

# Kin28 depletion increases association of TFIID subunits Taf1 and Taf4 with promoters in *Saccharomyces cerevisiae*

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## ABSTRACT

**Transcription of eukaryotic mRNA-encoding genes by RNA polymerase II (Pol II) begins with assembly of the pre-initiation complex (PIC), comprising Pol II and the general transcription factors. Although the pathway of PIC assembly is well established, the mechanism of assembly and the dynamics of PIC components are not fully understood. For example, only recently has it been shown that in yeast, the Mediator complex normally occupies promoters only transiently, but shows increased association when Pol II promoter escape is inhibited. Here we show that two subunits of TFIID, Taf1 and Taf4, similarly show increased occupancy as measured by ChIP upon depletion or inactivation of Kin28. In contrast, TBP occupancy is unaffected by depletion of Kin28, thus revealing an uncoupling of Taf and TBP occupancy during the transcription cycle. Increased Taf1 occupancy upon Kin28 depletion is suppressed by depletion of TBP, while depletion of TBP in the presence of Kin28 has little effect on Taf1 occupancy. The increase in Taf occupancy upon depletion of Kin28 is more pronounced at TFIID-dominated promoters compared to SAGA-dominated promoters. Our results support the suggestion, based on recent structural studies, that TFIID may not remain bound to gene promoters through the transcription initiation cycle.**

## INTRODUCTION

Transcription of mRNA genes in eukaryotes entails the formation of a pre-initiation complex (PIC) that includes the general transcription factors and Pol II. Although the

paradigm for PIC formation, Pol II initiation and promoter escape is well established (1), major mechanistic questions remain. For example, although one of the earliest steps in PIC formation is promoter binding by TBP, how this occurs is uncertain. In yeast, TBP can be delivered to promoters by either the SAGA complex or TFIID (2,3), and yeast promoters have been categorized as SAGA-dominated or TFIID-dominated based on relative occupancy by TFIID-specific subunits such as Taf1 and their response to mutations in SAGA or TFIID components (4–6). However, transcription of genes in both categories depends on both SAGA and TFIID components, and whether distinct mechanisms of PIC formation and transcription initiation operate at the two classes is unknown (4,7–9). Further complicating the picture, recent work suggests that Tafs (i.e. TBP-associated factors that are subunits of TFIID) may function in a step occurring post-initiation in the transcription cycle (10), while structural studies indicate major changes in TFIID configuration during and after TFIID recruitment and PIC assembly (11).

Another component central to assembly of the PIC is the Mediator complex. Mediator is a multiprotein complex that is conserved across eukaryotes and is important for transcription of essentially all genes transcribed by Pol II (12). Insight into Mediator recruitment and dynamics has been gained by a combination of genome-wide localization experiments, utilizing ChIP-chip (chromatin immunoprecipitation followed by microarray analysis), ChIP-seq (ChIP followed by high throughput sequencing) and ChEC-seq (chromosome endogenous cleavage followed by high throughput sequencing), together with genetic manipulations in yeast (13–18). These studies showed that although Mediator could be detected at upstream activating sequences (UASs) of many transcriptionally active genes in yeast, there were also many active genes at which little or no Mediator association was observed by ChIP, consistent with early studies using ChIP followed by qPCR at a more

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limited set of genes (19,20). The puzzle presented by these results was resolved by the discovery that prevention of promoter escape by Pol II resulted in increased Mediator ChIP signal at promoters genome-wide (15,18). These findings indicated that Mediator association with promoters is normally transient, with dissociation occurring rapidly upon Pol II escape.

Stabilization of Mediator at gene promoters was accomplished by inactivation or depletion of Kin28, a kinase that is a subunit of TFIID that phosphorylates the carboxy-terminal domain of the largest subunit of Pol II, thereby facilitating promoter escape (15,18). We recently showed that this stabilization depends on Pol II, as it is suppressed if Pol II is depleted using the anchor away technique (21). The transient occupancy of promoter regions by Mediator during the transcription cycle raises the question as to whether other components of the transcription machinery are stably bound or, like Mediator, bind and rapidly dissociate upon Pol II promoter escape. Here, we have used ChIP-seq to address this question with respect to TBP and Taf components of TFIID.

## MATERIALS AND METHODS

### Yeast strains and growth

Yeast strains used in this study are listed in Supplementary Table S1. Cultures were grown in CSM-ura (0.67% yeast nitrogen base without amino acids and 2% glucose supplemented with CSM-ura dropout mix (Bio101)) (Figure 1) or yeast peptone dextrose (YPD) media (1% bacto-yeast extract, 2% bacto-peptone extract, 2% glucose) at 30°C with shaking at 100 rpm. For experiments using the *kin28-as* analog sensitive mutant, 1-Naphthyl-PP1 (NaPP1) was added to 1 µg/ml and incubation continued for 30 min before cross-linking. For experiments using anchor away strains, rapamycin (LC Laboratories, Woburn, MA, USA) was added one hour prior to crosslinking to a final concentration of 1 µg/ml from a 1 mg/ml stock, stored in ethanol at -20°C for not more than one month. (Concentration of rapamycin stock solutions was determined using  $A_{267} = 42$  and  $A_{277} = 54$  for a 1 mg/ml solution).

### ChIP and ChIP-seq

Whole cell extracts (WCE) were prepared from 50 ml cultures as described previously, yielding 600–800 µl of WCE (16). Immunoprecipitations were performed using 180 µl of WCE for analysis by qPCR or the entire WCE less 36 µl saved as ‘input’ for ChIP-seq. Samples were incubated overnight at 4°C with 2.5–5 µg anti-TBP (58C9, Abcam, or 5 µl serum, generous gift from A. Weil, Vanderbilt University) or 2.0 µl anti-Taf1 or anti-Taf4 (serum, generous gift from J. Reese and Song Tan, Penn State University). Immunoprecipitated DNA was purified using 30 µl of protein A beads (Sigma), which were washed prior to DNA elution and cross-link reversal as previously described (16,22).

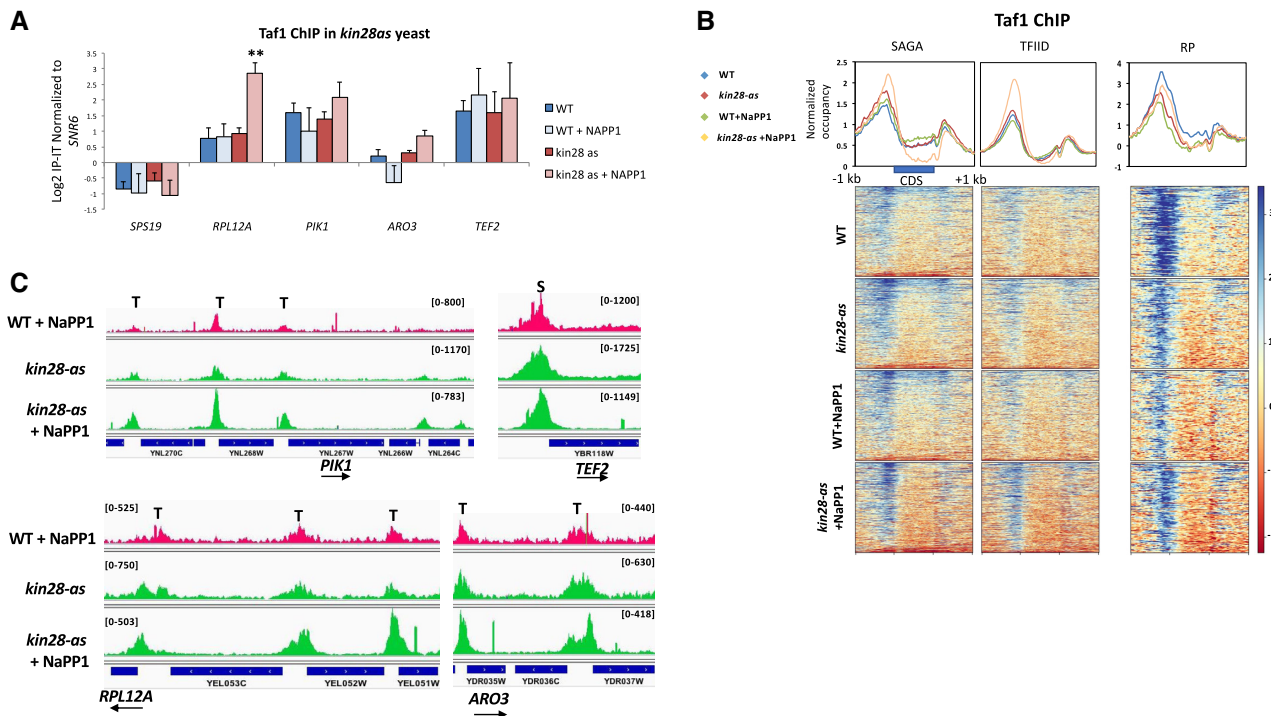
Analysis of ChIP samples by qPCR was performed on an Applied Biosystems StepOnePlus instrument, using SYBR Green master mix and ROX passive dye (ThermoFisher/USB/Affymetrix). Each reaction contained 0.5 µl of IP DNA or of a 1:100 dilution of input

DNA in a 12.5 µl volume, and was performed in duplicate. IP samples were normalized against input, and then against IP/input values for *SNR6* (a Pol III transcribed gene) or a non-transcribed region of ChrV (23). Oligonucleotides used for qPCR are shown in Supplementary Table S2.

Library preparation for Illumina paired-end sequencing was performed with the NEBNext Ultra II library preparation kit (New England Biolabs) according to manufacturer’s protocol and barcoded using NEXTflex barcodes (BIOO Scientific, Austin, TX, USA) or NEBNext Multiplex Oligos for Illumina. In some experiments, a size selection step was performed on barcoded libraries by isolating fragment sizes between 200 and 500 bp on a 2% E-Gel EX agarose gel apparatus (ThermoFisher Scientific). Sequencing was performed on the Illumina NextSeq 500 platform at the University of Buffalo next-generation sequencing and expression analysis core (University of Buffalo, State University of New York, Buffalo, NY, USA) or at the Illumina NextSeq platform at the Wadsworth Center, New York State Department of Health (Albany, NY, USA). Results of ChIP-seq experiments are representative of at least two biological replicate experiments, except for those of Figure 1 and the Taf4 ChIP experiments of Figure 2, which were performed only once (Supplementary Table S3).

### ChIP-Seq analysis

Unfiltered sequencing reads were aligned to the *S. cerevisiae* reference genome (Sacc3) using bwa (24). Up to one mismatch was allowed for each aligned read. Reads mapping to multiple sites were retained to allow evaluation of associations with non-unique sequences (24) and duplicate reads were retained. Calculation of coverage, comparisons between different data sets, and identification of overlapping binding regions were preceded by library size normalization, and were performed with the ‘chipseq’ and ‘GenomicRanges’ packages in BioConductor (25). Alternatively, reads were aligned and analysis conducted using the Galaxy platform (26) and Excel; results obtained using these two analysis pipelines were consistent. For meta-gene analysis, including heat maps, reads were normalized against input from *kin28-AA* yeast (KHW127) grown in the absence of rapamycin. Taf1 and TBP occupancy were determined as read depth over the 300 bp upstream of coding sequence using BedCov in SamTools (27). Ten genes exhibiting anomalous Taf1/Taf4 signal were removed prior to further analysis; these comprised five genes present in the rDNA locus and five additional genes displaying anomalous peaks. Genes proximate to tRNA genes (within 500 bp upstream of the 5’ end of the ORF) were removed in analyses of TBP occupancy. For analysis of Pol II/TBP ratios, 14 genes having ratios >20 were removed from consideration; these comprised nine dubious ORFs, a Ty element, *STE2* (which is expressed in the *Mat a* strain used for Pol II ChIP-seq but not in the *Mat α kin28-AA* strain), *CSS1*, *RPS29A* and *RPS29B*. The 1000 genes having highest Pol II occupancy, normalized to gene length, were obtained using BedCov to obtain read depth over coding sequences using Pol II ChIP-seq data (strain BY4741 grown at 30°C in YPD medium) (16,21). Genes designated as SAGA-dominated and TFIID-dominated were ob-



**Figure 1.** Increased occupancy of Taf1 at gene promoters upon inactivation of Kin28. (A) Normalized Taf1 occupancy at promoter regions of indicated genes was determined by ChIP followed by qPCR. ChIP was performed in wild type (WT) yeast (EPY4706) and *kin28-as* yeast (YFR763) grown in CSM-ura with or without 1 h treatment with NaPP1 as indicated. Error bars reflect s.d.;  $n = 3$ . Analysis of the four sets of data for each gene promoter by Anova followed by Tukey's posthoc test showed that only occupancy at *RPL12A* in *kin28-as* yeast in the presence of NaPP1 varied significantly ( $P < 0.05$ ) from occupancy in the other strains (indicated by  $***P < 0.01$ ). (B) Normalized Taf1 occupancy ( $\log_2$  ratio of IP reads to input reads) in WT and *kin28-as* yeast after 1 h treatment with NaPP1. Reads were mapped to all SAGA-dominated (567 genes), non-RP TFIID-dominated (4896 genes) and RP genes (137 genes). Genes were normalized for length and aligned by start and end of coding sequence (CDS) and sorted according to average signal intensity. Each horizontal line in the heat maps represents a gene, and the line graphs depict averages over all genes in the heat maps. (C) Browser scans showing Taf1 occupancy in *kin28-as* yeast with and without NaPP1 treatment, and in WT cells treated with NaPP1. Peaks at SAGA-dominated and TFIID-dominated gene promoters are indicated by 'S' and 'T', respectively. Scales, in reads, are shown in brackets, and have been adjusted relative to the total number of reads in each sample to allow direct comparison of occupancies. For example, the *kin28-as* sample yielded 19.3 million mapped reads, while the *kin28-as* + NaPP1 sample yielded 12.7 million mapped reads; the scales have therefore been adjusted to reflect the ratio of 0.66 in reads.

tained from (4), and genes designated as containing or not containing a consensus TATA element, and being Taf1-enriched or Taf1-depleted, were obtained from (28). Occupancy profiles were normalized for read depth and generated using the Integrative Genomics Viewer (29). Gene ontology analysis was performed using the Generic Gene Ontology Term Finder (<https://go.princeton.edu/cgi-bin/GOTermFinder/GOTermFinder>) (30). Hypergeometric test  $P$ -values were calculated using the online calculator at <http://www.alewand.de/stattab/tabdiske.htm>, and the Mann-Whitney  $U$  test was performed using the online calculators at <http://astatsa.com/WilcoxonTest/> and <https://www.socscistatistics.com/tests/mannwhitney/default2.aspx>.

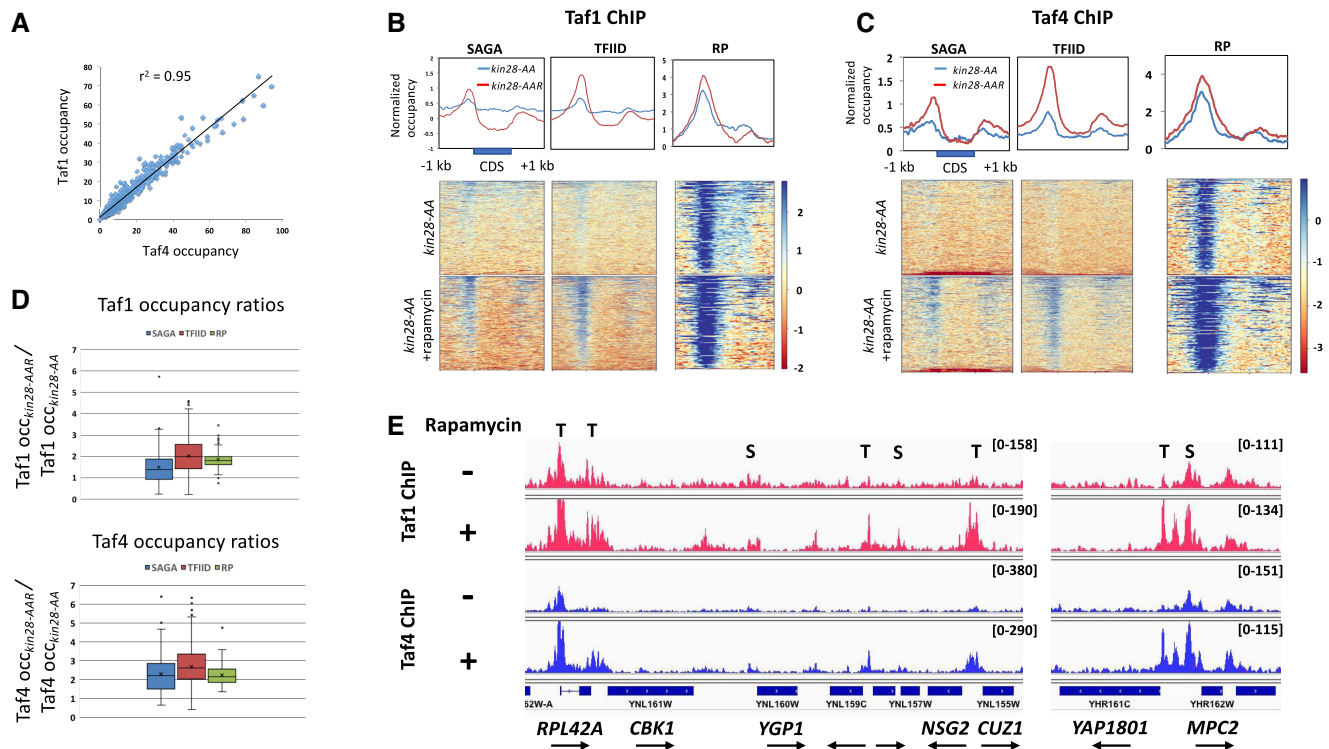
## RESULTS

### Inactivation of an analog-sensitive mutant of Kin28 stabilizes promoter occupancy by Taf1

Mediator association with gene promoters is difficult to detect by ChIP in yeast under normal growth conditions, but yields a clear ChIP signal upon depletion or inactivation of Kin28 (15,18). Kin28 phosphorylates Ser5 of the YSPTSPS heptad repeat of the carboxy terminal domain (CTD) of Rpb1, the largest subunit of Pol II, facilitating its asso-

ciation with factors involved in transcriptional elongation and associated processes (31). Inhibition of this phosphorylation by depletion or inactivation of Kin28, inhibits promoter escape by Pol II, and this evidently stabilizes Mediator association, thus implying that Mediator normally occupies active promoters only transiently (15,18).

To test whether TFIID exhibits similar behavior, we monitored Taf1 association by ChIP in wild type yeast and yeast harboring an analog sensitive *kin28-as* mutation, in which Kin28 can be inactivated by administration of NaPP1 (32). The *kin28-as* yeast strain harbors the *kin28-as* allele at the native chromosomal location as well as a copy of the *kin28-as* allele on a *URA3*-marked plasmid, which allows its expression at levels comparable to wild type Kin28 (32); therefore, strains for these experiments were grown in CSM-ura medium. ChIP against Taf1 followed by qPCR analysis revealed substantially increased Taf1 occupancy upon Kin28 inactivation at *RPL12A*, and modest, albeit not statistically significant, increases in ChIP signal at the *PIK1* and *ARO3* promoters (Figure 1A). No change in occupancy was apparent at the *TEF2* promoter, and a negative control, *SPS19* (not expressed in logarithmic growth in YPD), showed no enrichment for Taf1 whether Kin28 was active or inactive.



**Figure 2.** Increased occupancy of Taf1 and Taf4 at gene promoters upon depletion of Kin28. (A) Normalized occupancy of Taf1 plotted against Taf4 occupancy for the ~1000 genes (see Methods) most highly occupied by Pol II. (B, C) Normalized occupancy ( $\log_2$  ratio of IP reads to input reads) is depicted for Taf1 (B) and Taf4 (C) in *kin28-AA* yeast, without and with 1 h rapamycin treatment, mapped to all SAGA-dominated (567 genes), non-RP TFIID-dominated (4896 genes), and RP genes (137 genes). Genes were normalized for length, aligned by coding sequence (CDS) start and stop, and sorted according to average signal intensity. The 19 SAGA-dominated genes at the bottom of the Taf4 heat maps were removed before calculating averages used in the line graphs, as these were almost all Tyl elements that had higher intensity in the input control than in the Taf4 ChIP sample, and therefore yielded negative values in the heat map. Note that a different scale is shown for the RP gene line graphs than for SAGA- and TFIID-dominated genes. (D) Ratios of Taf1 (top) and Taf4 (bottom) occupancy in *kin28-AA* yeast in the presence and absence of rapamycin are shown in box and whisker plots for the ~1000 genes having highest occupancy by Pol II, sorted into SAGA-dominated (154 genes), non-RP TFIID-dominated (534 genes), and RP genes (136 genes) (see Materials and Methods). The boxes show the second and third quartiles, and the whiskers indicate the first and fourth quartiles; median values are indicated by the horizontal lines in the boxes separating second and third quartiles, and outliers are depicted as points above or below the whiskers. (E) Browser scans showing Taf1 and Taf4 occupancy in *kin28-AA* yeast with and without rapamycin treatment. Peaks at SAGA-dominated and TFIID-dominated gene promoters are indicated by 'S' and 'T', respectively. Scales, in reads, are shown in brackets, and have been adjusted relative to the total number of reads in each sample to allow direct comparison of occupancies within a given IP group (e.g. Taf1 with and without rapamycin).

To examine the effect of Kin28 inactivation on Taf1 occupancy on a genome-wide scale, we conducted ChIP followed by high throughput sequencing (ChIP-seq) against Taf1 in wild type yeast and in *kin28-as* yeast before and after treatment with NaPPI. Unlike Mediator subunits, Taf1 exhibits a clear ChIP signal near the transcription start site (TSS) of many genes under normal growth conditions (Figure 1B, WT). Taf1 occupancy was essentially identical in wild type yeast whether or not they were treated with NaPPI at SAGA-dominated and non-RP TFIID-dominated genes, but was reduced at RP genes by NaPPI treatment (Figure 1B; compare blue and green traces); possibly NaPPI administration induces a mild stress response that results in some RP gene repression. Taf1 occupancy in the *kin28-as* strain in the absence of NaPPI showed a very slight increase compared to wild type yeast (compare red and blue traces in Figure 1B), suggesting a modest effect on Taf1 occupancy of the *kin28-as* allele in the absence of NaPPI. Most notably, Taf1 occupancy increased in *kin28-as* yeast treated with NaPPI (orange traces and bottom heat maps in Figure 1B). This effect was strongest at non-RP TFIID-dominated

genes, but was also evident at SAGA-dominated genes. Taf1 occupancy was also increased at RP genes in *kin28-as* yeast treated with NaPPI compared to wild type yeast treated with NaPPI, or with the untreated *kin28-as* strain, but remained lower than in untreated wild type cells.

ChIP-seq results for *RPL12A*, *TEF2*, *ARO3* and *PIK1* were consistent with qPCR results, with all but *TEF2* showing increased occupancy of Taf1 upon Kin28 inactivation (Figure 1C). Increased Taf1 occupancy was seen at other promoters as well, consistent with heat map results. Altogether, the results depicted in Figure 1 suggest that Taf1 may, like Mediator, be stabilized at promoters when promoter escape by Pol II is inhibited, with the effect being strongest at TFIID-dominated promoters.

#### Depletion of Kin28 stabilizes promoter occupancy by Taf1 and Taf4

To test further the effect of Kin28 on Taf occupancy, we used the anchor away method to deplete Kin28 from the nucleus (33). This method employs a yeast strain in which

the ribosomal protein Rpl13A has been modified by addition of a C-terminal FKBP12 tag, while the protein of interest (Kin28) is C-terminally tagged with the FRB fragment, which binds tightly to FKBP12 upon addition of rapamycin, resulting in the FRB-tagged protein being transported out of the nucleus during ribosomal protein processing. The anchor away yeast strain also harbors a *tor1-1* mutation, which abrogates the normal stress response induced by rapamycin (33). Effective depletion of Kin28 from the nucleus after 1 h of rapamycin treatment in the yeast strain used here was previously demonstrated by ChIP analysis of Kin28-FRB binding and by the inability of the strain to grow on media containing rapamycin (18,21). In addition, previous studies, including our own, used this method to demonstrate increased association of Mediator with promoters, as monitored by ChIP, after depletion of Kin28 by 1 h of rapamycin treatment (14,17,18).

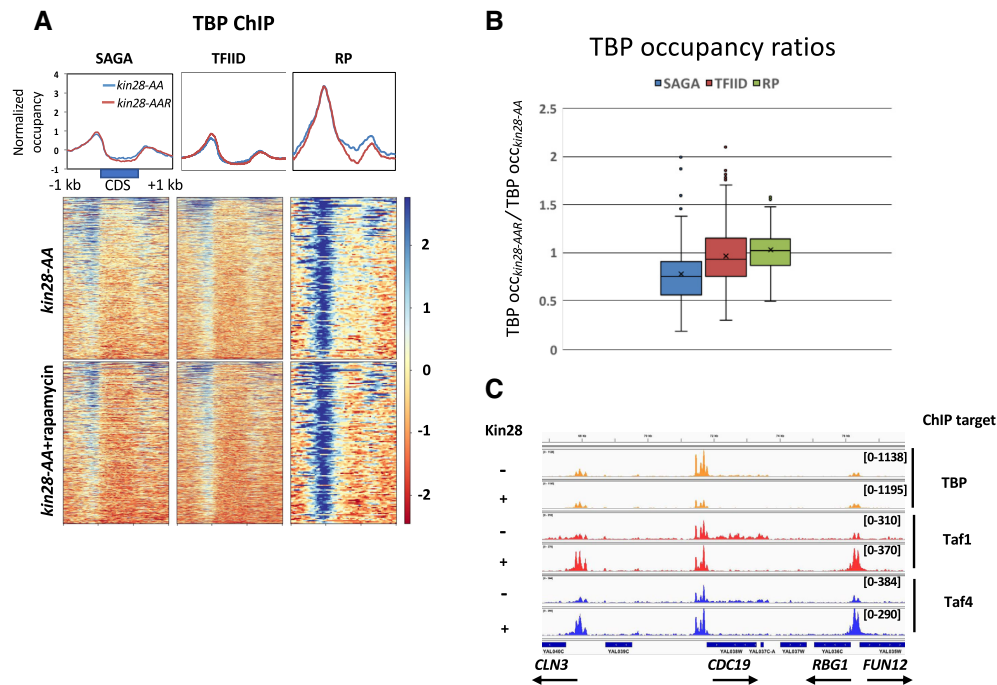
We conducted ChIP-seq using antibodies against the TFIID-specific subunits Taf1 and Taf4 and observed a strong correlation ( $r^2 = 0.95$ ) between Taf1 and Taf4 occupancy, measured over the promoter-proximal 300 bp, over a wide range of peak intensities (Figure 2A). Depletion of Kin28 by 1 h of rapamycin treatment resulted in increased ChIP signal for both Taf1 and Taf4 at most promoters (Figure 2B and C; results from biological replicates for Taf1 shown in Supplementary Figure S1A). (The lower signal for Taf1 observed in Figure 2B compared to Figure 1 may be related to the different growth media used in these experiments; CSM-ura in Figure 1, and YPD in Figure 2 and subsequent experiments reported here. Fan and Struhl reported lower ChIP signal for Mediator at gene promoters in yeast grown in YPD than in CSM media; it is possible that other PIC components likewise show differential association in different growth conditions (20).) To obtain a more quantitative assessment of the effect of Kin28 depletion on Taf1 and Taf4 occupancy, we measured the occupancy ratios in the presence and absence of rapamycin for the 1000 genes showing highest Pol II occupancy and plotted the results in boxplot format (Figure 2D; results for biological replicate experiments for Taf1 shown in Supplementary Figure S1B). The results indicate increased occupancy by Taf1 and Taf4 upon Kin28 depletion, supporting the results of Figure 1 with an independent method of Kin28 depletion and for an additional Taf. Consistent with the results for the *kin28-as* mutant, the increase in both Taf1 and Taf4 occupancy was more pronounced at TFIID-dominated promoters than for SAGA-dominated promoters (Figure 2D;  $P = 1.5 \times 10^{-12}$  for Taf1 and  $P = 1.5 \times 10^{-5}$  for Taf4 at SAGA versus TFIID promoters (Mann-Whitney *U* test)). This differential effect could be observed at individual promoters; compare the increase in signal upstream of the TFIID-dominated *NSG2/CUZ1* or *YAP1801* promoter to the SAGA-dominated *YGPI* or *MPC2* promoter (Figure 2E). Comparison of browser scans depicting Taf1 and Taf4 occupancy following depletion using either the analog-sensitive *kin28-as* mutant or anchor-away method revealed generally consistent results, with some differences (e.g. strong signal for Taf1 occupancy upstream of *YGPI*) likely due to differences in growth medium (CSM-ura for the *kin28-as* mutant experiments versus YPD for anchor-away experiments) (Supplementary Figure S2).

In contrast to Taf1 and Taf4, TBP occupancy did not increase upon depleting Kin28 (Figure 3A, B and Supplementary Figure S3). Rather, TBP occupancy decreased slightly at SAGA-dominated genes while showing almost no change on average for TFIID-dominated genes upon depletion of Kin28 (Figure 3B). Consistent with these results, a previous study showed a slight reduction in TFIIB promoter occupancy after depleting Kin28 using the anchor-away protocol, and our own re-analysis of that data confirmed that finding (Supplementary Figure S4) (18). Interestingly, genes showing the strongest (more than 2-fold) decrease in TBP signal following Kin28 depletion also showed little or no increase in Taf1 or Taf4 signal when Kin28 was depleted (Figure 3C; note decrease in TBP occupancy for *CDC19*; and Supplementary Figure S5). Analysis of the 56 genes for which TBP occupancy decreased by more than 2-fold indicated they were enriched ( $P < 10^{-4}$  in all cases) for SAGA-dominated genes (31 of 56), for several GO categories including cell wall (GO ID 5618, 7047 and 9277), translation (GO ID 6417), and glycolysis (GO ID 6096), and for promoter association of Ace2, Fkh2, Msn2, and Spt2 (34). Enrichment for Msn2 association suggests that some form of stress response might be involved in the observed decrease in TBP occupancy, but no GO categories involving stress response were enriched among this set of genes. These findings suggest that the effects of Kin28 depletion on Taf and TBP occupancy are variable and in some sense correlated, and likely depend on multiple variables (perhaps especially on the associated transcriptional activators). However, we do not have a mechanistic explanation for the variable decrease in TBP and Taf occupancy seen upon genome-wide examination of promoters following Kin28 depletion.

Taken together, the results presented here suggest that Tafs, like Mediator, bind transiently to most promoters under normal conditions, with their occupancy being stabilized by inhibition of promoter escape by Pol II. In contrast, occupancy by TBP and TFIIB is stable and is not sensitive to inhibition of Pol II promoter escape. Furthermore, occupancy by TBP and TFIIB must not require continued occupancy by Tafs or Mediator during the normal transcription cycle, since Tafs and Mediator appear to be associated only transiently.

### Increased occupancy by Taf1 upon Kin28 depletion is suppressed by TBP depletion

To test whether the increased association of Taf1 seen upon Kin28 depletion requires stable TBP occupancy, we performed ChIP-seq against Taf1 in *kin28-tbp-AA* yeast, in which rapamycin addition causes depletion of both Kin28 and TBP (21). We found that Taf1 occupancy was essentially unchanged upon simultaneous depletion of Kin28 and TBP (Figure 4A and Supplementary Figure S6A). We previously reported marked effects on Mediator and Pol II association in *kin28-tbp-AA* yeast upon rapamycin administration, providing evidence for efficient depletion of both Kin28 and TBP (Figure 5, Figure supplements S3–S4 and S7 in (21)). In addition, a conspicuous peak observed at the 3' ends of coding sequences is nearly abolished in the presence of rapamycin in *kin28-tbp-AA* yeast (Figure 4A). This peak is artifactual, as it is observed even in ChIP experi-



**Figure 3.** TBP occupancy is not affected by depletion of Kin28. (A) Normalized TBP occupancy ( $\log_2$  ratio of IP reads to input reads) in *kin28-ΔA* yeast, without and with 1 h rapamycin treatment, mapped to all SAGA-dominated, non-RP TFIID-dominated, and RP genes. Genes were normalized for length, aligned by coding sequence (CDS) start and stop, and sorted according to average signal intensity. (B) Ratios of TBP occupancy in *kin28-ΔA* yeast in the presence and absence of rapamycin are shown in box and whisker plots, as in Figure 2, for the ~1000 genes having highest occupancy by Pol II, sorted into SAGA-dominated (151 genes), non-RP TFIID-dominated (530 genes) and RP genes (136 genes) (see Materials and Methods). (C) Browser scan showing Taf1, Taf4 and TBP occupancy in *kin28-ΔA* yeast with and without rapamycin treatment. Scales, in reads, are shown in brackets, and have been adjusted relative to the total number of reads in each sample to allow direct comparison of occupancies within a given IP group.

ments against GFP, but it is dependent upon transcription (Supplementary Figure S7) (16,35). Thus, its disappearance in the presence of rapamycin corroborates effective depletion of Kin28 and TBP. Occupancy ratios for Taf1 and Taf4 in the presence and absence of rapamycin in *kin28-ΔA* yeast are increased by 1.5–3 fold at most genes upon depletion of Kin28, as discussed earlier (Figure 4B). Depletion of TBP together with Kin28 abrogates this increased occupancy at most genes in all categories (Figure 4B and Supplementary Figure S6B).

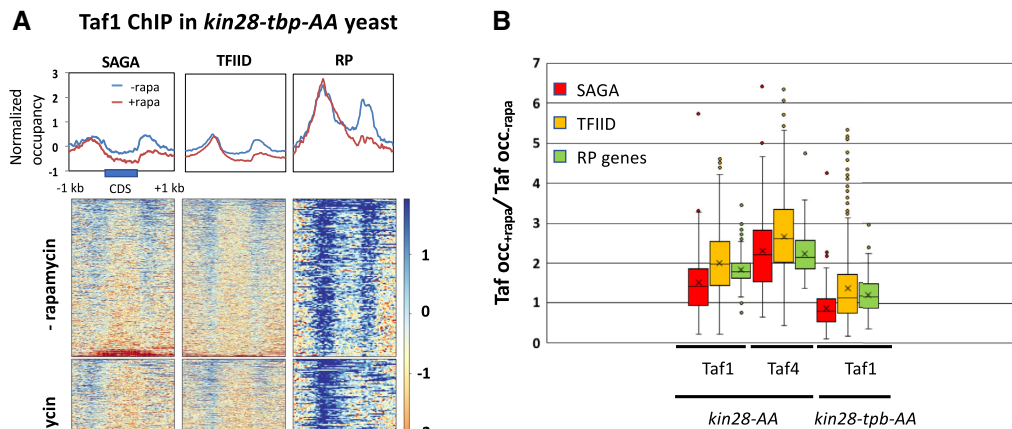
Although the increase in Taf1 occupancy seen upon depletion of Kin28 was suppressed by simultaneous depletion of TBP at most genes, we observed a substantial number of outliers among TFIID-dominated genes that showed increased Taf1 association upon depletion of Kin28 and TBP. Analysis of this cohort (101 non-RP, TFIID-dominated genes showing  $>2\times$  increase in Taf1 association in *kin28-tbp-ΔA* yeast after rapamycin treatment) revealed that it was enriched for genes involved in mRNA binding (21 genes; corrected  $P$ -value  $2.0 \times 10^{-11}$ ) and related functions (e.g. organic cyclic compound binding), and for genes involved in ribosome biogenesis (53 Ribi genes;  $P$ -value  $7.8 \times 10^{-34}$ ) (30). Furthermore, this gene set was enriched for promoters occupied by Abf1 but not by Rap1 or Reb1, all of which are enriched for binding at Ribi genes (36) ( $P$ -value  $1.5 \times 10^{-4}$  for Abf1;  $P > 0.05$  for Rap1 and Reb1 for genes with association  $P$ -value  $< 0.005$ ; hypergeometric test (37)). A recent study showed that Sfp1 is bound to nearly all Ribi gene promoters (38), and the cohort of 101 genes showing

increased Taf1 association upon simultaneous depletion of Kin28 and TBP is enriched for Sfp1-associated genes (55 genes;  $P < 10^{-29}$ , hypergeometric test), including 33 Ribi genes. No other transcription factors were found to be enriched for binding to this cohort (34). Possibly Taf1 is stabilized by factors associating with these promoters, including Abf1 and Sfp1, such that TBP is not needed for increased association upon Kin28 depletion.

Based on these results, we conclude that stable association of TBP is required at most promoters for increased association of Taf1 upon depletion of Kin28.

### Taf1 occupancy does not require TBP

We recently reported investigations of the interdependencies among the PIC components TBP, Taf1, and Pol II for occupancy of promoter regions, using ChIP-seq before and after conditional depletion of these same components (21). Examination of occupancy of PIC components at a subset of genes, dubbed ‘UAS genes’, that exhibit Mediator peaks at upstream activating sequences (UASs) in wild type yeast, revealed that depletion of TBP resulted in nearly complete loss of Pol II occupancy, but little change in occupancy by Taf1. These results are consistent with the generally accepted view that recruitment of Pol II strongly depends on TBP, and indicate that Taf components of TFIID can associate with promoters even in the absence of TBP. The latter observation is consistent with previous reports showing



**Figure 4.** Depletion of TBP suppresses increased occupancy of Taf1 seen upon depletion of Kin28. (A) Normalized TBP occupancy ( $\log_2$  ratio of IP reads to input reads) in *kin28-tbp-AA* yeast, without and with 1 h rapamycin treatment, mapped to all SAGA-dominated, non-RP TFIID-dominated, and RP genes. Genes were normalized for length, aligned by coding sequence (CDS) start and stop, and sorted according to average signal intensity. The 21 SAGA-dominated genes at the bottom of the heat maps were removed before calculating averages used in the line graphs, as these were almost Ty1 elements that had higher intensity in the input control than in the Taf1 ChIP sample, and therefore yielded negative values in the heat map. (B) Ratios of Taf1 and Taf4 occupancy in *kin28-AA* yeast in the presence and absence of rapamycin are shown in box and whisker plots, as in Figure 2, for the  $\sim 1000$  genes having highest occupancy by Pol II (see Materials and Methods), sorted into SAGA-dominated (151 genes), non-RP TFIID-dominated (530 genes) and RP genes (136 genes).

continued Taf1 association at several promoters after inactivation of a *tbp-ts* mutant (3,6).

We were interested in determining whether promoters categorized as ‘TFIID-dominated’ and ‘SAGA-dominated’ (4) differed in these interdependencies, as this could help address the question of whether such promoters differ mechanistically. We therefore re-examined our data to ascertain the effect of depletion of TBP on Taf1 occupancy at SAGA-dominated and TFIID-dominated genes. Examination of heat maps and line graphs indicated little change in Taf1 occupancy after depletion of TBP (Figure 5A and Supplementary Figure S8A). TBP depletion was efficient as shown by ChIP-seq of TBP (21). Quantitative comparison of Taf1 occupancy in the presence and absence of rapamycin in *tbp-AA* yeast revealed on average a decrease of about 30% at SAGA-dominated genes and no change at TFIID-dominated genes, including RP genes (Figure 5B and Supplementary Figure S8B). Variable effects of TBP depletion on Taf1 occupancy were observed in browser scans, although most Taf1 peaks were unaffected by TBP depletion (Figure 5C). We conclude that Taf1 occupancy does not generally depend on continued occupancy by TBP, and that SAGA-dominated genes show a mildly stronger dependence on TBP for normal levels of Taf1 association with promoters than do TFIID-dominated genes.

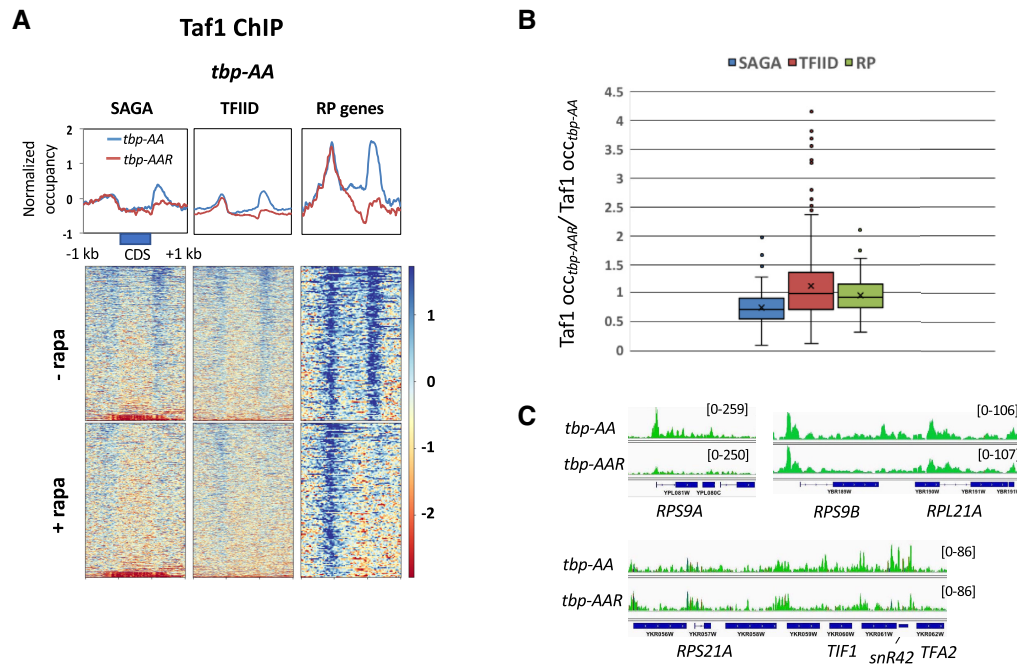
#### Variation in TBP/Taf1 ratio at gene promoters

Although essentially all genes transcribed by Pol II in yeast depend on both TFIID and the SAGA complex for normal levels of transcription, differential effects of conditional mutations in SAGA and TFIID components led to categorization of  $\sim 10\%$  of yeast genes as SAGA-dominated and  $\sim 90\%$  as being TFIID-dominated (4,8). This categoriza-

tion was supported by subsequent genome-wide ChIP results showing higher levels of TFIID components at TFIID-dominated than at SAGA-dominated genes (28,39). However, a more recent study using ChEC-seq reported comparable levels of Taf1 association at SAGA-dominated and TFIID-dominated promoters (13). Two other reports found that depletion of TFIID and SAGA components via auxin-induced degradation resulted in decreased Pol II association equally at SAGA- and TFIID-dominated genes; effects at genes having consensus TATA elements and those lacking consensus TATA elements were also indistinguishable when examined across all genes (7,9). However, these latter findings were disputed in a subsequent study that found preferential loss of Pol II at TFIID-dominated genes, both by independent experiments and in a re-analysis of data from the earlier study (40).

To gain further insight into whether categorization of genes as SAGA-dominated and TFIID-dominated cohorts reflects mechanistic differences, we compared the ratio of TBP and Taf1 occupancy for the 1000 genes having highest Pol II occupancy (normalized for gene length) as determined in previous ChIP-seq experiments (16,21). After removing genes showing anomalous ChIP peaks or being proximate to tRNA genes (see Methods), the remaining cohort included 154 SAGA-dominated genes, 534 TFIID-dominated genes and 136 RP genes that were considered separately from SAGA- or TFIID-dominated genes (4).

Plotting TBP occupancy against Taf1 occupancy for SAGA-dominated, TFIID-dominated, and RP genes revealed strong correlations for all three groups and slopes that differed considerably (Figure 6A). (Note that the slopes do not reflect molar ratio, as it is not possible to determine this directly from ChIP experiments using distinct antibodies for Taf1 and TBP.) RP genes clearly formed



**Figure 5.** Depletion of TBP has little effect on Taf1 occupancy. (A) Normalized Taf1 occupancy ( $\log_2$  ratio of IP reads to input reads) in *tbp-AA* yeast, without and with 1 h rapamycin treatment, mapped to all SAGA-dominated, non-RP TFIID-dominated, and RP genes. Genes were normalized for length, aligned by coding sequence (CDS) start and stop, and sorted according to average signal intensity. The 22 SAGA-dominated genes at the bottom of the heat maps were removed before calculating averages used in the line graphs, as these were almost Ty1 elements that had higher intensity in the input control than in the Taf1 ChIP sample, and therefore yielded negative values in the heat map. (B) Ratios of Taf1 occupancy in *tbp-AA* yeast in the presence and absence of rapamycin are shown in box and whisker plots, as in Figure 2, for the  $\sim 1000$  genes having highest occupancy by Pol II (see Materials and Methods), sorted into SAGA-dominated (151 genes), non-RP TFIID-dominated (530 genes) and RP genes (136 genes). (C) Browser scans showing normalized Taf1 occupancy in *tbp-AA* yeast with and without rapamycin treatment. Scales, in reads, are shown in brackets, and have been adjusted relative to the total number of reads in each sample to allow direct comparison of occupancies.

a distinct cohort and exhibited the lowest TBP/Taf1 ratios, consistent with previous studies indicating high TFIID occupancy and dependence of these genes (Figure 6A-B) (5,6,41). SAGA-dominated genes showed the lowest levels of Taf1 occupancy relative to TBP; TFIID-dominated genes were intermediate in their TBP/Taf1 ratios between SAGA-dominated and RP genes and significantly different as a class from both (respective medians were 3.2, 1.9 and 1.4;  $P$ -values  $< 1 \times 10^{-5}$  for all pairwise comparisons using Mann–Whitney  $U$ -test) (Figure 6B).

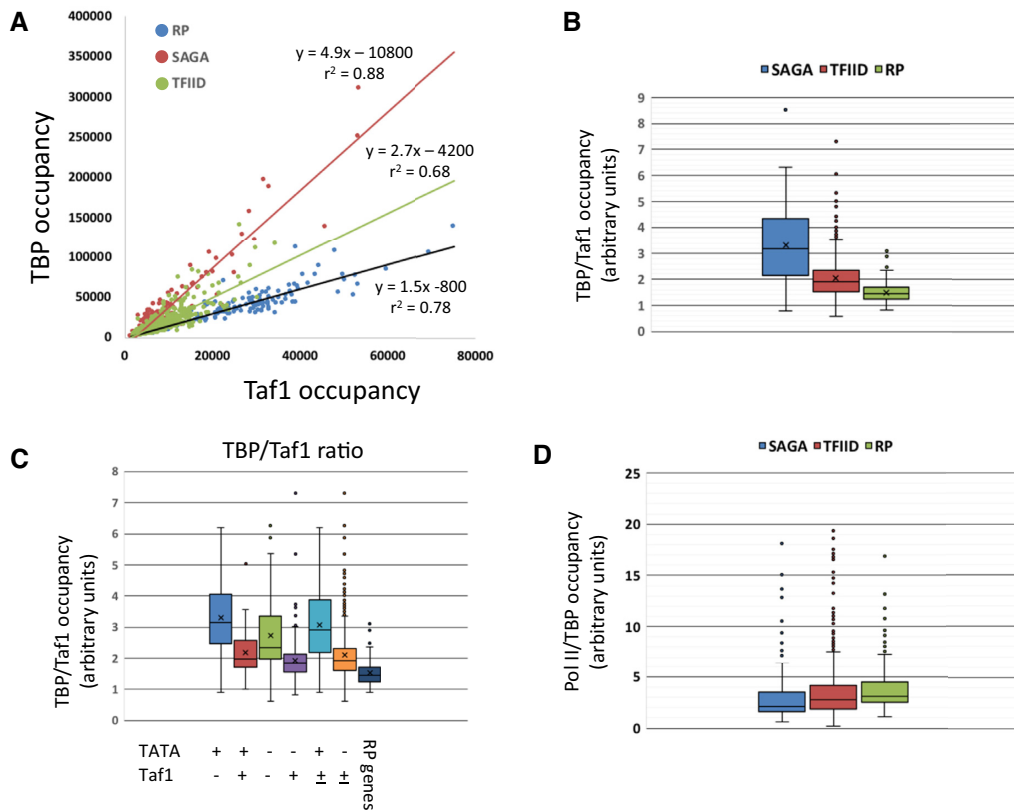
The category of RP genes is defined by function; the high Taf1 levels and low TBP/Taf1 ratios observed for this cohort therefore reflect properties independent of their categorization that likely reflect mechanistically distinct behavior. In contrast, the disparate TBP/Taf1 ratios observed for SAGA-dominated and TFIID-dominated genes corroborate their categorization but do not provide evidence independent of that categorization for distinct mechanisms of transcriptional activation. Rhee and Pugh measured occupancy of PIC components including Taf1 and TBP using ChIP-exo, and re-categorized yeast genes as enriched or depleted for Taf1, and containing or lacking a consensus TATA element (28). We plotted TBP/Taf1 ratios for these groups and noted that TATA-containing genes behaved distinctly (again with overlap) from genes lacking a consensus TATA element, consistent with previous analysis (Figure 6C) (28). Since the property of having or lacking a TATA element is independent of measurements of Taf1

and TBP occupancy, this provides additional evidence for distinct mechanisms of PIC assembly at these two gene categories. Finally, we note that the ratio of Pol II (normalized for gene length) to TBP occupancy displays a trend that is the opposite of that seen for TBP/Taf1 ratios (respective medians for SAGA-dominated, TFIID-dominated, and RP genes of 2.1, 2.7 and 3.1), suggesting that TFIID may be more effective than SAGA at facilitating Pol II recruitment.

## DISCUSSION

A principal conclusion of this work is that depletion or inactivation of Kin28 results in increased occupancy, as measured by ChIP, of Taf1 and Taf4 at promoters genome-wide. This conclusion presumes that ChIP signal accurately reflects occupancy. An alternative possibility is that conformational changes affect the efficiency of immunoprecipitation, and that inhibition of Pol II promoter escape by depletion or inactivation of Kin28 locks TFIID in a configuration in which ChIP of Taf subunits is more efficient than in the presence of Kin28. Consistent with this notion, human TFIID undergoes major conformational changes upon binding of TFIID (including its TBP subunit) to promoter DNA and TFIIA (11,42). However, Taf1 and Taf4 are situated in distinct regions of TFIID: TFIID comprises three lobes, with Taf4 situated at the apical ends of the two outer lobes in the promoter-unbound state while Taf1 is on the opposite side of the central lobe. It thus seems unlikely that





**Figure 6.** Differential TBP/Taf1 ratios at SAGA- and TFIID-dominated genes, and at gene promoters having and lacking consensus TATA elements. (A) Normalized occupancy of TBP plotted against Taf1 occupancy for the ~1000 genes (see Materials and Methods) most highly occupied by Pol II, separated into SAGA-dominated (151 genes), non-RP TFIID-dominated (530 genes), and RP genes (136 genes). (B) Ratios of TBP to Taf1 occupancy in *kin28-ΔA* yeast in the absence of rapamycin are shown in box and whisker plots, as in Figure 2, for the ~1000 genes having highest occupancy by Pol II, sorted into SAGA-dominated, non-RP TFIID-dominated, and RP genes. (C) As in (B), with genes sorted by the presence or absence of a consensus TATA element and enrichment or depletion of Taf1 (28). The designation '±' indicates that both Taf1 enriched and Taf1 depleted genes are included. RP genes were removed from these categories and are shown separately. (D) Ratios of Pol II, normalized for gene length, to TBP occupancy in *kin28-ΔA* yeast in the absence of rapamycin are shown in box and whisker plots, as in Figure 2, for the ~1000 genes having highest occupancy by Pol II (see Materials and Methods), sorted into SAGA-dominated, non-RP TFIID-dominated and RP genes.

conformational changes would have the same effect on accessibility of these two Tafs, while also having negligible effect on TBP accessibility. We therefore interpret our results as indicating that occupancy of Taf1 and Taf4, and likely TFIID as a unit, is stabilized when Pol II escape is inhibited by depletion or inactivation of Kin28. This could occur because inhibition of Pol II escape prevents clashes that could destabilize Taf association, as recently proposed (11), or could be caused by enhanced interactions with Mediator, whose occupancy at promoters also increases under these conditions (15,18).

Mediator occupancy at gene promoters is increased by depletion or inactivation of Kin28, and also in the presence of a Pol II CTD mutant in which Ser5 residues in the YSPTSPS repeat, which are targeted for phosphorylation by Kin28, are replaced by Ala (15,18,21). This increased occupancy is likely due to stabilization of a normally transient association that is lost upon Pol II promoter escape (15,18), and is suppressed by depletion of Pol II, Tafs, or TBP, which results in reduced Mediator occupancy at proximal promoter regions and, in the case of TBP depletion, an upstream shift of Mediator ChIP signal to the UAS regions that are sites of initial Mediator recruitment by gene-specific

activators (13,21). Here, we show that increased Taf1 occupancy caused by Kin28 depletion is suppressed by simultaneous depletion of TBP. In the case of Mediator, decreased occupancy caused by depletion of Pol II or TBP likely reflects loss of direct interactions between Mediator and PIC components, including Pol II (21,43). TFIID occupancy may similarly depend in part on interactions with other PIC components, including Mediator, and the decreased occupancy seen upon simultaneous depletion of TBP and Kin28 compared to Kin28 alone could reflect loss of interactions with TBP or with PIC components or other factors whose association depends on TBP. Alternatively, TFIID binding to promoter regions may be destabilized by clashes with elongating Pol II or with GTFs that bind subsequent to TFIID (11); inhibition of Pol II promoter escape could interfere with later steps in transcription initiation, thereby mitigating potential clashes and allowing continued occupancy by TFIID.

Interestingly, when Kin28 is present, depletion of TBP had little effect on Taf1 occupancy. The lack of effect on Taf1 occupancy of depletion of TBP indicates that recruitment of Tafs in TFIID does not depend much on TBP, consistent with previous reports and with biochemical studies

showing that the Taf subunits of TFIID co-fractionate as a stable complex under conditions that lead to loss of TBP from the complex (3,6,44). Given that TBP depletion interrupts the normal transcription cycle, one might have predicted increased Taf occupancy to accompany TBP depletion, analogous to interruption of the transcription cycle by depletion of Kin28. The fact that no such increase in Taf1 occupancy was observed indicates that TBP, or PIC components or other factors whose association with the proximal promoter depends on TBP, are required for such elevated Taf occupancy, consistent with TBP being required for elevated Taf1 occupancy caused by Kin28 depletion.

Unlike Mediator and TFIID, TBP occupancy measured by ChIP does not increase upon depletion of Kin28 (Figure 3). Thus, TBP occupancy does not strictly correlate with binding of Tafs through the transcription cycle. This does not mean that Tafs are not required for recruitment of TBP to promoters, as it may be that once TBP has been recruited, interactions with promoter DNA and other PIC components are sufficient to allow continued TBP occupancy. However, our findings do suggest that promoter occupancy by TBP and Tafs are uncoupled during the normal transcription cycle, with Taf occupancy being transient and TBP occupancy being stable. Such uncoupling has been suggested during transcription of metazoan genes based on structural and biochemical studies of TFIID (11). It should also be emphasized that although TBP occupancy is not affected by depletion of Kin28 and is therefore not transient in the same way as occupancy by Mediator and Taf1 and Taf4, this does not mean that TBP does not turn over at its binding sites. In fact, TBP binding is known to be dynamic (45), with turnover being more rapid at Pol II promoters than at promoters of genes transcribed by RNA polymerase I or III, and more rapid at TATA-containing than TATA-lacking promoters (46).

We also took advantage of our ChIP-seq data to examine TBP/Taf ratios at promoters across the yeast genome. Our data corroborate previous work showing increased Taf occupancy, relative to TBP, at TFIID-dominated promoters relative to SAGA-dominated promoters (Figure 6) (3–6,8,39). We also found a marked difference in TBP/Taf1 occupancy ratios at promoters having and lacking consensus TATA elements (Mann–Whitney *U* test *z*-score –12.5 for TATA+ versus TATA– promoters;  $P < 10^{-5}$ ; Figure 6), providing quantitative support for previous work (28). Because the presence or absence of a consensus TATA element is a property completely independent of occupancy by Tafs or TBP, this difference strongly suggests mechanistic differences between these two categories of promoters. At the same time, there is considerable overlap between the TBP/Taf ratios (and of other properties) between TATA+ and TATA– promoters; the most parsimonious explanation is that either of at least two distinct mechanisms or pathways can operate at both types of promoters, but their relative efficiency differs depending on the presence of a consensus TATA element. This notion is consistent with the idea that the categorization of genes as SAGA- and TFIID-dominated reflects a continuum in terms of regulation, rather than a rigid dichotomy (4,5,40).

We observed a greater increase in occupancy by Taf1 and Taf4 upon Kin28 depletion at TFIID-dominated than

at SAGA-dominated promoters (Figure 2), and at TATA– than at TATA+ promoters (not shown). This may simply reflect the higher occupancy at TFIID-dominated/TATA– promoters seen in the presence of Kin28, or it may be that Tafs are present in more than one configuration which differ in their response to Kin28 depletion and which vary in proportion at the two categories of promoters. This latter possibility is consistent with recent findings showing that depletion of Taf1 decreases Pol II occupancy at both SAGA-dominated and TFIID-dominated genes (indicating that TFIID contributes to Pol II occupancy at both categories of genes), but with larger effect at the latter genes when the most active ~1000 genes are examined (indicating that dependence of SAGA-dominated genes on TFIID is proportionally less than at TFIID-dominated genes) (40).

In conclusion, the results reported here expand our understanding of the dynamics of the PIC by showing that Tafs, and by extension TFIID, behave similarly to Mediator in exhibiting transient promoter occupancy during the normal transcription cycle, with occupancy being stabilized by inhibition of Pol II promoter escape. In contrast, TBP and TFIIB occupy promoters stably during transcription, thereby uncoupling TFIID dynamics from TBP. This uncoupling is consistent with a recent proposal made to reconcile structural data indicating clashes of TFIID with TFIIF and Pol II during transcription (11). Kin28 depletion affects Taf1 and Taf4 occupancy more at SAGA-dominated than at TFIID-dominated genes, supporting mechanistic differences in PIC formation or dynamics between genes in these two categories. The nature of these putative mechanistic differences remains an important topic for future studies.

## DATA AVAILABILITY

ChIP-seq reads have been deposited in the NCBI Short Read Archive under project number PRJNA541522. We also used previously published ChIP-seq data deposited at the NCBI Short Read Archive under accession numbers SRP047524 (16), PRJNA413080 (21) and SRP030670 (35).

## SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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