Genes Genet. Syst. (2020) **95**, p. 151–163

The function of Spt3, a subunit of the SAGA complex, in *PGK1* transcription is restored only partially when reintroduced by plasmid into *taf1 spt3* double mutant yeast strains

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(Received 24 January 2020, accepted 16 March 2020; J-STAGE Advance published date: 4 July 2020)

In *Saccharomyces cerevisiae*, class II gene promoters contain two classes of TATA elements: the TATA box and the TATA-like element. Functional loss of TFIID and SAGA transcription complexes selectively impacts steady-state mRNA levels expressed from TATA-like element-containing (i.e., TATA-less) and TATA box-containing promoters, respectively. While nascent RNA analysis has revealed that TFIID and SAGA are required for both types of promoters, the division of their roles remains unclear. We show here that transcription from the *PGK1* promoter decreased in some cases by more than half after disruption of the TATA box or *SPT3* (*spt3*Δ), whereas *spt3*Δ did not affect transcription from the TATA-less promoter, consistent with the prevailing view that Spt3 functions specifically in a TATA box-dependent manner. Transcription from this promoter was abolished in the *spt3*Δ *taf1-N568Δ* strain but unaffected in the *taf1-N568Δ* strain, regardless of TATA box presence, suggesting that TFIID was functionally dispensable for *PGK1* transcription at least in the *SPT3* strain. Furthermore, transcription from the TATA box-containing *PGK1* promoter was slightly reduced in the *taf1* strain lacking TAND (*taf1-ΔTAND*) upon temperature shift from 25 to 37 °C, with restoration to normal levels within 2 h, in an Spt3-dependent manner. Interestingly, when *SPT3* was reintroduced into the *spt3*Δ *TAF1*, *spt3*Δ *taf1-N568Δ* or *spt3*Δ *taf1-ΔTAND* strain, TATA box-dependent transcription from this promoter was largely restored, but TFIID independence in transcription was not restored, especially from the TATA-less promoter, and transient TAND/Spt3-dependent fluctuations of transcription after the temperature shift were also not recapitulated. Collectively, these observations suggest that Spt3 has multiple functions in *PGK1* transcription, some of which may be intimately connected to Taf1 function and may therefore become unrestorable when the TFIID and SAGA functions are simultaneously compromised.

Key words: SAGA, Spt3, Taf1, TFIID, transcriptional regulation

INTRODUCTION

During eukaryotic transcription of class II genes, a set of general transcription factors (GTFs; TFIIA, B, D, E, F and H) assemble at the core promoter region alongside Mediator and RNA polymerase II (Pol II) to form a pre-initiation complex (PIC) that directs accurate transcriptional initiation (Thomas and Chiang, 2006; Hahn and Young, 2011; Sainsbury et al., 2015). There are two types of core promoter in budding yeast. These contain either the TATA box or a TATA-like element (a 1- or 2-bp-mismatched version of the TATA box), the latter of which has been termed the "TATA-less" core promoter (Rhee and Pugh, 2012). TFIID is a large multiprotein complex composed of TATA-binding protein (TBP) and 14 TBP-associated factors (Tafs) (Thomas and Chiang, 2006). TFIID binds to the TATA box or TATA-like element as a first step during PIC assembly and then recruits other factors to the core promoter (Sainsbury et al., 2015; Gupta et al., 2016; Nogales et al., 2017; Bhuiyan

Edited by Hiroshi Iwasaki
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DOI: http://doi.org/10.1266/ggs.20-00004

and Timmers, 2019; Fischer et al., 2019). In previous studies, it was proposed that the Taf1 N-terminal domain (TAND) of TFIID plays an important role in TBP binding to the TATA box or TATA-like element (Kotani et al., 2000; Cheng et al., 2002b; Chitikila et al., 2002; Huisinga and Pugh, 2007; Patel et al., 2018; Kasahara et al., 2019), but the detailed mechanisms remained unelucidated (Gupta et al., 2017; Bhuiyan and Timmers, 2019; Patel et al., 2019; Wei et al., 2019).

SAGA (Spt-Ada-Gcn5-acetyltransferase) is composed of 19 subunits structurally organized into five distinct modules, namely the HAT (histone acetyltransferase), DUB (histone deubiquitinase), TBP-binding, TF (transcription factor)-binding, and core structural modules (Hahn and Young, 2011; Spedale et al., 2012; Han et al., 2014; Helmlinger and Tora, 2017). The core structural module contains five subunits that are identical (Taf5, 6, 9, 10 and 12) and two that are homologous (Spt7 and Ada1) to subunits of TFIID, indicating that SAGA and TFIID are closely related transcription complexes (Helmlinger and Tora, 2017). SAGA may play a similar role to TFIID in delivering TBP to the core promoter via the function of the TBP-binding module comprising Spt3 and Spt8, the former of which is homologous to the Taf11/13 heterodimer of TFIID (Birck et al., 1998; Bhaumik, 2011; Han et al., 2014; Helmlinger and Tora, 2017). Notably, Spt3 and Spt8 of SAGA bind to distinct surfaces of TBP, which are bound by Taf11/13 and TAND of TFIID, respectively (Anandapadamanaban et al., 2013; Han et al., 2014; Patel et al., 2018).

In general, transcription of stress-inducible genes is from TATA box-containing promoters and is dominated by SAGA, while transcription of housekeeping genes is from TATA-less promoters and is dominated by TFIID (Huisinga and Pugh, 2004). This suggests that TFIID and SAGA play distinct roles in transcription according to the presence of a TATA box in the core promoter (Huisinga and Pugh, 2004; Bhaumik, 2011; Rhee and Pugh, 2012; de Jonge et al., 2017). However, more recent studies have demonstrated that TFIID and SAGA are both involved in the transcription of nearly all class II genes (Baptista et al., 2017; Warfield et al., 2017; Fischer et al., 2019), although the effects of their deficiencies on steady-state mRNA levels are obscured by transcript buffering phenomena (Timmers and Tora, 2018).

A recent *in vitro* study showed that while transcription from both TATA box- and TATA-like element-containing (i.e., TATA-less) promoters was impaired by degron-mediated depletion of Taf1 from cell extracts, only the former could be restored by addition of recombinant TBP (Donczew and Hahn, 2017). Furthermore, transcription from TATA-less promoters could not be restored by addition of TBP, even when their TATA-like elements were substituted with TATA boxes (Donczew and Hahn, 2017), indicating that the mechanisms of transcription from these two types of promoters are fundamentally different. These observations also suggest that TATA-less promoters may contain as-yet-uncharacterized core promoter sequence(s) that specifically require TFIID function to promote transcription and/or prevent the promotive function of free TBP or SAGA in the absence of Taf1/TFIID. In higher eukaryotes, there are several well-characterized core promoter sequences other than the TATA box, such as Inr (Initiator), DPE (downstream core promoter element), MTE (motif ten element), and DCE (downstream core element), all of which are recognized by TFIID (Zabidi and Stark, 2016; Nogales et al., 2017; Vo Ngoc et al., 2017, 2019; Patel et al., 2019). Although similar sequences recognizable by TFIID remain unidentified in budding yeast (Hahn and Young, 2011; Lubliner et al., 2015), it was recently shown that yeast TFIID could bind to downstream DNA upon transcriptional initiation, especially on TFIID-dominated promoters, to facilitate re-initiation in an activator-independent manner (Joo et al., 2017). Such interaction of TFIID with downstream DNA, which presumably cannot be mediated by free TBP or SAGA, may play a more important role in transcription from TATA-less promoters than in transcription from TATA-containing promoters.

Consistent with recent observations that SAGA acts as a general cofactor for both TATA box-containing and TATA-less promoters (Baptista et al., 2017; Fischer et al., 2019), we previously showed that SAGA could facilitate transcription from the TATA-containing *AGP1* promoter in a Taf1-independent manner, even when its TATA box was substituted with TATA-like elements (Watanabe and Kokubo, 2017). Furthermore, chimeric promoter mapping analyses using the Taf1-dependent *CYC1* and Taf1-independent *AGP1* promoters demonstrated that while Taf1 dependence or independence was conferred by the upstream activating sequence (UAS), Spt3/SAGA dependence of the latter promoter was conferred by certain specific core promoter sequence(s) other than the TATA box or TATA-like element (Watanabe and Kokubo, 2017). These observations suggested that, like TFIID, SAGA could mediate transcription in a manner dependent on or independent of the TATA box and/or TATA-like element.

Previous research showed that addition of a TATA box abrogated or much reduced the Taf1 dependence of transcription from some promoters (Tsukihashi et al., 2000, 2001; Cheng et al., 2002a). Such observations clearly indicate that the mechanisms underlying TFIID-mediated transcription from TATA box-containing or TATA-less promoters are qualitatively different, at least in the requirement for a particular Taf1 function. To date, no SAGA mutants have been identified that affect transcription differently according to the presence or absence of the TATA box. Thus, it remains unknown whether SAGA can mediate transcription from both types of pro-

moter via the same or different mechanisms.

In this study, we found that introduction of plasmid-borne *SPT3* into *spt3*Δ or *spt3*Δ *taf1* mutant strains largely rescued TATA box-dependent transcription at the *PGK1* promoter, but did not restore TFIID independence in transcription, particularly from a TATA-less *PGK1* promoter. These observations indicate that Spt3/SAGA has a range of distinct functions, only some of which can be restored by reintroduction of *SPT3*, and that, like TFIID, Spt3/SAGA mediates transcription from TATA box-containing and TATA-less promoters by different mechanisms. Furthermore, we also found that TFIID independence of *PGK1* transcription could not be altered simply by changing the core promoter sequences, even though the promoter had the potential to be transcribed in a TFIID-dependent manner. Our observations lead us to propose that some functions of Spt3/SAGA are so closely connected with those of Taf1/TFIID that functional rescue is not possible when the two factors are compromised simultaneously.

MATERIALS AND METHODS

Yeast strains Standard techniques were used for yeast growth and transformation (Amberg et al., 2005). Yeast strains used in this study are listed in Supplementary Table S1. Oligonucleotide sequences used for strain construction are listed in Supplementary Table S2. All strains used in this study were generated from BY4741 or BY4742, as described below.

YTK11406 and YTK11411, both of which carry a deletion of the chromosomal *TAF1* coding region and the wild type *TAF1* gene in a *URA3*-based low copy vector (pYN1), were described previously (Ohyama et al., 2010).

To create YTK13834, two sub-fragments containing *His3MX6* (Longtine et al., 1998) (primers: TK3755 and TK3756; template: pFA6a-*His3MX6*; hereafter abbreviated as TK3755-TK3756/pFA6a-*His3MX6*) and the *PGK1* promoter [–598 to –1 bp] (TK11908-TK11909/genomic DNA of BY4741) were first amplified by PCR using the primer pair/template (genomic DNA or plasmid) combinations described above in parentheses, and then were PCR-fused using primers TK3755 and TK11909 to generate a 2-kb fragment that was used for transformation of BY4741. YTK11406 and YTK13834 were crossed and dissected to generate YTK13835.

To create YTK13837, two sub-fragments containing *His3MX6* + *PGK1* promoter [–598 to –147 bp] (TK3755-TK11659/genomic DNA of YTK13835) and the *PGK1* promoter [–170 to –1 bp] (TK11790-TK11909/genomic DNA of YTK13835) were first amplified by PCR using the primer pair/template combinations described above in parentheses, and then were PCR-fused using primers TK3755 and TK11909 to generate a 2-kb fragment that was used for transformation of YTK11411. YTK13839/16941/16943 and YTK13842/16945/16947 were generated from YTK13835 and YTK13837 by replacing pYN1 (*TAF1*/*URA3*) with pM7118 (*TAF1*/pRS315), pM7119 (*taf1-ΔTAND*/pRS315) and pM7188 (*taf1-N568Δ*/pRS315), respectively.

YTK11705 was described previously (Ohyama et al., 2010). YTK11705 and #4228 (obtained from Euroscarf) were crossed and dissected to generate YTK11870. YTK13834 and YTK11870 were crossed and dissected to generate YTK16787. YTK13837 and YTK11870 were crossed and dissected to generate YTK16791. YTK16805 was generated from YTK16787 by replacing pM4770 (*TAF1*/pRS313) with pYN1 (*TAF1*/*URA3*). YTK16825/16829/16827 and YTK16799/16803/16801 were generated from YTK16805 and YTK16791 by replacing pYN1 (*TAF1*/*URA3*) with pM7118 (*TAF1*/pRS315), pM7119 (*taf1-ΔTAND*/pRS315) and pM7188 (*taf1-N568Δ*/pRS315), respectively.

YTK16983/16969/16971/16987/16979 were generated from YTK13839/16941/16943/13842/16947, respectively, by transformation with pRS426. YTK17069/17075/17073/17079/17071/17077 and YTK16843/16831/16851/16839/16847/16835 were generated from YTK16825/16799/16829/16803/16827/16801 by transformation with pM7935 (*SPT3*/pRS426) and pRS426, respectively. YTK20353/20354/20355/20356 were generated from YTK16825/16827/16799/16801, respectively, by transformation with pM7936 (*SPT3*/pRS316).

To create YTK18606, two sub-fragments containing *VTC1* [–170 to –61 bp]+*His3MX6*+ *PGK1* promoter [–598 to –132 bp] (TK2496-TK13289/YTK13835) and the *PGK1* promoter [–141 to –1 bp]+*VTC1* [+1 to +305 bp] (TK13288-TK8263/YTK13835) were first amplified by PCR using the primer pair/template combinations described above in parentheses, and then PCR-fused using primers TK2496 and TK8263 to generate a 2.5-kb fragment that was used for transformation of YTK11411.

To create YTK18608, two sub-fragments containing *VTC1* [–170 to –61 bp]+*His3MX6*+ *PGK1* promoter [–598 to –110 bp] (TK2496-TK13286/YTK13835) and the *PGK1* promoter [–120 to –1 bp]+*VTC1* [+1 to +305 bp] (TK13287-TK8263/YTK13835) were first amplified by PCR using the primer pair/template combinations described above in parentheses, and then were PCR-fused using primers TK2496 and TK8263 to generate a 2.5-kb fragment that was used for transformation of YTK11411.

To create YTK18610, two sub-fragments containing *VTC1* [–170 to –61 bp]+*His3MX6*+ *PGK1* promoter [–598 to –131 bp] (TK2496-TK13730/YTK13835) and the *PGK1* promoter [–141 to –1 bp]+*VTC1* [+1 to +305 bp] (TK13291-TK8263/YTK18616) were first amplified by PCR using the primer pair/template combinations described above in parentheses, and then were PCR-fused using primers TK2496 and TK8263 to generate a 2.5-kb fragment that was used for transformation of YTK11411.

To create YTK18612, two sub-fragments containing *VTC1* [–170 to –61 bp]+*His3MX6*+ *PGK1* promoter [–598 to –110 bp] (TK2496-TK13286/YTK13837) and the *PGK1* promoter [–120 to –1 bp]+*VTC1* [+1 to +305 bp] (TK13287-TK8263/YTK13837) were first amplified by PCR using the primer pair/template combinations described above in parentheses, and then were PCR-fused using primers TK2496 and TK8263 to generate a 2.5-kb fragment that was used for transformation of YTK11411.

To create YTK18614, two sub-fragments containing *VTC1* [–170 to –61 bp]+*His3MX6*+ *PGK1* promoter [–598 to –132 bp] (TK2496-TK13731/YTK13837) and the *PGK1* promoter [–141 to –1 bp]+*VTC1* [+1 to +305 bp] (TK13288-TK8263/YTK13837) were first amplified by PCR using the primer pair/template combinations described above in parentheses, and then were PCR-fused using primers TK2496 and TK8263 to generate a 2.5-kb fragment that was used for transformation of YTK11411.

To create YTK18616, two sub-fragments containing *VTC1* [–170 to –61 bp]+*His3MX6*+ *PGK1* promoter [–598 to –131 bp] (TK2496-TK13290/YTK13837) and the *PGK1* promoter [–141 to –1 bp]+*VTC1* [+1 to +305 bp] (TK13291-TK8263/YTK13837) were first amplified by PCR using the primer pair/template combinations described above in parentheses, and then were PCR-fused using primers TK2496 and TK8263 to generate a 2.5-kb fragment that was used for transformation of YTK11411.

To create YTK18393/18397/18401/18405/18409/18413, 2.5-kb fragments containing *VTC1* [–170 to –61 bp]+*His3MX6*+*PGK1* promoter [–598 to –1 bp]+*VTC1* [+1 to +305 bp] were amplified from genomic DNA of YTK18606/18608/18610/18612/18614/18616 using primers TK2496 and TK8263, and used for transformation of YTK20346, a yeast strain derived from YTK11870 that carries pYN1 (*TAF1*/*URA3*) instead of pM4770 (*TAF1*/pRS313).

YTK16989/16995/18662/18664/18666/18668/18670/18672 and YTK17025/17031/18519/18523/18527/18531/18535/18539 were generated from YTK13835/13837/18606/18608/18610/18612/18614/18616 and YTK16805/16791/18393/18397/18401/18405/18409/18413, respectively, by replacing pYN1 (*TAF1*/*URA3*) with pM4201 (HA-tagged *TAF1*/pRS315).

YTK16991/16997/19046/19047/19042/19048/19049/19050 and YTK17027/17033/19051/19052/19044/19053/19054/19055 were generated from YTK13835/13837/18606/18608/18610/18612/18614/18616 and YTK16805/16791/18393/18397/18401/18405/18409/18413, respectively, by replacing pYN1 (*TAF1*/*URA3*) with pM4202 (HA-tagged *taf1-ΔTAND*/pRS315).

YTK16993/16999/18678/18680/18682/18684/18686/18688 and YTK17029/17035/18521/18525/18529/18533/18537/18541 were generated from YTK13835/13837/18606/18608/18610/18612/18614/18616 and YTK16805/16791/18393/18397/18401/18405/18409/18413, respectively, by replacing pYN1 (*TAF1*/*URA3*) with pM8201 (HA-tagged *taf1-N568Δ*/pRS315).

YTK20133/20134/20135 and YTK20136/20137/20138 were generated from YTK18610 and YTK18401 by replacing pYN1 (*TAF1*/*URA3*) with pM7118 (*TAF1*/pRS315), pM7119 (*taf1-ΔTAND*/pRS315) and pM7188 (*taf1-N568Δ*/pRS315), respectively.

Construction of plasmids Plasmids pYN1, pM1001, pM1169, pM1746, pM7118 and pM7119 were described previously (Kokubo et al., 1998; Takahata et al., 2003; Takahashi et al., 2009; Ohyama et al., 2010; Anandapadamanaban et al., 2013; Watanabe et al., 2015). Plasmid pM7118 (*TAF1*/pRS315) was subjected to site-specific mutagenesis (Kunkel et al., 1987) using the oligonucleotide TK176 (Tsukihashi et al., 2000) to generate pM7188 (*taf1-N568Δ*/pRS315). Plasmids pM4201 (HA-tagged *TAF1*/pRS315), pM4202 (HA-tagged *taf1-ΔTAND*/pRS315) and pM8201 (HA-tagged *taf1-N568Δ*/pRS315) were constructed by ligating NotI/PstI DNA fragments from pM1169, pM1001 and pM1746 into NotI/PstI sites of pRS315.

A 1.9-kb DNA fragment containing *SPT3* was amplified by PCR from genomic DNA of BY4741 using primers TK13229 and TK13214, and then was ligated into EcoRI/SpeI sites of pRS426 and pRS316, generating pM7935 (*SPT3*/pRS426) and pM7936 (*SPT3*/pRS316), respectively.

Northern analysis Northern analysis was performed as described previously (Tsukihashi et al., 2000). Briefly, total RNA (10–20 μg) was isolated from the indicated strains grown in synthetic complete (SC) medium to log-phase at 25 °C or after continued incubation at 37 °C for the indicated time periods. Total RNA was subjected to electrophoresis, blotted onto a nylon membrane, and hybridized with gene-specific probes.

For detection of *PGK1*, *VTC1* and *SCR1*, DNA fragments were amplified by PCR from genomic DNA of BY4741, purified, and ^{32}P-labeled by random priming with the Klenow fragment. The PCR primer pairs used were as follows: *PGK1*, TK1224 and TK1225; *VTC1*, TK9030 and TK9013; and *SCR1*, TK9507 and TK10081.

Immunoblot analysis Immunoblot analysis was conducted as described previously (Ohyama et al., 2010). Lysates containing 30 μg protein were resolved by SDS-PAGE, and transferred to a PVDF membrane. The blots were developed using a commercial kit (ChemiLumiOne, Nacalai Tesque) and quantified using a chemiluminescence image analyzer (ImageQuant LAS 4000 mini) and Multi Gauge software (Fujifilm). Polyclonal antibodies directed against Spt3 (amino acids (aa) 1–200) and Taf1 (aa 288–489) were described previously (Kotani et al., 2000; Takahata et al., 2004). An antibody against Tub1 (YOL1/34) was purchased from Santa Cruz

Biotechnology.

Primer extension analysis Primer extension analysis was performed as described previously (Watanabe et al., 2015). The primer used was TK8251 (+40 to +60 of *VTC1*). The cDNA products were analyzed on a 6% polyacrylamide DNA sequencing gel. Gels were exposed to imaging plates for visualization (Typhoon FLA 7000, GE Healthcare Life Sciences), scanning, and quantification of electrophoretic images (ImageQuant TL software version 8.1, GE Healthcare Life Sciences).

RESULTS

TFIID dependence of *PGK1* transcription is reliant upon the chromosomal or plasmid location of *SPT3* The *PGK1* promoter is one of the most extensively characterized promoters in *S. cerevisiae* (Chambers et al., 1989; Henry et al., 1994; Ogden et al., 1986; Rathjen and Mellor, 1990). Although the *PGK1* promoter contains a TATA box (TATATATAAA) located at –153 to –144, and a TATA-like element (TACATATT) located at –116 to –109, their roles in transcription remain unclear (Ogden et al., 1986; Rathjen and Mellor, 1990). Previously, *PGK1* transcription was decreased by inactivation of SAGA but not TFIID (Li et al., 2000; Huisinga and Pugh, 2004; Layer et al., 2010). Nevertheless, TFIID was detected at the *PGK1* promoter at 25 °C (Venters et al., 2011), and occupancy increased after heat shock (Kuras et al., 2000), suggesting that TFIID might yet be involved in *PGK1* transcription. On this basis, we reasoned that the kinetics of *PGK1* transcription after heat shock might be affected by loss of TFIID function.

During the preliminary stage of this work, we observed that the effects of *taf1* mutations on *PGK1* transcription differed between two distinct types of *taf1* strain: those carrying *SPT3* at the native locus and those with chromosomal *spt3*Δ complemented with plasmid-borne *SPT3*. We therefore sought to examine the detailed kinetics of *PGK1* transcription after temperature shift from 25 °C to 37 °C, a procedure which inactivates TFIID function in the temperature-sensitive *taf1-ΔTAND* and *taf1-N568Δ* strains carrying *SPT3* either on the chromosome or on the plasmid. In addition, a *VTC1* reporter system carried by the chromosome (Watanabe et al., 2015) was used to test the importance of the TATA box for *PGK1* transcription (Supplementary Fig. S1).

Consistent with previous results (Tsukihashi et al., 2000; Huisinga and Pugh, 2004; Takahata et al., 2004; Layer et al., 2010), endogenous *PGK1* transcription decreased markedly with the *spt3*Δ mutation ([b, d] in Fig. 1A, 1C) but not with the *taf1-ΔTAND* ([e, g] in Fig. 1A, 1C) or *taf1-N568Δ* ([i, k] in Fig. 1A, 1C) mutations, even 2 h after the temperature shift. Notably, *PGK1* mRNA levels decreased or increased transiently during the initial phase of the 2-h period following the temperature shift in the *taf1-ΔTAND* ([e, g] in Fig. 1A, 1C) or *taf1-N568Δ* ([i, k] in Fig. 1A, 1C) strains, respectively. Combination of the *spt3*Δ mutation with these *taf1* mutations both abolished the transient mRNA level fluctuations and largely conferred Taf1 dependence on *PGK1* transcription ([f, h, j, l] in Fig. 1A, 1C).

Levels of *VTC1* mRNA gradually decreased after the temperature shift even in the wild-type strain ([m] in Fig. 1A, 1C), indicating that this reporter was more heat-sensitive than the endogenous system, for unknown reasons. Accordingly, the transient increase of *PGK1* transcription in the *taf1-N568Δ* strain became unobservable ([u] in Fig. 1A, 1C), but its transient decrease in the *taf1-ΔTAND* strain was more apparent ([q] in Fig. 1A, 1C). Disruption of the TATA box by substitution with the GAGA sequence (see the Fig. 3A legend) decreased *PGK1* transcription to levels similar to those observed for *spt3*Δ in the *TAF1* strain (compare [o] and [n] in Fig. 1A, 1C). No additional decrease in *PGK1* transcription was observed with the disrupted TATA box in the *spt3*Δ *TAF1* strain (compare [n] and [p] in Fig. 1A, 1C), indicating that Spt3 functions in a TATA box-dependent manner. By contrast, while TATA disruption did not directly affect the fluctuating *PGK1* transcription profile (transient Spt3-dependent decrease and subsequent recovery) after the temperature shift in the *taf1-ΔTAND* strain ([q, s] in Fig. 1A, 1C), substantial further decreases in mRNA levels were observed in the *spt3*Δ *taf1-ΔTAND* strain (compare [r] and [t] in Fig. 1A, 1C), suggesting that the *taf1-ΔTAND* mutation rendered the Spt3 function less dependent on the TATA box.

Next, similar experiments were conducted using strains carrying *spt3*Δ on the chromosome and *SPT3* on a high-copy plasmid (*spt3*Δ + *SPT3*), or those containing an empty vector (*spt3*Δ + empty) (Fig. 1B, 1D). Transient fluctuations in mRNA level after the temperature shift were not observed with *PGK1* ([e, g] in Fig. 1B, 1D), and were much diminished with *VTC1* ([q, s] in Fig. 1B, 1D) in the *taf1-ΔTAND* strain, indicating that Spt3 expressed from the plasmid is not fully functional. Consistent with this, *PGK1* mRNA levels transiently increased but then decreased more rapidly during the later phase of the 2-h period following the temperature shift in the *taf1-N568Δ* strain ([i, k] in Fig. 1B, 1D), suggesting that Spt3 expressed from the plasmid could support initial increases in *PGK1* transcription but could not support transcription during the later phase in this particular *taf1* strain. This was supported by transcription results for *VTC1* mRNA, particularly when the TATA box of the *PGK1* promoter was disrupted ([w] in Fig. 1B, 1D). By contrast, a stimulatory function of Spt3 for TATA box-dependent transcription was observed even when expressed from the plasmid ([m, n] in Fig. 1B, 1D). Notably, *spt3*Δ strains carrying *SPT3* on a low-copy plasmid yielded similar results to *spt3*Δ

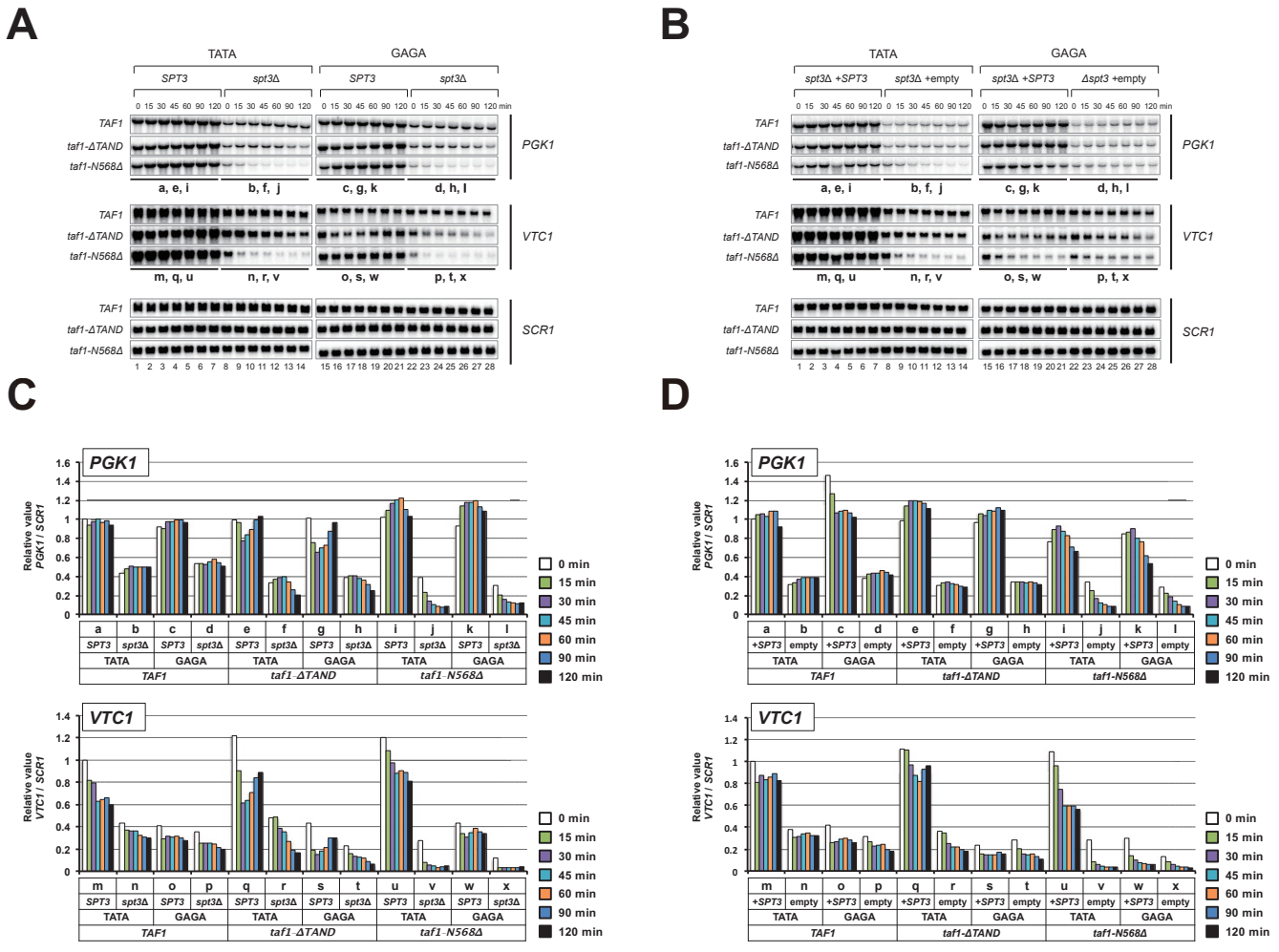


Fig. 1. Effect of *taf1* and *spt3*Δ mutations on the expression kinetics of the *PGK1* promoter after temperature shift from 25 °C to 37 °C, monitored using the *VTC1* reporter system. (A) Northern analysis of expression of *VTC1* (m, q, u, n, r, v, o, s, w, p, t, x in the middle panels), *PGK1* (a, e, i, b, f, j, c, g, k, d, h, l in the top panels) or *SCR1* (control; lanes 1–28 in the bottom panels) in 12 strains carrying the *VTC1* reporter driven by the *PGK1* promoter in which the TATA box (TATA) was intact or substituted with the GAGA sequence (GAGA) (see the Fig. 3A legend), as indicated in (C). In these strains, chromosomal *TAF1* was deleted and then substituted with *TAF1* (m, n, o, p), *taf1-ΔTAND* (q, r, s, t) or *taf1-N568Δ* (u, v, w, x) on a plasmid, as indicated in (C). Chromosomal *SPT3* was intact (m, o, q, s, u, w) or deleted (n, p, r, t, v, x), as indicated in (C). These strains were grown at 25 °C (0 min) and then further incubated at 37 °C for the time period indicated above the blot image (15, 30, 45, 60, 90, 120 min) in synthetic medium containing 2% glucose. (B) Northern analysis of expression of *VTC1* (m, q, u, n, r, v, o, s, w, p, t, x in the middle panels), *PGK1* (a, e, i, b, f, j, c, g, k, d, h, l in the top panels), or *SCR1* (control; lanes 1–28 in the bottom panels) in 12 strains carrying the *VTC1* reporter driven by the *PGK1* promoter in which the TATA box (TATA) was intact or substituted with the GAGA sequence (GAGA), as indicated in (D). In these strains, chromosomal *TAF1* was deleted and then substituted with *TAF1* (m, n, o, p), *taf1-ΔTAND* (q, r, s, t) or *taf1-N568Δ* (u, v, w, x) on the plasmid, as indicated in (D). Chromosomal *SPT3* was deleted and strains were transformed with either *SPT3*-containing (m, o, q, s, u, w) or empty (n, p, r, t, v, x) plasmid, as indicated in (D). Transformations were performed in strains in which chromosomal *TAF1* was substituted with *TAF1* or *taf1* on the plasmid. These strains were incubated as described in (A). (C) Raw expression data shown in (A) quantified and normalized to *SCR1*. Values for each transcript derived from *PGK1* or *VTC1* are summarized in the upper or lower panel, respectively. In each panel, data are presented relative to the value obtained for the strain indicated at the left-hand side. (D) Raw data shown in (B) and summarized as indicated in (C).

strains carrying *SPT3* on a high-copy plasmid (Supplementary Fig. S2), suggesting that such partial loss of Spt3 function was not due to differences in Spt3 expression levels, which varied over a wide range (Supplementary Fig. S3). Taken together, these results indicate that Spt3 performs multiple functions in the mediation of *PGK1* transcription, only some of which could be restored when Spt3 was reintroduced into *spt3*Δ *taf1* strains by plasmid expression.

The effects of plasmid-borne *SPT3* on the growth of *spt3*Δ *taf1-ΔTAND* and *spt3*Δ *taf1-N568Δ* strains In a previous study, *taf1-ts2* and *spt3*Δ mutants were found to display mild synthetic growth defects when cultured at

33 °C on SC medium (van Oevelen et al., 2005). Here, we examined whether *taf1-ΔTAND* and *taf1-N568Δ* mutants would display similar growth defects in the *spt3*Δ background. Strains were cultured for 3 days at 25, 37 and 38 °C on SC medium (Supplementary Fig. S4). Alone, the *spt3*Δ mutation resulted in a mild slow growth defect (compare lanes 1 and 4 in Supplementary Fig. S4). Severe growth defects were observed for *spt3*Δ in combination with *taf1-N568Δ* (compare lanes 3 and 6 at 25 °C in Supplementary Fig. S4) but not with *taf1-ΔTAND* (compare lanes 2 and 5 in Supplementary Fig. S4). Consistent with the results shown in Fig. 1, the introduction of plasmid-borne *SPT3* into the *spt3*Δ *taf1-N568Δ* strain only partially restored the synthetic growth defects (compare lanes 3, 6 and 9 in Supplementary Fig. S4). In addition, introduction of plasmid-borne *SPT3* into the *spt3*Δ *taf1-ΔTAND* strain was able to suppress, albeit weakly, the temperature-sensitive growth defects associated with *taf1-ΔTAND* (compare lanes 2, 5 and 8 at 37 or 38 °C in Supplementary Fig. S4). This observation suggested that ectopically expressed Spt3 was able to substitute, at least in part, for the loss of TAND at higher temperatures, even though the two molecules did not appear to be functionally redundant at 25 °C. Therefore, the suppressive effect of Spt3 on growth could explain the aforementioned reduced *PGK1* transcription fluctuations observed after the temperature shift in the *taf1-ΔTAND* (*spt3*Δ + *SPT3*) strain ([e, g, q, s] in Fig. 1B, 1D). Taken together, these results indicate that plasmid-borne *SPT3* could only partially compensate for the lack of Spt3, at least in the *spt3*Δ *taf1-N568Δ* strain, with respect to both *PGK1* transcription (Fig. 1) and cell growth (Supplementary Fig. S4).

Transcription start sites of the *PGK1* promoter are not altered by disruption of the TATA box or plasmid-borne *SPT3* Previous research showed that *PGK1* mRNA levels were not affected by deletion of either or both of the TATA box (TATATATAAA, labeled as #1 in Fig. 3A) and the TATA-like element (TACATATT, labeled as #3 in Fig. 3A) (Ogden et al., 1986; Rathjen and Mellor, 1990). Furthermore, one of these previous studies showed that deletion of both these elements caused a downstream shift in the transcription start sites (TSSs) of the *PGK1* promoter (Rathjen and Mellor, 1990). By contrast, we observed that *VTC1* mRNA levels were significantly reduced by disruption of the TATA box ([m, o] in Fig. 1A–1D). The reasons for this discrepancy are unclear, but one possibility is the difference in copy number of the reporter gene, which was placed on a multi-copy plasmid in earlier research (Ogden et al., 1986; Rathjen and Mellor, 1990) but integrated into the chromosome in this study.

We next conducted primer extension analyses to explore the possibility that disruption of the TATA box or introduction of plasmid-borne *SPT3* causes a TSS shift at the

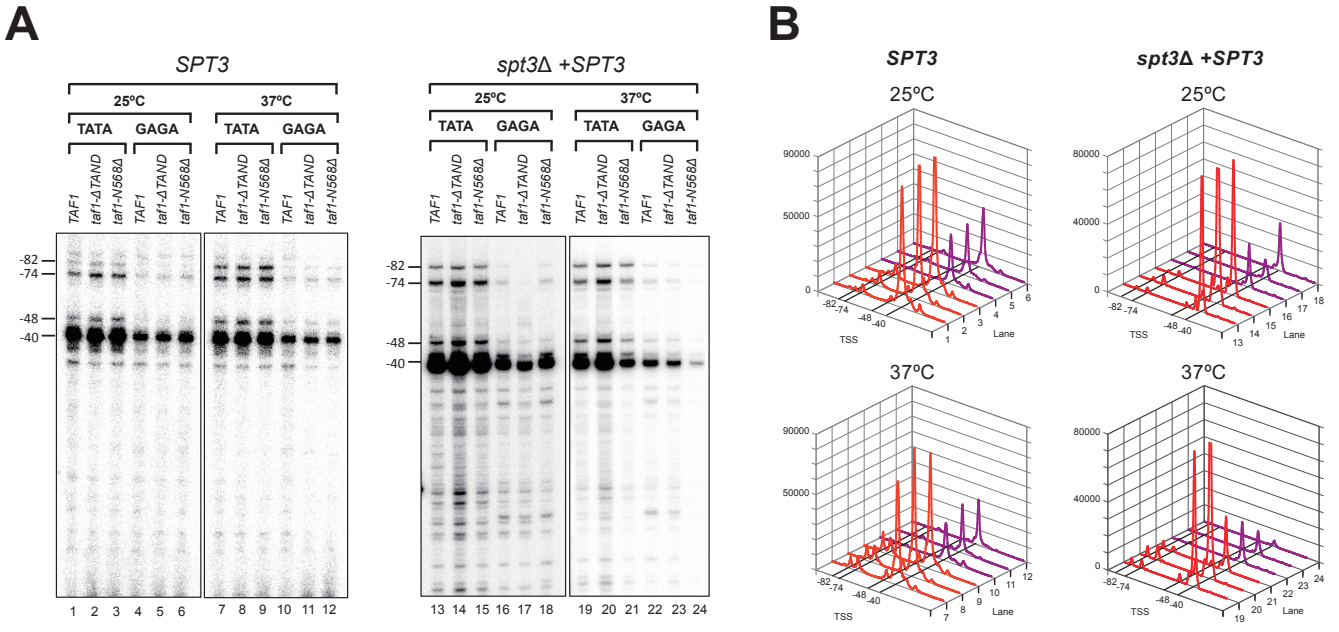
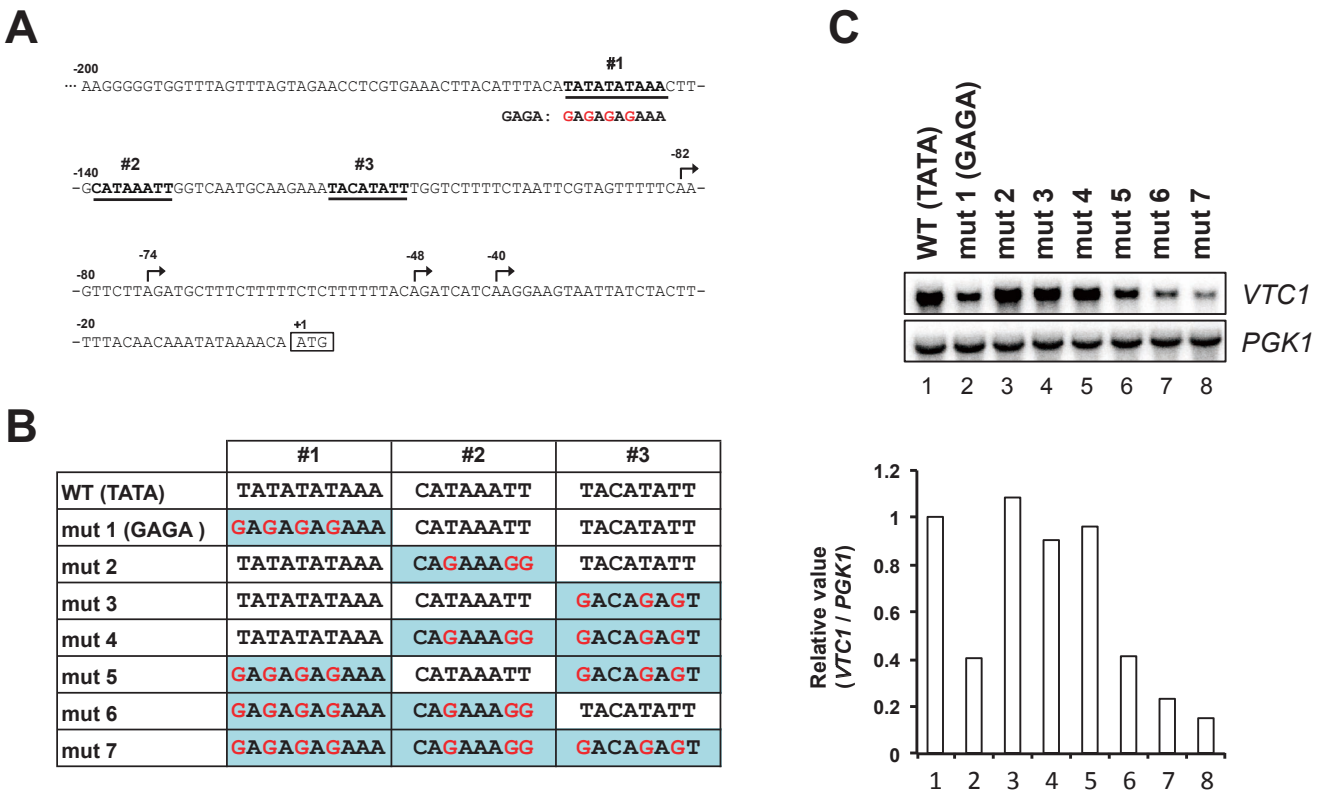


Fig. 2. Determination of transcriptional start site(s) (TSSs) of the *VTC1* reporter in the yeast strains used in Fig. 1 when cultured at 25 °C or 37 °C. (A) Primer extension analysis was performed to determine the TSSs of the *VTC1* reporter in the strains used in Fig. 1. Total RNA (20 μg) was isolated from strains that had been cultured at 25 °C (lanes 1–6 and 13–18) and further incubated at 37 °C for 2 h (lanes 7–12 and 19–24) in synthetic medium containing 2% glucose. The positions of TSSs are indicated at the left. Note that A of ATG is set as +1 in this figure. (B) Graphical summary of TSSs determined in (A). Each lane of the electropherograms shown in (A) was scanned, quantified by densitometry (ImageQuant TL software version 8.1, GE Healthcare), and is presented graphically.



	#1	#2	#3
WT (TATA)	TATATATAAA	CATAAATT	TACATATT
mut 1 (GAGA)	GAGAGAGAAA	CATAAATT	TACATATT
mut 2	TATATATAAA	CAGAAAGG	TACATATT
mut 3	TATATATAAA	CATAAATT	GACAGAGT
mut 4	TATATATAAA	CAGAAAGG	GACAGAGT
mut 5	GAGAGAGAAA	CATAAATT	GACAGAGT
mut 6	GAGAGAGAAA	CAGAAAGG	TACATATT
mut 7	GAGAGAGAAA	CAGAAAGG	GACAGAGT

Fig. 3. Characterization of the TATA box and TATA-like element(s) of the *PGK1* promoter using the *VTC1* reporter system. (A) Schematic diagram of the *PGK1* promoter. The positions of the three CE candidates are underlined and marked as #1 (TATATATAAA), #2 (CATAAATT) and #3 (TACATATT). Sequences #1 and #3 were described previously as CE (Ogden et al., 1986; Rathjen and Mellor, 1990). The TATA box (#1) was substituted with a GAGAGAGAAA sequence denoted as "GAGA" in Fig. 1. The black arrows above the sequence indicate the TSSs determined in Fig. 2. The initiation codon of *PGK1* is boxed, with A of ATG as +1. (B) Summary table of the promoter constructs tested in (C), harboring different combinations of the three CE sequences #1–#3. (C) Northern analysis of *VTC1* and *PGK1* expression in a wild-type (*TAF1 SPT3*) strain in which the *VTC1* reporter is driven by one of the series of promoter constructs depicted in (B). Strains were grown at 25 °C in synthetic medium containing 2% glucose. Raw expression data shown in the upper panel were quantified and are presented graphically in the lower panel, as described in Fig. 1B. Note that values for the *VTC1* transcript were normalized to *PGK1* here (instead of *SCR1*) and are presented relative to the value obtained for WT (TATA) (lane 1).

PGK1 promoter (Fig. 2A, 2B). The TSS profiles observed for the *SPT3* and [*spt3*Δ + *SPT3*] strains were similar, irrespective of the presence of the TATA box, with the exception that mRNA levels were lower with the *taf1-N568Δ* mutation at 37 °C in the latter strain (compare lanes 9, 12, 21 and 24 in Fig. 2A, 2B). This suggested that neither the function of the TATA box nor those specifically lost for plasmid-borne *SPT3* (Fig. 1) were required for accurate transcription initiation at TSSs of this promoter.

CATAAATT is a functional TATA-like element of the *PGK1* promoter Disruption of the TATA box decreased the levels of *PGK1* and/or *VTC1* mRNA, by more than half in some cases (Fig. 1A–1D and Fig. 2A, 2B), but a substantial proportion of transcription was unaffected. This suggested that a core promoter element(s) (CE) other than the TATA box was present and supported transcription from this promoter. Previously, multiple AT-rich sequences were shown to function as a CE in the *RPS5* promoter (Sugihara et al., 2011), and we therefore sought to determine whether two TATA-like elements, CATAAATT (#2 in Fig. 3A) and TACATATT (#3 in Fig. 3A) (Ogden et al., 1986; Rathjen and Mellor, 1990), could function as CEs in this promoter. Northern analysis was used to compare the levels of *VTC1* mRNA derived from *PGK1* promoters bearing combinations of the intact or mutated #1–3 sequences (Fig. 3B, 3C). The results showed that the two TATA-like elements (#2 and #3) were not functional in the presence of the intact TATA box (#1) (WT and mut 2–4 in Fig. 3C). However, in the presence of a mutated TATA box (#1), the CATAAATT (#2) sequence became functional (compare mut 1/lane 2 and mut 6/lane 7 in Fig. 3C), whereas the TACATATT (#3) sequence remained nonfunctional (compare mut 1/lane 2 and mut 5/lane 6 in Fig. 3C). Furthermore, when both the TATA box (#1) and CATAAATT (#2) sequence were mutated, the TACATATT (#3) sequence became

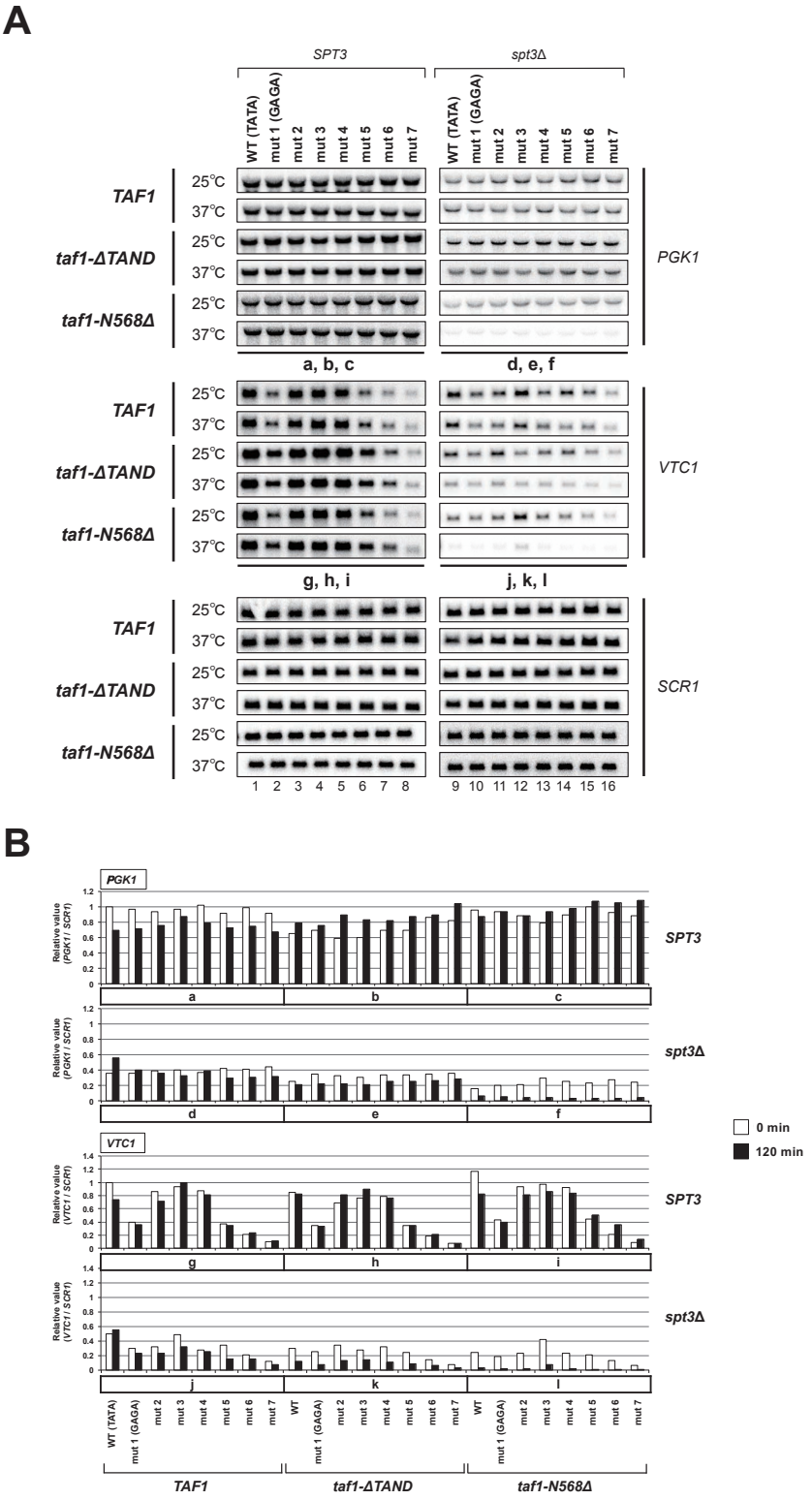


Fig. 4. Effect of changing the combinations of CEs on the Taf1 independence of *PGK1* transcription. (A) Northern analysis of expression of *VTC1* (g, h, i, j, k, l in the middle panels), *PGK1* (a, b, c, d, e, f in the top panels) or *SCR1* (control; lanes 1–16 in the bottom panels) in the strains used in Fig. 1A/1C (i.e., WT [TATA] and mut 1 [GAGA]), as well as those similarly constructed as in Fig. 1A/1C but harboring different promoter constructs, i.e., mut 2–7, as indicated above the blot images. Strains were grown at 25 °C and then further incubated at 37 °C for 2 h in synthetic medium containing 2% glucose. (B) Raw expression data shown in (A) were quantified and are presented graphically, as described in Fig. 1. Values for each transcript derived from *PGK1* and *VTC1* are presented relative to the value obtained for the *SPT3* strain indicated at the left-hand side.

functional (compare mut 6/lane 7 and mut 7/lane 8 in Fig. 3C). Therefore, we concluded that the three sequences (#1–3) functioned as CEs in the *PGK1* promoter only when located at the most upstream position among the potential CE sequences.

TFIID dependence or independence of *PGK1* transcription cannot be altered by changing the combinations of CE sequences *PGK1* transcription became TFIID-dependent when *SPT3* was introduced into the *spt3Δ taf1-N568Δ* strain on a plasmid (e.g., compare [w] in Fig. 1C, 1D), suggesting that this promoter had the potential to be transcribed in a TFIID-dependent manner. We suspected that TFIID dependence could be conferred on *PGK1* transcription by changing the combinations of CE sequences. To explore this possibility, the promoter activities of eight constructs, WT and mut 1–7 (Fig. 3B, 3C), were examined in the *TAF1* ([g] in Fig. 4A, 4B), *taf1-ΔTAND* ([h] in Fig. 4A, 4B), *taf1-N568* ([i] in Fig. 4A, 4B), *spt3Δ* ([j] in Fig. 4A, 4B), *spt3Δ taf1-ΔTAND* ([k] in Fig. 4A, 4B) and *spt3Δ taf1-N568Δ* ([l] in Fig. 4A, 4B) strains. Cells were harvested at 0 or 2 h after the temperature shift from 25 °C to 37 °C. None of the promoters exhibited TFIID dependence for *PGK1* transcription while chromosomal *SPT3* was intact ([g, h, i] in Fig. 4A, 4B). As expected, TFIID dependence was observed in the absence of chromosomal *SPT3* ([j, k, l] in Fig. 4A, 4B). Furthermore, the TATA box-specific stimulatory function of Spt3 was not affected by the absence of the CATAAATT (#2) and/or TACATATT (#3) sequences (WT and mut 2–4 in Fig. 4B). Together, these observations indicate that CE sequences cannot by themselves govern the TFIID dependence or independence of this promoter.

Transient fluctuation of *PGK1* transcription in the *taf1-ΔTAND* strain occurs independently of the TATA box and two TATA-like elements Transient fluctuation of *VTC1* mRNA levels was observed after the temperature shift in the *taf1-ΔTAND* strain, irrespective of the presence or absence of the TATA box (#1) ([q, s] in Fig. 1C). To explore whether the fluctuation was enabled by the presence of the two TATA-like elements (#2 and #3) in the promoter, Northern analysis was used to measure the accumulation kinetics of *VTC1* mRNA expressed from the "mut 4" promoter (Fig. 3B), which contained the TATA box (#1) but lacked the two additional TATA-like elements (#2 and #3), in the *TAF1*, *taf1-ΔTAND*, *taf1-N568Δ*, *spt3Δ*, *spt3Δ taf1-ΔTAND* and *spt3Δ taf1-N568Δ* strains (Supplementary Fig. S5A, S5B). Similar transient fluctuation was observed for the "mut 4"-derived *VTC1* mRNA specifically in the *taf1-ΔTAND* strain ([i] in Supplementary Fig. S5B), indicating that the fluctuation occurred independently of the two TATA-like elements (#2 and #3), as well as of the TATA box (#1). As mentioned previously, the *VTC1* reporter system was more heat-sensitive ([m], [u] in Fig. 1C) than the endogenous system ([a], [i] in Fig. 1C). However, this appeared to be alleviated when the *VTC1* mRNA was expressed from the "mut 4" promoter ([g], [k] in Supplementary Fig. S5B). This observation suggests that the two TATA-like elements (#2 and #3) affect the stability of *VTC1* mRNA at 37 °C by unknown post-transcriptional mechanisms.

DISCUSSION

In this study, we showed that plasmid-borne *SPT3* was functionally different from that *SPT3* carried on the chromosome. First, plasmid *SPT3* could not fully recapitulate the transient fluctuations of *PGK1* and/or *VTC1* mRNA levels observed during the initial 2-h phase, following the temperature shift from 25 °C to 37 °C, that was observed in the *taf1-ΔTAND* strain with chromosomal *SPT3* ([e, g, q, s] in Fig. 1B, 1D). However, only plasmid *SPT3* was able to suppress temperature-sensitive growth of the *taf1-ΔTAND* strain (lanes 2, 5 and 8 in Supplementary Fig. S4), and the differential effects of the two alleles on *PGK1* transcription might therefore be a simple consequence of suppression effects caused by copy number variation (the copy number of plasmid *SPT3* was approximately 50-fold higher than that of chromosomal *SPT3* when quantified by qPCR). Second, plasmid *SPT3* could not recapitulate the TFIID independence of *PGK1* transcription observed in the *taf1-N568Δ* strain with chromosomal *SPT3*, particularly when the promoter lacked the TATA box ([w] in Fig. 1B, 1D). As plasmid *SPT3* was unable to restore the growth defect of the *spt3Δ taf1-N568Δ* strain to a similar extent as chromosomal *SPT3* (lanes 3, 6 and 9 in Supplementary Fig. S4), the differential effect on transcription in the *taf1-N568Δ* strain may be due to intrinsically different properties of the two alleles, rather than to a simple suppression effect.

As stated above, introduction of plasmid-borne *SPT3* into the *spt3Δ taf1-N568Δ* strain failed to restore TFIID independence in transcription from the TATA-less *PGK1* promoter ([w] in Fig. 1B, 1D), whereas significant restoration of transcription from the TATA box-containing *PGK1* promoter occurred ([i, k, u] in Fig. 1B, 1D). Furthermore, the TATA box-dependent stimulatory function of *SPT3* for *PGK1* transcription was observed at similar levels both when *SPT3* was on the chromosome ([m, n, o, p] in Fig. 1A, 1C) and when provided ectopically via plasmid in the *spt3Δ TAF1* strain ([m, n, o, p] in Fig. 1B, 1D). These observations suggest two functions for Spt3: conferring TFIID independence on *PGK1* transcription, in particular from the TATA-less promoter when Taf1 function is impaired at 37 °C in the *taf1-N568Δ* strain ([w] in Fig. 1C, 1D), and stimulating *PGK1* transcription in a TATA box-dependent manner regardless of Taf1 functionality ([m–x] in Fig. 1C, 1D). Importantly, plasmid-expressed *SPT3* lacked the first of these two activities, thus indi-

cating that the two Spt3 functions were mediated by two different mechanisms.

An alternative interpretation of the different results observed with plasmid and chromosomal *SPT3* is that the alleles are not functionally different but that some unknown epigenetic mechanism hampers the restoration of TFIID independence of *PGK1* transcription when both Spt3 and Taf1 are compromised simultaneously in the *spt3Δ taf1-N568Δ* strain. This model is consistent with the observation that *SPT3* provided by the plasmid appears to be hypomorphic to the chromosomal counterpart, not only in *PGK1* transcription (Fig. 1) but also in growth (Supplementary Fig. S4), at least when introduced into the *spt3Δ taf1-N568Δ* strain. Further analysis is needed to discriminate between the two hypotheses, for example by introducing an *SPT3* expression plasmid prior to disruption of chromosomal *SPT3*.

The implications of our findings are two-fold. First, Spt3 has dual functions as described above. Second, plasmid *SPT3* was unable to support transcription from the TATA-less promoter in the *taf1-N568Δ* strain, indicating that Spt3/SAGA mediates *PGK1* transcription by different mechanisms depending on the presence or absence of the TATA box in the promoter. To our knowledge, this is the first demonstration that SAGA can mediate transcription by different mechanisms at a single promoter according to the presence of a TATA box or TATA-like element, indicating the requirement for particular subunit-specific functions.

Finally, although the *PGK1* promoter had the potential to be transcribed in a TFIID-dependent manner (Fig. 1), TFIID dependence of *PGK1* transcription could not be induced by changing the combinations of CE sequences (Fig. 4). This observation is consistent with our previous two-step model for the action of TFIID and SAGA, in which the UAS first specifies either TFIID or SAGA as the predominant factor for a specific promoter, and then the core promoter structure guides that factor to conduct transcription in an appropriate manner (Watanabe and Kokubo, 2017). If this model holds true for the *PGK1* promoter, TFIID dependence should not emerge unless the UAS is altered. However, TFIID dependence was evident, particularly at the TATA-less *PGK1* promoter, when plasmid *SPT3* was introduced into the *spt3Δ taf1-N568Δ* strain ([w] in Fig. 1B, 1D). Therefore, we propose that the initial "predominant factor-specifying" step mediated by the UAS is affected differently by the chromosomal and plasmid *SPT3* alleles, and/or that epigenetic events occur upon simultaneous functional loss of Spt3 and Taf1 as discussed above. We further suggest that the second step guided by core promoter structures is also affected differently by the two *SPT3* alleles, depending on the presence of the TATA box. This would suggest that involvement of the TATA box is in contrast to results obtained for the *AGP1* promoter, where CEs other than the TATA box or TATA-like element guided SAGA to conduct transcription in an appropriate manner (Watanabe and Kokubo, 2017). Further analyses are needed to identify the molecular mechanisms underpinning the different effects of the chromosomal and plasmid *SPT3* alleles, and to further elucidate the TFIID dependence of *PGK1* transcription.

We thank Dr. Y. Ohyama and Mr. K. Yonehara for constructing pM4201/pM4202/pM7188/YTK11406/YTK11411/YTK11705/YTK11870 and YTK13834/13835/13837/13839/13842, respectively. We also thank Dr. K. Kataoka, Dr. Y. Ohyama and other members of our laboratory for advice and helpful discussions. This work was supported by JSPS KAKENHI Grant Numbers 15K06953 and 18K06063.

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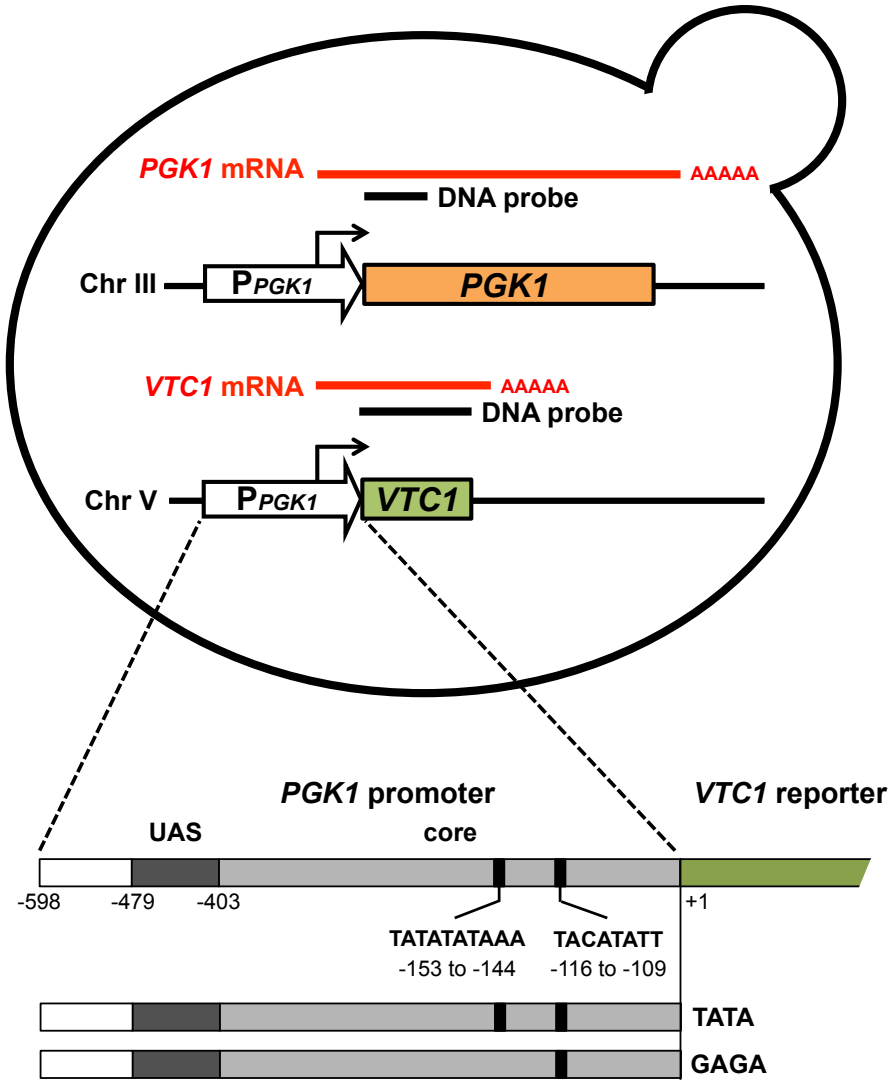
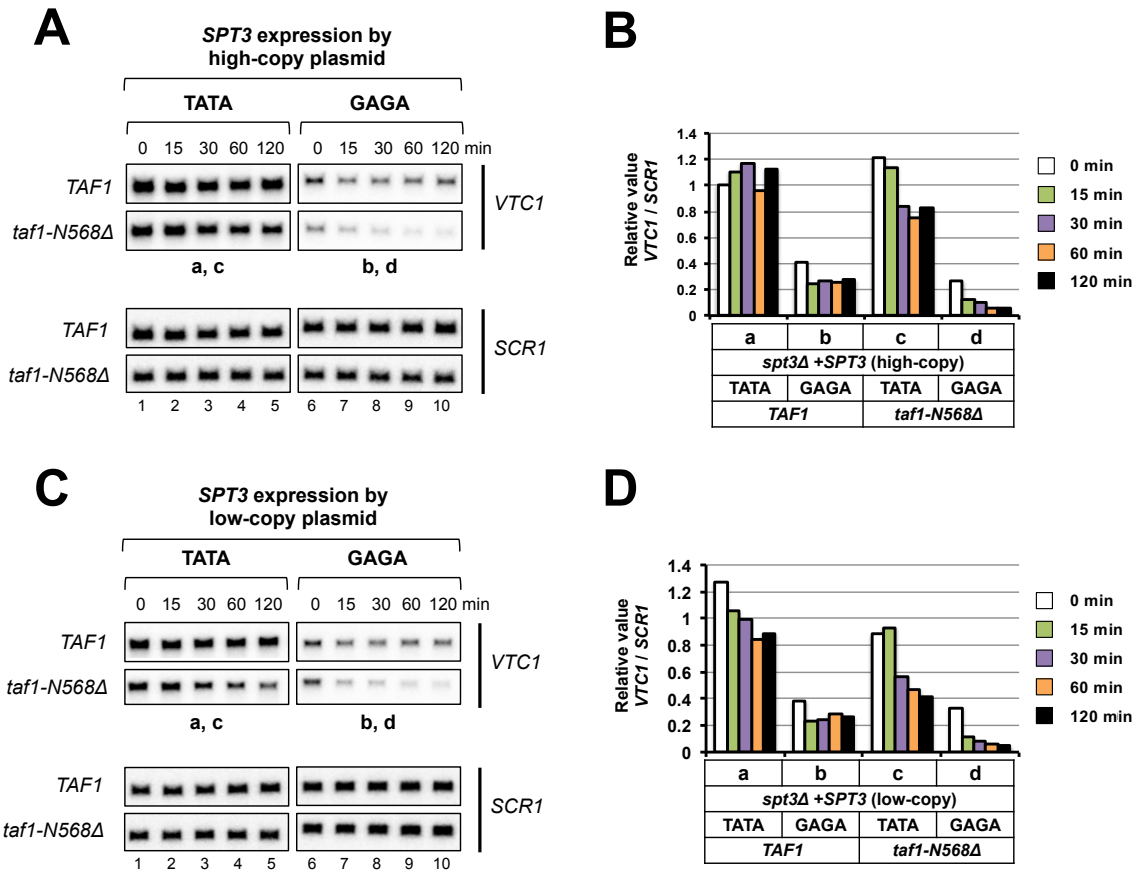
Supplementary Fig. S1



Supplementary Fig. S1. Schematic diagram of the *VTC1* reporter system under the control of the *PGK1* promoter.

The strains used in this study were constructed from the wild-type strain (WT, BY4741) by replacing the endogenous *VTC1* promoter with the *PGK1* promoter. Promoter activities were measured by Northern blot analysis using ^{32}P-labeled *PGK1* or *VTC1* DNA probes.

Supplementary Fig. S2



Supplementary Fig. S2. Effect of *SPT3* expression from high-copy or low-copy plasmids on the restoration of Taf1 independence of *PGK1* transcription.

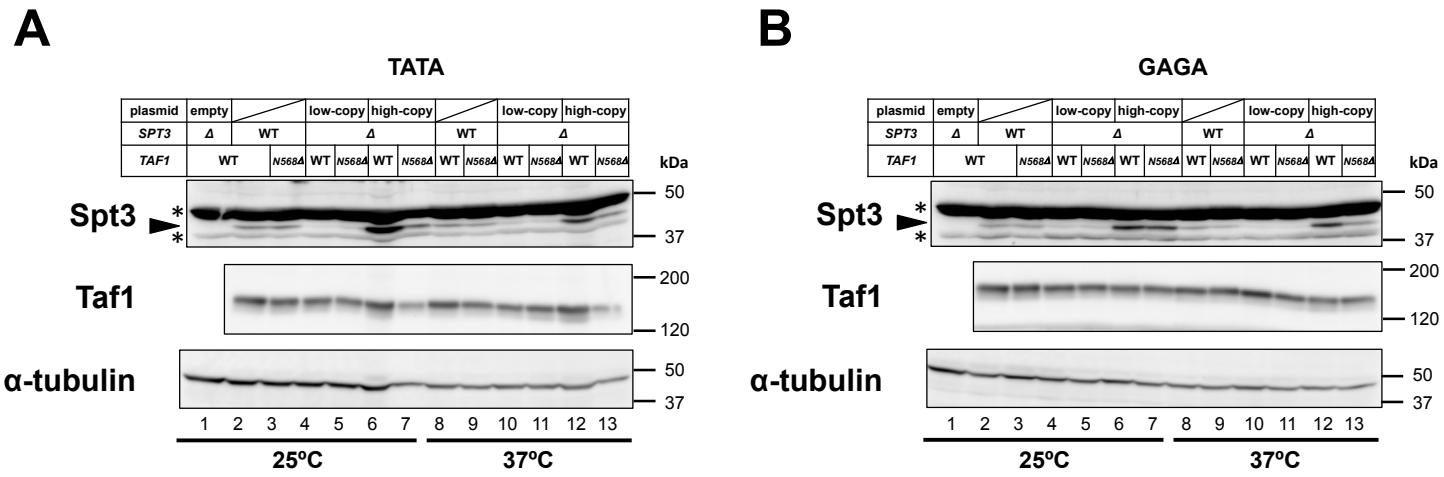
A. Northern analysis of expression of *VTC1* (a, c, b, d in the upper panels) or *SCR1* (control, lanes 1–10 in the lower panels) in the four strains used in Fig. 1B (a/m, c/o, i/u, k/w). Cultivation was performed as described in Fig. 1.

B. Raw expression data shown in **A** were summarized, as indicated in Fig. 1C.

C. Northern analysis of expression of *VTC1* (a, c, b, d in the upper panels) or *SCR1* (control, lanes 1–10 in the lower panels) in the four strains constructed similarly to those described in **A**, except that they carried *SPT3* on the low-copy plasmid instead of the high-copy plasmid.

D. Raw expression data shown in **C** were summarized, as indicated in Fig. 1C. In this panel, data are presented relative to the value obtained for the strain indicated at the left-hand side in **B**.

Supplementary Fig. S3

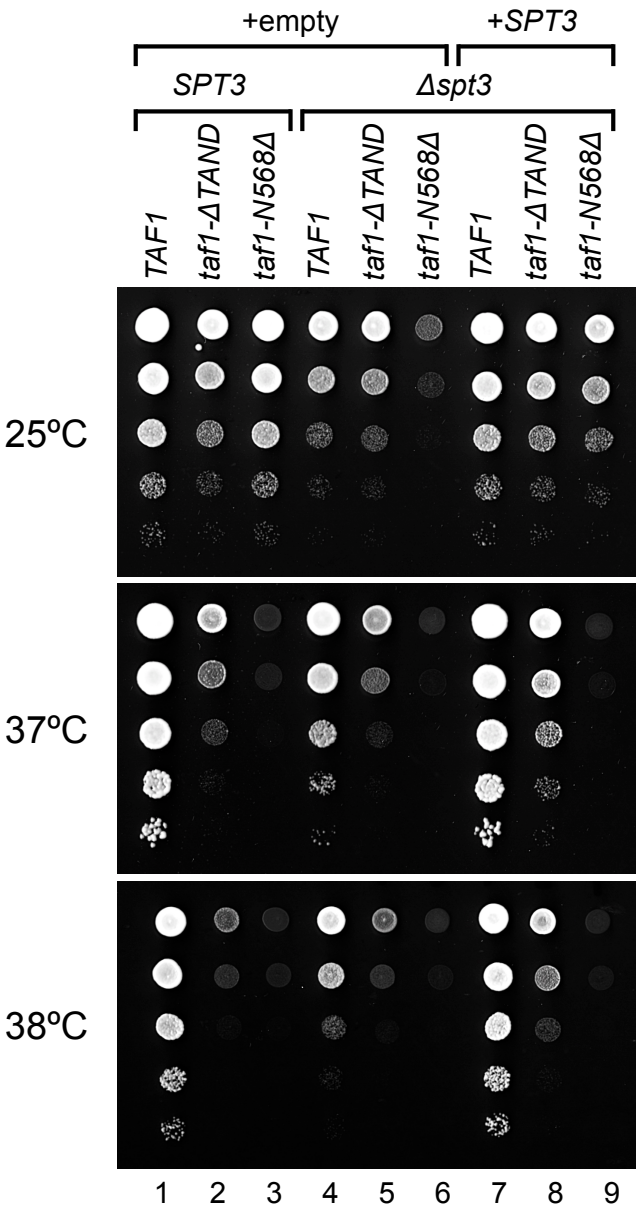


Supplementary Fig. S3. Expression levels of Spt3 in the strains used in Fig. 1 and Supplementary Fig. S2.

A. Immunoblot analysis of Spt3 (top panel), Taf1 (middle panel) and α-tubulin (loading control; bottom panel) in the seven strains carrying the *VTC1* reporter driven by the *PGK1* promoter in which the TATA box (TATA) was intact. Note that an arrowhead indicates the position of Spt3, whereas two asterisks indicate non-specific bands. The strains tested here are the same as those used in Fig. 1 or Supplementary Fig. S2. The details of strain correspondence are as follows: lane 1 (= b/n in Fig. 1B), lane 2/8 (= a/m in Fig. 1A), lane 3/9 (= i/u in Fig. 1A), lane 4/10 (= a in Supplementary Fig. S2C), lane 5/11 (= c in Supplementary Fig. S2C), lane 6/12 (= a in Supplementary Fig. S2A) and lane 7/13 (= c in Supplementary Fig. S2A). Whole-cell extracts were prepared from cells cultured at 25 °C (lanes 1–7) or at 37 °C for 1 h after the shift from 25 °C (lanes 8–13).

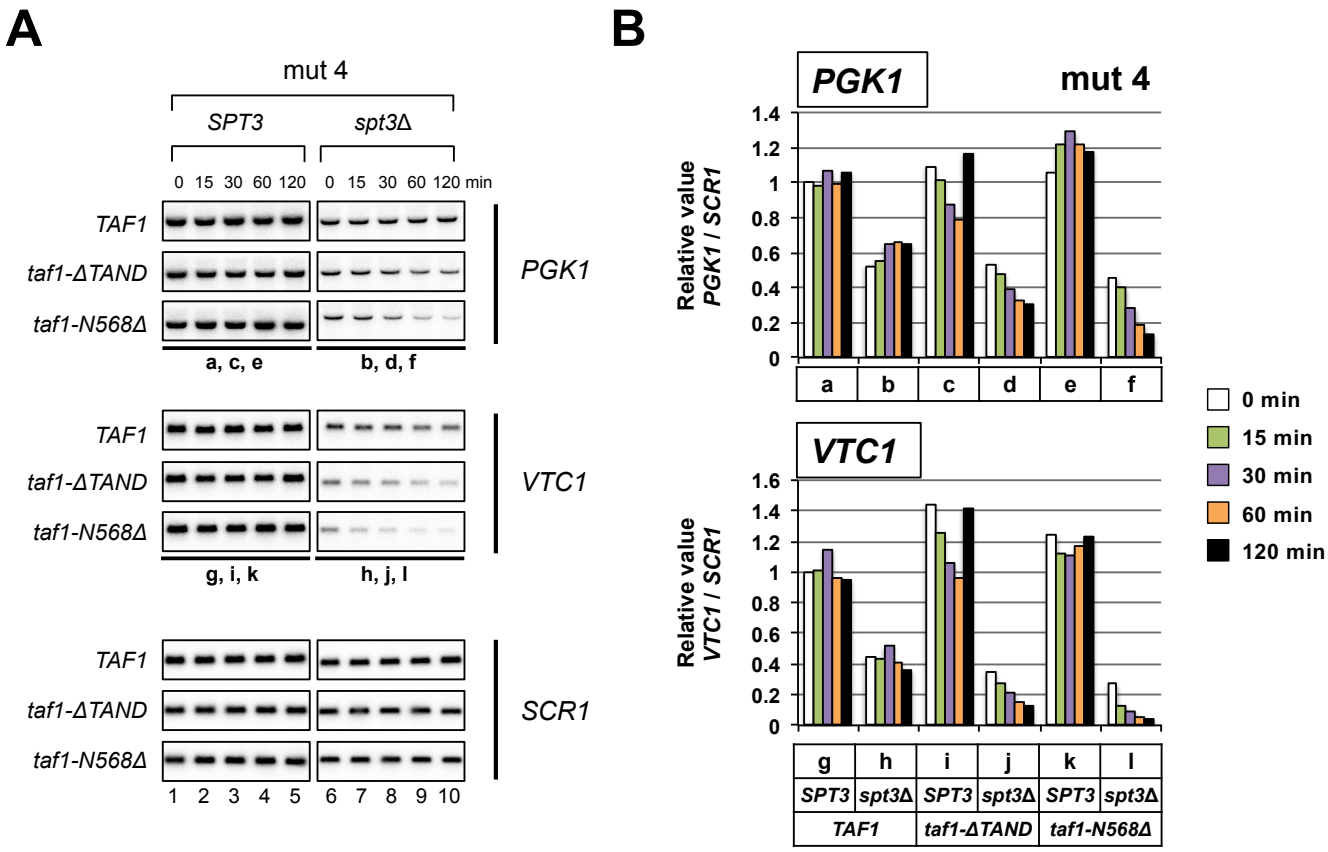
B. Immunoblot analysis was conducted as in **A** in the seven strains carrying the *VTC1* reporter driven by the *PGK1* promoter in which the TATA box was substituted with the GAGA sequence (GAGA). The strains tested here are the same as those used in Fig. 1 or Supplementary Fig. S2. The details of strain correspondence are as follows: lane 1 (= b/n in Fig. 1B), lane 2/8 (= c/o in Fig. 1A), lane 3/9 (= k/w in Fig. 1A), lane 4/10 (= b in Supplementary Fig. S2C), lane 5/11 (= d in Supplementary Fig. S2C), lane 6/12 (= b in Supplementary Fig. S2A) and lane 7/13 (= d in Supplementary Fig. S2A).

Supplementary Fig. S4



Supplementary Fig. S4. Growth phenotypes of the yeast strains used in Fig. 1.
The six strains used in Fig. 1 harboring the *VTC1* reporter driven by the TATA box-containing *PGK1* promoter were spotted on agar plates (synthetic medium containing 2% glucose) with serial dilutions (10-fold) and then grown for 3 days at the indicated temperatures.

Supplementary Fig. S5



Supplementary Fig. S5. Testing the requirement of the two TATA-like elements #2 and #3 for TAND/Spt3-dependent transient fluctuation of *PGK1* or *VTC1* mRNA levels.

A. Northern analysis of expression of *VTC1* (g, i, k, h, j, l in the middle panels), *PGK1* (a, c, e, b, d, f in the top panels) or *SCR1* (control; lanes 1–10 in the bottom panels) in the "mut 4" strains. Cultivation was performed as described in Fig. 1.

B. Raw expression data shown in **A** were quantified and are presented graphically, as described in Fig. 1C. Values for each transcript derived from *PGK1* and *VTC1* are presented relative to the value obtained for the strain indicated at the left-hand side.

Supplementary Table S1. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Source
BY4741	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	Euroscarf
BY4742	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	Euroscarf
#4228	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 spt3Δ::kanMX4*	Euroscarf
YTK11406	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 taf1Δ::kanMX6* pYN1/*TAF1*	Ohyama et al. (2010)
YTK11411	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 taf1Δ::kanMX6* pYN1/*TAF1*	Ohyama et al. (2010)
YTK11705	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 taf1Δ::KanMX6* pM4770/*TAF1*	Ohyama et al. (2010)
YTK11870	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4770/*TAF1*	this study
YTK13834	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1*	this study
YTK13835	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK13837	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK13839	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 taf1Δ::kanMX6* pM7118/*TAF1*	this study
YTK13842	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 taf1Δ::kanMX6* pM7118/*TAF1*	this study
YTK16787	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4770/*TAF1*	this study
YTK16791	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK16799	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7118/*TAF1*	this study
YTK16801	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7188/*taf1-N568Δ*	this study
YTK16803	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7119/*taf1-ΔTAND*	this study
YTK16805	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pYN1/*TAF1*	this study
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YTK16827	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7188/*taf1-N568Δ*	this study
YTK16829	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7119/*taf1-ΔTAND*	this study
YTK16831	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7118/*TAF1* pRS426	this study
YTK16835	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7188/*taf1-N568Δ* pRS426	this study
YTK16839	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7119/*taf1-ΔTAND* pRS426	this study
YTK16843	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7118/*TAF1* pRS426	this study
YTK16847	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7188/*taf1-N568Δ* pRS426	this study
YTK16851	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7119/*taf1-ΔTAND* pRS426	this study
YTK16941	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 taf1Δ::kanMX6* pM7119/taf1-ΔTAND	this study
YTK16943	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 taf1Δ::kanMX6* pM7188/taf1-N568Δ	this study
YTK16945	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 taf1Δ::kanMX6* pM7188/*taf1-N568Δ*	this study
YTK16947	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 taf1Δ::kanMX6* pM7188/taf1-N568Δ	this study
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YTK16987	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 taf1Δ::kanMX6* pM7118/*TAF1* pRS426	this study
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YTK16995	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 taf1Δ::kanMX6* pM4201/*TAF1*	this study
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YTK17069	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7118/*TAF1* pM7935/*SPT3*	this study
YTK17071	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7188/*taf1-N568Δ* pM7935/*SPT3*	this study
YTK17073	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7119/*taf1-ΔTAND* pM7935/*SPT3*	this study
YTK17075	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7118/*TAF1* pM7935/*SPT3*	this study
YTK17077	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7188/*taf1-N568Δ* pM7935/*SPT3*	this study
YTK17079	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7119/*taf1-ΔTAND* pM7935/*SPT3*	this study
YTK18393	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18397	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18401	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18405	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18409	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 2 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18413	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1, 2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18519	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18521	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18523	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18525	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18527	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18529	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18531	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18533	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18535	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 2 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18537	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 2 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18539	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1, 2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18541	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1, 2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18606	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 mut]-*VTC1 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18608	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#3 mut]-*VTC1 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18610	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18612	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 3 mut]-*VTC1 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18614	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 2 mut]-*VTC1 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18616	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1, 2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK18662	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 mut]-*VTC1 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18664	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#3 mut]-*VTC1 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18666	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18668	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18670	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 2 mut]-*VTC1 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18672	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1, 2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM4201/*TAF1*	this study
YTK18678	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 mut]-*VTC1 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18680	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#3 mut]-*VTC1 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18682	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18684	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 3 mut]-*VTC1 taf1Δ::kanMX6*pM8201/*taf1-N568Δ*	this study
YTK18686	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 2 mut]-*VTC1 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK18688	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1, 2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM8201/*taf1-N568Δ*	this study
YTK19042	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19044	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 spt3Δ::kanMX4* pM4202/*taf1-ΔTAND*	this study
YTK19046	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 mut]-*VTC1 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19047	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#3 mut]-*VTC1 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19048	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19049	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 2 mut]-*VTC1 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19050	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1, 2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19051	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19052	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19053	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19054	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1 & 2 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK19055	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#1, 2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM4202/*taf1-ΔTAND*	this study
YTK20133	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM7118/*TAF1*	this study
YTK20134	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM7119/*taf1-ΔTAND*	this study
YTK20135	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 taf1Δ::kanMX6* pM7188/*taf1-N568Δ*	this study
YTK20136	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7118/*TAF1*	this study
YTK20137	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7119/*taf1-ΔTAND*	this study
YTK20138	*MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [#2 & 3 mut]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7188/*taf1-N568Δ*	this study
YTK20346	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 spt3Δ::kanMX4 taf1Δ::kanMX6* pYN1/*TAF1*	this study
YTK20353	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7118/*TAF1* pM7936/*SPT3*	this study
YTK20354	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [TATATATAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7188/*taf1-N568Δ* pM7936/*SPT3*	this study
YTK20355	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7118/*TAF1* pM7936/*SPT3*	this study
YTK20356	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 His3MX6::PGK1* promoter [GAGAGAGAAA]-*VTC1 spt3Δ::kanMX4 taf1Δ::kanMX6* pM7188/*taf1-N568Δ* pM7936/*SPT3*	this study

Supplementary Table S2. Oligonucleotides used in this study

ID	Sequence
TK176	5'-GCCAATAAATTAATTTATTATCGGAAAGCC-3'
TK1224	5'-ATGTCTTTATCTTCAAAGTT-3'
TK1225	5'-TCGATGTGGTAACGCAAGTT-3'
TK2496	5'-AAATAGATATTAGCACGTGTCTCGG-3'
TK3755	5'-TTAAGAGTCCATATTTCCATAACCAGCGACTTGCATACACCGGATCCCCGGGTTAATTAA-3'
TK3756	5'-GTCGACGGATCCCCGGGAATTCATGCAGGTTAACCTGGCTTA-3'
TK8251	5'-TCGTGTGGGCAAAGCGATCT-3'
TK8263	5'-GTGGGCCCCAATCTGTCATC-3'
TK9013	5'-TAACTTAGTGTTAGCGTCATT-3'
TK9030	5'-ATGTCTTCAGCACCATTATTACAA-3'
TK9507	5'-AGGCTGTAATGGCTTTCT-3'
TK10081	5'-CCACAATGTGCGAGTAAATCC-3'
TK11659	5'-CTCTCTCTGTAAATGTAAGTTTCACGAG-3'
TK11790	5'-TGAAACTTACATTTACAGAGAGAGAAACTTGCATAAATTG-3'
TK11908	5'-GAATTCCCGGGGATCCGTCGACGCCAGAAAAAGGAAGTGT-3'
TK11909	5'-TTTTCCCAGGTGTTCTTTGTAATAATGGTGCTGAAGACATTGTTTTATATTTGTTGTAAAAAGTA-3'
TK13214	5'-CACACATCTAGAACTAGTTCGCCCCATAGTATCTCAAC-3'
TK13229	5'-CACACAGAATTCGAGTTGGCAGTTTGTTCTCG-3'
TK13286	5'-CTCTGTCTTTCTTGCATTGACCAATTTATGC-3'
TK13287	5'-GAAAGACAGAGTTGGTCTTTTCTAATTCGTAG-3'
TK13288	5'-TGCAGAAAGGGGTCAATGCAAGAAATACATATTTGGTC-3'
TK13289	5'-CCTTTCTGCAAGTTTATATATATGTAAATGTAAGTTTCAC-3'
TK13290	5'-CCCTTTCTGCAAGTTTCTCTCTCTGTAAATGTAAGTTTCACG-3'
TK13291	5'-TGCAGAAAGGGGTCAATGCAAGAAAGACAGAGTTGGTC-3'
TK13730	5'-CCCTTTCTGCAAGTTTATATATATGTAAATGTAAGTTTCACG-3'
TK13731	5'-CCTTTCTGCAAGTTTCTCTCTCTGTAAATGTAAGTTTCAC-3'