The transcription elongation factor TFIIS is a component of RNA polymerase II preinitiation complexes

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In this article, we provide direct evidence that the evolutionarily conserved transcription elongation factor TFIIS functions during preinitiation complex assembly. First, we identified TFIIS in a mass spectrometric screen of RNA polymerase II (Pol II) preinitiation complexes (PICs). Second, we show that the association of TFIIS with a promoter depends on functional PIC components including Mediator and the SAGA complex. Third, we demonstrate that TFIIS is required for efficient formation of active PICs. Using truncation mutants of TFIIS, we find that the Pol II-binding domain is the minimal domain necessary to stimulate PIC assembly. However, efficient formation of active PICs requires both the Pol II-binding domain and the poorly understood N-terminal domain. Importantly, Domain III, which is required for the elongation function of TFIIS, is dispensable during PIC assembly. The results demonstrate that TFIIS is a PIC component that is required for efficient formation and/or stability of the complex.

isotope-coded affinity tagging | quantitative mass spectrometry | *Saccharomyces cerevisiae* | stable isotopes

Transcription of protein-coding genes by RNA polymerase II (Pol II) is a dynamic process that begins with the formation of a preinitiation complex (PIC) at the promoter and proceeds through initiation, elongation, termination, and, finally, reinitiation (1). Proper regulation of transcription during this cycle involves interactions between a number of factors with Pol II and chromatin. During PIC formation, interactions between gene-specific activator proteins, chromatin remodeling complexes, coactivator complexes, and general transcription factors (GTFs) result in the recruitment of an initiation-competent Pol II to the promoter (2, 3). Importantly, both the exact composition and the order of factor recruitment at specific promoters are still unresolved questions (4, 5).

During the transition from initiation to elongation, promoter-specific contacts between Pol II and the PIC are disrupted as the polymerase begins messenger RNA synthesis and traverses into the ORF (1, 6). The efficiency of elongation by Pol II is regulated by a number of additional factors such as TFIIS, Spt4-Spt5, FACT, and Paf (6). At least one PIC component, TFIIF, stimulates elongation *in vitro*. The elongation factors can exert their effects through a number of distinct mechanisms, which include controlling the rate of elongation or the processivity of Pol II, facilitating transcription through nucleosomes, or by reactivating Pol II that has become arrested (6).

TFIIS is perhaps the best characterized elongation factor (7). *In vitro*, TFIIS stimulates arrested Pol II to cleave the nascent transcript that has fallen out of register with template DNA (7, 8). This generates a new 3′ end in the nascent mRNA that is used by Pol II to resume transcription. The recently solved structure of a TFIIS–Pol II complex has provided important details regarding TFIIS function that are consistent with many of the known biochemical activities of TFIIS (9).

Consistent with the biochemical activities defined *in vitro*, a number of *in vivo* studies indicate that TFIIS plays a role in elongation. These include the sensitivity of *dst1* mutant cells to the nucleotide-depleting drug 6-azauracil (6-AU) (10), ChIP experiments that localize TFIIS over the coding regions of a number of genes under conditions of stress or in the presence of 6-AU (11), and genetic interactions with genes encoding elongation factors such as *SPT4*, *SPT5*, *SPT6*, *SPT16*, and *RTF1* (12–14). In addition, a recent report indicates that the role of TFIIS in elongation stems from its ability to stimulate the processivity of Pol II in the presence of 6-AU (15).

TFIIS is composed of three independently folding domains (16–19). Domain I (amino acids 1–130) is composed of a four-helix bundle that is not required for the known biochemical and biological functions of TFIIS (19). Intriguingly, however, a number of biochemical and genetic interactions between Domain I and initiation factors have been described (see below) (20–24). Domain II (amino acids 131–240) forms a three-helix bundle and is tethered to Domain III (amino acids 265–309) through a short, unstructured linker (9, 18). Domain III is composed of three antiparallel β-sheets that form a zinc ribbon. Domain II and the linker are required for Pol II binding, whereas Domain III is essential for stimulation of RNA cleavage (17). As such, Domains II-III (amino acids 131–309) are sufficient to carry out all of the known activities of TFIIS *in vitro* and *in vivo* (7).

Although the role of TFIIS in Pol II elongation is well established, some studies have suggested that TFIIS may also function during early events in transcription. One of the first indications of this came from experiments in which immobilized GST–TFIIS was used to purify a holoenzyme complex containing GTFs, TAFs, and Mediator components (20). Interestingly, efficient interaction of this holoenzyme complex with TFIIS required only the N-terminal region of TFIIS. More recently, the N-terminal Domain I was shown to interact with SAGA component Spt8 and Mediator component Med13 (Srb9) in a two-hybrid screen. and these factors copurified with TFIIS when it was overexpressed (21). Perhaps the most suggestive evidence comes from a study that used ChIP assays to demonstrate cross-linking of TFIIS to the *GAL1* UAS (25). This study also showed that TFIIS was required for efficient recruitment of TATA box-binding protein (TBP) and Pol II to the promoter, and, at high temperature, efficient recruitment of SAGA and Mediator components to the UAS was TFIIS-dependent. In addition to these molecular and biochemical studies, a number of

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Abbreviations: GTF, general transcription factor; ICAT, isotope-coded affinity tagging; PIC, preinitiation complex; TBP, TATA box-binding protein.

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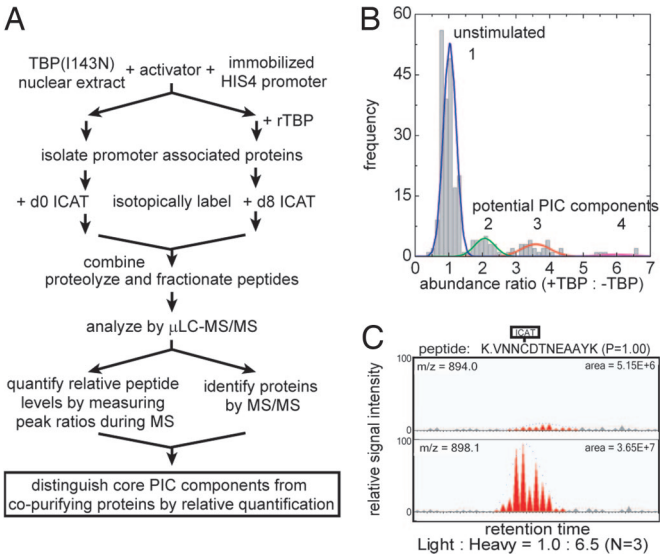

Fig. 1. Identification of TFIIS by quantitative MS analysis of promoter DNA-purified Pol II PICs. (*A*) Schematic of the quantitative MS approach for the analysis of Pol II PICs. (*B*) Histogram of abundance ratios (+rTBP:−TBP) for 252 quantified proteins. The distributions were modeled by using a mixture model expectation–maximization algorithm. The model predicts five clusters: four clusters of TBP-stimulated proteins and one cluster of unstimulated proteins. TFIIS is a component of cluster 4. Cluster 5 (highest degree of enrichment) is not shown. (*C*) Quantification of isotopically labeled peptides derived from TFIIS. A SEQUEST database search (52) matched the MS/MS spectrum of an ion with mass-to-charge ratio (*m/z*) of 898.1 to the indicated ICAT-labeled peptide sequence corresponding to TFIIS. Relative quantification of the isotopically heavy (*Lower*) and normal (*Upper*) ICAT-labeled peptides was determined from the summed signal intensities for each peptide ion during the elution time with XPRESS software (53). The abundance ratio is the average from three independent measurements. P is the ProteinProphet score that describes the probability that the identification is correct (54).

genetic studies have uncovered functional interactions between *DST1* and genes encoding factors that can function during initiation such as components of Mediator, Swi/Snf, and TFIIF (22–24). Although these results suggest that TFIIS may play a role during early events in transcription, no direct biochemical evidence has been presented to support this claim.

We were alerted to a potential role for TFIIS before elongation when we identified TFIIS in a quantitative MS screen of Pol II PICs (26). Based on this observation, we tested directly whether TFIIS was a bona fide component of PICs and whether it was required for the activity of PICs. Our results confirm that TFIIS can associate with PICs at a promoter, and its stable recruitment to the promoter depends on TBP, SAGA, and Mediator. We demonstrate that TFIIS is required for efficient formation and/or stability of active PICs. Furthermore, we find that the Pol II-binding domain (amino acids 133–265) is the minimal domain necessary to stimulate formation of PICs. However, efficient formation of active PICs requires both the Pol II-binding domain and the poorly understood N-terminal domain (amino acids 1–265). Importantly, Domain III, which is required for the elongation function of TFIIS, is dispensable during PIC assembly. Together, the results reveal a previously uncharacterized role for TFIIS in PIC assembly and/or stability and uncover an important function for the evolutionarily conserved N-terminal domain.

Results

Identification of TFIIS in a Quantitative MS Screen of Pol II PICs. To characterize the composition of Pol II PICs, we conducted a quantitative MS screen of promoter DNA affinity-purified complexes (Fig. 1*A*) (26). In this screen, the composition of complexes formed at a modified core yeast *HIS4* promoter in the presence and absence of functional TBP was monitored by differentially incorporating isotope-coded affinity tagging (ICAT) reagents (27) into the proteins from the two samples, followed by MS analysis to identify and quantify the proteins. Potential PIC components were distinguished from copurifying proteins by monitoring their relative enrichment in the presence and absence of recombinant (r)TBP. A histogram of enrichment ratios (+TBP:−TBP) for 252 quantified proteins identified with high confidence showed a multimodal distribution consisting of a large number of proteins with ratios close to 1:1 and a pronounced shoulder extending in the direction of higher enrichment ratios (Fig. 1*B*). To determine each protein's probability of being enriched in the sample containing rTBP, the distribution of ICAT ratios was modeled by using a varying number of Gaussian distributions, and the mixture model was fitted to the data by using an expectation–maximization algorithm. The optimal number of clusters was determined by using the Bayesian Information Criterion (28), and each protein was assigned to one of the clusters. Interestingly, in addition to the cluster of proteins showing no enrichment (ICAT ratios close to 1:1), the model predicts the presence of four clusters of proteins that are enriched to a different degree in the presence of rTBP [Fig. 1*B* and supporting information (SI) Table 2]. These clusters contain 57 of the 252 quantified proteins. Importantly, TFIIS was found in the second most significantly enriched cluster 4, with an average enrichment ratio of 6.5 (Fig. 1*C* and SI Table 2). The other proteins in cluster 4 are the GTFs: TFIIB, TFG1, and TFG2, and MOT1. In fact, all but two of the proteins in the enriched clusters are PIC components (29, 30), transcriptional regulators (31–33), or they have been shown to interact with PIC components (34–36). The strong TBP-dependent stimulation of TFIIS levels at the promoter, along with the rest of the core transcription machinery, suggests that TFIIS can associate with Pol II PICs.

Activator-Stimulated and Promoter-Dependent Recruitment of TFIIS. Previously, we used immobilized templates to demonstrate that activators stimulated the extent of PIC formation in a manner that correlated with their ability to stimulate transcription (37). In addition, we showed that, in the presence of a weak activator such as Gal4-AH, recruitment of PIC components depended on promoter sequences. To characterize the specificity of TFIIS recruitment to immobilized templates, we tested whether TFIIS levels depended on activation domains and promoter sequences. Nuclear extracts were incubated with immobilized templates that contained the modified core *HIS4* promoter with a single upstream Gal4-binding site or a single Gal4-binding site alone in the presence or absence of the activators Gal4-AH, Gal4-GCN4, and Gal4-VP16. We find that activators stimulated the recruitment of TFIIS to the HIS4 promoter along with the other PIC components except TBP (Fig. 2*A*, lanes 1–4). We have previously shown that TBP is present in excess in these reactions and is likely present in nonfunctional complexes (38). Quantitative Western blotting revealed that TFIIS was present at a level comparable with that of TFIIB (data not shown). In addition, in basal reactions and in the presence of Gal4-AH, TFIIS recruitment depended on promoter sequences (lanes 1 and 2 vs. lanes 5 and 6). We previously reported that the strong activator Gal4-VP16 can stably recruit many PIC components in the absence of promoter sequences. Like these PIC components, TFIIS is also recruited in these reactions (lane 7). Consistent with the proposal that TFIIS can associate with Pol II PICs, the results show that TFIIS recruitment to immobilized templates is stimulated by activators and depends on promoter sequences.

Recruitment of TFIIS to a Promoter Depends on SAGA and Mediator. A recent report demonstrated that recruitment of TFIIS to the *GAL1* UAS depended on SAGA and Mediator (25). To further define the requirements for TFIIS recruitment to promoters *in*

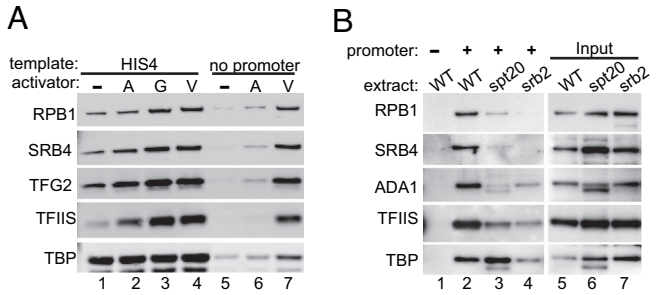

Fig. 2. TFIIS is a component of Pol II PICs. (*A*) Activator-stimulated recruitment of TFIIS to a promoter. Immobilized template assays were performed by incubating nuclear extract with the indicated templates for 40 min with or without Gal4-AH (A, 6 pmol), Gal4-VP16 (V, 4 pmol), or Gal4-GCN4 (G, 4 pmol). (*B*) Efficient recruitment of TFIIS and other PIC components to a promoter requires functional SAGA and Mediator complexes. Immobilized template assays were performed by incubating the indicated nuclear extracts with the HIS4 immobilized template for 40 min in the presence of Gal4-AH (1.5 pmol) (lanes 2–4). In lane 1, Dynabeads lacking DNA were incubated with Gal4-AH and nuclear extract. Five micrograms of the indicated nuclear extracts was resolved by SDS/PAGE and analyzed by Western blotting (lanes 5–7).

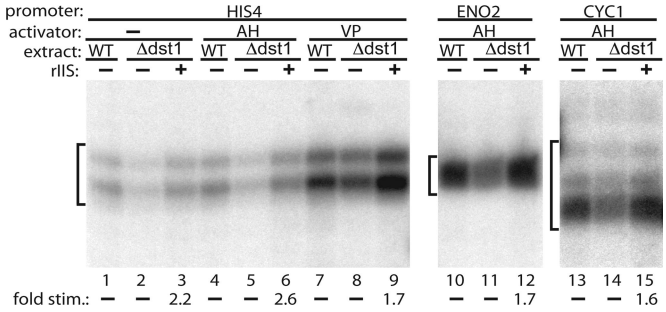

Fig. 3. TFIIS stimulates formation of active PICs. PICs were assembled by incubating nuclear extracts from either a wild-type strain or a strain lacking *DST1* with the indicated promoters in the presence or absence of Gal4-AH (1.5 pmol) or Gal4-VP16 (1 pmol) for 60 min. rTFIIS (3 pmol) was included where indicated. Transcription was initiated by the addition of NTPs, and reactions were stopped after 2 min. Brackets indicate correctly initiated transcripts that were detected by primer extension. The fold stimulation was calculated by comparing the transcription signal in reactions supplemented with rTFIIS to those lacking TFIIS.

vitro, we monitored TFIIS recruitment to promoters in extracts prepared from strains carrying mutant alleles of genes encoding components of SAGA (*spt20* deletion), and Mediator (*srb2* deletion). In agreement with the situation at the *GAL1* UAS (25), recruitment of TFIIS strongly depended on functional SAGA and Mediator complexes (Fig. 2*B*). The dependence of TFIIS recruitment on activators and functional components of the transcription machinery for efficient promoter recruitment strongly suggests that TFIIS is a bona fide PIC component at some promoters.

TFIIS Functions at an Early Step in Transcription. Early studies with partially purified factors failed to detect a requirement for TFIIS during PIC assembly (39, 40). However, a recent study using chromatin templates and purified GTFs, Mediator, Pol II, and p300 reported a modest, but reproducible stimulation (<2-fold) by TFIIS in initiation assays (41). Thus, it is possible that a requirement for TFIIS during PIC assembly was not observed because of the composition of the purified system in the earlier work. To test for a role of TFIIS at an early step in transcription, we performed transcription assays using nuclear extracts prepared from a *DST1* deletion strain in the presence or absence of rTFIIS and the activators Gal4-AH or Gal4-VP16. PICs were allowed to form by preincubating the extracts and recombinant proteins with the modified *HIS4* promoter for 60 min, and transcription was initiated by addition of NTPs. To assay a single round of transcription, the reactions were stopped after 2 min, and transcripts were analyzed by either primer extension or S1 nuclease protection (37). These assays detect transcripts that are at least ≈100 and ≈30 nucleotides, respectively. TFIIS reproducibly stimulated transcription by ≈2-fold in both assays (Fig. 3, lanes 2, 5, and 8 vs. lanes 3, 6, and 9; and see Fig. 5*B*, lane 2 vs. 3). This effect is not promoter-specific because a similar result was observed when templates containing the core CYC1 and ENO2 promoters were used (Fig. 3, lane 11 vs. lane 12 and lane 14 vs. lane 15). These results demonstrate that TFIIS can play a functional role during early events in transcription that is independent of chromatin.

TFIIS Is Required for Efficient PIC Formation and/or Stability. Because we monitor the synthesis of ≈30 and 100 nucleotide transcripts in our transcription assays, it is not possible to determine exactly which of the early steps in transcription is affected by TFIIS in this system. To directly test whether the observed defect in transcription activity could be attributed to a defect in PIC formation or stability. we monitored PIC assembly using immobilized promoters and nuclear extracts prepared from a *DST1* deletion strain. Compared with a wild type extract, recruitment of all PIC components analyzed except TBP was defective in the *DST1* deletion extract (Fig. 4, lane 2 vs. lane 4). Furthermore, addition of increasing amounts of rTFIIS stimulated recruitment of each of the affected PIC components. The high levels of TFB1 observed in the absence of TFIIS and the weak stimulation of TFB1 levels by TFIIS could be due to an interaction between TFB1 and the Gal4-VP16 activator used in this experiment (see below) (42). The results show that TFIIS is required for the stable recruitment of Pol II, Mediator, and TFIIB and suggest that TFIIS is required for efficient PIC assembly and/or stability.

To obtain a more comprehensive understanding of the role of TFIIS in PIC assembly, we analyzed the composition of PICs formed in the presence and absence of rTFIIS by quantitative MS (SI Fig. 6*A*) (26). In this analysis, we measured the abundance ratios for 87 proteins (Table 1 and SI Table 3), and the distribution of

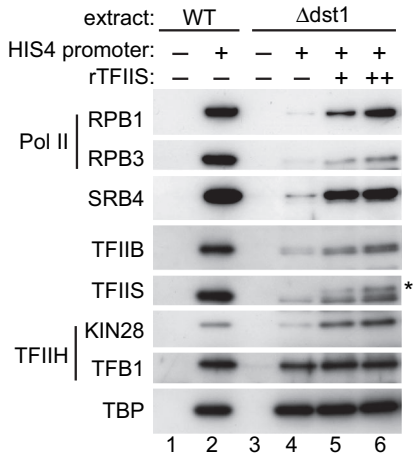

Fig. 4. TFIIS is required for efficient formation and/or stability of PICs. Western blot analysis of PIC formation in the presence and absence of TFIIS. Nuclear extract from a wild-type or a *DST1*-deletion strain was incubated with the immobilized HIS4 promoter and the activator Gal4-VP16 (1 pmol) in the presence or absence of rTFIIS (1.5 pmol, lane 5; 3 pmol, lane 6). After 60 min, the immobilized templates were washed, and protein composition was analyzed by SDS/PAGE, followed by Western blotting. Background binding to Dynabeads is shown in lanes 1 and 3. The asterisk indicates the band corresponding to rTFIIS that closely migrates with TFIIB, which was probed for in a previous experiment.

Table 1. Summary of proteins that were significantly stimulated (abundance ratio ≥2.0, probability P_{stim} > 0.95) in the quantitative MS analysis of the effect of TFIIS on PIC composition

Protein	H/L ratio	SD	P_{stim}	Description
DST1	>2.7		1	TFIIS
SIN4	3.0	0.1	1	Mediator
RGR1	2.6	0.0	1	Mediator
MED1	2.5	0.1	1	Mediator
NUT1	2.5	0.2	1	Mediator
MED2	2.5	0.2	1	Mediator
SRB6	2.4	0.1	1	Mediator
SRB8	2.4		1	Mediator
MED4	2.2	0.2	0.99	Mediator
GAL11	2.2	0.2	0.99	Mediator
SSN2/SRB9	2.1		0.97	Mediator
SRB2	2.0	0.1	0.96	Mediator
RPB4	2.7	0.1	1	RNA pol II
RPB7	2.3	0.1	0.99	RNA pol II
RPB2	2.2	0.2	0.99	RNA pol II
RBP1/RPO21	2.1	0.0	0.98	RNA pol II
RPB9	2.1	0.1	0.97	RNA pol II
RPB3	2.0	0.1	0.97	RNA pol II
TFA2	>2.4	0.1	1	TFIIE complex
SUA7	2.5	0.2	1	TFIIB
TFG2	2.2	0.1	0.99	TFIIF complex
RAD3	>2.0		0.96	TFIIH complex
CDC19	>2.6	0.1	1	Pyruvate kinase

abundance ratios was modeled by using an expectation–maximization mixture modeling algorithm (SI Fig. 6*B*) (43). The analysis predicts the presence of two populations of proteins: group one contains proteins with abundance ratios that are unaffected by the presence of TFIIS (average ratio = 1.1 ± 0.2), and group two contains proteins with abundance ratios that are stimulated by rTFIIS (average ratio = 2.3 ± 0.3). From this analysis, a probability P_{stim} is calculated for each protein that estimates the likelihood that the levels of a protein are stimulated by rTFIIS. Within the population of proteins that were clearly stimulated by rTFIIS (abundance ratio ≥2.0, probability $P_{stim} > 0.95$) were 11 Mediator subunits, six Pol II subunits and the GTF subunits TFIIB, TFA2 (TFIIE), TFG2 (TFIIF), and RAD3 (TFIIH) (Table 1). PIC components that were not significantly stimulated by rTFIIS (abundance ratio <2, $P_{stim} < 0.9$) included TBP, TOA1 (TFIIA), TFIIH subunits TFB1, TFB5, SSL1, and CCL1, the shared Pol II subunit RPB8, and shared subunits of TFIID and SAGA. In addition, subunits of chromatin-remodeling complexes such as SAGA, SWI/SNF, NuA4, RSC, INO80, and ISW1 were not significantly stimulated by rTFIIS. In general, these results confirm and extend the results from the Western blot analysis of selected PIC components (Fig. 4). It is interesting to note that TFIIH subunits showed different tendencies toward enrichment in both analyses. KIN28 and RAD3 were clearly stimulated by TFIIS in the Western blot and MS analyses, respectively, whereas four other TFIIH subunits were not significantly stimulated in these analyses. As mentioned above, the lack of stimulation of some TFIIH subunits is likely due to an interaction between TFB1 and the Gal4-VP16 activator used in this experiment (42). Overall, the results show that TFIIS is required for efficient PIC formation by stimulating the stable recruitment of Pol II, Mediator, and most of the GTFs to the promoter. Together with the results from transcription assays (Figs. 3 and 5 and SI Fig. 7), the data provides direct evidence for a role of TFIIS in PIC assembly.

TFIIS Stimulates Both the Rate and Extent of PIC Formation. The ability of TFIIS to stimulate PIC formation could be explained by

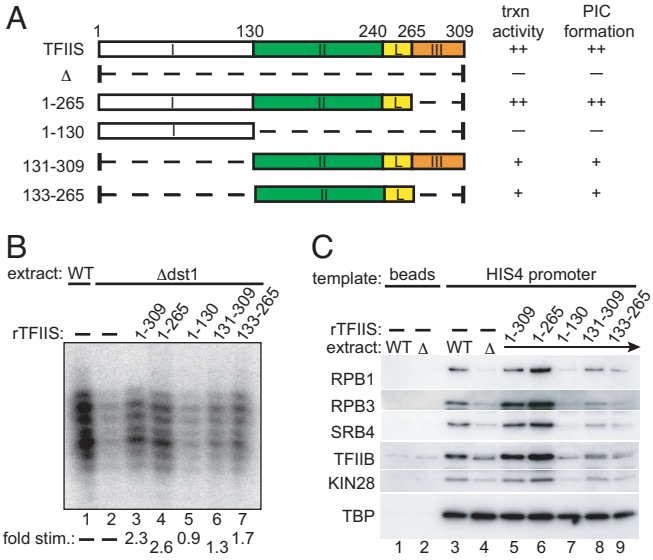

Fig. 5. Role of the N-terminal domain and the Pol II-binding domain of TFIIS during PIC assembly. (*A*) Schematic representation of the TFIIS truncation mutants used in this study and summary of their activities in PIC formation and transcription assays. The amino acids defining the endpoints of each truncation mutant are indicated on the left. (*B*) PIC activity in the presence of TFIIS truncation mutants. PICs were assembled by incubating nuclear extracts from either a wild-type strain or a strain lacking *DST1* with the HIS4 promoter template and Gal4-AH (1.5 pmol) in the absence or presence of either full-length rTFIIS (3 pmol) or the indicated rTFIIS truncation mutants (3 pmol) for 60 min. Transcription was initiated by the addition of NTPs, reactions were stopped after 2 min, and correctly initiated transcripts were detected by S1 nuclease protection. The fold stimulation was calculated by comparing the transcription signal in each reaction to that obtained in the reaction lacking TFIIS (lane 2). (*C*) PIC assembly in the presence of TFIIS truncation mutants. Nuclear extract from a wild-type or a *DST1*-deletion strain was incubated with the immobilized HIS4 promoter and the activator Gal4-AH (6 pmol) in the absence or presence of the indicated rTFIIS proteins (12 pmol). After 60 min, the immobilized templates were washed, and protein composition was analyzed by SDS/PAGE, followed by Western blotting. Background binding to Dynabeads is shown in lanes 1 and 2.

an effect on the rate of complex assembly and/or other parameters such as the stability of PICs. To probe the mechanism of TFIIS action, we measured the rate of active PIC formation in the presence and absence of TFIIS (SI Fig. 7). Nuclear extracts from wild-type or *DST1* deletion strains were incubated with the modified *HIS4* promoter in the presence or absence of rTFIIS for increasing amounts of time, after which transcription was initiated by addition of NTPs. Single-round measurements of transcription were obtained as described for Fig. 3. Consistent with the results from previous transcription assays, ≈3-fold fewer active PICs formed in the absence of TFIIS (SI Fig. 7*A*, lane 10 vs. lanes 5 and 15, and 7*B*). Although TFIIS stimulated the rate of active PIC formation ($t_{1/2}$ wild type = 24 min, $t_{1/2}$ *dst1* + rTFIIS = 18 min, $t_{1/2}$ *dst1* = 30 min), the requirement for TFIIS could not be overcome by prolonged incubation of extract with the template. This suggests that in addition to stimulating the rate of PIC formation, TFIIS may also increase PIC stability.

Domain III of TFIIS Is Not Required During PIC Assembly. TFIIS is composed of three independently folding domains (7, 16). To gain further insight into the mechanism of TFIIS action during PIC assembly, we generated truncation mutants of TFIIS that are composed of different combinations of its domains and assayed the ability of the mutants to stimulate PIC assembly and activity (Fig. 5 and SI Fig. 8). Interestingly, all of the proteins except Domain I (1–130) alone retained some ability to stimulate synthesis of short

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transcripts (Fig. 5*B*). Whereas the Domain I-II-linker (I-II-L) construct (1–265) retained full activity (Fig. 5*B*, lane 3 vs. lane 4), the Domain II-L-III construct (131–309) and the Domain II-L construct (133–265) only retained partial activity (57% and 74% of full-length TFIIS, respectively, Fig. 5*B*, lane 3 vs. lanes 6 and 7). These results show that Domain II and the linker are necessary and sufficient to provide the transcriptional stimulatory affect of TFIIS during early events in transcription. However, the presence of Domain I enhances the stimulatory affect. The observation that Domain III is dispensable for the synthesis of short transcripts is significant because it shows that TFIIS function during early events in transcription is distinct from its role in elongation, which requires Domain III.

To test whether the stimulatory affect of the truncation mutants in transcription assays correlates with an ability to enhance PIC formation, we monitored the ability of the truncation mutants to stimulate recruitment of PIC components to a promoter using the immobilized promoter assay (Fig. 5*C*). Interestingly, the I-II-L construct (1–265) retained the ability to efficiently stimulate PIC assembly (lane 6). Domain I (1–130) was inactive in this assay (lane 7) and the II-L (133–265) and II-L-III constructs (131–309) weakly stimulated factor recruitment (lanes 8 and 9). In agreement with the results of transcription assays, the results show that Domain II and the linker are necessary to promote efficient PIC assembly, whereas Domain III, which harbors the RNA cleavage stimulatory activity, is not required. Interestingly, the results uncover a function for the poorly understood N-terminal domain of TFIIS in stimulating stable PIC formation. This provides direct biochemical evidence for the role of TFIIS in stimulating the formation and/or stability of PICs, and its N-terminal domain contributes to this activity.

Discussion

In this study, we show that TFIIS has an important role during early events in transcription that is distinct from its role in elongation. By monitoring the affect of TFIIS on PIC formation and activity, we demonstrated that TFIIS stimulates the rate and extent of PIC assembly. Quantitative MS and Western blot analysis of transcription complexes revealed that TFIIS stimulates the stable recruitment of Mediator, Pol II, TFIIB, TFIIE, and TFIIF to the promoter. Finally, analysis of truncation mutants of TFIIS revealed that Domain III, which is required for the antiarrest activity of TFIIS, is dispensable for its role in PIC assembly. Surprisingly, the poorly understood Domain I, in conjunction with Domain II and the linker region, was required for efficient activity in PIC assembly assays. Our results are supported by those of Werner and colleagues (accompanying article, ref. 44) who demonstrate a functional interaction between Mediator and the Pol II-binding domain of TFIIS that is linked to a requirement for TFIIS for efficient recruitment of Pol II to several promoters *in vivo*).

These results provide direct biochemical evidence for a role of TFIIS in stimulating PIC formation and/or stability. Several recent studies are consistent with our findings. First, TFIIS was shown to interact with initiation factors Spt8 of SAGA and Med13 of Mediator (21). Although the authors interpreted these results as evidence that SAGA and Mediator may participate in elongation, they are consistent with our findings that TFIIS interacts with the PIC and promotes efficient complex formation. Second, a GST-fusion of TFIIS was used to purify initiation factors such as Mediator, TAFs, and some GTFs from HeLa nuclear extracts (20). Third, *DST1* interacts genetically with numerous genes encoding factors with roles in initiation such as *MED31*, *MED3*, *SIN4*, *SRB5*, *SPT8*, *SWI1*, *SNF2*, *SNF5*, and *KIN28* (21–23, 45) and with genes encoding factors involved in both initiation and elongation such as *RPB1*, *RPB4*, *TFG1*, *TFG2*, *TAF14*, and *HTZ1* (21, 22, 24, 45). In light of the well characterized role of TFIIS in stimulating elongation *in vitro*, the results of some of these genetic studies were interpreted as evidence that TFIIS could function at a step after PIC assembly (21, 22, 24). Although this may be true, the interactions also fit well with our model in which TFIIS interacts directly with initiation factors to promote PIC assembly. Finally, TFIIS was recently shown to localize to *GAL* promoter regions under inducing conditions, and it was required for optimal recruitment of TBP, Pol II, and Mediator (25). Our results are consistent with these findings, and, moreover, they provide direct biochemical evidence in support of a model in which TFIIS interacts with PIC components to promote stable complex formation.

What is the mechanism by which TFIIS stimulates PIC assembly? Our results demonstrate that TFIIS stimulates the stable association of Mediator, Pol II, TFIIB, TFIIE, and TFIIF with the promoter. Importantly, we find that the evolutionarily conserved N-terminal domain of TFIIS stimulates stable recruitment of PIC components to the promoter. Although this domain is not present in the cocrystal structure available between TFIIS and RNA polymerase II (9), it is reasonable to suggest that it would be on the periphery of the Pol II structure and would thus be available for interaction with other Pol II factors such as SAGA and Mediator components (21). In addition, although this domain is dispensable for the known *in vitro* and *in vivo* activities of TFIIS, it was recently shown to be required to complement the lethality of yeast cells lacking *TAF14* and *DST1* (24). This and other evidence suggested an undefined function of TFIIS mediated by its N-terminal region. Together with our findings, the results suggest that TFIIS stimulates the extent and stability of PIC formation through direct interactions with PIC components such as SAGA and Mediator via its N-terminal domain in addition to its well characterized interaction with Pol II via Domain II and the linker (7, 9). These direct interactions are proposed to contribute to PIC stability and would be most apparent under conditions where the stability of the PIC is compromised, such as during stress or in mutant cells where the activity of another PIC component is compromised [see accompanying article (44)].

Despite the compelling evidence that TFIIS plays important roles during PIC assembly and elongation, strains lacking *DST1* are viable. Notably, however, *DST1* deletion strains exhibit growth defects in the presence of nucleotide-depleting drugs (10), microtubule-destabilizing drugs (22), and at elevated temperature (46, 47). In addition, TFIIS becomes essential for normal growth in strains carrying mutant alleles of factors with roles in initiation such as Med31, Sin4, Srb5, Tfg3, Rpb1, Rpb4, Htz1, and Swi/Snf components (21–24, 45). It is likely that TFIIS is required for normal transcription initiation under conditions where efficient PIC assembly is compromised. These conditions would include situations where the activity of other PIC components, such as Med31 or Swi/Snf is limited (22, 23). It is also possible that the growth defect of *DST1* deletion strains at elevated temperatures is a consequence of inefficient PIC assembly under these conditions. In addition, TFIIS may become required for normal transcription under conditions of high transcriptional activity where efficient PIC assembly could be limiting. This appears to be the case at *GAL* promoters (25) and the *DST1* deletion strain's sensitivity to microtubule destabilizing drugs may be due to inefficient PIC assembly at promoters of genes that are normally induced in the presence of these drugs (22). Of course it is also possible that elongation defects contribute to these phenotypes. Careful examination of the affect of TFIIS on Pol II density along the length of TFIIS-dependent genes by ChIP may help to elucidate the role(s) of TFIIS in transcription at these genes. Finally, several reports have demonstrated a transcriptional requirement for TFIIS during the induction of specific genes *in vivo*, but these studies did not determine the step(s) in the transcription cycle that was affected by TFIIS. In light of our findings and those of other groups (21–25), it will be important to consider the potential role of TFIIS in PIC assembly as well as elongation at these genes.

One key question for future work will be to determine the generality of the requirement for TFIIS during PIC assembly. Recent studies shown that TFIIS can be cross-linked to the pro-

moters of several genes (25, 44). In addition, the ability of TFIIS to associate with the PIC suggests the possibility that TFIIS may remain associated with Pol II during initiation so that it can function during subsequent steps in transcription. This would provide a way for TFIIS to rapidly function at promoter proximal arrest sites (48). However the fact that TFIIS can function after initiation *in vitro* suggests that its association with the PIC is not a prerequisite for it to function after initiation. Future biochemical and genetic tests with initiation- and elongation-specific TFIIS mutants should help to unravel the diverse functions of TFIIS throughout the transcription cycle at specific genes.

Materials and Methods

Yeast Strains and Extracts. Yeast strains used in this study are listed in SI Table 4. Yeast nuclear extracts were prepared as described online at www.fhcrc.org/scince/basic/labs/hahn. Protein concentrations were determined by using the BioRad dye binding assay.

Recombinant Proteins. Gal4-AH, Gal4-VP16, and Gal4-GCN4 have been described (37, 49). An NdeI/BamHI fragment containing full-length *DST1* (encoding TFIIS) was subcloned from plasmid pRF108 (gift from C. Kane, University of California, Berkeley) into pET15b (Novagen, San Diego, CA) to create *Escherichia coli* expression plasmid pBK1 containing N-terminally tagged 6XHis *DST1*. *DST1* truncation mutants were generated by PCR by using pBK1 and primers containing NdeI and BamHI restriction sites. All constructs were verified by sequencing. 6XHis TFIIS and derivates were expressed and purified as described in *SI Text*.

Immobilized Promoter Templates. Immobilized promoter templates were prepared as described in ref. 37 and in *SI Text*.

Immobilized Template Assays. Immobilized template assays were performed as described in refs. 26 and 37, in *SI Text*, and online at www.fhcrc.org/scince/basic/labs/hahn.

Transcription Assays. *In vitro* transcription reactions with plasmid and immobilized templates were performed as described in refs. 37 and 50, in *SI Text* and online at www.fhcrc.org/scince/basic/labs/hahn.

Preparation of Purified Pol II PICs for Quantitative MS Analysis. Samples were prepared for quantitative MS analysis essentially as described in refs. 26 and 30 and in *SI Text*.

μLC-MS/MS and Data Analysis. μLC-MS/MS and data analysis was performed as described in ref. 26 and in *SI Text*.

Computational Modeling of Quantitative Protein Expression Ratios. To assess the statistical significance of the data and to allow more transparent interpretation of protein groups with various degrees of enrichment in the affinity-purified samples compared with control, quantitative MS data were analyzed by using the model-based unsupervised clustering approach (for review, see ref. 51). Details are provided in *SI Text*.

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