TFIIB/*SUA7*(E202G) is an allele-specific suppressor of *TBP1*(E186D)

Boon Shang CHEW and Norbert LEHMING¹

Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, 5 Science Drive 2, Singapore 117597, Singapore

The TBP (TATA-box-binding protein), Tbp1p, plays a vital role in all three classes of transcription by RNA polymerases I–III. A *TBP1*(E186D) mutation had been described that affected interaction of Tbp1p with TFIIB (transcription factor IIB) and that caused slow-growth, temperature-sensitivity, 3-aminotriazole-sensitivity as well as a gal[−] phenotype. We used the *TBP1*-(E186D) mutant for suppressor screens, and we isolated TFIIB/*SUA7*(E202G) as an allele-specific suppressor of all phenotypes caused by the *TBP1*(E186D) mutation. Our results show that the *SUA7*(E202G) mutation restored binding of TFIIB to Tbp1(E186D)p. In addition, we observed that Tbp1(E186D)p was expressed at a lower level than wild-type Tbp1p, and that

SUA7(E202G) restored the protein level of Tbp1(E186D)p. This suggested that the *TBP1*(E186D) mutation might have generated its phenotypes by making Tbp1p the limiting factor for activated transcription. DNA microarray analysis indicated that the *TBP1*(E186D) temperature-sensitivity and slow-growth phenotypes might have been caused by insufficient amounts of Tbp1p for efficient transcription of the rRNA genes by RNA polymerase I.

Key words: allele-specific suppressor, protein interaction, RNA polymerase, split-ubiquitin, TATA-box-binding protein 1 (TBP1), transcription factor IIB (TFIIB).

INTRODUCTION

Activated transcription of protein-coding genes by RNA polymerase II in eukaryotic cells involves the recruitment and interaction of various proteins [1–3]. According to the recruitment model, activators of transcription function by raising the local concentration of limiting factors at the promoter in order for transcription to begin [4]. According to the reverse recruitment hypothesis, transcription factors form gene expression machines in the nuclear periphery, while uninduced genes are located in the centre of the nucleus. Upon recruitment of a gene to a machine associated with a nuclear pore, the gene is transcribed, and the mRNA is exported out of the nucleus [5]. Gal4p is an example of a well-studied activator in yeast [6]. Gal4p binds to the UAS (upstream activating sequence) elements found in the *GAL1*–*GAL10*, *GAL7* and *GAL80* promoters. Gal4p recruits the transcription machinery and co-activators such as the SAGA (Spt–Ada–Gcn5–acetyltransferase complex) and the TBP (TATA-box-binding protein) Tbp1p for initiation of gene transcription from these promoters upon induction. Reports suggested that the recruitment of SAGA to these promoters acts as a scaffold for the assembly of the PIC (pre-initiation complex) [7] and is needed for the recruitment of the mediator [8]. The histone acetyltransferase activity of chromatin remodelling complexes like SAGA also helps to facilitate transcription by acetylation of histone tails [9,10].

TBP1 was identified in a genetic selection for suppressors of a Ty insertion in the *HIS4* promoter, and it plays an important role in gene regulation [11–13]. Tbp1p is a general transcription factor that is found in a variety of complexes in yeast such as SL1 (selectivity factor 1), TFIID (transcription factor IID) and TFIIB (transcription factor IIB), which are involved in gene regulation by the three classes of RNA polymerases [14]. The SL1 complex is required for the transcription of rRNA by polymerase I [15], and TFIIB is required for the transcription of tRNA and 5S

rRNA by polymerase III [16,17]. Tbp1p, together with the TAFs (TBP1-associated factors), forms TFIID, which is needed for the transcription of polymerase II promoters [18]. Tbp1p functions as a co-activator, which is recruited by activators and binds directly to the minor groove of the TATA-box sequence to nucleate the assembly of the PIC [19,20]. Mutations in Tbp1p that hinder its binding to the TATA-box cause the inability for transcription of regulated genes to begin. This hints to the importance of the recruitment of Tbp1p for transcription [21]. Following the binding of TFIID, recruitment of other general transcription factors occurs in the order of TFIIA, TFIIB (Sua7p), TFIIF, polymerase, TFIIE and TFIIH [22]. We used *TBP1*(E186D) in the present study, which has previously been described for its inability to support galactose-induced *GAL1* transcription [23]. Yeast cells carrying the *TBP1*(E186D) mutation not only displayed a gal[−] phenotype but also grew slowly and showed sensitivity towards 3-aminotriazole and high temperature.

Sua7p is a 38 kDa general transcription factor, and it plays a key role in the initiation and start site selection by RNA polymerase II [24–26]. It autoacetylates itself in the presence of acetyl-CoA at Lys²³⁸, which is thought to stabilize its interaction with TFIIF and activate transcription [27]. Sua7p is an essential component of the PIC and enters the complex after binding of Tbp1p to the TATA-box and before the recruitment of RNA polymerase II takes place. Once the PIC is formed at the promoter, transcription begins with the phosphorylation of the C-terminus of RNA polymerase II and promoter clearance [28].

We made use of the Split-ubiquitin assay [29] to study the interactions between Tbp1p and other transcription factors. The Split-ubiquitin assay has previously been used to detect interactions between nuclear proteins [30], and between integral membrane proteins [31,32]. This method is based on the ability to split ubiquitin into two halves, namely the N_{ub} and the C_{ub} (N-terminal and C-terminal half of ubiquitin respectively), which can be reconstituted into a native-like ubiquitin moiety if two

Abbreviations used: C_{ub}, C-terminal half of ubiquitin; FOA, 5-fluoro-orotic acid; GST, glutathione S-transferase; HA, haemagglutinin; N_{ub}, N-terminal half of ubiquitin; ORF, open reading frame; PIC, pre-initiation complex; SAGA, Spt–Ada–Gcn5–acetyltransferase complex; TBP1, TATA-box-binding protein 1; TFI, transcription factor I; UAS, upstream activating sequence.

¹ To whom correspondence should be addressed (email michn@nus.edu.sg).

proteins fused to these two halves interact inside the living cell. This brings about the N-end rule degradation of the reporter gene, ornithine decarboxylase (Ura3), which had been modified to start with an arginine residue (RUra3), and was linked to the C-terminus of the C_{ub}. The interaction between the two proteins fused to N_{ub} and C_{ub} respectively results in the cells being uracil auxotroph- and FOA (5-fluoro-orotic acid)-resistant [33].

Using the Split-ubiquitin assay, we found that the interactions of Tbp1p with the transcription factors Sua7p, Srb4p, Rpb1p, Rpb4p and Rpb8p were affected by the *TBP1*(E186D) mutation [34]. In the present study, we isolated Sua7(E202G)p through a suppressor screen, searching for mutants among these five proteins that would suppress the temperature-sensitivity of the *TBP1*(E186D) mutation. Sua7(E202G)p was found to be an allele-specific suppressor of the phenotypes of *TBP1*(E186D). The *TBP1*(E186D) mutation had been found to affect one of the three residues bridging Tbp1p to Sua7p and was proposed to affect activated transcription due to the inability to recruit Sua7p into the PIC [23]. Glu²⁰² of Sua7p is not a residue linking Sua7p to Tbp1p. According to the results presented here, Tbp1(E186D)p was expressed at lower protein levels than wild-type Tbp1p, and the *SUA7*(E202G) mutation restored the protein level of Tbp1(E186D)p. Taken together, our results suggest that the *TBP1*(E186D) mutation not only affects the interaction between Tbp1p and Sua7p but also the stability of Tbp1p. Sua7(E202G)p was able to suppress the phenotypes of *TBP1*(E186D) due to its ability to stabilize Tbp1(E186D)p so that transcription of ribosomal genes and other affected genes was restored.

MATERIALS AND METHODS

Yeast strains

Parental *Saccharomyces cerevisiae* strain used in Figures 1, 2, 4(a) and 4(c) was NLY2 (*matα Δgal4 Δgal80 ura3-52 leu2-1 his3Δ200 lys2Δ3 trp1-1*) [36]. The endogenous *TBP1* gene in NLY2 had been replaced with the *HIS3* gene by homologous recombination in the presence of YCplac33 [37] containing the wild-type *TBP1* gene. Mutant *TBP1* alleles were introduced into competent yeast cells with the respective expression plasmids followed by the removal of the wild-type *TBP1* allele by plating the cells on FOA plates.

The parental yeast strains used in Figure 3 (BY4741ΔW), Figure 1(b), line 1 and Figure 2, line 21 (BY4741ΔWΔGCN4) were derived from EUROSCARF (the European *S. cerevisiae* Archive for Functional Analysis; http://web.uni-frankfurt.de/fb15/mikro/euroscarf/col_index.html) by deleting the *TRP1* gene with the help of NKY1009 [38]. For Figure 4(b), JD52 (*Mata ura3-52 leu2-3 his3Δ200 lys2-801 trp1Δ63*) [39] was used.

Screening for suppressors of the temperature-sensitivity and gal⁻ phenotype of *TBP1*(E186D)

The single-copy vectors expressing the N_{ub} fusions were under the control of the *ADHI* promoter/terminator cassette [30]. We amplified *SUA7*, *SRB4*, *RPB1-CTD*, *RPB4* and *RPB8* borne on PACNX-N_{ub} by PCR using Promega Taq polymerase. The PCR fragments of individual genes were transformed into competent NLY2Δ*TBP1*::*HIS3* + YCplac22-*TBP1*(E186D) via gapped repair. To isolate suppressors of temperature-sensitivity, the transformants were incubated at 35°C for 1 week. All candidates acquired were tested for plasmid linkage. To investigate if *SUA7*(E202G) could suppress the gal⁻ phenotype of *TBP1*(E186D), ASZ11-GAL4, an *ADE2*-marked single-copy

vector containing the *GAL4* gene, was transformed into NLY2Δ*TBP1*::*HIS3* + YCplac22-*TBP1*(E186D) together with PACNX-N_{ub}-*SUA7*(E202G) or PACNX-N_{ub}-*SUA7*. Ten-fold serial dilutions of the transformants were grown on galactose plates containing 1 μg/ml antimycin A.

Split-ubiquitin assay

In Figure 3, N_{ub} and N_{ub} fusions of Sua7p or Sua7(E202G)p were transformed into BY4741ΔW expressing Tbp1-C_{ub}-RUra3p or Tbp1(E186D)-C_{ub}-RUra3p under the control of the *CUP1* promoter. Ten-fold serial dilutions of the transformants were grown on control medium lacking tryptophan and leucine and on medium additionally lacking uracil in the absence and presence of 100 μM CuSO₄ at 39°C for 3 days.

GST (glutathione S-transferase) pull-down and Western blot

The GST-Tbp1p fusions were cloned into YG1μ, a multi-copy vector expressing GST under the control of the *ADHI* promoter. JD52 cells were transformed with GST, GST-Tbp1p or GST-Tbp1(E186D)p, as well as with PACNX-N_{ub}, PACNX-N_{ub}-Sua7p or PACNX-N_{ub}-Sua7(E202G)p. The HA (haemagglutinin)-Sua7p fusions were cloned into PACNX-N_{ub} and expressed under the control of the *ADHI* promoter. The transformants were grown in medium lacking tryptophan and leucine until a *D*₆₀₀ (attenuance) of 1.0 was reached, and GST pull-down assays were performed with cell extracts as described in [40], followed by Western-blot analysis with anti-GST and anti-HA antibodies.

Real-time PCR

Plasmids expressing N_{ub} fusions of Sua7p and Sua7(E202G)p together with ASZ11 or ASZ11-GAL4 were transformed into competent NLY2Δ*TBP1*::*HIS3* cells expressing Tbp1p or Tbp1(E186D)p. Transformants were inoculated into glucose-selective medium lacking adenine, tryptophan and leucine, and were incubated at 28°C until they reached a *D*₆₀₀ of 1.0. The cells were harvested and washed with sterile water before they were induced in 2% galactose-selective medium lacking adenine, tryptophan and leucine for 1 h. The cells were harvested and total RNA was isolated from individual samples using a Qiagen RNeasy Mini kit. Real-time PCR was performed with SYBR Green with *GAL1* ORF (open reading frame) primers (forward 5'-ACTTGCACCGGAAAGGTTTG-3'; reverse 5'-TTGGTACA-TCACCCTCACAGAAGA-3') and *ACT1* ORF primers (forward 5'-AAACCGCTGCTCAATCTTCT-3'; reverse 5'-AATACCGGCAGATTCCAAAC-3') as the internal control. The readings were normalized against NLY2Δ*TBP1*::*HIS3* cells co-expressing Sua7p and Tbp1p or Tbp1(E186D)p without Gal4p.

DNA microarray analysis

Knock-in strains expressing *SUA7*(E202G) in the *TBP*, *TBP1*(E186D) and *TBP1*(I143N) backgrounds were created (see Supplementary Table S1 at <http://www.BiochemJ.org/bj/406/bj4060265add.htm>). DNA microarray experiments were performed with the yeast genome S98 array from Affymetrix GeneChip® probe array. To investigate genes affected by various *TBP1* mutant backgrounds contributing to the slow-growth phenotype, cells were precultured at 28°C in glucose medium overnight and diluted 50 times the next day. Each sample was harvested at a *D*₆₀₀ of between 0.8 and 1.0. Samples were prepared for analysis

Figure 1 The *TBP1*(E186D) mutation affects activation by Gal4p and Gcn4p and causes temperature-sensitivity

(a) Ten-fold serial dilutions of yeast cells expressing the depicted proteins were titrated on glucose and galactose plates containing 1 µg/ml antimycin A (AA) and incubated on the indicated medium at 28 °C for 10 days. (b) Ten-fold serial dilutions of yeast cells expressing the depicted proteins were titrated on the indicated medium and incubated at 28 °C for 10 days. (c) Ten-fold serial dilutions of yeast cells expressing the depicted proteins were titrated on glucose medium and incubated at 28 and 35 °C for 10 days.

based on the protocol found in the Affymetrix GeneChip® probe array user manual. The results of the DNA microarray experiments have been deposited into the ArrayExpress database (accession number E-MEXP-1020).

RESULTS

The transcriptional activators Gal4p and Gcn4p activate the *GAL* and *HIS3* genes in *S. cerevisiae* respectively. Cells lacking *GAL4* failed to grow on galactose medium in the presence of the respiration inhibitor antimycin A (Figure 1a, line 1), and cells lacking *GCN4* failed to grow on histidine-lacking medium in the presence of the competitive inhibitor 3-aminotriazole (Figure 1b, line 1). Cells expressing the Tbp1(E186D)p mutant in place of wild-type Tbp1p failed to grow on galactose medium containing antimycin A (Figure 1a, line 3), indicating that Gal4p failed to activate the *GAL* genes. The *TBP1*(E186D) mutant cells also failed to grow on histidine-lacking medium containing 3-aminotriazole, indicating that Gcn4p failed to activate the *HIS3* gene as well (Figure 1b, line 3). The *TBP1*(E186D) mutant cells also displayed slow-growth and temperature-sensitivity (Figure 1c, line 3). Both Gal4p and Gcn4p were able to activate their respective targeted genes in the presence of the temperature-sensitive *TBP1*(I143N) allele (Figure 1c, line 4), and the cells were able to grow on the respective selection plates (Figures 1a and 1b, line 4).

Using the Split-ubiquitin assay, we had found that the interactions of Tbp1p with the transcription factors Sua7p, Srb4p, Rpb1p, Rpb4p and Rpb8p were affected by the *TBP1*(E186D) mutation [34]. We generated libraries of point mutations in these five proteins using PCR and performed suppressor screens in search of mutants that would allow yeast expressing Tbp1(E186D)p in place of wild-type Tbp1p to grow at the restrictive temperature. The screens resulted in the Sua7p point

mutant E202G that allowed the *TBP1*(E186D) mutant to grow at 35 °C. The *SUA7*(E202G) mutant was isolated independently five times. For the other four Tbp1p-interacting proteins affected by the E186D mutation, no suppressor mutant was isolated.

Figure 2 shows that *SUA7*(E202G) was able to partially suppress the temperature-sensitive phenotype of *TBP1*(E186D) (compare lines 4 and 5). The Figure further shows that the *SUA7*(E202G) mutation was allele-specific for the *TBP1*(E186D) mutation, since *SUA7*(E202G) did not suppress the temperature-sensitive phenotype of *TBP1*(I143N) (compare lines 5 and 7).

SUA7(E202G) had been isolated from the screen for temperature resistance of *TBP1*(E186D), and we asked if the suppressor was able to suppress the gal⁻ phenotype of *TBP1*(E186D) as well. Figure 2 shows that *SUA7*(E202G) was also able to suppress the gal⁻ phenotype of *TBP1*(E186D). *TBP1*(E186D) mutants were unable to grow on galactose plates in the presence of the respiration inhibitor antimycin A (line 11). Yeast cells expressing Sua7(E202G)p and Tbp1(E186D)p were able to utilize galactose for growth (compare lines 11 and 12). Cells carrying the *TBP1*(I143N) mutation did not display a gal⁻ phenotype and hence the growth was not affected on galactose antimycin A plates (line 13). Table 1 shows that transcript levels of *GAL1* mRNA in galactose were reduced approx. 20-fold in yeast cells carrying the *TBP1*(E186D) mutation. Transcription of *GAL1* in galactose was increased by approx. 4-fold by the presence of Sua7(E202G)p. This coincides with Figure 2, where *SUA7*(E202G) had enabled yeast cells carrying the *TBP1*(E186D) mutation to grow on galactose plates in the presence of antimycin A.

The *TBP1*(E186D) mutation had also caused sensitivity to 3-aminotriazole, indicating that Gcn4p failed to activate the *HIS3* promoter. We asked if the *SUA7*(E202G) mutant was able to suppress this phenotype as well. Figure 2 shows that *SUA7*(E202G) was able to restore growth of *TBP1*(E186D) mutants in the presence of 50 mM 3-aminotriazole (compare lines 17 and 18).

Figure 2 *SUA7(E202G)* is an allele-specific suppressor of *TBP1(E186D)*

Ten-fold serial dilutions of yeast cells expressing the depicted proteins were dropped on to the indicated medium and incubated at the respective temperatures for 10 days.

Table 1 *SUA7(E202G)* partially restores transcriptional activation of *GAL1* by Gal4p in the *TBP1(E186D)* mutant background

Yeast cells expressing the indicated proteins were grown in glucose-selective medium (lacking adenine, tryptophan and leucine) to a D_{600} of 1.0 and induced in 2% galactose-selective medium (lacking adenine, tryptophan and leucine) for 1 h. Real-time PCR was performed using *GAL1* ORF and *ACT1* ORF primers. Values shown are *GAL1* mRNA levels normalized to *ACT1* and relative to *GAL1* mRNA levels in the absence of Gal4p. The experiments were performed in duplicate.

Strain	Fold activation
Tbp1p/Sua7p	198 ± 16
Tbp1p/Sua7(E202G)p	169 ± 32
Tbp1(E186D)p/Sua7p	8.5 ± 0.1
Tbp1(E186D)p/Sua7(E202G)p	36.3 ± 1.4

This indicated that *SUA7(E202G)* was able to restore transcriptional activation of both Gal4p and Gcn4p in the *TBP1(E186D)* mutant background.

We used the Split-ubiquitin assay to determine if *Sua7(E202G)p* was able to restore the interaction with *Tbp1(E186D)p*. *Tbp1-C_{ub}-Rura3p* and *Tbp1(E186D)-C_{ub}-Rura3p* were expressed from single-copy vectors under the control of the *CUP1* promoters. Yeast cells expressing *N_{ub}* and *Tbp1-C_{ub}-Rura3p* were able to grow on uracil-depleted medium in the absence of CuSO_4 (Figure 3, line 1). This indicated that the fusion of wild-type *Tbp1p* to *C_{ub}-Rura3p* was stable

and enzymatically active. Yeast cells expressing *N_{ub}-Sua7p* and *Tbp1-C_{ub}-Rura3p* were unable to grow in the absence of uracil (Figure 3, line 2). This indicated that *Sua7p* and *Tbp1p* interacted inside the cell, raising the local concentrations of *N_{ub}* and *C_{ub}*. A native-like ubiquitin was formed and recognized by the UBPs (ubiquitin-specific proteases). The *Rura3* reporter was cleaved off and degraded by the enzymes of the N-end rule, causing uracil auxotrophy.

Yeast cells expressing *Tbp1(E186D)-C_{ub}-Rura3p* displayed only little growth on uracil-depleted medium in the absence of CuSO_4 (results not shown). In the presence of 100 μM CuSO_4 , however, the yeast cells expressing *Tbp1(E186D)-C_{ub}-Rura3p* were able to grow on the uracil-depleted medium to a level comparable with that of yeast cells expressing *Tbp1-C_{ub}-Rura3p* (Figure 3, compare lines 1 and 4). This indicated that the fusion of the mutant *Tbp1(E186D)p* to *C_{ub}-Rura3p* was less stable than the fusion of wild-type *Tbp1p*. Yeast cells expressing *N_{ub}-Sua7p* and *Tbp1(E186D)-C_{ub}-Rura3p* were able to grow on uracil-depleted medium in the presence of 100 μM CuSO_4 (Figure 3, line 5), indicating that the *TBP1(E186D)* mutation affected the interaction with *Sua7p*. Yeast cells expressing *Tbp1(E186D)-C_{ub}-Rura3p* and *N_{ub}-Sua7(E202G)p* were uracil auxotroph, indicating that the *Sua7(E202G)p* mutation restored the protein interaction with *Tbp1(E186D)p* (Figure 3, compare lines 5 and 6).

To confirm the interaction between *Tbp1p* and *Sua7p* *in vitro*, we co-expressed both proteins in yeast cells using different tags. *Tbp1p* was fused to GST, whereas *Sua7p* was fused to *N_{ub}* and an HA tag. Figure 4(a) shows that GST-*Tbp1p* and GST-*Tbp1(I143N)p* were able to complement a *TBP1* deletion

Figure 3 The *SUA7*(E202G) mutation restores the protein interaction with *TBP1*(E186D) *in vivo*

Ten-fold serial dilutions of BY4741 Δ W cells expressing the depicted proteins were titrated on the indicated plates and incubated at 39 °C for 3 days. Yeast cells expressing Tbp1(E186D)-Cub-RUra3p were incubated on a uracil-depleted plate containing 100 μ M CuSO₄ so that the growth of cells expressing Nub and Tbp1(E186D)-Cub-RUra3p was comparable with yeast cells expressing Nub and Tbp1-Cub-RUra3p.

Figure 4 The *SUA7*(E202G) mutation restores the protein levels of *TBP1*(E186D)

(a) Complementation of GST-fusion proteins in NLY2 strain. A yeast strain carrying a chromosomal deletion of *TBP1* and expressing *TBP1* from a *URA3*-marked single-copy vector was transformed with plasmids expressing the depicted proteins. Ten-fold serial dilutions of cells were titrated on glucose medium with and without FOA. The plates were incubated at 28 °C for 7 days. Growth on FOA indicated that the respective GST-Tbp1p fusion was able to complement the chromosomal *TBP1* deletion. (b) GST pull-down and Western blot for the analysis of the *in vitro* protein-protein interaction between Sua7(E202G)p and Tbp1(E186D)p. Yeast cells expressing the depicted proteins were grown in a medium lacking tryptophan and leucine, and GST pull-down assays were performed with cell extracts followed by Western-blot analysis with anti-GST and anti-HA antibodies. (c) Western blot for the analysis of the expression of endogenous Tbp1p and Tbp1(E186D)p. Yeast cells expressing the depicted proteins were grown in medium lacking tryptophan and leucine, and Western-blot analysis was performed with anti-Tbp1p antibodies. As a loading control, all proteins were visualized with Coomassie Brilliant Blue.

(lines 3–4 and 7–8). GST-Tbp1(E186D)p, on the other hand, was only able to complement a *TBP1* deletion in the presence of Nub-Sua7(E202G)p (compare lines 5 and 6). Therefore we performed the GST pull-down with JD52 cells, expressing GST-Tbp1p in addition to endogenous Tbp1p. Figure 4(b) shows that GST-Tbp1p, but not GST, was able to precipitate Nub-Sua7p and Nub-Sua7(E202G)p (compare lanes 2–5). GST-Tbp1(E186D)p was

expressed at lower levels than GST-Tbp1p, and the amount of precipitated Nub-Sua7p was reduced. The co-expression with Nub-Sua7(E202G)p increased protein levels of GST-Tbp1(E186D)p and precipitation of Nub-Sua7(E202G)p was observed (compare lines 6 and 7). GST-Tbp1(I143N)p interacted with Nub-Sua7p like GST-Tbp1p, and the interaction was not affected by the E202G mutation. Figure 4(c) shows that untagged Tbp1(E186D)p

expressed from a single-copy vector under the control of its own promoter was found at lower levels than wild-type Tbp1p as well (compare lanes 1 and 3), and that the Sua7(E202G)p suppressor restored the expression level of Tbp1(E186D)p (compare lanes 3 and 4).

We hypothesized that the temperature-sensitivity and slow-growth phenotype observed in *TBP1*(E186D)-expressing cells might be due to defects in transcription of genes required for good growth and survival. To test this hypothesis, we performed a genome-wide expression analysis using arrays of oligonucleotides.

We compared gene expression in yeast strains carrying the *TBP1* wild-type and the *TBP1*(E186D) mutant alleles by using the yeast genome S98 array from Affymetrix. This array contained approx. 6400 well-characterized genes recognized by SGD (*Saccharomyces* Genome Database) and MIPS (Munich Information Center for Protein Sequences) together with approx. 600 probe sets representing putative ORFs, mitochondria proteins, Ty proteins, ORFs from 2 micron plasmids and ORFs from strains other than S288C. Of the 7145 genes analysed, 481 (6.73 %) were decreased 2-fold or more in cells expressing *TBP1*(E186D) in place of wild-type. Of these 481 genes significantly affected by the *TBP1*(E186D) mutation, 125 were restored back to at least 50 % of wild-type expression level in the presence of *SUA7*(E202G), while not being significantly affected by the *SUA7*(E202G) mutation in the *TBP1*(I143N) background. The top ten genes that were sorted out from our data according to the criteria mentioned above were listed in Supplementary Table S2 (<http://www.BiochemJ.org/bj/406/bj4060265add.htm>).

Supplementary Table S3 (<http://www.BiochemJ.org/bj/406/bj4060265add.htm>) lists several genes of interest for the present study that were not affected by the *TBP1*(E186D) and *SUA7*(E202G) mutations. *ADH1*, which had been used to express the GST–Tbp1p fusions, as well as *CUP1*, which had been used to express the Tbp1–C_{ub}–RUra3p fusions, were left unchanged by the mutations. Supplementary Table S3 further shows that the endogenous *TBP1* promoter and the *ACT1* gene were not affected by the *TBP1*(E186D) or the *SUA7*(E202G) mutations. We conclude that the decrease in Tbp1p protein level caused by the *TBP1*(E186D) mutation was probably due to a decrease in protein stability, and that the *SUA7*(E202G) mutation was able to suppress the *TBP1*(E186D) mutation by stabilizing the mutant Tbp1p protein.

DISCUSSION

We have analysed the protein–protein interactions between Tbp1p and other transcription factors by making use of a Tbp1p mutant. This Tbp1p mutant carries a point mutation at the amino acid position 186. Tbp1(E186D)p has been described previously as causing a severe slow-growth and a gal[−] phenotype [23]. In addition, we found that yeast cells carrying this mutation showed sensitivity to 3-aminotriazole and high temperature. We have made use of the Split-ubiquitin assay and we found that the interactions of Tbp1p with the transcription factors Sua7p, Srb4p, Rpb1p, Rpb4p and Rpb8p were affected by the Tbp1(E186D)p mutation [34]. In the present study, we used libraries of PCR-generated mutations in these five proteins to search for mutants that suppressed the temperature-sensitivity of Tbp1(E186D)p. We isolated a Sua7p (TFIIB) mutant carrying a substitution at the amino acid position 202 from glutamic acid residue to glycine residue. Sua7(E202G)p was isolated as an allele-specific suppressor of the temperature-sensitivity of *TBP1*(E186D). *SUA7*(E202G) also suppressed the slow-growth, gal[−] and 3-aminotriazole-sensitivity of *TBP1*(E186D). Split-

ubiquitin and GST pull-down assays showed that *SUA7*(E202G) restored the interaction between Tbp1p and Sua7p, which was eliminated by the Tbp1(E186D)p mutation.

Glu¹⁸⁶ is one of the three amino acids bridging Tbp1p to Sua7p [23]. Tbp1(E186D)p was found to be capable of binding to the TATA-box, but it was not able to support the entry of Sua7p into the PIC [23]. Sua7(E202G)p suppressed Tbp1(E186D)p, and one possible mechanism of suppression is the restoration of a protein–protein interaction lost by the primary mutation. However, glycine does not have a side chain that could account for this restoration, and the crystal structure of *Arabidopsis thaliana* (thale cress) Tbp1p and human Sua7p [35] shows that the point of mutation in the Sua7p suppressor is not a residue linking Sua7p to Tbp1p. Hence, we assume that Sua7(E202G)p might restore the interaction with Tbp1(E186D)p via an allosteric manner.

The E186D mutation appeared to affect the protein stability of Tbp1p. Contrary to wild-type Tbp1p, the C_{ub}–RUra3p fusion of Tbp1(E186D)p expressed from the *CUP1* promoter was unable to support growth on medium lacking uracil unless the expression of the fusion protein was increased by the addition of CuSO₄. The protein level of the GST–Tbp1p fusion expressed from the *ADH1* promoter was also reduced by the E186D mutation, as shown by Western-blot analysis with an anti-GST antibody. Furthermore, Western-blot analysis with an antibody against endogenous Tbp1p showed that Tbp1(E186D)p protein levels were significantly reduced as well. The mRNA levels of *CUP1*, *ADH1* and *TBP1*, on the other hand, were not affected by the E186D mutation, as revealed by the microarray analysis. Therefore the inability of Tbp1(E186D)p to support activated transcription appeared not only to be due to the inability to recruit Sua7p into PIC, but also due to the lack of availability of Tbp1(E186D)p, making Tbp1p the limiting factor of activated transcription. The microarray analysis showed further that the mRNA level of *TBP1*(E186D) was not affected by the presence of Sua7(E202G)p. This indicated that Sua7(E202G)p suppressed *TBP1*(E186D) by stabilizing Tbp1(E186D)p.

The microarray analysis revealed that yeast cells carrying the *TBP1*(E186D) mutation had significantly reduced transcription of RDN37-1, RDN18-1 and RDN25-1. These ribosomal RNAs are important precursors for the assembly of ribosomal subunits required for protein synthesis of vital proteins. Hence, the phenotypes observed for the *TBP1*(E186D) mutation might have been caused by a reduced level of proteins or enzymes that are vital for good growth or survival. The lack of stability of Tbp1(E186D)p might have caused the reduced transcription of the ribosomal genes. With the stabilization of Tbp1(E186D)p in the presence of Sua7(E202G)p, transcription of the ribosomal genes was restored back to near wild-type level. The suppression of the various phenotypes of the *TBP1*(E186D) mutation by *SUA7*(E202G) coincided with the restoration of Tbp1(E186D)p expression as detected by Western blot.

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