MOLECULAR AND CELLULAR BIOLOGY, Apr. 2005, p. 2650–2659 Vol. 25, No. 7
0270-7306/05/$08.00+0 doi:10.1128/MCB.25.7.2650–2659.2005

Evidence that the Elongation Factor TFIIS Plays a Role in Transcription Initiation at *GAL1* in *Saccharomyces cerevisiae*

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Received 23 November 2004/Returned for modification 21 December 2004/Accepted 27 December 2004

TFIIS is a transcription elongation factor that has been extensively studied biochemically. Although the in vitro mechanisms by which TFIIS stimulates RNA transcript cleavage and polymerase read-through have been well characterized, its in vivo roles remain unclear. To better understand TFIIS function in vivo, we have examined its role during Gal4-mediated activation of the *Saccharomyces cerevisiae GAL1* gene. Surprisingly, TFIIS is strongly associated with the *GAL1* upstream activating sequence. In addition, TFIIS recruitment to Gal4-binding sites is dependent on Gal4, SAGA, and Mediator but not on RNA polymerase II (Pol II). The association of TFIIS is also necessary for the optimal recruitment of TATA-binding protein and Pol II to the *GAL1* promoter. These results provide strong evidence that TFIIS plays an important role in the initiation of transcription at *GAL1* in addition to its well-characterized roles in transcription elongation.

TFIIS is the best-characterized transcription elongation factor at the biochemical level, with several defined biochemical activities (53). TFIIS promotes the elongation of arrested RNA polymerase II (Pol II) by stimulating the inherent RNA cleavage activity of Pol II (13, 18). This cleavage allows Pol II to recover from arrest by placing the 3′ end of the nascent mRNA in the Pol II active site. TFIIS has also been shown to bind Pol II and nucleic acids in vitro (2, 57). A recent structural analysis of TFIIS complexed with Pol II suggested a detailed mechanism for TFIIS function consistent with its biochemical activities. In this structure, the C terminus of TFIIS is able to reach deep into the Pol II secondary channel, approaching the catalytic site of the polymerase (20). This proximity of TFIIS to the nascent RNA allows TFIIS to stimulate the endogenous cleavage activity of Pol II, allowing arrested polymerase complexes to restart elongation by realigning the transcript with the polymerase catalytic site.

Although TFIIS is well characterized in vitro, its role in vivo is less well understood. Consistent with the results of biochemical experiments, several genetic and molecular studies with *Saccharomyces cerevisiae* have also indicated a role for this protein in transcription elongation. First, mutations in *DST1*, the gene that encodes TFIIS, cause sensitivity to 6-azauracil, a compound that reduces intracellular GTP and UTP levels (17). This sensitivity is believed to be conferred by mutations in elongation factors because the mutant strains are no longer able to efficiently complete transcripts when nucleotide pools are decreased (12). Second, mutations in the *DST1* gene exhibit genetic interactions with mutations in other *S. cerevisiae* genes that encode elongation factors, such as *SPT4*, *SPT5*, *SPT6*, *SPT16*, and *RTF1* (9, 15, 31, 36). Third, TFIIS is required for efficient transcription elongation through the *lacZ* gene when fused to the yeast *GAL1* promoter (24). Finally, chromatin immunoprecipitation (ChIP) experiments have suggested that, under stress conditions, such as cold temperature, heat shock, or the presence of 6-azauracil in the growth medium, TFIIS is localized over the open reading frames (ORFs) of several genes (40).

In addition to its role in transcription elongation, some studies have indicated that TFIIS may also play a role in transcription initiation. For example, glutathione *S*-transferase–TFIIS has been shown to interact with general transcription factors in vitro (16, 38). More recently, TFIIS was found to interact with the Spt8 subunit of SAGA and the Med13 (Srb9) subunit of Mediator (52). Furthermore, TFIIS localizes to Cajal bodies in *Xenopus* oocytes (46), sites that recruit transcription initiation factors (14). Finally, genetic tests have suggested a functional interaction between *DST1* and genes encoding components of the Mediator and Swi/Snf complexes, both of which facilitate transcription initiation (10, 32). Together, these results suggest that TFIIS may play an important role in transcription initiation and the transition to productive elongation. However, direct evidence of a role for TFIIS in initiation in vivo is lacking.

Several reports previously demonstrated a transcriptional requirement for TFIIS in vivo but did not define whether the requirement occurs during initiation or elongation (29, 44, 54). In this work, we addressed the requirement for TFIIS during Gal4-mediated activation of the *S. cerevisiae GAL1* gene. Surprisingly, we observed that TFIIS associates with the upstream activating sequence (UAS) of *GAL1* to a higher degree than the coding region. The recruitment of TFIIS to the *GAL1* promoter is dependent on the SAGA and Mediator complexes and is necessary for the optimal recruitment of TATA-binding protein (TBP) and Pol II to *GAL1*. We also observed that TFIIS associates with isolated Gal4-binding sites independently of Pol II. These results strongly suggest that TFIIS is normally recruited by Gal4 to the *GAL1* promoter and is required for proper transcription initiation.

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TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype
FY1976	*MAT*α *his4-917*δ *lys2-173R2 leu2Δ0 ura3Δ0*
FY2352	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 leu2Δ1 RPB3*-HA::*LEU2*
FY2353	*MAT***a** *his4-912*δ *lys2-128*δ *ura3-52 leu2Δ1 RPB3*-HA::*LEU2 dst1*Δ::*KANMX*
FY2354	*MAT***a** *his4-917*δ *lys2-173R2 leu2Δ0 ura3Δ0 gal4*Δ::*KANMX* 3×Myc-*DST1* 3×HA-*SPT20*
FY2355	*MAT***a** *SPT20*-3×HA 3×Myc-*DST1 his4-917*δ *lys2-173R2 leu2Δ0 ura3Δ0*
FY2356	*MAT*α *spt20Δ200*::*ARG4* 3×Myc-*DST1 his4-917*δ *lys2-173R2 leu2Δ0 ura3-52*
FY2357	*MAT*α *med15*Δ::*TRP1* 3×Myc-DST1 *his4-917*δ *lys2-173R2 ura3Δ0 arg4-12 trp1Δ63*
FY2358	*MAT***a** *SPT20*-3×HA *MED13*-13×Myc::*KANMX dst1*Δ::*LEU2 leu2Δ0 his4-917*δ *lys2-173R2 ura3Δ0*

MATERIALS AND METHODS

Yeast strains and plasmids. All *S. cerevisiae* strains (Table 1) are isogenic to a *GAL2*$^+$ derivative of S288C (55). To epitope tag TFIIS, three copies of a sequence encoding the Myc epitope were integrated at the 5′ end of the *DST1* gene (encoding TFIIS) (43). The 3×Myc-TFIIS fusion was fully functional in vivo, as assayed by several phenotypes, including growth on medium containing galactose, caffeine, or mycophenolic acid. The carboxy-terminally tagged TFIIS-9×Myc allele described previously (40) has impaired function, as indicated by the inability of the fusion protein to grow in a manner similar to that of the wild-type protein on medium containing galactose (at 37°C) or mycophenolic acid (data not shown); this finding is likely to explain the differences between earlier data and data from this work. The *dst1*Δ::*KANMX* allele was constructed by one-step PCR-mediated disruption, which replaced the entire ORF with the *KANMX4* cassette (7). Plasmid SGP4, containing the three consensus Gal4-binding sites, was described previously (5).

ChIP. All ChIP experiments were carried out as previously described (27). Antibodies used in these experiments included mouse monoclonal antibody 12CA5 against the hemagglutinin (HA) epitope (1 μl for Spt20 and 0.5 μl for Rpb3), anti-Med19 rabbit polyclonal antibody (1 μl; gift from R. Kornberg), anti-Myc rabbit polyclonal antibody A14 (5 μl; Santa Cruz), anti-Gal4 DNA-binding domain antibody (1 μl; Santa Cruz), anti-TBP antibody (5 μl) (22), anti-Spt16 antibody (2 μl; gift from T. Formosa), anti-TFIIS antibody (1 μl; gift from C. Kane), anti-Rpb3 antibody (1 μl; NeoClone Biotechnology), and monoclonal antibody 8WG16 against the C-terminal domain of Rpb1 (2 μl; Covance).

Immunoprecipitations were carried out with either FA lysis buffer (150 mM NaCl, 50 mM HEPES-KOH [pH 7.5], 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride) containing 150 mM NaCl and no sodium dodecyl sulfate (for antibodies to Myc, HA-Rpb3, Spt16, Rpb3, and TFIIS and for 8WG16) or FA lysis buffer containing 300 mM NaCl and 0.1% sodium dodecyl sulfate (for HA-Spt20, Med19, Gal4, and TBP). Dilutions of input DNA (1/50 and 1/100) and immunoprecipitated DNA (1/2 and 1/4) were subjected to quantitative PCR by the incorporation of [α-^{32}P]dATP. The products were separated on a 6% nondenaturing polyacrylamide gel, and quantification was carried out by PhosphorImager (Molecular Dynamics) analysis. The percent immunoprecipitation was calculated for each sample, and values for all samples were normalized to the value for a control PCR product amplified in each reaction. The primers for this control PCR product amplify a region of chromosome V that is not contained in any ORFs (22).

The *GAL1*, *GAL2*, and *GAL3* primer sets (27) were described previously, and the sequences are available upon request. The relative positions of the primer sites are as follows: *GAL1*-UAS, −536 to −276; TATA, −190 to +54; 5′, +590 to +877; and 3′, +1330 to +1657; *GAL2*-UAS, −464 to −195; *GAL3*-UAS, −420 to −157; *ARG1*-UAS, −441 to −213; TATA, −176 to +64; and ORF, +230 to +474; *AHP1*-UAS, −530 to −348; TATA, −151 to +80; and ORF, +267 to +486; *TEF1*-UAS, −459 to −259; promoter (Prom.), −149 to +49; and ORF, +813 to +1043; *PDC1*-Prom., −485 to −118; 5′, +48 to +424; middle of ORF (Mid.), +411 to +799; and 3′, +1226 to +1602; and *PMA1*-UAS, −623 to −390; Prom., −370 to −90; 5′, +584 to +807; 3′, +2018 to +2290; and 3′ untranslated region, +529 to +742 (relative to stop codon).

All values are relative to that for the translation start site (ATG, +1) except where noted otherwise. All experiments were carried out at least three times, and standard error bars are shown on the graphs. Western analysis revealed no differences in protein levels determined by ChIP between any of the mutants and the wild type.

Northern analysis. RNA isolation and Northern hybridization experiments were carried out as described previously (3, 50). Northern hybridization analysis was conducted with probes to *GAL1* and *SCR1*. At least three independent Northern analysis experiments were carried out.

RESULTS

TFIIS is present at the UAS of Gal4-activated genes. Although TFIIS has been shown to be required for the proper induction of *GAL1* transcription, it was not known whether this requirement occurs during initiation or elongation in vivo (54). To better define the role for TFIIS in transcriptional regulation at Gal4-activated genes, we performed ChIP analysis of TFIIS over the *GAL1* gene (Fig. 1A). Surprisingly, we observed that when cells were grown at 30°C under inducing conditions (galactose-grown cultures), TFIIS was present over the entire *GAL1* locus, with the highest level of association over the UAS (Fig. 1B and C). This localization was dependent on transcription because TFIIS was not present at *GAL1* under noninducing conditions (raffinose-grown cultures) (Fig. 1B). Similar results were observed in ChIP experiments with either amino-terminally Myc-tagged TFIIS or untagged TFIIS and anti-TFIIS antisera (Fig. 1B). A previous study did not find TFIIS present at *GAL1* under these same conditions (40). However, the epitope-tagged version of TFIIS used in those experiments (nine copies of the Myc epitope at the carboxy-terminal end) has impaired function because it causes a mutant phenotype, sensitivity to mycophenolic acid, likely explaining the different results (D. Prather, E. Larschan, D. Pokholok, R. Young, and F. Winston, unpublished data). Our results obtained with both a functional, epitope-tagged version of TFIIS and wild-type TFIIS showed that TFIIS is physically associated with the *GAL1* UAS and coding region.

To compare the distribution of TFIIS across *GAL1* to that of another well-studied elongation factor, we assayed the association of Spt16 with *GAL1*. Previous studies showed that Spt16 and other elongation factors, including Spt6 and the Paf1 complex, are generally present at significantly higher levels over coding regions than over promoters (21, 33, 40, 45). As expected, our ChIP experiments showed that Spt16 associated at a higher level with the *GAL1* ORF than with the promoter region (Fig. 1E). These results are in contrast to those obtained for TFIIS, which showed a higher level of association with the *GAL1* UAS than with the coding region. Thus, the distribution of TFIIS is distinct from those of other elongation factors or initiation factors, such as SAGA and Mediator, which are present only over promoter regions (27, 28). This pattern of TFIIS localization suggests that TFIIS may play roles in both transcription initiation and elongation at *GAL1*.

We next investigated whether TFIIS is specifically recruited to the *GAL1* UAS or whether it is also recruited to other galactose-inducible promoters in *S. cerevisiae*. We found a significant level of association with the *GAL2* UAS (3-fold) but at most only very weak enrichment at the *GAL3* UAS (1.3-fold) (Fig. 1F). These levels of TFIIS association correlate with the level of SAGA occupancy at the *GAL2* UAS and the *GAL3* UAS (27) and suggest a general role for TFIIS in Gal4-activated transcription.

TFIIS is required for the rapid induction of *GAL1* transcription. TFIIS was previously shown to be required for full induc-

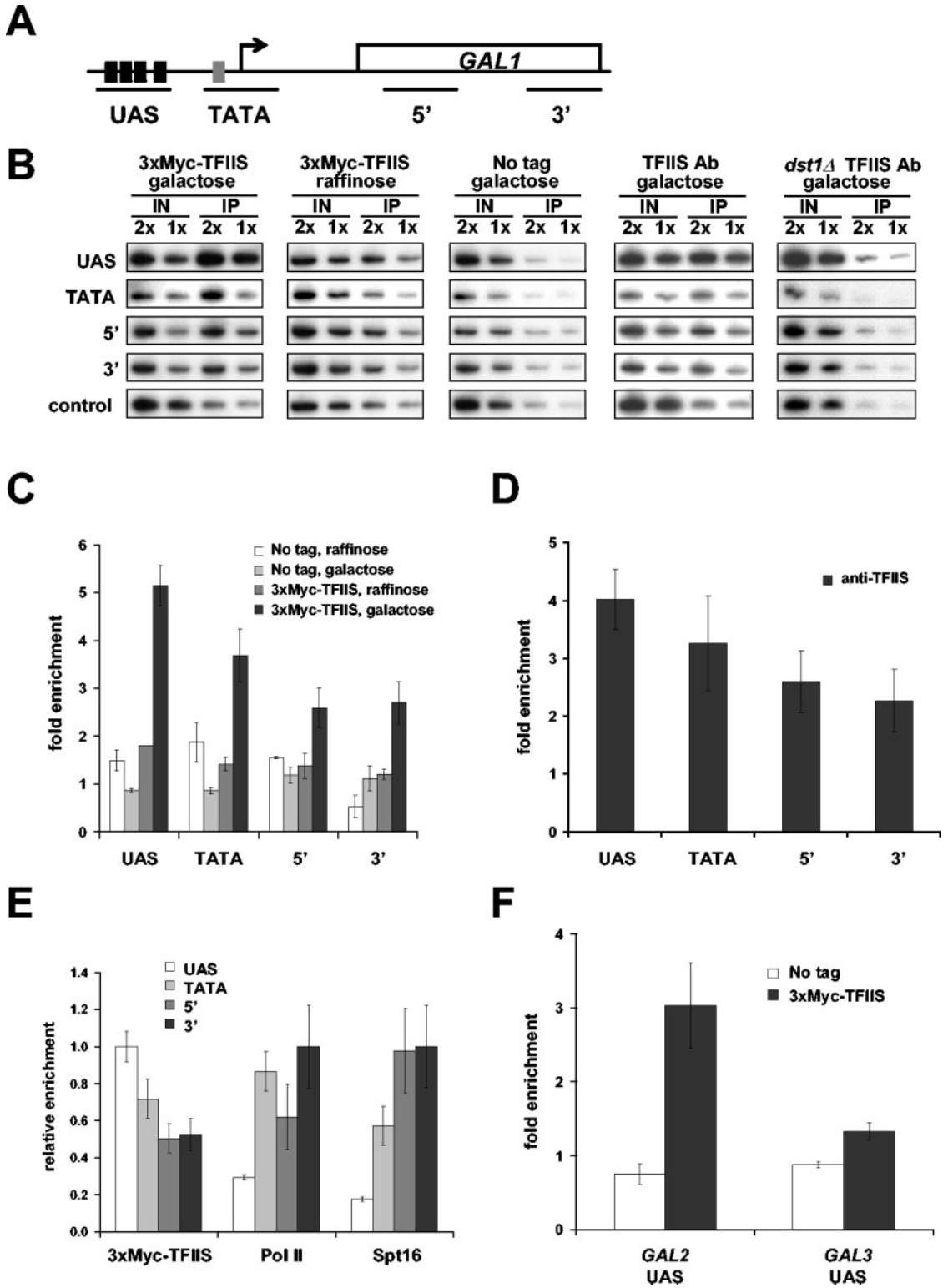

FIG. 1. TFIIS is recruited to both the UAS and coding regions of *GAL1* upon induction by galactose. (A) Schematic representation of the *GAL1* gene. The Gal4-binding sites are represented as black boxes (−460 to −330, where the A of the ATG is +1), the TATA box is represented by a gray box (−140), and the transcription initiation site is denoted by the arrow (−60). Regions amplified in the ChIP PCRs are noted below the gene. (B) ChIP analysis of TFIIS at *GAL1*. Strains containing 3×Myc-TFIIS, wild-type TFIIS, or *dst1*Δ were grown in YP medium–2% raffinose at 30°C to 1×10^7 to 2×10^7 cells/ml and induced for 20 min with 2% galactose. Cells were cross-linked before galactose addition (raffinose sample) or after galactose addition (galactose sample). In the left three panels, TFIIS was immunoprecipitated from chromatin with anti-Myc polyclonal antibody (Ab) A14 against the 3×Myc epitope. In the right two panels, TFIIS was immunoprecipitated with anti-TFIIS antibody. PCRs were conducted with two dilutions to ensure linearity. IN, input chromatin; IP, immunoprecipitated chromatin. (C) Quantitation of 3×Myc-TFIIS ChIP

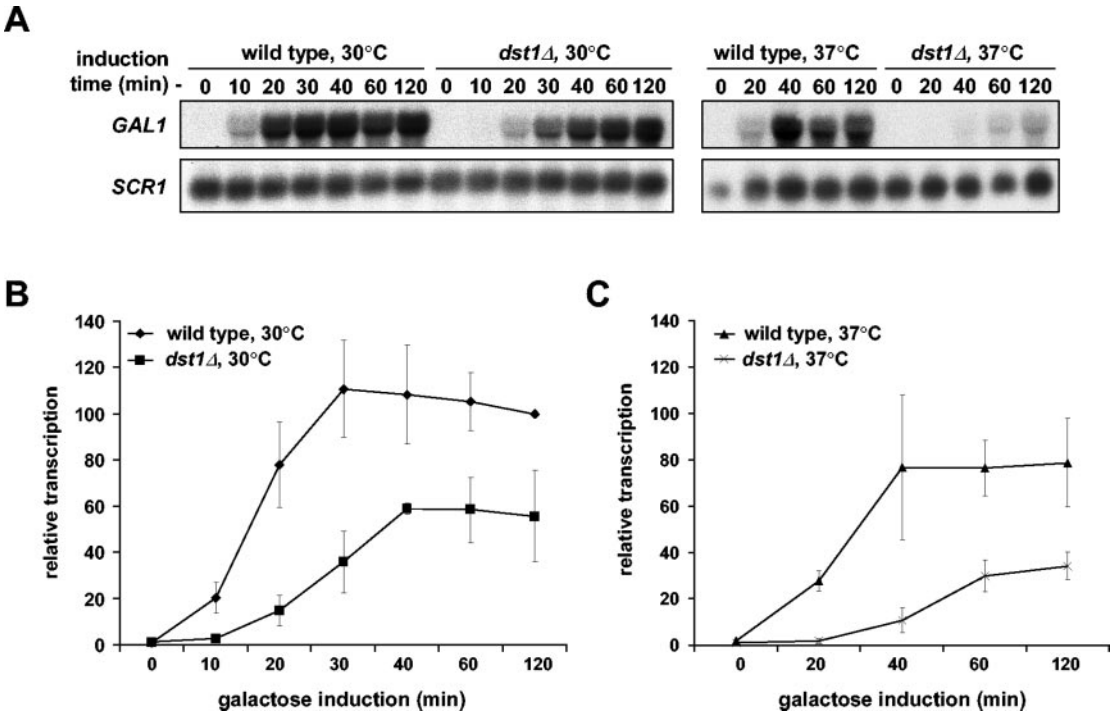

FIG. 2. TFIIS is necessary for the rapid induction of *GAL1* transcription. (A) Northern analysis of *GAL1* mRNA levels in response to galactose induction. Wild-type and *dst1*Δ strains were grown in YP medium–2% raffinose at 30 or 37°C to 1×10^7 to 2×10^7 cells/ml and induced for 20 min with 2% galactose at either 30 or 37°C. RNA was isolated at different times after galactose addition. *SCR1* served as a loading control. (B) Quantitation of Northern analysis at 30°C. Levels of *GAL1* mRNA were quantitated and normalized to the level of *SCR1* mRNA. The level of *GAL1* mRNA at 120 min in the wild-type strain at 30°C was set to a value of 100, and all other values are shown relative to this time point. Each value represents the mean and standard deviation from at least three independent experiments. (C) Quantitation of Northern analysis at 37°C. Northern analysis was performed as described for panel B.

tion of the *GAL1* gene in synthetic complete (SC) medium (54). Since we used different growth conditions (yeast extract-peptone [YP] medium with galactose rather than SC medium with galactose), we performed experiments to measure the requirement for TFIIS during *GAL1* induction. When strains were grown at 30°C, *GAL1* mRNA levels were clearly reduced in the *dst1*Δ mutant, particularly at early time points (Fig. 2A and B). At later time points, *GAL1* mRNA levels in the *dst1*Δ mutant were approximately 60% wild-type levels. When strains were grown at a higher temperature, 37°C, we found a significantly more severe defect in the *dst1*Δ mutant over the entire 2-h time course of the experiment (Fig. 2C). These results suggest that TFIIS plays an important role in the robust and rapid induction of *GAL1*, both under optimal growth conditions and at higher temperatures.

TFIIS is required for TBP and Pol II association with the *GAL1* promoter. Our observations that TFIIS is necessary for normal levels of *GAL1* transcription and is physically associated with the *GAL1* UAS suggest that TFIIS may be important for initiation at *GAL1*. To determine whether TFIIS functions to promote transcription initiation, we performed ChIP experiments to measure the levels of TBP and Pol II at the *GAL1* promoter. We observed that the levels of TBP and Pol II at the *GAL1* promoter were significantly reduced in the *dst1*Δ mutant, with TBP being recruited at only 34% and Pol II being recruited at 37% the levels found in a wild-type strain (Fig.

results at 30°C. Strains containing 3×Myc-TFIIS or wild-type TFIIS were cross-linked before (raffinose) or after (galactose) the addition of galactose. With anti-Myc antibody A14, the percent immunoprecipitation of each *GAL1* region was calculated and normalized to the percent immunoprecipitation of a noncoding region of chromosome V. Each value represents the ratio reported as the mean and standard error from at least three independent experiments. In this experiment, wild-type TFIIS was included as a negative control, as it is not recognized by the anti-Myc antibody. (D) Quantitation of TFIIS ChIP results at 30°C. Strains containing wild-type TFIIS were cross-linked after the addition of galactose. With the anti-TFIIS antibody, the percent immunoprecipitation of each *GAL1* region was calculated and normalized to the percent immunoprecipitation of a noncoding region of chromosome V. Each value represents the ratio reported as the mean and standard error from at least three independent experiments. (E) Distributions of TFIIS, Pol II, and Spt16 across *GAL1*. Antibodies to Spt16 and Pol II (antibody 8WG16 against the C-terminal domain of Rpb1) were used to immunoprecipitate these proteins from the same wild-type chromatin samples as those used in panel B. The relative distributions of TFIIS, Pol II, and Spt16 are shown; the highest level of enrichment for each protein was arbitrarily set to 1. (F) TFIIS is recruited to the *GAL2* and *GAL3* UAS regions. Strains containing 3×Myc-TFIIS or wild-type TFIIS were grown in YP medium–2% raffinose at 30°C to 1×10^7 to 2×10^7 cells/ml and induced for 20 min with 2% galactose. Cells were cross-linked after galactose induction. TFIIS was immunoprecipitated from chromatin with anti-Myc polyclonal antibody A14 against the 3×Myc epitope. PCRs amplified the UAS regions of either *GAL2* or *GAL3* (see Materials and Methods).

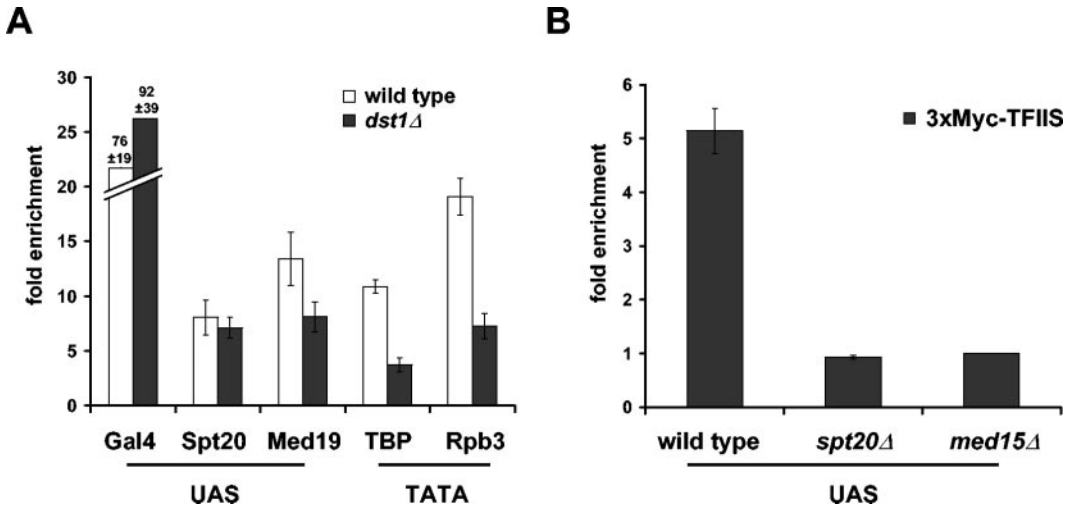

FIG. 3. TFIIS is required for the association of TBP and Pol II with *GAL1*. (A) TFIIS is required for the association of TBP and Pol II with *GAL1* at 30°C. ChIP experiments were conducted with wild-type and *dst1*Δ strains containing Spt20-3×HA or Rpb3-HA. Strains were grown in YP medium–2% raffinose at 30°C to 1×10^7 to 2×10^7 cells/ml and induced for 20 min with 2% galactose. Primers were directed to the *GAL1* TATA for TBP and Pol II and to the *GAL1* UAS for Gal4, SAGA (Spt20-3×HA), and Mediator (Med19). Percent immunoprecipitation was calculated relative to that for the control region. Values shown represent the mean and standard error from at least three independent experiments. (B) SAGA and Mediator are required for TFIIS association with the *GAL1* UAS. ChIP experiments were conducted with wild-type, *spt20*Δ, and *med15*Δ (*gal11*Δ) strains containing 3×Myc-TFIIS. Strains were grown in YP medium–2% raffinose at 30°C and induced for 20 min with galactose. Cells were cross-linked after the addition of galactose, and 3×Myc-TFIIS was immunoprecipitated with anti-Myc antibody A14. Primers were directed to the *GAL1* UAS region, and percent immunoprecipitation was calculated relative to that for the control region. Values shown represent the mean and standard error from at least three independent experiments.

3A). Previous studies suggested that some elongation mutants, such as *spt6* and *spt16*, have an indirect effect on TBP recruitment at promoters due to aberrant recruitment of TBP to cryptic TATA elements (19, 33). This possibility seems unlikely for the *dst1*Δ mutant for two reasons. First, unlike *spt6* and *spt16* mutations, *dst1*Δ mutations do not cause transcription to initiate from cryptic TATA elements (data not shown). Second, unlike Spt6 and Spt16, TFIIS shows a high level of physical association with the *GAL1* UAS. Thus, the most likely explanation for the reduced levels of TBP and Pol II at *GAL1* in the *dst1*Δ mutant is that TFIIS functions directly in transcription initiation, likely at the level of the formation of the preinitiation complex (PIC).

To define further the role for TFIIS in initiation at *GAL1*, we compared wild-type and *dst1*Δ strains for the recruitment of three factors known to act during *GAL1* transcription initiation: Gal4, SAGA (Spt20), and Mediator (Med19/Rox3). Gal4 is bound to the *GAL1* UAS even under noninducing conditions (11, 42), while SAGA and Mediator are recruited early after induction by galactose (5, 6, 8, 27, 28). Mediator was also recently shown to be recruited to the *GAL1* UAS independently of Pol II, suggesting a role for Mediator in transcription initiation prior to its association with Pol II (25, 28). In our experiments, Gal4 and SAGA were recruited to wild-type levels in *dst1*Δ cells, whereas the association of Mediator was only modestly affected (Fig. 3A). These results show that under these conditions, TFIIS is required for normal levels of TBP and Pol II association with the *GAL1* promoter, yet it is not significantly required for the association of Gal4 or SAGA and has only a modest effect on Mediator association.

Because SAGA and Mediator were recruited to significant levels in the absence of TFIIS, we tested whether these factors are required for TFIIS association with the *GAL1* promoter. To do this, we measured the level of TFIIS association in an *spt20*Δ mutant, in which the integrity of the SAGA complex is abolished (47, 56), and a *med15*Δ (*gal11*Δ) mutant, in which the recruitment of Mediator to the *GAL1* promoter is eliminated (8, 28, 35, 39, 48). We found that TFIIS association was completely dependent upon both SAGA and Mediator because in the absence of either, TFIIS was not detectable at *GAL1* (Fig. 3B). These results suggest that the association of TFIIS is dependent upon both SAGA and Mediator.

Higher temperatures increase the requirement for TFIIS in initiation at *GAL1*. Since TFIIS has been implicated as playing a role in the stress response (32, 40), we also measured its level of association with *GAL1* when cells were grown at an elevated temperature, 37°C (Fig. 4A). Under these conditions, TFIIS was still associated with the *GAL1* UAS, although the levels were apparently lower than those seen at 30°C (compare Fig. 4A to Fig. 1C). However, the association of TFIIS with the *GAL1* coding region was no longer detected. These results raise the possibility that TFIIS plays a more limited role in *GAL1* transcription at 37°C than at 30°C, possibly confined to initiation.

Because the overall level of TFIIS associated with *GAL1* was decreased at 37°C, we reexamined the effects of *dst1*Δ on the recruitment of Gal4, SAGA, Mediator, TBP, and Pol II at this temperature to determine whether this decreased level was functionally significant. Under these conditions, the association of Gal4 with the *GAL1* UAS was not affected (Fig. 4B). However, the levels of both SAGA and Mediator associated with the *GAL1* locus were reduced although not completely abolished (Fig. 4B). Furthermore, the recruitment of TBP and Pol II to the *GAL1* promoter became completely dependent on TFIIS at 37°C because the levels of association of both of these

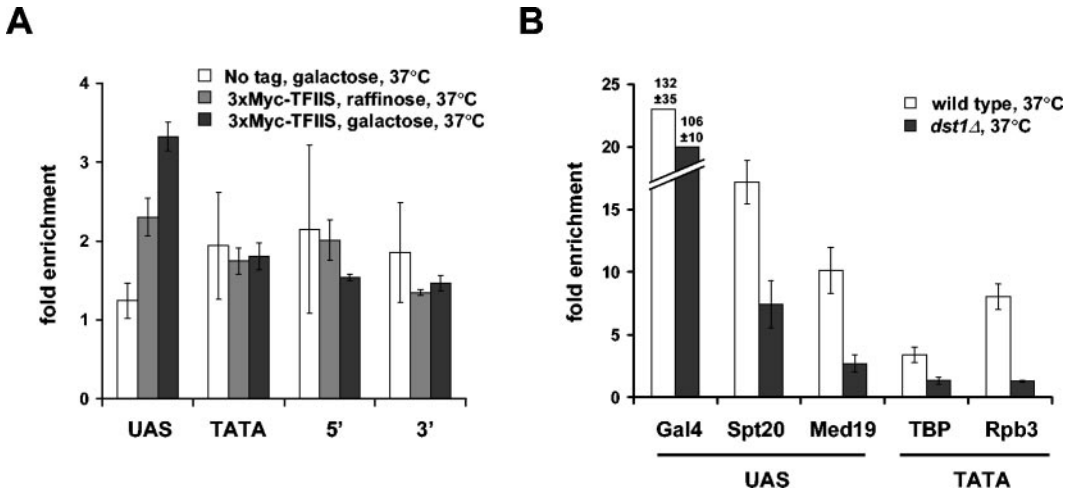

FIG. 4. Higher temperatures increase the requirement for TFIIS for initiation at *GAL1*. (A) Levels of TFIIS at *GAL1* at high temperatures. Strains containing 3×Myc-TFIIS or wild-type TFIIS were grown in YP medium–2% raffinose at 37°C to 1×10^7 to 2×10^7 cells/ml and induced for 20 min with 2% galactose at 37°C. ChIP experiments were performed as described in the legend to Fig. 1 with antibody A14. (B) TFIIS is partially required for SAGA and Mediator recruitment and becomes essential for the association of TBP and Pol II with *GAL1* at 37°C. ChIP experiments were conducted with wild-type and *dst1*Δ strains containing Spt20-3×HA or Rpb3-HA. Strains were grown in YP medium–2% raffinose at 30°C to 1×10^7 to 2×10^7 cells/ml and induced for 20 min with 2% galactose. Primers used were directed to the *GAL1* TATA for TBP and Rpb3-HA and to the *GAL1* UAS for Gal4, SAGA (Spt20-3×HA), and Mediator (Med19). Percent immunoprecipitation was calculated relative to that for the control region. Values shown represent the mean and standard error from at least three independent experiments.

factors were at background levels. This increased dependence on TFIIS for TBP and Pol II association compared to the effect observed at 30°C likely was caused by the defects in SAGA and Mediator recruitment seen in *dst1*Δ cells at this temperature. These results could have been due to an increased requirement for TFIIS in stabilizing SAGA and Mediator at 37°C or to some other indirect effect of TFIIS at a more stressful temperature. Thus, at 37°C, the loss of TFIIS caused additional defects at the *GAL1* promoter, consistent with the more severe transcriptional defects that we observed at high temperatures (Fig. 2).

TFIIS can be recruited to Gal4-binding sites independently of Pol II. The localization of TFIIS to the *GAL1* UAS and the defects in TBP and Pol II recruitment in the *dst1*Δ mutant suggest a role for TFIIS in transcription initiation. However, these results do not rule out the possibility that TFIIS is recruited to the promoter by its well-characterized association with Pol II. To gain additional evidence of a role for TFIIS in initiation, we tested whether it can be recruited to a UAS in the absence of Pol II. To do this, we used a plasmid containing three consensus Gal4-binding sites and no other promoter elements (5). Previous results showed that these three Gal4-binding sites are sufficient to recruit Gal4 and SAGA; however, TBP and Pol II do not associate with this plasmid (5). Our ChIP results (Fig. 5) showed that TFIIS was recruited to the three Gal4-binding sites, while Pol II did not associate with this plasmid, providing strong evidence that TFIIS can be recruited to genes in a Pol II-independent fashion. These results are consistent with the results obtained at *GAL1* (Fig. 3) because both SAGA and Mediator were also recruited in this experiment and the recruitment of all of these factors was dependent upon Gal4 (Fig. 5). We conclude that TFIIS is recruited to isolated Gal4-binding sites by the Gal4 activator and that this recruitment can occur independently of Pol II.

TFIIS is recruited to the coding regions of several constitutive and inducible genes. To examine the association of TFIIS with other genes not regulated by Gal4, we performed several additional ChIP experiments. First, we examined two genes that have been shown to be SAGA dependent, *ARG1* and *AHP1* (41, 49). *ARG1* is regulated by Gcn4 and is induced by the addition of sulfometuron methyl, which mimics nutrient starvation. *AHP1* is regulated by osmolarity and is induced by the addition of 0.4 M NaCl. When we performed ChIP analyses of TFIIS at these two genes, we found that the association of TFIIS with these genes increased under activating conditions (Fig. 6A). However, in contrast to the association of TFIIS with *GAL1*, we found high levels of TFIIS associated with the coding regions but very little associated with the promoters.

We extended our analysis of TFIIS to three constitutively expressed genes; *TEF1*, *PMA1*, and *PDC1*. Similar to what we found at *ARG1* and *AHP1*, TFIIS was present at higher levels over the coding regions of all three of these genes than over the promoter regions (Fig. 6B). A previous study showed a similar distribution of TFIIS at *TEF1* but suggested that TFIIS was recruited only under stressful conditions, such as 14°C (40). However, we found that even when cells were grown in rich media at 30°C, TFIIS was recruited to each of the genes examined here. This difference in results likely was due to the different epitope tags used in these studies (as discussed above). We also analyzed the association of TFIIS with *TEF1* and *PMA1* by using anti-TFIIS antibody and obtained results very similar to those obtained with Myc-tagged TFIIS (data not shown). These results suggest that TFIIS is cotranscriptionally recruited to the coding regions of most genes even under nonstressful conditions. In contrast, TFIIS appears to play an additional, more specialized role in initiation at Gal4-regulated genes.

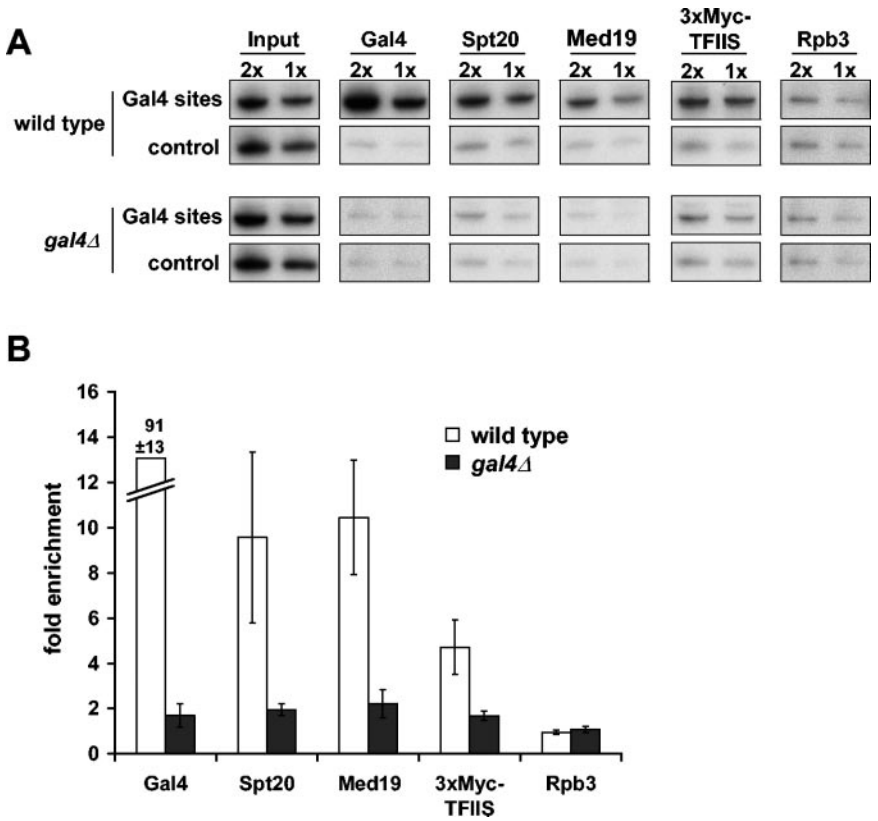

FIG. 5. TFIIS can be recruited to Gal4-binding sites independently of Pol II. (A) ChIP experiments were conducted with wild-type and *gal4*Δ strains containing plasmid SGP4 with three consensus Gal4-binding sites. Strains were grown in SC raffinose medium without Ura at 37°C to 1×10^7 to 2×10^7 cells/ml and induced for 20 min with 2% galactose at 37°C. Cells were cross-linked after the addition of galactose. Immunoprecipitation was performed with anti-HA antibody 12CA5 (Spt20-3×HA), anti-Myc antibody A14 (3×Myc-TFIIS), and antibodies to Gal4, Med19, and Rpb3. Primers used amplified a region of the plasmid containing the three consensus Gal4-binding sites. PCRs were conducted with two dilutions to ensure linearity. These experiments were conducted at 37°C, and similar results were seen for strains grown at 30°C (data not shown). (B) Quantitation of recruitment to the plasmid containing the Gal4-binding sites shown in panel A. Percent immunoprecipitation was calculated relative to that for the control region. Values shown represent the mean and standard error from three independent experiments.

DISCUSSION

In this report, we have provided several lines of evidence that elongation factor TFIIS plays a direct role in transcription initiation at *GAL1*. First, our ChIP analyses demonstrated that TFIIS is physically associated with the *GAL1* UAS. This localization is in clear contrast to that of other elongation factors, such as Spt16, Spt6, and the Paf1 complex, which are localized to coding regions (21, 33, 40, 45). In addition, mutant analyses showed that SAGA and Mediator are both required for the recruitment of TFIIS to the *GAL1* UAS, while TFIIS in turn is necessary for optimal levels of TBP and Pol II and for normal levels of *GAL1* mRNA. Importantly, we found that TFIIS can be recruited to isolated Gal4-binding sites independently of Pol II. Taken together, our results suggest a model in which TFIIS functions subsequent to SAGA and Mediator in initiation at *GAL1* by increasing the levels of TBP and Pol II associated with the promoter (Fig. 7).

Our model of a role for TFIIS in initiation is supported by several previous molecular and genetic results. Directly relevant to our studies, TFIIS was recently shown by two-hybrid analysis to interact with Spt8 of SAGA and Med13 (Srb9) of Mediator, and TFIIS could be coimmunoprecipitated with these factors when it was overexpressed (52). These interactions were interpreted by Wery et al. (52) as evidence of roles for SAGA and Mediator in transcription elongation. However, their results also fit well with our model, in which TFIIS functions in initiation at *GAL1* to recruit TBP and Pol II, a role closely related to what was previously shown for both SAGA and Mediator (4, 5, 25, 27). We note that SAGA is strongly localized over the *GAL1* UAS, suggesting that its role is limited to initiation (27). Additionally, glutathione *S*-transferase–TFIIS was found to copurify with several general transcription initiation factors in HeLa cells, such as TFIIF, TFIIH, Mediator, and the *S. cerevisiae* Cdk8 (Srb10) kinase homologue CDK8 (38). Finally, mutations in *S. cerevisiae DST1* exhibit genetic interactions with mutations in several genes that encode factors important for transcription initiation, including Swi/Snf and Mediator (10, 32, 52), and factors involved in both initiation and elongation, including Htz1, Rpb4, and Rpb1 (1, 23, 31, 52).

Our results suggest two possible roles for TFIIS in initiation at *GAL1*. First, TFIIS may function in the assembly or stability of the fully assembled PIC, as previously described for the SAGA and Mediator complexes at *GAL1* (4, 5, 25, 27). This possibility is supported by the recently described interactions of TFIIS with Spt8 of SAGA and Med13 (Srb9) of Mediator

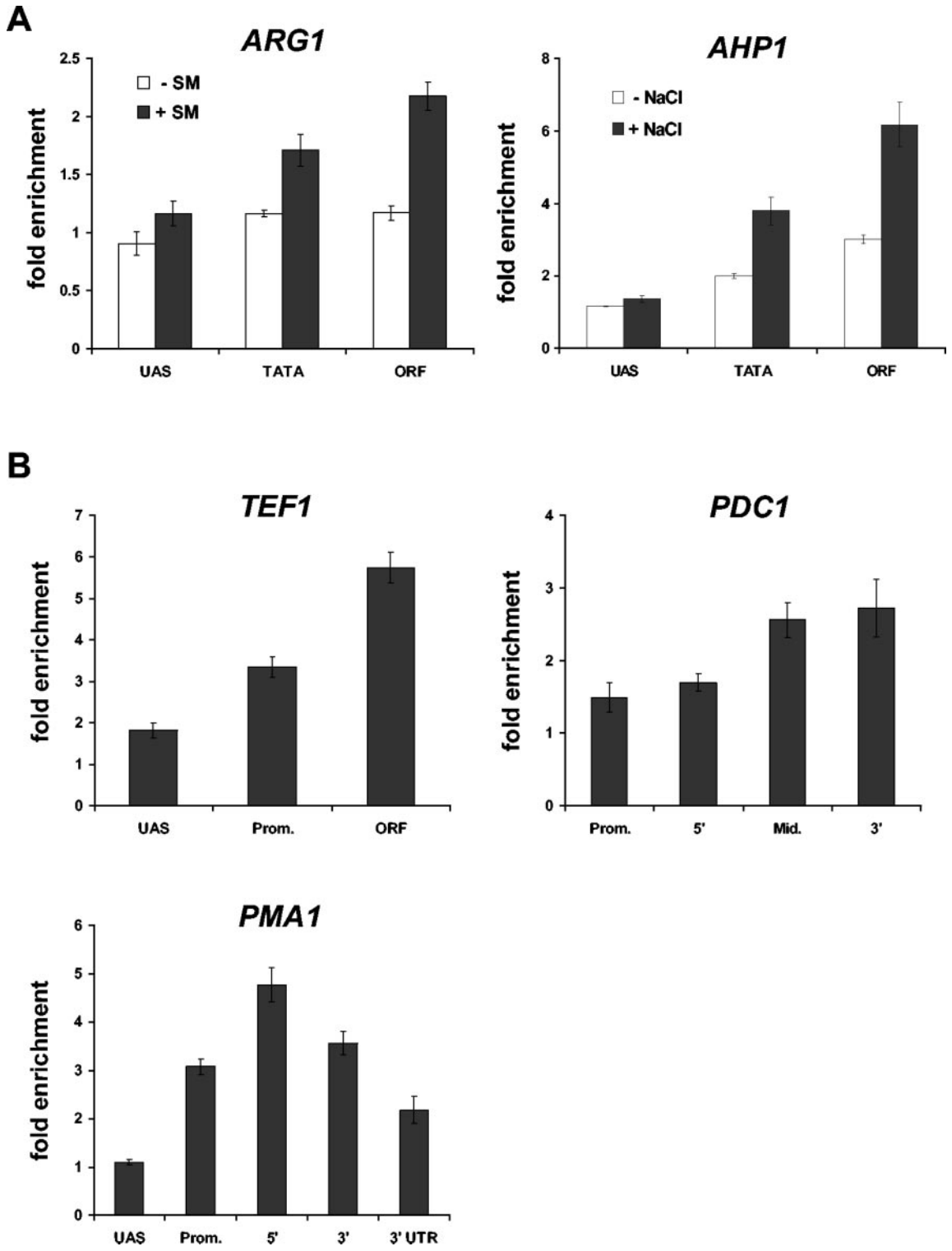

FIG. 6. TFIIS is recruited to several inducible and constitutive genes even under nonstress conditions. (A) TFIIS is recruited to the inducible genes *ARG1* and *AHP1*. For *ARG1* induction, strains containing 3×Myc-TFIIS were grown in SC medium lacking isoleucine and valine at 30°C to 10^7 cells/ml. Half of the culture was induced with 0.6 μg of sulfometuron methyl (SM)/ml for 2 h, and the other half was left untreated. For *AHP1* induction, strains containing 3×Myc-TFIIS were grown in YP glucose (YPD) medium at 30°C to 1×10^7 to 2×10^7 cells/ml. Half of the culture was induced with 0.4 M NaCl for 5 min, and the other half was left untreated. ChIP experiments were performed with antibody A14, and the percent immunoprecipitation was calculated relative to that for the control region. Values shown represent the mean and standard error from three independent experiments. (B) TFIIS is recruited to constitutively expressed genes under nonstress conditions. Strains containing 3×Myc-TFIIS were grown in YPD medium at 30°C to 1×10^7 to 2×10^7 cells/ml, and cells were cross-linked. TFIIS localization at the *TEF1*, *PDC1*, and *PMA1* genes was analyzed by ChIP with antibody A14, and the percent immunoprecipitation was calculated relative to that for the control region. Values shown represent the mean and standard error from three independent experiments.

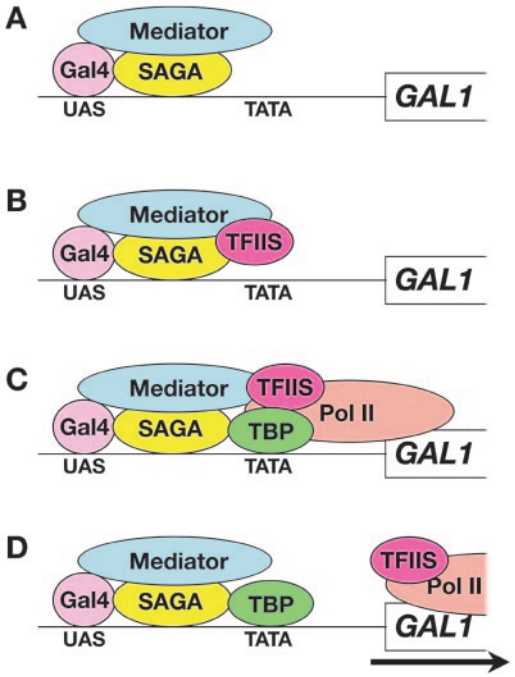

FIG. 7. Model for TFIIS function during transcription initiation. (A) Upon the addition of galactose to induce the transcription of the *GAL1* gene, the Gal4 activator recruits the SAGA histone acetyltransferase complex, followed by recruitment of the Mediator complex to the UAS (5, 8, 25, 27). (B) The association of TFIIS with the *GAL1* UAS is dependent on the SAGA and Mediator complexes but can occur independently of PIC assembly. (C) SAGA, Mediator, and TFIIS all facilitate optimal recruitment of the PIC, including TBP and Pol II. (D) TFIIS accompanies Pol II into the elongation phase of transcription, while SAGA and Mediator are left behind at the promoter.

(52) and the well-established interaction of TFIIS with Pol II (20, 53). The interactions of TFIIS with both coactivators and Pol II may assist in the stabilization of the PIC under conditions of high transcriptional activity. In addition, our finding that TFIIS becomes partially required for the association of SAGA and Mediator with *GAL1* at 37°C suggests that under some conditions, TFIIS may also stabilize these coactivators at the *GAL1* UAS.

A second possible role for TFIIS consistent with our results was suggested by Malagon et al. (32), who hypothesized a role for TFIIS after PIC assembly. In this role, TFIIS enhances promoter escape by preventing pausing and arrest during initiation, as suggested by in vitro studies (37, 51). Based on the reduced level of TBP at *GAL1* in the *dst1*Δ mutant, we would extend this model to postulate that, in the absence of TFIIS, a number of Pol II complexes that fail to escape the promoter are removed, destabilizing PIC components, including TBP. Consistent with the idea that PIC stability affects the level of the TBP-promoter interaction, studies have shown that TFIIB mutants affect the levels of TBP and Pol II at promoters (6, 25, 30). This role for TFIIS is also supported by recent evidence that TFIIS plays a positive role in an early transcription elongation checkpoint at the *MET16* gene (34). Although previous in vitro studies showed that TBP, as part of the TFIID complex, remains stably bound at a promoter after initiation (58), the situation was different in our in vivo studies at *GAL1*, where TBP but not TFIID was present (30). Further analysis of the role for TFIIS in transcription at *GAL1* is likely to produce new insights into the mechanisms that regulate the transition of Pol II from initiation to productive elongation.

One important question that our studies have also addressed is whether TFIIS is a general elongation factor in vivo. In our investigation of TFIIS at *GAL1*, we found TFIIS localized to the coding region as well as the regulatory region. We also found that TFIIS was localized to the coding region of each of the Gal4-independent genes that we examined. These included two inducible genes as well as three constitutively transcribed genes. At the inducible genes, the level of TFIIS association was correlated with the level of transcription (41, 49). The physical association of TFIIS with each of the constitutive genes, even under normal conditions of cell growth, was initially surprising because TFIIS was previously described as being recruited to such genes only when yeast cells were stressed (40). However, the tagged version of TFIIS used in the previous study is not fully functional, likely explaining the difference in the results (Prather et al., unpublished). Our finding that TFIIS is recruited to the coding region of each gene examined suggests that TFIIS is a more general factor than previously thought and is not just recruited by stalled elongation complexes.

Among the genes that we examined, TFIIS was preferentially localized to the upstream regulatory region only at Gal4-dependent genes. There are several possible reasons for this apparent specificity. First, although TFIIS is a general elongation factor, it may play a more specific role in initiation, solely as a Gal4-specific factor. Second, TFIIS may not be specific for Gal4 but rather may be required for initiation only under conditions of rapid and high-level induction. Third, the association of TFIIS may be more transient at other promoters, making it less detectable. Finally, TFIIS may not be measurable at other SAGA-dependent genes because the levels of SAGA associated with most regulatory regions are lower than those at *GAL1*. It was previously noted that SAGA levels appear to be very low at several SAGA-dependent promoters (26). The localization of TFIIS to the five Gal4-independent genes examined here is far from an exhaustive characterization; therefore, the degree of specificity and the nature of the roles for TFIIS in initiation and elongation require further study.

ACKNOWLEDGMENTS

We thank C. Kane, T. Formosa, S. Bhaumik, M. Green, and R. Kornberg for antibodies and plasmids. We thank D. Pokholok and R. Young for valuable discussion of results. We are grateful to A. Duina, J. Martens, and A. Nourani for helpful comments on the manuscript.

This work was supported by NIH grant GM45720 to F. Winston. D. M. Prather and E. Larschan were supported by Regulation of Gene Expression in Prokaryotes and Eukaryotes and Genetics of Cancer and Inherited Diseases training grants from the NIH.

REFERENCES

1. **Adam, M., F. Robert, M. Larochelle, and L. Gaudreau.** 2001. H2A.Z is required for global chromatin integrity and for recruitment of RNA polymerase II under specific conditions. Mol. Cell. Biol. **21:**6270–6279.
2. **Agarwal, K., K. H. Baek, C. J. Jeon, K. Miyamoto, A. Ueno, and H. S. Yoon.** 1991. Stimulation of transcript elongation requires both the zinc finger and RNA polymerase II binding domains of human TFIIS. Biochemistry **30:** 7842–7851.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A.**

Smith, and K. Struhl (ed.). 1988. Current protocols in molecular biology. John Wiley & Sons, Inc., New York, N.Y.
4. **Bhaumik, S. R., and M. R. Green.** 2002. Differential requirement of SAGA components for recruitment of TATA-box-binding protein to promoters in vivo. Mol. Cell. Biol. **22:**7365–7371.
5. **Bhaumik, S. R., and M. R. Green.** 2001. SAGA is an essential in vivo target of the yeast acidic activator Gal4p. Genes Dev. **15:**1935–1945.
6. **Bhaumik, S. R., T. Raha, D. P. Aiello, and M. R. Green.** 2004. In vivo target of a transcriptional activator revealed by fluorescence resonance energy transfer. Genes Dev. **18:**333–343.
7. **Brachmann, C. B., A. Davies, G. J. Cost, E. Caputo, J. Li, P. Hieter, and J. D. Boeke.** 1998. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast **14:**115–132.
8. **Bryant, G. O., and M. Ptashne.** 2003. Independent recruitment in vivo by Gal4 of two complexes required for transcription. Mol. Cell **11:**1301–1309.
9. **Costa, P. J., and K. M. Arndt.** 2000. Synthetic lethal interactions suggest a role for the Saccharomyces cerevisiae Rtf1 protein in transcription elongation. Genetics **156:**535–547.
10. **Davie, J. K., and C. M. Kane.** 2000. Genetic interactions between TFIIS and the Swi-Snf chromatin-remodeling complex. Mol. Cell. Biol. **20:**5960–5973.
11. **Dudley, A. M., C. Rougeulle, and F. Winston.** 1999. The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. Genes Dev. **13:**2940–2945.
12. **Exinger, F., and F. Lacroute.** 1992. 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. Curr. Genet. **22:**9–11.
13. **Fish, R. N., and C. M. Kane.** 2002. Promoting elongation with transcript cleavage stimulatory factors. Biochim. Biophys. Acta **1577:**287–307.
14. **Gall, J. G.** 2000. Cajal bodies: the first 100 years. Annu. Rev. Cell Dev. Biol. **16:**273–300.
15. **Hartzog, G. A., T. Wada, H. Handa, and F. Winston.** 1998. Evidence that Spt4, Spt5, and Spt6 control transcription elongation by RNA polymerase II in Saccharomyces cerevisiae. Genes Dev. **12:**357–369.
16. **Hirst, M., M. S. Kobor, N. Kuriakose, J. Greenblatt, and I. Sadowski.** 1999. GAL4 is regulated by the RNA polymerase II holoenzyme-associated cyclin-dependent protein kinase SRB10/CDK8. Mol. Cell **3:**673–678.
17. **Hubert, J. C., A. Guyonvarch, B. Kammerer, F. Exinger, P. Liljelund, and F. Lacroute.** 1983. Complete sequence of a eukaryotic regulatory gene. EMBO J. **2:**2071–2073.
18. **Izban, M. G., and D. S. Luse.** 1992. The RNA polymerase II ternary complex cleaves the nascent transcript in a 3′-5′ direction in the presence of elongation factor SII. Genes Dev. **6:**1342–1356.
19. **Kaplan, C. D., L. Laprade, and F. Winston.** 2003. Transcription elongation factors repress transcription initiation from cryptic sites. Science **301:**1096–1099.
20. **Kettenberger, H., K. J. Armache, and P. Cramer.** 2003. Architecture of the RNA polymerase II-TFIIS complex and implications for mRNA cleavage. Cell **114:**347–357.
21. **Kim, M., S. H. Ahn, N. J. Krogan, J. F. Greenblatt, and S. Buratowski.** 2004. Transitions in RNA polymerase II elongation complexes at the 3′ ends of genes. EMBO J. **23:**354–364.
22. **Komarnitsky, P., E. J. Cho, and S. Buratowski.** 2000. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev. **14:**2452–2460.
23. **Krogan, N. J., M. C. Keogh, N. Datta, C. Sawa, O. W. Ryan, H. Ding, R. A. Haw, J. Pootoolal, A. Tong, V. Canadien, D. P. Richards, X. Wu, A. Emili, T. R. Hughes, S. Buratowski, and J. F. Greenblatt.** 2003. A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol. Cell **12:**1565–1576.
24. **Kulish, D., and K. Struhl.** 2001. TFIIS enhances transcriptional elongation through an artificial arrest site in vivo. Mol. Cell. Biol. **21:**4162–4168.
25. **Kuras, L., T. Borggrefe, and R. D. Kornberg.** 2003. Association of the Mediator complex with enhancers of active genes. Proc. Natl. Acad. Sci. USA **100:**13887–13891.
26. **Kuras, L., P. Kosa, M. Mencia, and K. Struhl.** 2000. TAF-Containing and TAF-independent forms of transcriptionally active TBP in vivo. Science **288:**1244–1248.
27. **Larschan, E., and F. Winston.** 2001. The S. cerevisiae SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. Genes Dev. **15:**1946–1956.
28. **Larschan, E., and F. Winston.** 2005. The *Saccharomyces cerevisiae* Srb8-Srb11 complex functions with the SAGA complex during Gal4-activated transcription. Mol. Cell. Biol. **25:**114–123.
29. **Lennon, J. C., III, M. Wind, L. Saunders, M. B. Hock, and D. Reines.** 1998. Mutations in RNA polymerase II and elongation factor SII severely reduce mRNA levels in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **18:**5771–5779.
30. **Li, X. Y., S. R. Bhaumik, and M. R. Green.** 2000. Distinct classes of yeast promoters revealed by differential TAF recruitment. Science **288:**1242–1244.
31. **Lindstrom, D. L., and G. A. Hartzog.** 2001. Genetic interactions of Spt4-Spt5 and TFIIS with the RNA polymerase II CTD and CTD modifying enzymes in *Saccharomyces cerevisiae*. Genetics **159:**487–497.
32. **Malagon, F., A. H. Tong, B. K. Shafer, and J. N. Strathern.** 2004. Genetic interactions of DST1 in *Saccharomyces cerevisiae* suggest a role of TFIIS in the initiation-elongation transition. Genetics **166:**1215–1227.
33. **Mason, P. B., and K. Struhl.** 2003. The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. Mol. Cell. Biol. **23:**8323–8333.
34. **Morillon, A., N. Karabetsou, J. O'Sullivan, N. Kent, N. Proudfoot, and J. Mellor.** 2003. Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. Cell **115:**425–435.
35. **Myers, L. C., and R. D. Kornberg.** 2000. Mediator of transcriptional regulation. Annu. Rev. Biochem. **69:**729–749.
36. **Orphanides, G., W. H. Wu, W. S. Lane, M. Hampsey, and D. Reinberg.** 1999. The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature **400:**284–288.
37. **Pal, M., D. McKean, and D. S. Luse.** 2001. Promoter clearance by RNA polymerase II is an extended, multistep process strongly affected by sequence. Mol. Cell. Biol. **21:**5815–5825.
38. **Pan, G., T. Aso, and J. Greenblatt.** 1997. Interaction of elongation factors TFIIS and elongin A with a human RNA polymerase II holoenzyme capable of promoter-specific initiation and responsive to transcriptional activators. J. Biol. Chem. **272:**24563–24571.
39. **Park, J. M., H. S. Kim, S. J. Han, M. S. Hwang, Y. C. Lee, and Y. J. Kim.** 2000. In vivo requirement of activator-specific binding targets of Mediator. Mol. Cell. Biol. **20:**8709–8719.
40. **Pokholok, D. K., N. M. Hannett, and R. A. Young.** 2002. Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. Mol. Cell **9:**799–809.
41. **Proft, M., and K. Struhl.** 2002. Hog1 kinase converts the Sko1-Cyc8-Tup1 repressor complex into an activator that recruits SAGA and SWI/SNF in response to osmotic stress. Mol. Cell **9:**1307–1317.
42. **Ren, B., F. Robert, J. J. Wyrick, O. Aparicio, E. G. Jennings, I. Simon, J. Zeitlinger, J. Schreiber, N. Hannett, E. Kanin, T. L. Volkert, C. J. Wilson, S. P. Bell, and R. A. Young.** 2000. Genome-wide location and function of DNA binding proteins. Science **290:**2306–2309.
43. **Schneider, B. L., W. Seufert, B. Steiner, Q. H. Yang, and A. B. Futcher.** 1995. Use of polymerase chain reaction epitope tagging for protein tagging in Saccharomyces cerevisiae. Yeast **11:**1265–1274.
44. **Shaw, R. J., and D. Reines.** 2000. *Saccharomyces cerevisiae* transcription elongation mutants are defective in PUR5 induction in response to nucleotide depletion. Mol. Cell. Biol. **20:**7427–7437.
45. **Simic, R., D. L. Lindstrom, H. G. Tran, K. L. Roinick, P. J. Costa, A. D. Johnson, G. A. Hartzog, and K. M. Arndt.** 2003. Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. EMBO J. **22:**1846–1856.
46. **Smith, A. J., Y. Ling, and G. T. Morgan.** 2003. Subnuclear localization and Cajal body targeting of transcription elongation factor TFIIS in amphibian oocytes. Mol. Biol. Cell **14:**1255–1267.
47. **Sterner, D. E., P. A. Grant, S. M. Roberts, L. J. Duggan, R. Belotserkovskaya, L. A. Pacella, F. Winston, J. L. Workman, and S. L. Berger.** 1999. Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Mol. Cell. Biol. **19:**86–98.
48. **Suzuki, Y., Y. Nogi, A. Abe, and T. Fukasawa.** 1988. GAL11 protein, an auxiliary transcription activator for genes encoding galactose-metabolizing enzymes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**4991–4999.
49. **Swanson, M. J., H. Qiu, L. Sumibcay, A. Krueger, S. J. Kim, K. Natarajan, S. Yoon, and A. G. Hinnebusch.** 2003. A multiplicity of coactivators is required by Gcn4p at individual promoters in vivo. Mol. Cell. Biol. **23:**2800–2820.
50. **Swanson, M. S., E. A. Malone, and F. Winston.** 1991. *SPT5*, an essential gene important for normal transcription in *Saccharomyces cerevisiae*, encodes an acidic nuclear protein with a carboxy-terminal repeat. Mol. Cell. Biol. **11:**3009–3019.
51. **Ujvari, A., M. Pal, and D. S. Luse.** 2002. RNA polymerase II transcription complexes may become arrested if the nascent RNA is shortened to less than 50 nucleotides. J. Biol. Chem. **277:**32527–32537.
52. **Wery, M., E. Shematorova, B. Van Driessche, J. Vandenhaute, P. Thuriaux, and V. Van Mullem.** 2004. Members of the SAGA and Mediator complexes are partners of the transcription elongation factor TFIIS. EMBO J. **23:**4232–4242.
53. **Wind, M., and D. Reines.** 2000. Transcription elongation factor SII. Bioessays **22:**327–336.
54. **Wind-Rotolo, M., and D. Reines.** 2001. Analysis of gene induction and arrest site transcription in yeast with mutations in the transcription elongation machinery. J. Biol. Chem. **276:**11531–11538.
55. **Winston, F., C. Dollard, and S. L. Ricupero-Hovasse.** 1995. Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. Yeast **11:**53–55.
56. **Wu, P. Y., and F. Winston.** 2002. Analysis of Spt7 function in the *Saccharomyces cerevisiae* SAGA coactivator complex. Mol. Cell. Biol. **22:**5367–5379.
57. **Yoon, H., A. S. Sitikov, C. Jeon, and K. Agarwal.** 1998. Preferential interaction of the mRNA proofreading factor TFIIS zinc ribbon with rU.dA base pairs correlates with its function. Biochemistry **37:**12104–12112.
58. **Yudkovsky, N., J. A. Ranish, and S. Hahn.** 2000. A transcription reinitiation intermediate that is stabilized by activator. Nature **408:**225–229.