The TATA-binding protein is not an essential target of the transcriptional activators Gal4p and Gcn4p in *Saccharomyces cerevisiae*

Christine BONGARDS, Boon Shang CHEW and Norbert LEHMING¹

Department of Microbiology, Faculty of Medicine, National University of Singapore, Block MD4, 5 Science Drive 2, Singapore 117597

According to the recruitment model, transcriptional activators work by increasing the local concentration of one or several limiting factors for the transcription process at the target promoter. The TATA-binding protein Tbp1 has been considered as a likely candidate for such a limiting factor. We have used a series of Gal4p and Tbp1 mutants to correlate the *in vivo* interaction between the two proteins with the strength of activation. We find a clear correlation between activation strength and *in vivo* interaction for the series of Gal4p mutants. Consistently, the weaker activator Gcn4p does not interact with

Tbp1. However, a corresponding analysis of the series of Tbp1 mutants revealed that Tbp1 is not an essential target of the acidic activators Gal4p and Gcn4p. Furthermore, detailed analysis of a Tbp1 mutant deficient for transcriptional activation by Gal4p revealed that the mutant is defective in interactions with five other proteins involved in the process of transcription.

Key words: activation, mutation, protein–protein interaction, split-ubiquitin, transcription.

INTRODUCTION

Recruitment of the RNA polymerase II holoenzyme of transcription is an important step in transcriptional activation [1]. Transcriptional activators consist of at least two domains: a DNA-binding domain that confers promoter specificity, and an activating domain that is believed to make protein contacts with various components of the transcription machinery. According to this model, the activator works by raising the local protein concentration of one or several limiting factors at its target promoter. For several transcription factors, protein interactions with transcriptional activators have been demonstrated *in vitro*. The TATA-binding protein Tbp1, for example, has been shown to interact *in vitro* with activators like *Saccharomyces cerevisiae* Gal4p [2–4], human nuclear factor κ B [5] and viral VP16 [6]. Gal4p mutants defective for activation *in vivo* were also defective for the interaction with Tbp1 *in vitro* [3]. Chromatin immunoprecipitation experiments suggested that binding of Tbp1 to promoters is stimulated by activators *in vivo* [7–9]. Likewise, activators enhance the occupancy of TFIIB at promoters [7], and a clear correlation for Gal4p mutants to activate transcription *in vivo* and to interact with TFIIB *in vitro* has been established [3]. Other components of the transcription machinery for which protein interactions with activators have been demonstrated include TFIIF [10], Tbp1-associated factors (TAFs) [11], Srb4p [12], Med3p and Gal11p [13]. Therefore, it has been concluded that an activator can recruit the holoenzyme of transcription by making multiple contacts with its components [1]. Transcriptional activators have further been shown to interact with chromatin remodelling complexes like the SAGA complex [14,15]. The recruitment of these chromatin remodelling complexes is thought to change the acetylation status of the nucleosomes at the promoter *in vivo* [16].

Tbp1 is a well-studied protein. It is essential for cell viability, and transcription is abolished in temperature-sensitive mutants [17]. Tbp1 has been crystallized in several forms, including as a complex with TFIIA and DNA [18,19], and as a complex with TFIIB and DNA [20]. Numerous point mutations in Tbp1 have been described. We used the four mutants I143N, T153I, E186D and E236P in this study. E236P and T153I are defective for their response to acidic activators [21]. I143N is defective for the formation of a stable pre-initiation complex (PIC) *in vitro* [22]. E186D affects one of the three residues contacting TFIIB [20,23] and cannot support galactose-inducible *GAL1* transcription [7].

We wanted to analyse the protein–protein interaction between Tbp1 and Gal4p *in vivo*. Since the classical two-hybrid approach [24] is not suitable to measure interactions between the transcription factors that are functional in *S. cerevisiae*, we decided to use the split-ubiquitin method [25]. This method is based on the ability of N_{ub} and C_{ub}, the N- and C-terminal halves of ubiquitin, to assemble into a native-like ubiquitin [26]. Ubiquitin-specific proteases (Ubps), which are present in all eukaryotic cells, recognize the reconstituted ubiquitin, but not its halves, and release the ubiquitin moiety from a reporter protein which is linked to the C-terminus of C_{ub}. The assay is designed in a way that the association of N_{ub} and C_{ub} is only efficient if the two halves of ubiquitin are linked to two proteins that interact *in vivo*. The release of the reporter serves as a readout indicating that the reconstitution of ubiquitin has occurred. Previously, the split-ubiquitin assay has been shown to detect the *in vivo* interactions between nuclear transcriptional activators and repressors [27,28] and between membrane proteins [29,30]. As a reporter for the reconstitution of native-like ubiquitin we chose Ura3p or the green fluorescent protein (GFP) with an Arg residue in position 1 (RUra3p or RGFP). Arg is a destabilizing residue in the N-end rule pathway of protein degradation [31]. The reporter that is

Abbreviations used: C_{ub}, C-terminal half of ubiquitin; CFP, cyan fluorescent protein; FOA, 5-fluoro-orotic acid; GFP, green fluorescent protein; GST, glutathione S-transferase; HA, haemagglutinin; N_{ub}, N-terminal half of ubiquitin; PIC, pre-initiation complex; TAF, Tbp1-associated factor; Tbp1, TATA-binding protein; Ubp, ubiquitin-specific protease; YFP, yellow fluorescent protein.

¹ To whom correspondence should be addressed (e-mail nicln@nus.edu.sg).

Figure 1 Tbp1 interacts with Gal4p *in vivo*

(A) The split-ubiquitin system. *S. cerevisiae* cells expressing the Tbp1-Cub-RUra3p fusion are uracil prototrophic (panel 1). If Gal4p is fused to Nub, the protein-protein interaction between Gal4p and Tbp1 leads to the formation of a ubiquitin-like moiety, which is recognized and cleaved by the UbPs (panel 2). The free RUra3p is degraded so rapidly by the enzymes of the N-end rule pathway of protein degradation that the *S. cerevisiae* cells become uracil auxotrophic. (B) The effect of mutations upon the protein-protein interaction between Gal4p and Tbp1. 10-fold serial dilutions of cells expressing the depicted fusions were spotted on to control plates lacking leucine and tryptophan to select for the presence of the fusions, and on to test plates lacking uracil, leucine and tryptophan. Lack of growth on the test plates reveals the protein-protein interaction.

released from C_{ub} is therefore degraded rapidly by the enzymes of the N-end rule. Phenotypically, the protein-protein interaction inside the living cell leads to uracil auxotrophy and 5-fluoroorotic acid (FOA) resistance if RUra3p has been used as the reporter, and to the disappearance of green fluorescence if RGFP has been used as the reporter (Figure 1A).

Here we show that Gal4p interacts with Tbp1 *in vivo*, and that there is a clear correlation between the activation strength of a series of Gal4p mutants and their *in vivo* interaction with Tbp1. Gcn4p fails to interact with Tbp1, but it is also a much weaker activator than Gal4p. However, the analysis of a series of Tbp1

mutants defective for the interaction with Gal4p, artificial recruitment experiments, and a Gal4p derivative that interacts with Tbp1 but fails to stimulate transcription, reveal that Tbp1 is not an essential target for acidic activators like Gal4p and Gcn4p.

MATERIALS AND METHODS

Parental *S. cerevisiae* strains were JD52 (*mata ura3-52 leu2-3 his3Δ200 lys2-801 trp1Δ63*) [32] and NLY2 (*matα Δgal4 Δgal80 ura3-52 leu2-1 his3Δ200 lys2Δ3 trp1-1*) [33]. *GAL4* and *TBP1* mutants were constructed by PCR using standard techniques and sequenced by the ADIS (Automatische DNS Isolierung und Sequenzierung) DNA-sequencing unit of the Max-Planck Institut für Züchtungsforschung (MPIZ), Cologne, Germany. Mutant *TBP1* alleles were introduced into NLY2 by the plasmid-shuffle method. *TBP1* was deleted with a linearized construct consisting of the *TBP1* promoter, the *HIS3* gene and the *TBP1* terminator by homologous recombination in the presence of YCplac33 [34] containing the *TBP1* gene. Mutant *TBP1* alleles were introduced by transformation with the respective expression plasmids and removal of wild-type *TBP1* on FOA plates. The *GCN4* deletion strain was obtained from EUROSCARF (the European *Saccharomyces cerevisiae* Archive for Functional Analysis; <http://www.uni-frankfurt.de/fb15/mikro/euroscarf/index.html>). The single-copy vectors expressing the N_{ub} fusions under the control of the *ADH1* promoter and the C_{ub}-RUra3p fusions under the control of the *CUP1* promoter have been described previously [35]. The *TRP1*-marked single-copy vector expressing the C_{ub}-RYFP fusions was constructed like the C_{ub}-RGFP vector described in [36], except that yellow fluorescent protein (YFP; Clontech) was used as a PCR template instead of GFP. The *HIS3*-marked single-copy vector expressing the C_{ub}-RCFP fusions was constructed like the C_{ub}-RGFP vector described in [36], except that cyan fluorescent protein (CFP; Clontech) was used as a PCR template instead of GFP and that pRS313 was used as a backbone instead of pRS314 [37].

Plasmids expressing glutathione S-transferase (GST)-Gal4(1-100+840-881)p, wild-type Tbp1 and the E236P and I143N mutants fused to six histidines and a haemagglutinin (HA) tag in *Escherichia coli* were constructed by cloning the respective PCR fragments into GEX-5X-1 (Pharmacia) and pET11a (Invitrogen). GST pull-down assays were performed as described previously [27], with 40 mg of BSA/ml of PBS to prevent non-specific binding of Tbp1 to GST. β -Galactosidase activities were determined as described in [38]. Data shown were derived from at least four independent cultures. S.D. values were less than 20%.

RESULTS

Figure 1(B) shows that Gal4p interacts with Tbp1 *in vivo*. *S. cerevisiae* cells expressing N_{ub} together with Tbp1 fused to the C_{ub}-RUra3p reporter were able to grow on uracil-depleted medium (Figure 1B, line 1). When N_{ub} was fused to a minimal version of Gal4p, comprised of just the N-terminal DNA-binding and the C-terminal activation domain, Gal4(1-100+840-881)p, the cells became uracil auxotrophic, reflecting the interaction between Tbp1 and Gal4p inside of the cell, the assembly of the native-like ubiquitin, the cleavage by the Ubps and the degradation of the free RUra3p by the enzymes of the N-end rule (Figure 1B, line 2). The Figure also shows that there is a clear correlation between the interaction of a series of N_{ub}-Gal4p derivatives with Tbp1 and their strength of activation (compare

Table 1 Activation of transcription derived from Gal4p-based activators

The depicted *GAL4* alleles were transformed into *S. cerevisiae* cells carrying an integrated *GAL1-lacZ* reporter. Cells were grown in liquid culture, and β -galactosidase activities were determined as described in [38].

No.	Gal4p-based activator	Activity (units/min)
1	N _{ub}	< 1
2	N _{ub} -Gal4(1-100 + 840-881)p	2049
3	N _{ub} -Gal4(1-100 + 840-881(F856A; F869A))p	1281
4	N _{ub} -Gal4(1-100 + 840-857)p	518
5	N _{ub} -Gal4(1-100 + 840-857(F856A))p	71
6	C _{ub} -RUra3p	< 1
7	Gal4(1-100 + 840-881)-C _{ub} -RUra3p	< 1
8	Gal4(1-100 + 840-857)-C _{ub} -RUra3p	< 1
9	Gal4(1-100 + 840-857(F856A))-C _{ub} -RUra3p	< 1
10	Gal4(1-147)p	1
11	Gal4(1-147 + 768-881)p	1446
12	Gal4(1-147) + Tbp1	8
13	Gal4(1-147) + Gcn4p	106

Figure 1B, lines 2–5, with Table 1, lines 2–5). N_{ub}-Gal4(1-100 + 840-881)p, the strongest activator, interacted with Tbp1-C_{ub}-RUra3p best. Mutation of residues 856 and 869 of Gal4p from Phe to Ala residues (F856A, F869A) reduced the interaction with Tbp1 and the activation to 63 %. Removal of the last 24 amino acids from the activation domain of Gal4p reduced the interaction with Tbp1 further and the activation down to 25 %. Changing residue 856 of the truncated activation domain of Gal4p from Phe to Ala (F856A) nearly abolished the interaction with Tbp1 and reduced the activation to 3 %. Consistently, Gcn4p did not interact with Tbp1 in the split-ubiquitin assay (Figure 1, line 6), and showed only about 5 % of the activation strength of Gal4p (Table 1, line 13). Figure 1(B) further shows a series of Tbp1 mutants with decreasing interaction with Gal4(1-100 + 840-881)p (Figure 1B, lines 7–14). E186D interacted with Gal4p-like wild-type Tbp1, T153I was slightly deficient in the interaction, E236P was severely deficient and I143N showed no sign of interaction with Gal4p. All N_{ub} fusions were expressed at comparable levels, as determined by Western blotting with the help of an HA tag present in the N_{ub} part of the fusions (results not shown).

Figure 2(A) shows that Tbp1 and Gal4p still interacted in the split-ubiquitin assay when the fusions were swapped. Gal4(1-100 + 840-881)p was fused to the C_{ub}-RUra3p reporter, Tbp1 was fused to N_{ub}, and cells expressing both fusions were unable to grow on plates lacking uracil (Figure 2A, line 2). Introduction of the E236P or the I143N mutation into N_{ub}-Tbp1 abolished the interaction (Figure 2A, lines 3 and 4). Truncating the activation domain of Gal4p decreased the interaction with Tbp1 (Figure 2A, line 6), and mutating residue 856 from Phe to Ala in the truncated activation domain reduced the interaction even further (Figure 2A, line 10). The Tbp1 mutants I143N and E236P showed no sign of interaction with the Gal4p derivatives (Figure 2A, lines 7, 8, 11 and 12). Figure 2(B) shows that the interaction between Tbp1 and Gal4p was observed when the cells were grown with glucose or galactose as a carbon source (Figure 2B, lines 2 and 6), and that Tbp1(I143N) failed to interact in both cases (Figure 2B, lines 4 and 8). The N_{ub}-Tbp1 mutants were expressed at comparable levels as N_{ub}-Tbp1 wild-type, as determined by Western blotting (results not shown).

Figure 3(A) shows that the interaction between Gal4p and Tbp1 takes place in the nucleus of the cell, and confirms that the

Figure 2 Tbp1 interacts with Gal4p *in vivo* independent of the carbon source and when the fusions are swapped

(A) 10-fold serial dilutions of cells expressing the depicted fusions were spotted on to control plates lacking leucine and tryptophan to select for the presence of the fusions, and on to test plates lacking uracil, leucine and tryptophan. Lack of growth on the test plates reveals the protein–protein interaction. (B) Upper panel: 10-fold serial dilutions of cells expressing the depicted fusions were spotted on to glucose control plates lacking leucine and tryptophan to select for the presence of the fusions, and on to glucose test plates lacking uracil, leucine and tryptophan. Lower panel: 10-fold serial dilutions of cells expressing the depicted fusions were spotted on to galactose control plates lacking leucine and tryptophan to select for the presence of the fusions, and on to galactose test plates lacking uracil, leucine and tryptophan. Lack of growth on the test plates reveals the protein–protein interaction.

E236P and I143N Tbp1 mutants are deficient for this interaction. *S. cerevisiae* cells expressing N_{ub} together with Tbp1 fused to a C_{ub}-RCFP reporter displayed blue nuclear staining. As a control, the cells were also expressing Tbp1 fused to a C_{ub}-RYFP reporter, and so the cells displayed yellow nuclear staining as well (Figure 3A, column 1). CFP and YFP are excited at different wavelengths and can be observed independently. When N_{ub} was fused to Gal4(1-100 + 840-881)p, the nuclear CFP and YFP staining disappeared, presumably reflecting the interaction between Tbp1 and Gal4(1-100 + 840-881)p inside of the nucleus, the assembly of the native-like ubiquitin, the cleavage by the Ubps and the degradation of the free RCFP and RYFP by the enzymes of the N-end rule (Figure 3A, column 2). Figure 3(A) further shows that when the Tbp1(E236P) mutant was fused to the C_{ub}-RCFP reporter, while the C_{ub}-RYFP reporter remained linked to wild-type Tbp1, co-expression with N_{ub}-Gal4(1-100 + 840-881)p resulted in only partial loss of the CFP signal, while the YFP signal completely disappeared (Figure 3A, compare columns 3 and 4).

Figure 3 Tbp1 interacts with Gal4p *in vivo* and *in vitro*

(A) Cells expressing the depicted fusions were spotted on to slides and analysed under a Leitz fluorescence microscope. Shown are CFP staining (excitation, 436 nm; emission, 480 nm), YFP staining (excitation, 500 nm; emission, 535 nm) and bright-field illumination for each sample. Lack of fluorescence reveals a protein–protein interaction. (B) The depicted purified proteins were incubated with glutathione beads, precipitated and separated on a protein gel. After Western blotting, bound proteins were detected with the help of an anti-HA antibody (upper panel) and an anti-GST antibody (lower panel).

Figure 4 Nub-Tbp1 wild-type and mutants, as well as Tbp1-Cub-RCFP wild-type and mutants, are able to complement a *TBP1* deletion

Upper panel: 10-fold serial dilutions of cells with a chromosomal *TBP1* deletion balanced by a *URA3*-marked plasmid that are expressing the depicted fusion were spotted on to control plates lacking leucine and on to test plates containing FOA. Lower panel: 10-fold serial dilutions of cells with a chromosomal *TBP1* deletion balanced by a *URA3*-marked plasmid that are expressing the depicted fusion were spotted on to control plates lacking histidine and on to test plates containing FOA. The ability of the cells to grow on FOA reveals the ability of the respective fusion to complement the *TBP1* deletion.

When the Tbp1 I143N mutant was fused to the C_{ub}-RCFP reporter, the CFP signal remained unchanged upon co-expression with Nub-Gal4(1-100+840-881)p, whereas the wild-type Tbp1 YFP signal completely disappeared again (Figure 3A, compare columns 5 and 6). Therefore, we were able to observe the interaction between wild-type Tbp1 and Gal4p and the effect of the respective mutation on this interaction in the same nucleus.

Figure 5 Gal4-Cub-RUra3p and Gal4(1-147)-Tbp1 fail to complement a *GAL4* deletion

(A) 10-fold serial dilutions of cells carrying a chromosomal *GAL4* deletion that are expressing the depicted proteins were grown on glucose plates and on galactose plates containing 0.1 ng of antimycin A/ml. (B) 10-fold serial dilutions of cells carrying a chromosomal *GCN4* deletion expressing the depicted proteins were grown on plates lacking histidine and on plates lacking histidine and containing 20 mM 3-aminotriazole (3AT). (C) 10-fold serial dilutions of cells carrying chromosomal *GAL4* and *TBP1* deletions that are expressing the depicted proteins were grown on glucose plates and on galactose plates containing 0.1 ng of antimycin A/ml.

The split-ubiquitin assay reveals close proximity of two proteins *in vivo*, but not necessarily a direct protein contact. To confirm that Tbp1 interacted directly with Gal4p, we expressed the proteins in *E. coli*. Gal4(1-100+840-881)p was fused to GST. Tbp1 wild-type as well as the E236P and the I143N mutants were fused to six histidines and an HA tag. The proteins were purified with the help of the attached tags. Figure 3(B) shows that Tbp1 interacted with Gal4(1-100+840-881)p in a GST pull-down assay (Figure 3B, lane 3) and that the E236P and the I143N mutants, as suggested by the split-ubiquitin assay, were deficient for this *in vitro* interaction (Figure 3B, lanes 6 and 9).

Figure 4 shows that Nub-Tbp1 and Tbp1-Cub-RCFP were both able to complement a *TBP1* deletion. An *S. cerevisiae* strain with a chromosomal deletion of the entire *TBP1* open reading frame carrying a *URA3*-marked plasmid with the *TBP1* gene on it was not able to grow on FOA plates (Figure 4, lines 1 and 5). Expression of either Nub-Tbp1 or Tbp1-Cub-RCFP allowed the cells to grow in the presence of FOA, demonstrating that both fusions were able to complement the *TBP1* deletion (Figure 4, lines 2 and 6). The Figure further shows that the E236P and the I143N mutants, which were defective for the Gal4p interaction, were both able to complement the *TBP1* deletion as Nub- and C_{ub}-RCFP fusions (Figure 4, lines 3, 4, 7 and 8).

Figure 5(A) shows that Nub-Gal4(1-100+840-881)p, but not Gal4(1-100+840-881)-Cub-RUra3p, was able to complement a *GAL4* deletion. *S. cerevisiae* cells lacking *GAL4* were unable to grow with galactose as the sole carbon source in the presence of the respiration inhibitor antimycin A (Figure 5A, line 1). Cells transformed with a plasmid containing the entire *GAL4* gene were able to utilize galactose (Figure 5A, line 2), as were cells expressing Nub-Gal4(1-100+840-881)p (Figure 5A, line 4). How-

Figure 6 Gal4p activates transcription in the E236P and I143N Tbp1 mutant backgrounds

(A) 10-fold serial dilutions of cells with the indicated *TBP1* allele, lacking *GAL4*, containing an integrated *GAL1-lacZ* reporter, and expressing the depicted fusions were plated on glucose plates and on galactose plates containing 0.1 ng of antimycin A/ml. (B) The same cells were grown in liquid medium and assayed for β -galactosidase activity (determined as described in [38]).

ever, cells expressing Gal4(1-100+840-881)-C_{ub}-Rura3p were unable to grow on galactose plates, indicating that the C_{ub}-Rura3p part of the fusion blocked the activation domain of Gal4p (Figure 5A, line 6). We quantified the difference in activation strength between N_{ub}-Gal4(1-100+840-881)p and Gal4(1-100+840-881)-C_{ub}-Rura3p with the help of an integrated *GAL1-lacZ* reporter in a strain lacking *GAL4*. Table 1 shows that whereas N_{ub}-Gal4(1-100+840-881)p activated transcription by more than 2000-fold, Gal4(1-100+840-881)-C_{ub}-Rura3p was not able to activate transcription (Table 1, compare lines 2 and 7). Similar results were obtained for a Gal4(1-100+840-881)-C_{ub}-RGFP fusion (results not shown). Figure 5(B) shows that N_{ub}-Gcn4p and Gal4(1-147)-Gcn4p were able to complement a *GCN4* deletion, demonstrating that Gcn4p remained functional in the context of the fusions.

Both the transcriptionally active (N_{ub}-Gal4p) and the transcriptionally inactive Gal4p derivatives (Gal4-C_{ub}-Rura3p) were able to interact with Tbp1 *in vivo*, but recruitment of Tbp1 by Gal4(1-

Figure 7 The E186D, but not the I143N, Tbp1 mutation prevents activation by the acidic activators Gal4p and Gcn4p

(A) 10-fold serial dilutions of cells with the indicated *TBP1* and *GAL4* alleles were plated on glucose plates and on galactose plates containing 0.1 ng of antimycin A/ml. (B) The same cells were grown in liquid medium and assayed for β -galactosidase activity (determined as described in [38]). (C) 10-fold serial dilutions of cells with the indicated *TBP1* and *GCN4* alleles were spotted on plates lacking histidine and on plates lacking histidine and containing 50 mM 3-aminotriazole (3-AT).

100+840-881)-C_{ub}-Rura3p was not sufficient to stimulate transcription. Thus a strong interaction with Tbp1 is not likely to be a limiting factor for transcriptional activation by Gal4p. To test this hypothesis, the chromosomal *TBP1* was replaced by a Gal4(1-147)-Tbp1 fusion in a strain lacking *GAL4*. Since Tbp1 is essential for growth, the growth of this strain on glucose plates showed that the Tbp1 moiety of the fusion is functional (Figure 5C, line 1). However, the strain was only able to grow on galactose plates if transformed with a single-copy vector containing the entire *GAL4* gene (Figure 5C, line 2). This demonstrated that while the Gal4(1-147)-Tbp1 fusion was able to fully complement *TBP1*, it was not able to complement *GAL4*. Table 1 shows that Gal4(1-147)-Tbp1 had only about 0.5% of the activation strength of Gal4(1-147+768-881)p (Table 1, compare lines 11 and 12). As Gal4p was able to activate when *TBP1* was replaced by Gal4(1-147)-Tbp1, the Tbp1 part of the fusion had to be fully functional. Apparently, recruitment of Tbp1 alone to the *GAL1* promoter is not sufficient to stimulate transcription significantly.

Figure 6(A) shows that N_{ub}-Gal4(1-100+840-881)p, the same molecule that failed to interact with Tbp1(I143N)-C_{ub}-Rura3p, was still able to activate transcription when the chromosomal

TBP1 was replaced by the I143N mutant (Figure 6A, line 15). Consistently, N_{ub} -Gal4(1-100+840-881)p was also able to activate when *TBP1* was replaced by the E236P mutant (Figure 6A, line 9). Figure 6(B) shows that the activation of an integrated *GAL1-lacZ* reporter by N_{ub} -Gal4(1-100+840-881)p was reduced only about 2-fold when TBP1 was replaced by either the E236P or the I143N mutant. Similar results were obtained when full-length Gal4p expressed from its own promoter was tested for its ability to activate transcription in the Tbp1(I143N) mutant background (Figure 7A, line 8). Figure 7(A) further shows that Gal4p was also able to activate transcription in the Tbp1(T153I) mutant background (Figure 7A, line 6). However, when we replaced *TBP1* by the E186D mutant, the cells were unable to grow on galactose plates (Figure 7A, line 4). Figure 7(B) shows the quantification with the help of the integrated *GAL1-LacZ* reporter. No correlation between the interaction of Gal4p with the different Tbp1 alleles and the ability of Gal4p to activate transcription in these genetic backgrounds was observed. The I143N mutant showed no interaction with Gal4p, but Gal4p was able to stimulate transcription. The T153I mutant was only slightly reduced for the interaction with Gal4p, and Gal4p was able to activate in its background. Finally, the E186D mutant interacted with Gal4p like wild-type Tbp1, but Gal4p was not able to activate in its sole presence. The second activator we tested behaved like Gal4p. Figure 7(C) shows that activation by Gcn4p was impaired in the E186D mutant background (Figure 7C, line 3). However, as for Gal4p, activation by Gcn4p was not impaired in the I143N and T153I mutant backgrounds (Figure 7C, lines 4 and 5).

Recently, the defects of the E186D mutant have been attributed to its failure to interact with Sua7p (= TFIIB) [23]. We have tested wild-type Tbp1 and the E186D mutant with more than 100 proteins involved in transcription (results not shown). We have found that wild-type Tbp1 interacts significantly with 28 of these (Toa1p, Toa2p, Sua7p, Taf1p, Taf4p, Taf6p, Taf9p, Taf11p, Taf12p, Taf14p, Tfg2p, Srb4p, Srb10p, Rpb1p, Rpb3p, Rpb4p, Rpb5p, Rpb6p, Rpb8p, Rpb9p, Rpb10p, Spt8p, Ada2p, Nhp6Bp, Hta1p, Htb1p, Hhf1p and Rap1p). The E186D mutation abolishes the interaction of Tbp1 with five of those proteins (Sua7p, Srb4p, Rpb1p, Rpb4p and Rpb8p).

DISCUSSION

We have shown that Tbp1 is not an essential target of Gal4p. Our *in vivo* results are consistent with previous results obtained *in vitro* [39–41]. Transcriptional activators work by raising the concentration of one or several limiting components for the transcription process. The series of Gal4p mutants showed that there is a clear correlation between activation strength and *in vivo* interaction with Tbp1. However, other transcription factors like TFIIB interact better with stronger Gal4p derivatives too [3]. The series of Tbp1 mutants, on the other hand, showed that the *in vivo* interaction between Gal4p and Tbp1 is dispensable for transcription. Gal4p was able to activate transcription in the Tbp1(I143N) mutant background, even though Gal4p failed to interact with this Tbp1 mutant *in vivo* and *in vitro*. Furthermore, Gal4p was unable to activate transcription in the Tbp1(E186D) mutant background, even though this Tbp1 mutant interacted with Gal4p like wild-type Tbp1. Finally, Gal4(1-100+840-881)- C_{ub} -Rura3p is a Gal4p derivative not capable of stimulating transcription, but able to interact with Tbp1. We assume that the C-terminal C_{ub} -Rura3p moiety blocks the activation domain of Gal4p similar to Gal80p bound in the same position. We argue that the interaction between Gal4p and Tbp1 cannot be necessary for transcription, as Tbp1 is not able to discriminate between the

transcriptionally active and inactive Gal4p derivatives. Chromatin immunoprecipitation experiments have shown that activators enhance Tbp1 binding *in vivo* [7,8]. We conclude that Tbp1 is essential for transcription and that it is recruited as part of the holoenzyme. The recruitment presumably involves the interaction of Gal4p with holoenzyme components other than Tbp1, like TFIIB [3], TFIIF [10], certain TAFs [11], Srb4p [12], SAGA [15,42], Med3p [13] or Gal11p [43].

N_{ub} -Gal4(1-100+840-881)p complemented the growth defect of a *GAL4* deletion on galactose plates in wild-type Tbp1, and in the E236P and I143N mutant backgrounds (Figure 6A, lines 3, 9 and 15). The weaker Gal4p derivatives tested were unable to complement the *GAL4* deletion in the genetic background of all three Tbp1 alleles. However, quantification with the help of the integrated *GAL1-lacZ* reporter showed that the Gal4p double mutant activated the *GAL1* promoter in the wild-type Tbp1 background at least as strongly as N_{ub} -Gal4(1-100+840-881)p in the *TBP1* mutant backgrounds (Figure 6B). This discrepancy can be explained by the different carbon sources used in the two assays. The complementation analysis was performed on plates containing galactose minimal medium, and the determination of the β -galactosidase activity was performed with cells grown in liquid glucose minimal medium. Glucose was used for the determination of the β -galactosidase activities because the cultures expressing truncated versions of Gal4p were unable to grow in galactose medium. Since the cells were also deficient for *GAL80*, Gal4p was able to activate transcription in glucose. However, glucose repression mediated by Mig1p was still in place, and one possible explanation could be that Gal4(1-100+840-881)p had problems to overcome glucose repression in the *TBP1* mutant backgrounds. An alternative explanation would be that the quantification was performed with the *GAL1* promoter only, and that some of the other *GAL* genes might respond differently to mutations in Tbp1 and Gal4p.

Most of the Gal4p-Tbp1 interaction assays were performed on glucose plates, where Gal4p is normally not active. However, when over-expressed, Gal4p can activate transcription in glucose. We expressed the N_{ub} fusions from single-copy vectors, but under the control of the strong constitutive *ADHI* promoter. Therefore, we tested N_{ub} -Gal4p under conditions where it is active.

The Tbp1(I143N) mutant failed to interact with Gal4p and Gcn4p, but supported transcriptional activation by these activators. This suggests that the direct recruitment of Tbp1 by an activator is not a prerequisite for transcriptional activation *in vivo*. Gal4p interacts with other components of the holoenzyme of transcription as well (see the Introduction). This might lead to redundancy of the individual protein–protein interactions. We assume that the Tbp1(I143N) mutant is recruited together with the rest of the holoenzyme of transcription and functions like wild-type Tbp1 in the PIC. The Tbp1(E186D) mutant, on the other hand, is recruited to the PIC like wild-type Tbp1. However, the mutation presumably affects a post-recruitment step of transcriptional activation, and the PIC containing the Tbp1 (E186D) mutant is not functional. This could be due to any or all of the five protein–protein interactions found to be affected by the E186D mutation. We conclude that Tbp1 is important for transcriptional activation, and that it interacts with Gal4p inside the nucleus of the wild-type cell. However, our genetic studies have revealed that Tbp1 is not an essential target of the acidic activators Gal4p and Gcn4p.

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