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RESEARCH ARTICLE

# Mechanistic Differences in Transcription Initiation at TATA-Less and TATA-Containing Promoters

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**ABSTRACT** A yeast *in vitro* system was developed that is active for transcription at both TATA-containing and TATA-less promoters. Transcription with extracts made from cells depleted of TFIID subunit Taf1 demonstrated that promoters of both classes are TFIID dependent, in agreement with recent *in vivo* findings. TFIID depletion can be complemented *in vitro* by additional recombinant TATA binding protein (TBP) at only the TATA-containing promoters. In contrast, high levels of TBP did not complement Taf1 depletion *in vivo* and instead repressed transcription from both promoter types. We also demonstrate the importance of the TATA-like sequence found at many TATA-less promoters and describe how the presence or absence of the TATA element is likely not the only feature that distinguishes these two types of promoters.

**KEYWORDS** *Saccharomyces cerevisiae*, TATA, TATA-less, promoters, transcription

An early step in transcription initiation by RNA polymerase II (Pol II) is formation of the transcription preinitiation complex (PIC) (1–4). In this step, Pol II and the basal transcription factors TBP (TATA binding protein), TFIIA, TFIIB, TFIIF, TFIIE, and TFIIH form a complex with double-stranded promoter DNA. This step is followed by ATP-dependent DNA opening, transcription start site (TSS) recognition, and initiation of RNA synthesis. PIC formation is often regulated by gene-specific transcription factors.

Much effort has been directed at determining DNA sequences that constitute a functional Pol II basal promoter, which contains sequence elements directing formation of an active PIC (5, 6). At some promoters, the 8-bp TATA element (consensus TATAW AWR) provides a specific binding site for TBP to nucleate PIC formation. At many TATA-containing promoters, mutations in this element are detrimental to transcription (see, e.g., reference 7). However, the TATA element is present at only ~20% of eukaryotic promoters, and mutations in the *Saccharomyces cerevisiae* (yeast) TBP DNA binding surface that inhibit TBP-TATA binding are viable (8). These findings suggest alternative mechanisms rather than sequence-specific DNA binding for the role of TBP in PIC formation at TATA-less promoters (9).

TBP is a subunit of the TFIID complex, comprised of TBP and 14 Taf (TBP-associated factor) subunits (2, 10, 11). In metazoans, some TFIID subunits are known to recognize short promoter elements such as INR, MTE, and DPE. It is thought that sequence-specific recognition of these motifs by TFIID provides a mechanism to nucleate PIC formation in the absence of a consensus TATA (12–14). While analogous TFIID recognition sequences have not been identified in *S. cerevisiae*, genome-wide analysis of yeast PICs found that at TATA-less promoters, PICs are often found coincident with "TATA-like" sequences, typically containing 1 or 2 mismatches from the consensus (15). The role of these sequences and whether they are direct targets of TBP have not been systematically investigated.

The coactivator SAGA is also known to bind TBP and has been proposed to assist recruitment of TBP to many TATA-containing promoters (16–20). Prior studies have

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characterized the apparent TFIID and SAGA dependence of promoters using chromatin immunoprecipitation (ChIP) to map TFIID binding (15, 21, 22) and by monitoring genome-wide changes in steady-state mRNA levels upon inactivation of TFIID or SAGA subunits (22–25). These studies suggested that SAGA and TFIID contribute to expression of many genes but that expression of most genes is dominated by either TFIID or SAGA. Genes proposed to be dominated by SAGA were generally correlated with TATA-containing promoters, while TFIID-dominated genes typically correlated with TATA-less genes (26). In contrast with this model, we recently used rapid TFIID and SAGA subunit depletion and monitoring of nascent transcription changes to show that *in vivo* transcription from nearly all yeast genes is dependent on both TFIID and SAGA (27, 28). Consistent with this finding, genome-wide mapping of both coactivators using chromatin endogenous cleavage and high-throughput sequencing (ChEC-seq) finds TFIID at most promoters (29) and SAGA at most upstream activation sequence (UAS) elements (28).

In light of these new findings, it is important to understand the role of TFIID and TBP at TATA-containing and TATA-less promoters. For example, can TBP promote transcription independent of TFIID *in vivo*? Nearly all structural studies on PIC architecture and function using purified components have been conducted on TATA-containing promoters using TBP in the absence of TFIID (see, e.g., references 11 and 30 to 32). As a consequence, there is little direct information on how TFIID is arranged within the PIC, the position and role of TBP at TATA-less promoters, and what promoter elements contribute to the dependence on TFIID.

To facilitate mechanistic studies, we developed a yeast *in vitro* system for analysis of transcription at both TATA-containing and TATA-less promoters. Here we show that both classes of yeast promoters require TFIID for transcription *in vitro*, validating our recently reported *in vivo* findings. We also investigated mechanistic differences between several TATA-containing and TATA-less promoters, including the requirements for TFIID versus TBP and the role of TATA-like sequences in regulating transcription.

## RESULTS

**An *in vitro* system for transcription of yeast TATA-containing and TATA-less promoters.** To analyze mechanistic differences between yeast TATA-less and TATA-containing promoters, we first established an *in vitro* system to achieve robust transcription from TATA-less promoters. To simplify extract preparation and to facilitate depletion of specific factors using the auxin-inducible degron system (33), we developed a whole-cell extract (WCE) preparation that generated robust Pol II transcription at the well-characterized *HIS4* TATA-containing promoter, comparable to levels previously observed with yeast nuclear extracts (34). We used this WCE to compare transcription at three TATA-containing (*HIS4*, *CYC1*, and *SNR20*) and three TATA-less (*EFB1*, *HSP10*, and *RPS5*) promoters, which were selected based on high levels of *in vivo* transcription (35), the presence or absence of a consensus TATA, and prior characterization of transcription factor and nucleosome binding patterns (15, 36). The latter three promoters contained a TATA-like sequence with a one-base mismatch to the TATA consensus (TATAWAWR) (15, 26). *HIS4* is a well-studied promoter which contains a single mismatch to the TATA consensus in the last position (TATATAAT). Since the *HIS4* TATA box is recognized and bound by TBP with high affinity and because variations in the last position of the 8-bp TATA sequence were shown to have a minimal impact on TBP-DNA interactions (37), we define this promoter as TATA containing. For this paper, we refer to the TATA consensus as TATAWAW.

Since the core promoter is responsible for basal transcription and was suggested to be the major determinant of TFIID dependence (38, 39), we prepared a set of templates with core promoter sequences having ~40 bp of DNA upstream from the TATA or TATA-like element. The *HIS4* template used in this work contained 62 bp of *HIS4* DNA downstream of the TSS, while the others contained between 226 and 534 bp of downstream DNA. *In vitro* transcription reactions were performed with supercoiled plasmid DNA templates and assayed by primer extension (Fig. 1). For all these promot-

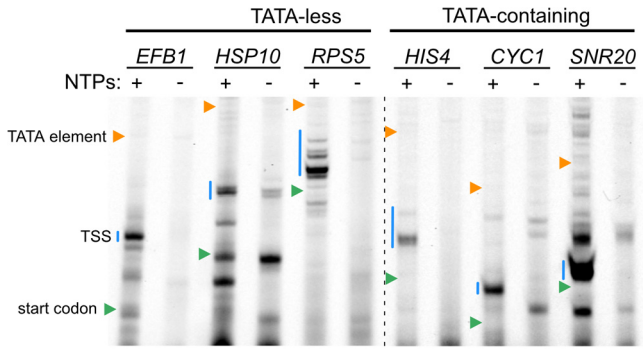


**FIG 1** *In vitro* transcription from selected TATA-less and TATA-containing promoters. The transcription products were visualized by primer extension with fluorescently labeled primers. Nucleoside triphosphate mix (NTP) was added or omitted as indicated. The positions of promoter features were estimated by running the samples next to $^{32}$P-labeled standard ladders (not shown). The most prominent *in vivo* TSS (40) is marked by a blue bar. The positions of a TATA/TATA-like element and start codon are represented by orange and green arrowheads, respectively.

ers, we observed a clear nucleoside triphosphate (NTP)-dependent transcription signal in the area corresponding to the previously mapped *in vivo* TSS (indicated by blue in Fig. 1) (40). Templates containing the respective promoters and several hundred base pairs of upstream DNA resulted in similar patterns and levels of basal transcription (data not shown). Signals observed in the absence of NTPs are likely due to primer extension of nucleic acids in the WCE. Our results show that the yeast WCE is active for basal transcription of these TATA-less and TATA-containing core promoters.

**Transcription from both TATA-less and TATA-containing promoters is dependent on TFIID.** Although prior work suggested that transcription of most yeast genes was dominated by either TFIID or SAGA, we recently found that most *in vivo* transcription from nearly all yeast promoters requires both TFIID and SAGA (27, 28). To biochemically validate this finding and to explore mechanistic differences between TATA-containing and TATA-less promoters, we utilized our *in vitro* transcription system to test TFIID dependence for both promoter types. First, we prepared WCEs from a strain expressing TFIID subunit Taf1 tagged with an auxin-inducible degron (AID) (27, 33). Based on the Western blot analysis, we estimate that at least 90% of Taf1 present in the cell was degraded (Fig. 2A). The WCE was prepared from cultures treated for 30 min with either dimethyl sulfoxide (DMSO; control) or the auxin indole-3-acetic acid (3-IAA) to induce rapid degradation of the tagged protein. Although there is a small subset of Taf1-independent genes, nearly all TATA-containing and TATA-less genes are sensitive to Taf1 depletion *in vivo* (27). Consistent with this result, we found that the WCE depleted of Taf1 was deficient in transcription from all six promoters (Fig. 2B). A 2-fold or greater decrease was observed, with TATA-less promoters being slightly more sensitive to Taf1 depletion. Addition of TAP-tagged purified TFIID (TAP-TFIID) recovered transcription at all promoters to wild-type (WT) or higher levels, which indicates that the observed decrease is a direct result of TFIID depletion.

To test whether TFIID is a limiting factor in the WCE from wild-type cells, transcription was performed in the presence of additional TFIID. Purified TFIID was added to a final concentration ~2-fold higher than that in WT WCEs (Fig. 2C). We observed a modest (1.5- to 2-fold) increase in transcription with excess TFIID, suggesting that TFIID in fact is a limiting factor in the WCEs. Collectively, our *in vitro* findings validate the *in vivo* results showing that TFIID is required for most transcription from both TATA-less and TATA-containing promoters. In addition, we demonstrate that the *in vitro* transcription system presented here is well suited for mechanistic studies of yeast transcription.

**TBP can stimulate TFIID-independent transcription *in vitro* only at TATA-containing promoters.** The essential role of TBP in supporting eukaryotic transcription is well documented. However, important mechanistic details of its function, e.g.,

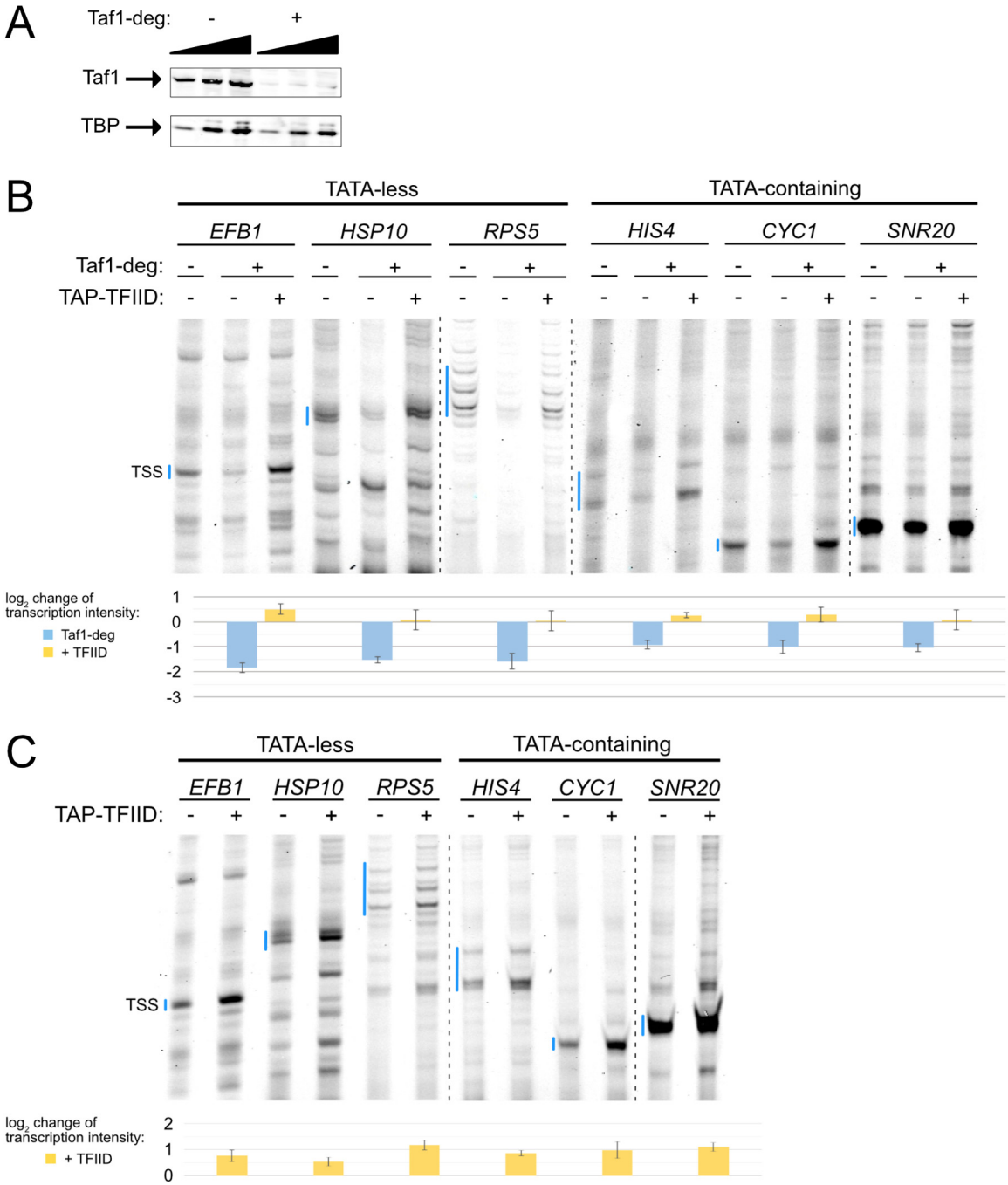


**FIG 2** TFIID dependence of selected TATA-less and TATA-containing promoters. (A) Western blot analysis of whole-cell extracts (WCE) prepared from cultures of a strain carrying auxin-inducible degron tag on Taf1. Cultures were either left untreated (Taf1-deg −) or treated (Taf1-deg +) with auxin indole-3-acetic acid (3-IAA) to induce rapid degradation of Taf1. The Western blot was probed with anti-Taf1 and anti-TBP antibodies. (B) *In vitro* transcription using either WT (Taf1-deg −) or Taf1-depleted (Taf1-deg +) WCE. Reactions were supplemented with 240 ng of TAP-TFIID as indicated. (C) *In vitro* transcription using WT WCE. TAP-TFIID was added (240 ng) or omitted as indicated. The $\log_2$ relative change in transcription intensity is represented as a bar plot. Standard deviation was calculated based on three independent experiments.

the role of TBP in TATA-less transcription (8), are still unclear. To address this issue, we probed the function of TBP in extracts depleted of TFIID. We observed that high levels of recombinant TBP (rTBP; ~4-fold-higher TBP concentration than in WCEs) added to Taf1-depleted extracts can only weakly complement transcription at TATA-less promoters (Fig. 3A). In contrast, adding a 0.5-fold excess of rTBP was able to completely

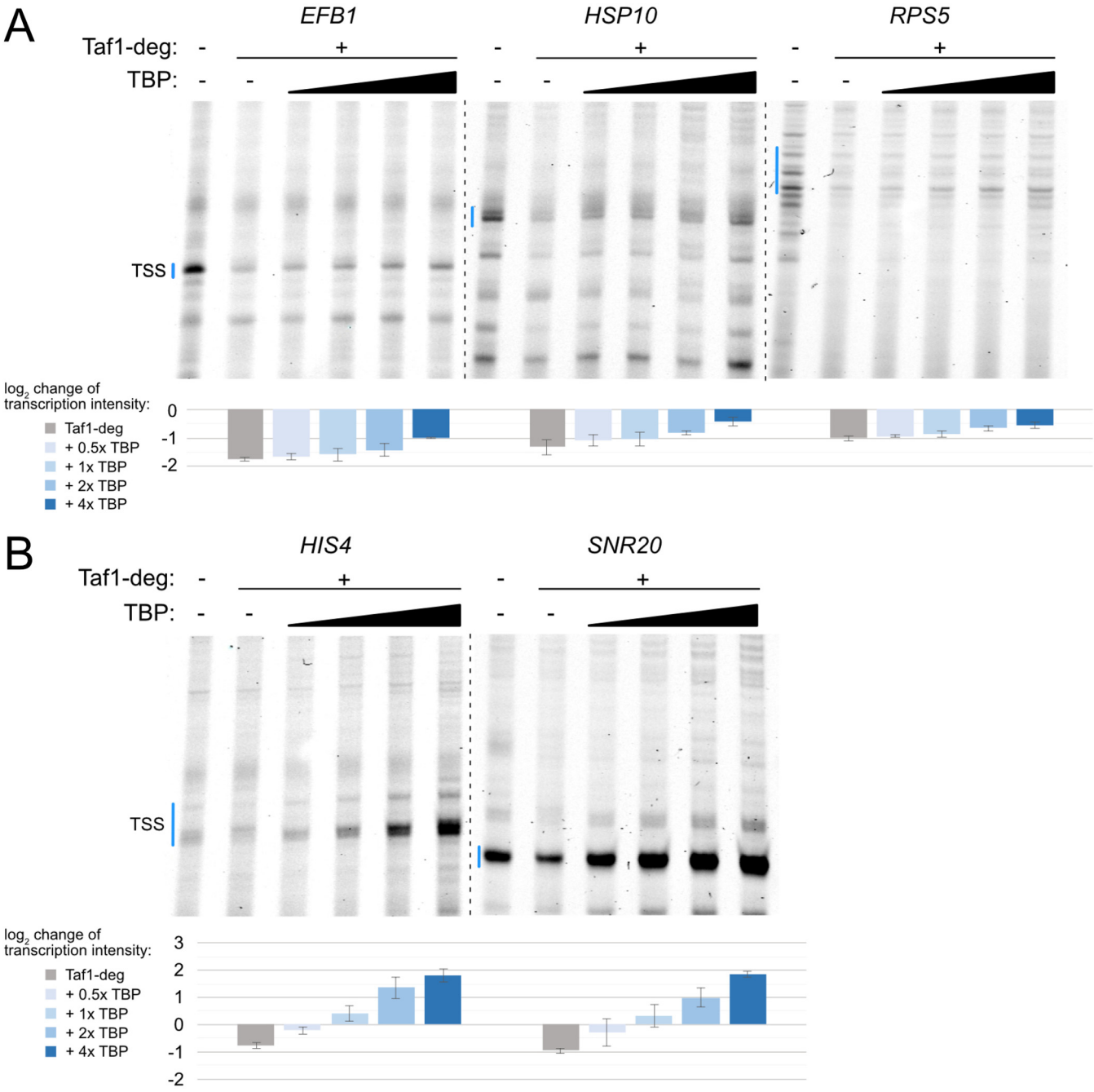


**FIG 3** rTBP is efficient in complementing Taf1-depleted transcription only on TATA-containing promoters. *In vitro* transcription using either WT (Taf1-deg −) or Taf1-depleted (Taf1-deg +) WCE was carried out. Selected TATA-less (A) and TATA-containing (B) promoters were analyzed. The following amounts of rTBP were used: 10, 20, 40, and 80 ng; these correspond to approximately 0.5, 1, 2, and 4 times the amount of TBP in WT WCE. The $\log_2$ relative change in transcription intensity is represented as a bar plot. Standard deviation was calculated based on three independent experiments.

compensate for the lack of TFIID on two TATA-containing promoters. At higher concentrations (up to ~4-fold higher than WT levels), rTBP significantly stimulated transcription above levels observed in extracts containing TFIID (Fig. 3B). To test if this result derived from inherent differences in responsiveness to TBP between TATA-less and TATA-containing promoters, we used a wild-type WCE and supplemented reactions with the highest rTBP concentration used in the previous experiment (Fig. 4). Transcription from both TATA-containing promoters (*HIS4* and *SNR20*) was induced 2- to 4-fold. In contrast, we did not observe significant stimulation at three TATA-less

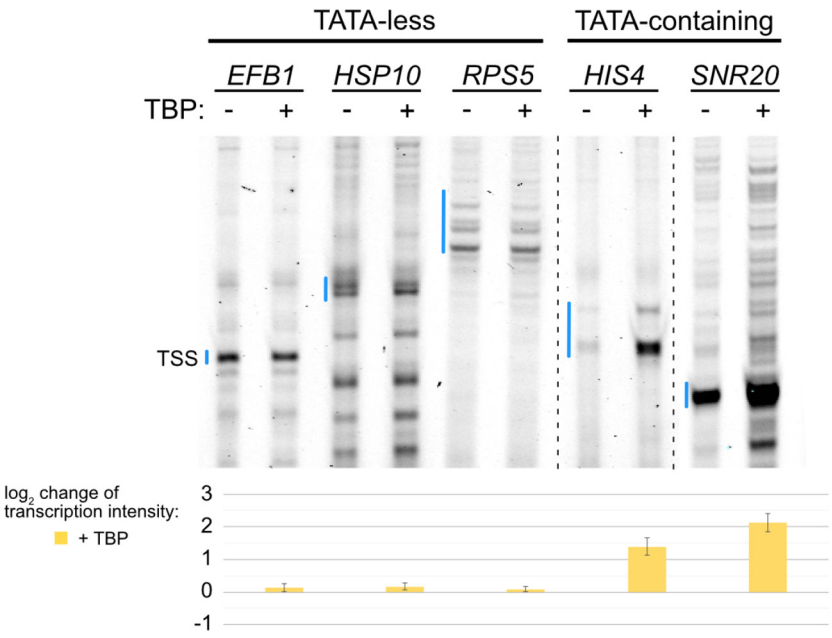


**FIG 4** Excess rTBP can stimulate basal transcription only on TATA-containing promoters. *In vitro* transcription was performed with WT WCE. Selected samples were supplemented with rTBP (4 times the amount of TBP in WT WCE). The $\log_2$ relative change in transcription intensity is represented as a bar plot. Standard deviation was calculated based on three independent experiments.

promoters (*EFB1*, *HSP10*, and *RPS5*). Our results illustrate differences in formation of functional PICs between TATA-less and TATA-containing promoters.

**High *in vivo* levels of TBP do not compensate for TFIID depletion at either TATA-containing or TATA-less promoters.** To test if overexpression of TBP can compensate for TFIID-depletion *in vivo*, we generated a Taf1-AID strain with TBP expressed from its WT promoter on a high-copy-number plasmid (SHY1131). Western blot quantitation showed that TBP was expressed at ~5-fold-higher levels than in the comparable WT strain (Fig. 5A). Using 4-thiouracil RNA labeling (41–43), we isolated nascent RNA from cultures either treated or not treated with IAA and with or without TBP overexpression. Transcription at four TATA-less and four TATA-containing promoters was assayed by reverse transcription-quantitative PCR (RT-qPCR) (Fig. 5B). As expected from published results (27) and our *in vitro* data, we observed a significant reduction in transcription for all tested promoters following Taf1 depletion. In contrast to our *in vitro* observations, overexpression of TBP *in vivo* resulted in an ~2-fold decrease in transcription at all genes, suggesting that excess TBP is generally detrimental to cellular Pol II transcription. Furthermore, TBP overexpression did not compensate for the loss of TFIID function due to depletion of Taf1 at either TATA-containing or TATA-less genes.

**The consensus TATA sequence is not the only determinant of promoter sensitivity to TBP.** While nearly all yeast TATA-less promoters contain a TATA-like sequence (15), the role of this sequence is not clear. For example, TBP binds many TATA-like sequences with lower affinity than for the consensus TATA (37, 44). However, many nonconsensus TATA sequences can promote transcription *in vivo* (45). Furthermore, mutations in the TBP DNA binding surface that abolish TBP-TATA binding are viable (8), suggesting that transcription at many promoters may not require TBP-DNA interactions such as those observed in the TBP-DNA crystal structure (46, 47).

To explore the role of the TATA-like sequence in yeast transcription, we modified *EFB1*, *HSP10*, and *RPS5* promoters by either randomizing the TATA-like sequence (to ACCTCGAG) or by changing the TATA-like motif to a consensus TATA (TATAWAW) (26). We first compared transcription between promoters with native (unmodified), random-

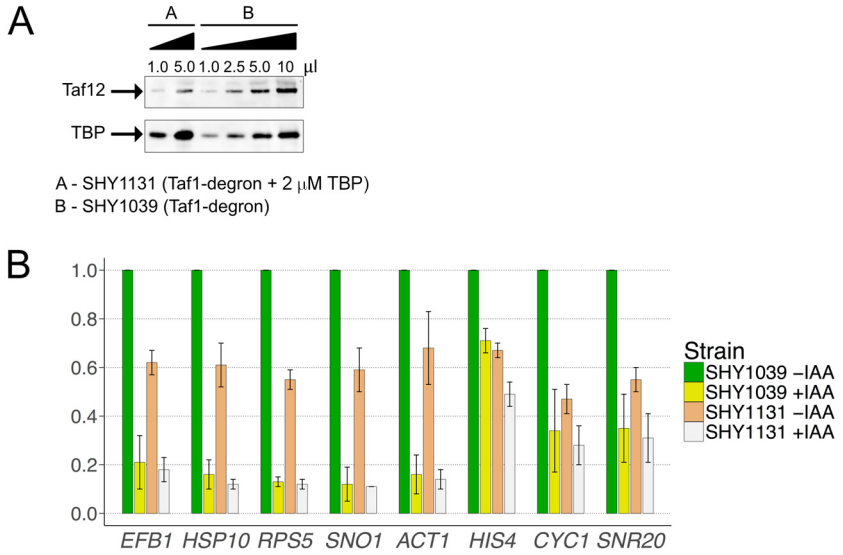


**FIG 5** High TBP levels do not compensate for Taf1 depletion *in vivo*. (A) Western blot analysis of TBP levels in Taf1-degron strain (SHY1039) and in its derivative carrying a high-copy-number 2$\mu$ plasmid containing the gene encoding TBP (SHY1131). Samples were collected before addition of 3-IAA. Increasing volumes of SHY1039 extract were loaded to estimate the level of TBP compared to that of the SHY1131 strain. The Western blot was probed with anti-TBP and anti-Taf12 antibodies. (B) RT-qPCR analysis of changes in nascent RNA levels following Taf1 degradation and/or TBP overexpression. 4-Thiouracil-labeled RNA was isolated from cells treated with either 3-IAA (+IAA) or DMSO (−IAA). Samples were normalized by spike-in of labeled *S. pombe* cells before RNA purification. Results are presented as percentages of signal for a sample without 3-IAA and TBP overexpression (SHY1039-IAA). Error bars represent the standard deviations between two independent experiments.

ized, or consensus TATA sequences (Fig. 6A). In all cases, randomization of the TATA-like sequence decreased transcription at least 2-fold. Derivatives of the *EFB1* and *HSP10* promoters with a consensus TATA showed ~2-fold-increased levels of transcription compared to the wild-type promoters, while *RPS5* with a consensus TATA showed no reproducible increase over unmodified *RPS5*. Interestingly, randomization of the TATA-like sequence in the EFB1 promoter resulted in increased transcription from a weak transcription start site located upstream from the main TSS. We next examined the properties of these modified TATA-less promoters carrying a consensus TATA (*EFB1 TATA*, *HSP10 TATA*, and *RPS5 TATA*). We found that all promoters still required TFIID (Fig. 6B), which agrees with the global role of TFIID in regulating transcription independently of the promoter class.

Excess TBP did not further increase transcription of the TATA-less promoters *EFB1*, *HSP10*, and *RPS5* (Fig. 4). This raised the question of whether the stimulatory effect of TBP was dependent on the TATA element. To test this idea, we assayed whether excess TBP stimulated transcription of the *EFB1 TATA*, *HSP10 TATA*, and *RPS5 TATA* promoter variants. We found that up to 4-fold excess rTBP did not stimulate transcription at *EFB1* and *HSP10* TATA-containing derivatives (Fig. 7A). At the *RPS5* TATA-containing derivative, the excess rTBP increased the usage of an alternative downstream TSS while failing to stimulate transcription in the region overlapping the main *in vivo* TSS (Fig. 7A). Similarly, rTBP did not efficiently complement a TFIID-depleted extract for transcription at these promoters while still increasing transcription originating from the downstream area at *RPS5* promoter (Fig. 7B). Our results suggest that other features of promoters, in addition to the TATA element, determine the ability of TBP to substitute for TFIID *in vitro*.

## DISCUSSION

There is much evidence suggesting that there are different types of Pol II promoters, including highly regulated and housekeeping promoters (23, 48–52). These two promoter types typically differ in promoter sequence elements, UAS-to-transcription start

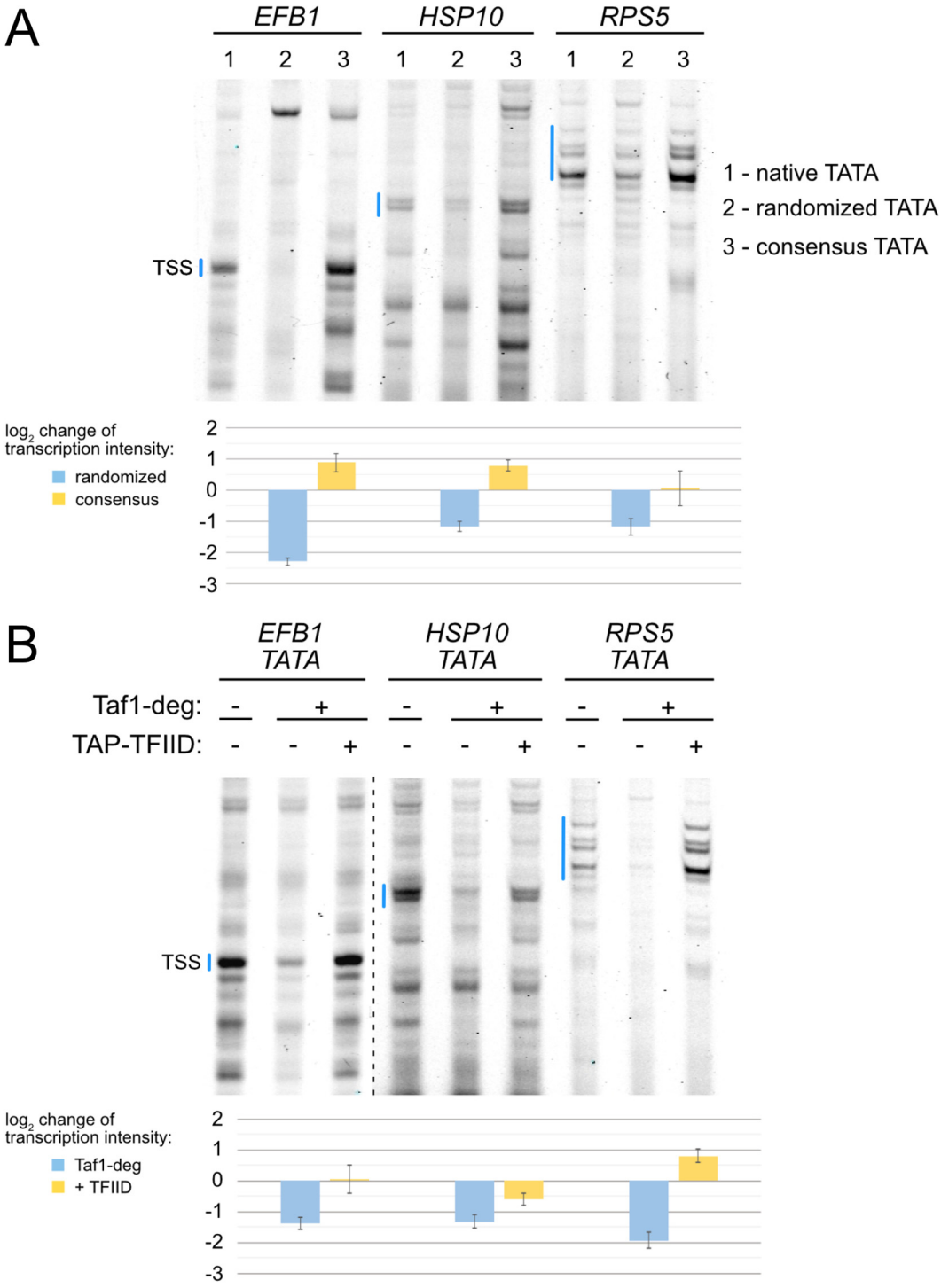


**FIG 6** The sequence of the TATA element is important for TATA-less transcription but does not affect the promoter dependence on TFIID. (A) *In vitro* transcription using WT WCE on promoters with native, randomized, and consensus TATA sequences. (B) TFIID dependence of TATA-less promoters carrying a consensus TATA box. *In vitro* transcription was done with either WT (Taf1-deg −) or Taf1-depleted (Taf1-deg +) WCE. Reactions were supplemented with 240 ng of TAP-TFIID as indicated. The $\log_2$ relative change in transcription intensity is represented as a bar plot. Standard deviation was calculated based on three independent experiments.

site (TSS) distance, chromatin modifications, nucleosome positioning, and the response to stress and signaling pathways. Yeast TATA-containing promoters are typically targeted by signaling pathways and often utilize regulated chromatin remodeling and modifications for efficient activation. Yeast promoters lacking the TATA consensus are

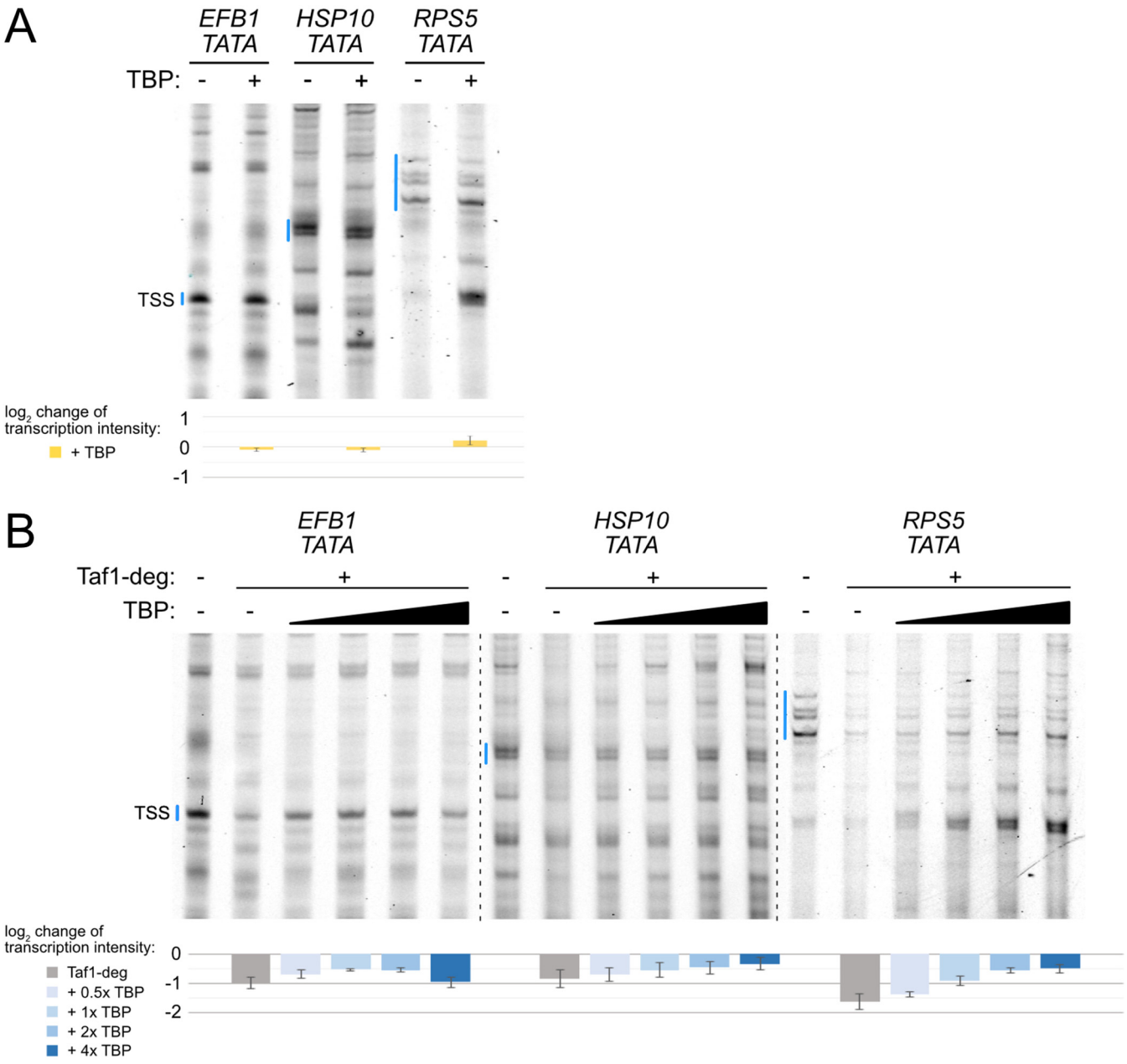


**FIG 7** Consensus TATA is not the sole determinant of promoter sensitivity to TBP. (A) *In vitro* transcription using WT WCE. Selected samples were supplemented with 80 ng of rTBP, which corresponds to 4 times the amount of TBP in WT WCE. (B) *In vitro* transcription using either WT (Taf1-deg −) or Taf1-depleted (Taf1-deg +) WCE. The following amounts of rTBP were used: 10, 20, 40, and 80 ng; these correspond approximately to 0.5, 1, 2, and 4 times the amount of TBP in WT WCE. The $\log_2$ relative change in transcription intensity is represented as a bar plot. Standard deviation was calculated based on three independent experiments.

often classified as housekeeping genes, which are typically less regulated and contain a TATA-like sequence (1- or 2-bp mismatch from the consensus) overlapping the site of PIC formation (15, 26). These TATA-less promoters also typically respond differently to transcription activators, and are often arranged with the TSS adjacent to the +1 nucleosome.

From a combination of genetic, biochemical, and structural approaches, much is known about the function and architecture of the minimal Pol II PIC at TATA-containing promoters assembled with TBP. However, much less is known about the role of TBP at TATA-less promoters and how TFIID assembles within the PIC and modulates transcriptional regulation at these promoters. As more than 80% of genes do not contain a TATA

element, it is essential to understand how transcription initiation at these genes is facilitated and regulated. One barrier to the study of TATA-less promoters has been the lack of a suitable *in vitro* transcription system. While efficient yeast *in vitro* transcription from TATA-containing promoters was observed many years ago (53), demonstration of *in vitro* transcription from TATA-less promoters is scarce (8, 54).

In this study, we optimized a rapid whole-cell extract system that is active for basal transcription at examples of both promoter types. The method also allows for testing of the role of specific factors and subunits *in vitro* by efficient *in vivo* depletion methods, followed by rapid extract preparation. Using this approach, we depleted cells of TFIID subunit Taf1 using the auxin-dependent degron system and created WCEs that allowed us to test the role of TFIID, TBP, and promoter features. In agreement with recent *in vivo* findings that show that most transcription is TFIID dependent (27) and that near equivalent TFIID levels are observed at nearly all promoters (29), we found that most *in vitro* transcription from TATA-containing and TATA-less promoters was decreased at least 2-fold upon depletion of TFIID from these extracts.

Based on the ratio of TBP/Tafs observed at different promoters in ChIP assays, it was proposed that there are at least two forms of TBP that can promote Pol II transcription *in vivo*: TFIID and a Taf-independent form that might be TBP alone (21, 22). To test this model, we examined TFIID-independent expression *in vitro* after Taf1 depletion and whether excess TBP could complement these depleted extracts. These experiments revealed a distinction between the tested TATA-containing and TATA-less promoters. While transcription in WCEs is largely TFIID dependent at both promoter types, rTBP can restore transcription from the TFIID-depleted extracts only at TATA-containing promoters. The ability of TBP to promote transcription at TATA-containing promoters is in agreement with earlier findings showing that functional PICs can be assembled at TATA-containing promoters using TBP in the absence of TFIID (55).

However, in contrast to earlier models, overexpression of TBP *in vivo* repressed transcription at both TATA-containing and TATA-less genes. Upon Taf1 depletion, neither gene class showed increased transcription compared to that in wild-type cells. This result contradicts the simple idea that there is a balance between TBP and TFIID-driven transcription. If there is TFIID-independent transcription *in vivo*, our results suggest that this is promoted by TBP in a more complex form than TBP alone. We do not yet understand why high levels of TBP decrease specific mRNA transcription. One possibility is that high TBP levels overwhelm Mot1, the ATP-dependent factor that mobilizes TBP (56). In this model, overexpression of TBP could lead to much longer-lived nonspecifically bound TBP-DNA complexes resulting in less specific transcription initiation.

Our system allowed testing of the role of the TATA-like sequences found at most TATA-less promoters. We found that this sequence is important for normal levels of initiation and proper TSS selection, as randomizing the sequence at three promoters led to at least a 2-fold decrease in transcription and resulted in utilization of an alternative upstream TSS at *EFB1* promoter. In contrast, converting the TATA-like sequence to a consensus TATA increased *in vitro* transcription at two out of three promoters. The importance of the TATA-like sequence does not prove that this is a direct TBP binding site. However, one model consistent with our results is that this sequence presents a weak TBP interaction site which is supplemented by Taf-promoter interactions. In this model, a consensus TATA would increase overall TFIID affinity and transcription at promoters where TFIID binding is limiting. Although we found that the TATA-like sequence is important, at the three promoters tested, it is not the only sequence feature that distinguishes the two promoter classes. When TFIID-depleted extracts were supplemented with high TBP levels, the TATA-less promoter derivatives with a consensus TATA did not show efficient TBP-driven transcription. Thus, we propose that there are additional promoter features that distinguish these two gene classes, consistent with earlier observations (39). In summary, we have developed a new *in vitro* system that can be utilized to examine transcription at both TATA-containing and TATA-less promoters, clarify the mechanism of TFIID-dependent and TFIID-

**TABLE 1** Strains and plasmids used in this work

| Strain or plasmid | Genotype or features | Reference or source |
|---|---|---|
| Strains | | |
| *S. cerevisiae* | | |
| BY4705 | *matα ade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0* | 58 |
| SHY1039 | *matα ade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 RPB3-3×Flag::NAT MX* pGPD1-*OSTIR::HIS3 TAF1-3×V5 IAA7::KanMX* | 27 |
| SHY1131 | *matα ade2Δ::hisG his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 RPB3-3×Flag::NAT MX* pGPD1-*OSTIR::HIS3 TAF1-3×V5 IAA7::KanMX*/pSH223 (2*μ LEU2 SPT15*) | This work |
| *Schizosaccharomyces pombe* 972h (ATCC 24843) | | ATCC |
| Plasmids | | |
| pSH223 | YEp351 derivative with the gene coding for TBP (2*μ LEU2 SPT15*) | 59 |
| pSH515 | *HIS4* core promoter transcription template | 34 |
| pEFB1_txn | pSH515 derivative, *EFB1* core promoter transcription template | This work |
| pHSP10_txn | pSH515 derivative, *HSP10* core promoter transcription template | This work |
| pRPS5_txn | pSH515 derivative, *RPS5* core promoter transcription template | This work |
| pCYC1_txn | pSH515 derivative, *CYC1* core promoter transcription template | This work |
| pSNR20_txn | pSH515 derivative, *SNR20* core promoter transcription template | This work |

independent transcription, and be used to identify promoter regions in addition to TATA element that distinguish these promoter types.

## MATERIALS AND METHODS

**Yeast strains and culture.** Strains and plasmids utilized are listed in Table 1. All *Saccharomyces cerevisiae* strains were grown in YPD medium (1% [wt/vol] yeast extract, 2% [wt/vol] peptone, 3% [wt/vol] dextrose, and 0.004% [wt/vol] adenine) or, for SHY1131, glucose complete (GC) medium (0.17% [wt/vol] yeast nitrogen base, 0.5% [wt/vol] ammonium sulfate, 2% [wt/vol] dextrose, and amino acid mix lacking leucine to sustain the selection for cells carrying the pSH223 plasmid). SHY1039 strain carries the IAA7 degron tag on Taf1 (27), which allows for fast and controlled protein degradation. For whole-cell-extract preparation and nascent RNA isolation, strains were grown to an optical density at 600 nm ($OD_{600}$) of ~1.0 and cells were treated with either indole-3-acetic acid (3-IAA; 500 μM final concentration in DMSO) or an equal volume of DMSO for 30 min before further processing. Following incubation with 3-IAA, cultures grown for nascent RNA isolation were additionally treated with 4-thiouracil (5 mM final concentration) or an equal volume of DMSO for 6 min. *Schizoaccharomyces pombe*, used as a spike-in control for nascent RNA isolation, was grown in YE medium (0.5% [wt/vol] yeast extract, 3% [wt/vol] dextrose) and treated with 4-thiouracil as described above.

**WCE preparation.** Whole-cell extracts (WCE) were prepared as previously described (8), with the following modifications. For cell lysis, cells were resuspended in equal weight/volume ratios of cold lysis buffer and transferred to 5-ml capped tubes (57 by 15.3 mm; Sarstedt) half-filled with 0.5-mm zirconia-silica beads. The lysis buffer had the following composition: 200 mM Tris-acetate (pH 7.9; 23°C), 390 mM ammonium sulfate, 20% glycerol, 1 mM EDTA. 3 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM benzamidine, 0.28 μg/ml of leupeptin, and 1.37 μg/ml of pepstatin were added immediately before use. The cells were lysed in a Mini-Beadbeater-96 (BioSpec Products) with five 3-min pulses and 8-min rests in an ice bath to keep the cells cool during the procedure. Tubes were centrifuged for 2 min at 2,000 × *g*, and crude extracts were removed. Pelleted beads were washed with a 0.5× volume of cold lysis buffer, and the two supernatants were combined. Crude extracts were supplemented with 5 M potassium acetate to a final concentration of 0.5 M, followed by a 15-min incubation on a tube roller at 4°C. Samples were centrifuged in an FL21 rotor (12,000 × *g* for 15 min) to remove cell debris, followed by centrifugation for 2 h at 200,000 × *g* in a Beckman 50.2 Ti rotor. Proteins in the clarified extract were precipitated by addition of 0.337 g/ml of ammonium sulfate. The mixtures were stirred for 1 h at 4°C. After centrifugation, proteins in the pellet were resuspended in cold resuspension buffer (20 mM HEPES-KOH [pH 7.9], 20% glycerol, 10 mM magnesium sulfate, 10 mM EGTA, 5 mM DTT, and protease inhibitors as listed for the composition of the lysis buffer) at 0.1 ml of buffer per gram of starting cell pellet. The suspension was dialyzed at 4°C against three 0.5-liter changes of dialysis buffer (resuspension buffer plus 75 mM ammonium sulfate) using a 10,000-molecular-weight-cutoff (MWCO) dialysis chamber. The first dialysis was carried out overnight, followed by two 1-h dialyses the next day. The resulting WCE was clarified by centrifugation for 5 min at 10,000 × *g*, flash-frozen, and stored at −80°C.

**TFIID and TBP purification.** TAP-TFIID was purified as described previously (8) from strain SHY626 harboring a tandem affinity purification tag on Taf13. Recombinant yeast TBP (rTBP) was purified as described previously (57).

***In vitro* transcription and primer extension.** *In vitro* transcription mixtures (25 μl) contained 10 mM HEPES (pH 7.6), 100 mM potassium glutamate, 10 mM magnesium acetate, 3.5% glycerol, 3 mM DTT, 38 mM creatine phosphate, 0.03 U of creatine phosphokinase, and 4 U of RNase Out (Invitrogen). A total of

**TABLE 2** Oligonucleotides used in this work

| Purpose and oligonucleotide | Sequence |
|---|---|
| Primer extension analysis of transcription assays | |
| EFB1_txn | GTATGACTTGTCAGCCAAAGAAGCG |
| HSP10_txn | CTTGTGCCTTGATTCTTTGGACAAG |
| RPS5_txn | CTTCTGGAATTGGAGTAGCCAAG |
| HIS4_txn | TTCACCAGTGAGACGGGCA |
| CYC1_txn | CATCTAGTCTTGAAAAGTGTAGC |
| SNR20_txn | GGTAATGAGCCTCATTGAGGTCAT |
| qPCR | |
| EFB1_qfw | ATCCACCGATTTCTCCAAGATT |
| EFB1_qRv | AGTAAACTAAATCGGAACATACCCT |
| HSP10_qFw | GTCCAAAGAATCAAGGCACAAG |
| HSP10_qRv | GCAACAACTTCAGCTTGGTTTA |
| RPS5_qFw | CACCGAAGCTCCAGTTGAA |
| RPS5_qRv | GTTTGAGCTTGTTGGACTTCTTC |
| SNO1_qFw | CCTGAGGATCTAGCCCAGT |
| SNO1_qRv | CAAGGATATAAGCCTGTTCTTTGAG |
| ACT1_qFw | GCACCATCCCATTTAACTGTAAG |
| ACT1_qRv | TAGGAGGTTATGGGAGAGTGAA |
| HIS4_qFw | CTCCAGTTCTCCAAAGAGGAAG |
| HIS4_qRv | CGTTGTTCAAGAAGGCAATGAT |
| CYC1_qFw | TAGATGTCTACAATGCCACACC |
| CYC1_qRv | TACGAATACCCTTCAGCTTGAC |
| SNR20_qFw | AAATGGACGGGAAGAGACTTT |
| SNR20_qRv | TGAAGAAACCATGAGCGAAGA |

150 ng of supercoiled plasmid DNA and 100 $\mu$g of whole-cell extract were added to each mixture, followed by 10 min of preincubation at 23°C. Transcription was initiated by the addition of nucleoside triphosphate mix (0.4 mM final concentration of each nucleoside triphosphate) and allowed to proceed for 30 min at room temperature. Reactions were stopped by the addition of 10 mM sodium acetate, 1 mM EDTA, and 0.05% SDS. Samples were assayed by primer extension as described previously (57). Each experiment was repeated at least three times. Sequences of oligonucleotides used in primer extension assays are listed in Table 2.

**Nascent RNA purification and RT-qPCR analysis.** Nascent RNA was purified and assayed by RT-qPCR as described previously (27). Sequences of oligonucleotides used are listed in Table 2.

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