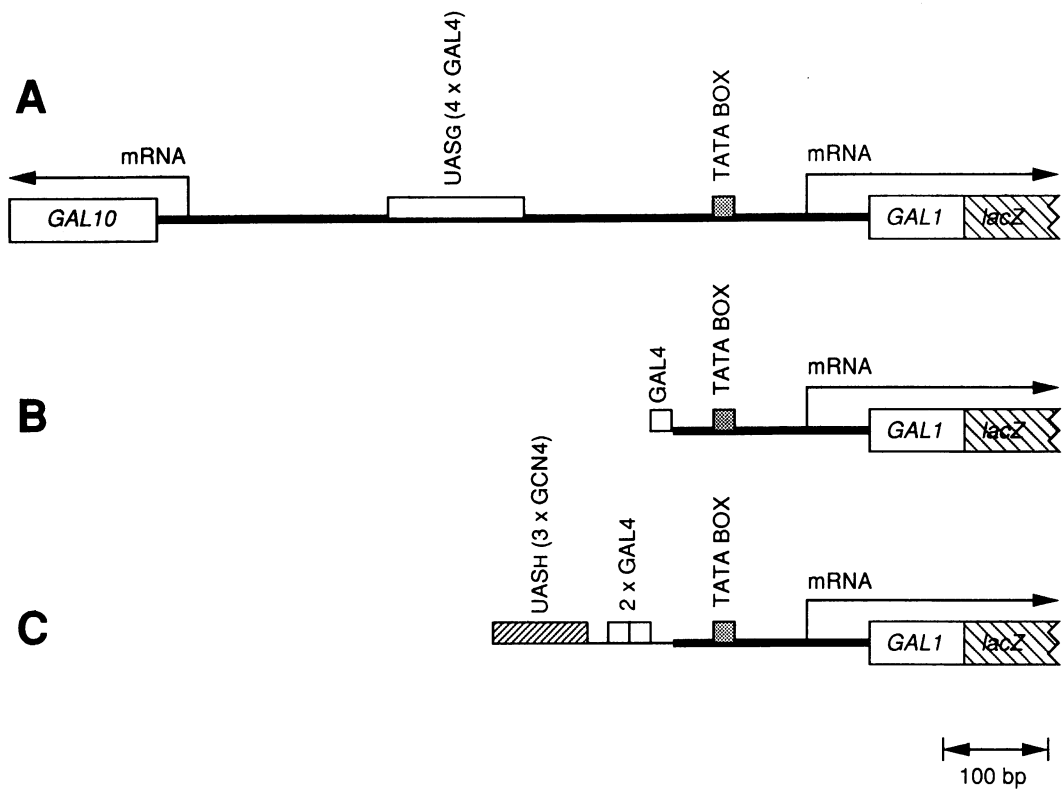


**Fig. 1.** The arrangement of GAL4 binding sites (GAL4) in the promoters of the *GAL1–lacZ* reporter genes. Plasmids pRY171 (**A**), pSV15 (**B**) and pGG9 (**C**), carrying the different reporter constructs have been described previously (Yocum *et al.*, 1984; Giniger *et al.*, 1985; Keegan *et al.*, 1986). In pRY171 (Yocum *et al.*, 1984), *lacZ* gene expression is directed by the whole chromosomal *GAL1* promoter region with its intact $UAS_G$ containing four GAL4 binding sites positioned 310 bp upstream of the *GAL1* transcription start site or 226 bp upstream of the *GAL1* TATA box, respectively. The pSV15 construct (Giniger *et al.*, 1985) bears a single synthetic 17 bp GAL4 binding site 128 bp upstream of the *GAL1* transcription start site or 44 bp upstream of the *GAL1* TATA box, respectively. In pGG9 (Keegan *et al.*, 1986) two synthetic 17 bp GAL4 binding sites are positioned between the *HIS4* UAS ($UAS_H$) containing three GCN4 binding sites and the *GAL1* start site of transcription. The GAL4 binding sites were inserted upstream of the same portion of the *GAL1* promoter as present in pSV15. All plasmids have been integrated at the *URA3* locus of *S.cerevisiae* strain GGY1 (Gill and Ptashne, 1987), resulting in strains GGY1::RY171, GGY1::SV15 and GGY1::GG9, respectively.

## Results and discussion

### *Expression of 10 different mammalian activation domains fused to the GAL4 DNA binding domain in yeast*

We have previously analyzed activation domains of different well-characterized mammalian transcription factors fused to the DNA binding domain of yeast GAL4 (amino acid residues 1–93) for transactivation from different *cis*-positions in mammalian cells (Seipel *et al.*, 1992). From this collection of chimeric activators we have selected representatives of all three classes identified in mammalian cells for a similar analysis in yeast (Table I). Different portions of the VP16 activation domain were characteristic acidic-type activation domains. Different portions of the AP-2 activation domain and the activation domain of

CTF/NF1 represented the proline-rich class of activation domains. Finally, different portions of the Sp1 activation domain and the activation domains of the octamer factors Oct1 and Oct2 exemplified the glutamine-rich activation domains. The GAL4 DNA binding domain without any activation domain fused to it was used as control. The fusion protein constructs were cloned into a yeast centromeric plasmid under the control of the yeast *ADH1* (alcohol dehydrogenase) promoter to allow expression in yeast. All of the GAL4 fusion proteins were able to bind specifically to GAL4 recognition sequences *in vitro* and were stably produced in similar amounts in yeast, as judged by gel retardation experiments (data not shown).

The GAL4 fusion proteins were tested for transactivation by introducing plasmids carrying the various constructs into three different yeast reporter strains. All three strains were derivatives of a strain lacking endogenous GAL4, and differed only in the arrangement of GAL4 binding sites upstream of an integrated *GAL1−lacZ* fusion gene (Figure 1). The different arrangements allowed us to test the various GAL4 fusion proteins for transactivation from a remote position (Figure 1A) and from a proximal position close to the TATA box (Figure 1B and C). Since mammalian octamer (Oct1 and Oct2) and Sp1 factors are able to respond to a remote enhancer, without considerable activity of their own (Schatt *et al.*, 1990; Seipel *et al.*, 1992), we tested a similar configuration in yeast, where a GCN4-responsive upstream activation sequence (UAS) (Struhl, 1989) is located immediately upstream of a GAL4 recognition element in a TATA-proximal position. This construct should reveal any ability of the chimeric activators to respond to the upstream bound yeast activator protein GCN4 (Figure 1C). Transactivation of all reporter genes was determined by assaying the specific β-galactosidase activity of the various transformants (Figure 2).

### *Mammalian acidic-type domains stimulate transcription from both remote and TATA-proximal positions in the yeast GAL1 promoter*

Acidic-type activators, exemplified by different portions of the VP16 activation domain, were capable of a 1000-fold transcription activation from remote upstream elements located 310 bp upstream of the *GAL1* transcription start site or 226 bp upstream of the *GAL1* TATA box, respectively. Activation from a TATA-proximal GAL4 recognition element located 128 bp upstream of the *GAL1* transcription start site or 44 bp upstream of the *GAL1* TATA box, respectively, resulted in an up to 250-fold activation of the yeast *GAL1* promoter (Figure 2A and B). Only a 6-fold activation by the acidic-type domains relative to the GAL4 DNA binding domain without an activation domain was observed with the *GAL1* reporter construct, where three additional binding sites for the yeast activator protein GCN4 are located upstream of two GAL4 binding sites in TATA-proximal position (Figure 2C). From a position close to the TATA box both N- (amino acids 413−454) and C-terminal (amino acid residues 454−490) segments of VP16 are maximally active, while from a remote position the complete VP16 domain (amino acid residues 413−490) is required for full activity. Overall, acidic-type domains conferred the strongest activation to all reporter constructs. From a remote position transactivation was only observed with this class of domains.

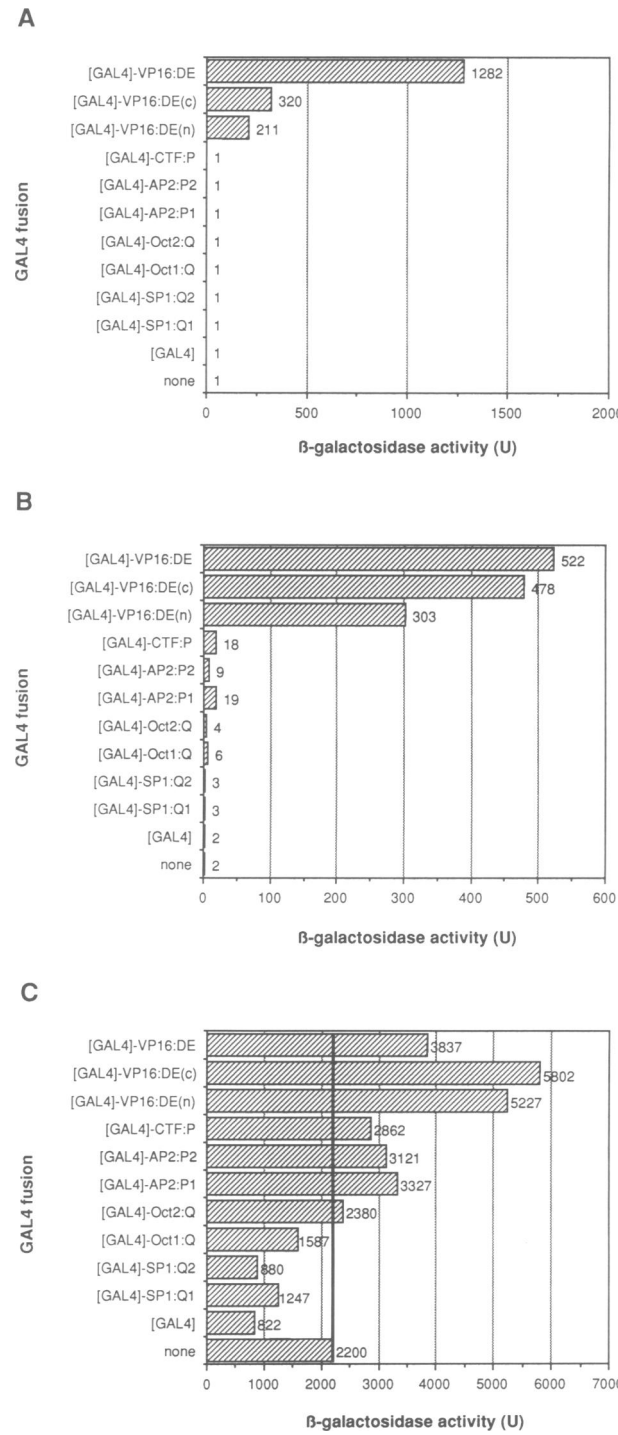


**Fig. 2.** Specific β-galactosidase activities of the reporter genes driven by the various GAL4 fusion proteins. Acidic-type and proline-rich, but not glutamine-rich domains stimulate transcription in yeast. Specific β-galactosidase activities of strains GGY1::RY171 (**A**), GGY1::SV15 (**B**) and GGY1::GG9 (**C**) expressing the various GAL4 fusion proteins are shown. The standard error did not exceed 30%. Values < 1 U could not be determined with accuracy and were assigned the value 1 U. The thick line in (C) indicates the GCN4-mediated β-galactosidase level in strain GGY1::GG9 in the absence of any GAL4 fusion protein.

These results are consistent with those obtained in mammalian cells (Seipel *et al.*, 1992). Thus acidic-type domains are quite universal and can function from promoter−distal as well as proximal positions in both

organisms. They may represent the prototypes of eukaryotic activation domains.

### *Proline-rich domains only activate transcription from a TATA-proximal position in the yeast GAL1 promoter*

The proline-rich domains of CTF/NF1 and AP-2 factors did not stimulate transcription from a remote position in the *GAL1* promoter (Figure 2A). However, from a TATA-proximal position they were capable of a 3- to 6-fold activation relative to the level conferred by the GAL4 DNA binding domain (Figure 2B). This level of activation is significant and comparable with the stimulation of yeast transcription factors such as GCN4 (Hinnebusch, 1988). An ~4-fold activation by the proline-rich activation domains relative to the GAL4 DNA binding domain without an activation domain was also observed from a position downstream of a GCN4-responsive UAS (Figure 2C). This activation resulted in a transcription level greater than the one mediated by GCN4 without additional activator protein. In contrast, the level of transcription in the presence of the GAL4 DNA binding domain without an activation domain was 3-fold lower than with GCN4 alone.

In summary, proline-rich activation domains reveal considerable promoter activity, but only low enhancer activity in mammalian cells (Seipel *et al.*, 1992), and seem to activate transcription from the yeast *GAL1* promoter only in a narrow window close to the TATA box. The role of proline-rich activation domains in transcription remains to be elucidated. It is possible that acidic-type and proline-rich domains do interact with different parts of the basal eukaryotic transcriptional machinery and activate transcription by distinct mechanisms.

### *Glutamine-rich domains do not stimulate transcription from the yeast GAL1 promoter*

Whereas activation of transcription was observed with the acidic-type and the proline-rich domains, we did not find any significant transcriptional activation from the various *GAL1* promoter derivatives with the glutamine-rich activation domains (Figure 2). The glutamine-rich activation domains of the mammalian transcription factors Oct1, Oct2 and Sp1 were inactive, both from remote and proximal positions in the *GAL1* promoter (Figure 2A and B). When a GCN4-responsive UAS was located upstream of the GAL4 binding site, the Oct2 domain did not activate transcription to a level that was significantly higher than with GCN4 alone, whereas the Oct1 and Sp1 domains even repressed transcription almost 3-fold. This reduction of GCN4-mediated transcription is comparable with the repression observed with the GAL4 DNA binding domain without any activation domain fused to it. It has been shown previously that GAL4 derivatives that are able to bind DNA but unable to activate transcription repress transcription from this construct (Keegan *et al.*, 1986).

The inability of yeast to efficiently use glutamine-rich activation domains in either of the three promoter contexts might reflect basic differences in the organization of *cis*-regulatory sequences in yeast and mammals. In general, yeast transcriptional elements seem to be fewer and more scattered (Struhl, 1988) than in mammals, where they typically occur in blocks of multiple transcription factor binding sites (Cochran and Weissmann, 1984; Müller *et al.*, 1988). In addition, although yeast UASs share some features in common with mammalian enhancers, they are usually located within a few hundred base pairs upstream and do not stimulate transcription from a position downstream of the TATA box (Struhl, 1988). In mammalian cells, glutamine-rich activation domains require an extra enhancer for strong activation, even from a position close to the TATA box, whereas acidic-type activation domains can be very active by themselves (Schatt *et al.*, 1990; Seipel *et al.*, 1992). In vertebrates a given gene can be independently controlled by several remote enhancers. Therefore the glutamine-rich activation domains might provide the efficient channelling of an enhancer effect mediated by acidic-type or possibly proline-rich activation domains to the responsive promoter. In yeast, the distances between the open reading frames of functional genes are often in the range of only several hundred base pairs (Oliver *et al.*, 1992). The yeast genome is not only condensed by small intergenic regions, but also by the elimination of introns, presumably by means of the reverse transcriptase activity of Ty elements (Fink, 1987). Although we cannot exclude the possibility that the failure of mammalian glutamine-rich domains is a peculiarity of the transactivator and reporter genes used, we consider it likely that the function of glutamine-rich stretches as promoter-proximal activation domains has been abandoned by yeast, together with truly remote enhancers, in an attempt to become a genetically streamlined, unicellular microorganism such as the rapidly growing bacterium *Escherichia coli*. Therefore, it will be interesting to test whether other eukaryotic model organisms, such as *Schizosaccharomyces pombe* or *Aspergillus nidulans*, which appear to be more closely related to mammals, are able to use mammalian glutamine-rich activation domains.

## Materials and methods

### *Plasmids, yeast strains and media*

For analysis in yeast various GAL4 fusion constructs (see Table I) were cloned into a pBRHAC (Gill and Ptashne, 1987) derived *HIS3* centromeric vector under the control of the yeast *ADH1* promoter. Recipient *S.cerevisiae* strains, GGY1::RY171, GGY1::SV15 and GGY1::GG9, harboring the different reporter constructs have been described previously (Gill and Ptashne, 1987). All three strains are derivatives of strain GGY1 (*gal4−542 gal80−538 tyr1 ade leu2−3,-112 his3−200 ura3−52*) and differ only in elements upstream of a *GAL1-lacZ* fusion (see Figure 1) integrated at the *URA3* locus. Yeast cells were made competent for plasmid transformation by treatment with lithium acetate (Ito *et al.*, 1983). Selection for pBRHAC derived plasmids was performed on MV minimal medium (Miozzari *et al.*, 1978) containing supplementing amounts of isoleucine, leucine, valine, arginine, lysine, methionine, phenylalanine, tyrosine and tryptophan. Glucose was used as sole carbon source for the cultivation of all yeast strains. Strain GGY1::GG9 was grown in the presence of 30 mM 3-aminotriazole to fully induce GCN4 (Hinnebusch, 1988).

### *Gel retardation experiments*

Yeast crude extracts were prepared according to Arndt *et al.* (1987). The double stranded oligonucleotides used for shift and competition assays are described elsewhere (Seipel *et al.*, 1992). Binding assays were performed as described in Braus *et al.* (1989). 5 μg extract were incubated with 10 fmol $^{32}$P-end-labelled probe. For competition assays 1 pmol unlabelled competitor DNA was added to the incubation mix.

### *β-galactosidase assay*

Specific β-galactosidase activities were determined as described earlier (Künzler *et al.*, 1993). Plasmid-carrying yeast strains were grown in 5 ml selective medium at 30°C to an optical density at 546 nm ($OD_{546}$) of between 1 and 2. GGY1::GG9 transformants were cultivated in the presence of 30 mM 3-aminotriazole to fully induce GCN4. Each assay was performed at least in triplicate from independent transformants. The standard error was <30%. Values <1 U could not be determined with accuracy and were assigned the value 1 U.

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