Saccharomyces cerevisiae has a TFIIS-related transcription elongation factor, originally called P37 (Sawadogo, M., Sentenac, A., and Fromageot, P. (1979) J. Biol. Chem. 255, 12-15; Nakashisi, T., Nakano, A., Nomura, K., Sekimizu, K., and Natori, S. (1992) J. Biol. Chem. 267, 13200-13204), which binds directly to RNA polymerase II and stimulates read-through of intrinsic blocks to elongation. To elucidate functional features of this protein-protein interaction, we tested the ability of several forms of RNA polymerase II to respond to either full-length or an amino-terminal truncation of TFIIS. The variants of the polymerase differed in the structure of the carboxyl-terminal domain of the largest subunit or lacked two of the smaller subunits. No differences in ability to recognize intrinsic blocks to elongation or to read through them in response to either form of TFIIS were detected among these variants.

Furthermore, ternary complexes containing each variant form of RNA polymerase cleave the 3′ end of the nascent transcripts in response to TFIIS, a reaction previously reported for mammalian and Drosophila TFIIS (Kassavetis, G. A., and Geiduschek, E. P. (1993) Science 259, 944–945) and likely to be important in TFIIS function. Thus the carboxyl-terminal domain of the largest subunit and subunits four and seven of the polymerase, required in vitro, are not required in vitro for recognition of intrinsic blocks to elongation, read-through in response to TFIIS, or TFIIS-stimulated cleavage of the nascent transcript.

Many eukaryotic genes contain regulated blocks to transcript elongation within the transcription unit (12, 13). Generally, these blocks to elongation are found early in the transcription unit, often within the first intron in mammalian genes. When the polymerase stops at such sites, expression of the gene is prevented. For the gene product to be expressed, the polymerase must read through these sites and synthesize full-length RNA. One strategy that might permit synthesis of full-length RNA involves a protein factor (TFIIS) that promotes read-through of such blocks to elongation (12, 14–19).

TFIIS-related proteins which stimulate elongation by RNA polymerase II have been purified and the genes cloned from a variety of organisms (1–5, 18, 20–26).1 Sequence analysis of these clones predicts significant amino acid similarities, especially in the COOH-terminal region of the proteins (20). In most cases, these TFIIS-related proteins have also been shown to promote read-through of specific blocks to elongation in vitro (14–19).

The mechanism by which these factors stimulate elongation and promote read-through is not understood, but some important features of the reaction have been described. The TFIIS-related proteins can bind to RNA polymerase II (1, 15, 18, 27, 28), although some data suggest that they do not remain associated throughout elongation (18). Mutants in mouse SII (29) and human TFIIS (27) unable to bind the polymerase neither stimulate elongation nor promote read-through.

Recent work with mammalian and Drosophila RNA polymerase II has shown that ternary elongation complexes blocked in elongation cleave the 3′ end of the nascent transcript in response to TFIIS (6–11). Following cleavage, the 5′ fragment is retained in the ternary complex and can be elongated. This cleavage precedes and may be necessary for efficient read-through.

To dissect the molecular mechanism by which this factor stimulates elongation and promotes transcript cleavage, it will be important to define the binding interaction between TFIIS and RNA polymerase. An identification of the subunits of RNA polymerase necessary for TFIIS activity would permit an analysis of the more specific contacts. This problem is complex; TFIIS apparently can function as a single polypeptide (11, 17, 26, 27, 30), but catalytically active RNA polymerase II purifies as a complex of 9–12 polypeptides (31, 32).

Immunological, genetic, and biochemical experiments have been reported which begin to assess the RNA polymerase subunits involved in TFIIS function. Much of this work has pointed to the largest subunit of RNA polymerase II. Antibodies directed against a fusion peptide containing sequences from the largest subunit of mammalian RNA polymerase II interfere with TFIIS function (33). Conditional mutations in a different region of the largest subunit of the yeast RNA polymerase II interfere with TFIIS function (34). Also, the yeast TFIIS analogue apparently binds more efficiently to RNA polymerase II when its largest subunit contains the highly conserved COOH-terminal domain (CTD) of heptapeptide repeats (1). This domain, unique to RNA polymerase II (34, 35), is often removed by proteolysis during purification to generate form IIb (31). In this work, we examine the TFIIS-stimulated read-through activity of purified RNA polymerase II with or without the conserved COOH-terminal domain of the largest subunit.

1 A. GUYONVARCH, A. RUET, AND F. LACROUTE, personal communication.
2 The abbreviations used are: CTD, conserved carboxyl-terminal domain; kb, kilobase(s); ORF, open reading frame; PCR, polymerase chain reaction; DTT, dithiothreitol.
Subunits other than the largest subunit may also be important for TFIIS function. In particular, the yeast TFIIS analogue, P37, can protect against inhibition of transcription by antibodies to the 23-kDa subunit of yeast RNA polymerase II (2). Here, we test the requirement for two different small subunits, designated four and seven, in TFIIS function; deletion of the gene for subunit seven renders yeast cells inviable (36). Purified yeast RNA polymerase II contains substoichiometric amounts of these two subunits (37, 38). Although they are required for promoter-specific initiation in vitro, they are not required for efficient elongation or recognition of some specific blocks to elongation in vitro (38). However, it was not known whether TFIIS could stimulate polymerase lacking these subunits to read through these blocks to elongation.

One such block to elongation falls within the first intron of the human histone H3.3 gene (39), and mammalian TFIIS stimulates mammalian polymerase to read through this block (16, 17). Thus a comparison of the effect of TFIIS on read-through of this site with each variant of yeast RNA polymerase II could establish which structural features of the polymerase are required for TFIIS function.

**EXPERIMENTAL PROCEDURES**

**Materials**—Inhibit-ACE was obtained from 5′-3′ Phosphocellulose (P11) and DEAE-cellulose (DE52) were obtained from Whatman. CM-Sephadex, C-25 was obtained from Pharmacia LKB Biotechnology Inc. Nucleotides were obtained from Pharmacia. ([α-32P]CTP, >400 Ci/mmol), was obtained from Amersham Corp. Bio-Gel C30 columns were obtained from Bio-Rad.

RNA polymerase II forms IIa and A4.7 were purified by immunoafinity as described (40, 41). RNA polymerase IIb, (ACTD) was the generous gift of Y. Li and R. Kornberg, Stanford University. It was prepared by treating enzyme containing a recombinant largest subunit with Factor Xa; this treatment removed the CTD from the largest subunit and generated form IIb. The specific activities of the IIa and A4.7 polymerases were comparable. The specific activity of the ACTD form was approximately 2-4-fold lower, probably due in part to the Factor Xa cleavage conditions used to generate this polymerase (data not shown).

**Plasmid Constructions**—The 3.7-kb Saccharomyces cerevisiae genomic BamHI fragment containing the PPR2 gene, encoding TFIIS, from pFL44D (generously provided by A. Guyonvarch, A. Ruef, and F. LaCour) was ligated into the BamHI site of pBS KS+ (Stratagene) to generate pK3. Plasmids were then constructed encoding fusion proteins between an amino-terminal histidine-rich region with either the full-length open reading frame (ORF) of TFIIS or a 113-amino acid amino-terminal deletion of the ORF.

**Preparation of Yeast Extract**—S. cerevisiae strain CB018 (the generous gift of Robert Fuller, Stanford University), and partial fractionation was carried out by ammonium sulfate precipitation. Proteins were fractionated by column chromatography as described (1). Active fractions were pooled, and glycerol was added to 15%. The pooled fraction was stored at 5–20°C. Activity was measured using the stringent assay as described (1). Briefly, a clarified cell lysate was prepared from 80 g of S. cerevisiae strain CBO18 (the generous gift of Robert Fuller, Stanford University), and partial fractionation was carried out by ammonium sulfate precipitation. Proteins were fractionated by column chromatography as described (1). Active fractions were pooled, and glycerol was added to 15%. The pooled fraction was stored at -80°C. Activity was measured using the stringent assay as described (1). 

**SDS-Polyacrylamide Gel Electrophoresis**—RNA polymerase II preparations were resolved on 7–12% acrylamide gradient gels. Proteins were visualized by silver staining.

**Read-through Assay**—The ability of TFIIS to stimulate read-through by RNA polymerase II stalled at intrinsic blocks to elongation was assayed using a 3′-deoxyxycytidine-extended template (42) of the 5′UTR fragment containing the human histone H3.3 gene (39). In this template are three characterized blocks to elongation, TII, TIB, and TIA (39, 43). Reactions (50 μl) in transcription buffer (60 mM Tris-Cl, pH 7.5, 1 mM MgOAc; 5% glycerol; 100 mM (NH4)2SO4; 5 mM spermidine HCl; 0.8 mM each ATP, GTP, and UTP, 1 μM [α-32P]CTP; 1 unit of Inhibit-ACE; and yeast RNA polymerase II) were incubated for 1 min at 30°C. A 5-μl aliquot was diluted into 100 μl of stop buffer (20 mM EDTA, 0.5 mM NHEt, and 12 μg/ml carrier RNA) on ice for analysis. At 1 min 15
This procedure labels the 5' proximal region of all transcripts with resolved on a 7-12% gradient SDS-PAGE gel.

IIa. equivalent amounts of 32P such that quantitation of cpm in each transcript allows a direct comparison of the number of transcripts of each molecular weight protein standards from Bio-Rad, sizes are in kilodaltons, as indicated.

size that are present, regardless of length. Next, a 30-pl aliquot was diluted into 100 pl of stop buffer. The remainder of the reaction was diluted as described for the read-through assay and were incubated for 1 min at 30 °C. The reactions were then diluted 5-fold into chase buffer at which almost completely removed unincorporated nucleotides from the mixture. When a-amanitin was used, it was added to a final concentration of 30 columns prepared as described above and centrifuged for 15 minutes at a setting of 8000 g.

Isolated complexes were incubated at 30 °C in the presence of either TFIIS protein or TFIIS storage buffer (200 nm Tris-HCl, pH 8.0, 10 mM β-mercaptoethanol, 5 mM EDTA, 250 mM (NH4)2SO4, and 15% glycerol). Aliquots (30 μl) were removed to 100 μl of stop buffer at various time points, and RNAs were ethanol-purified, resuspended in formamide load buffer (80 mM Tris borate, pH 8.0, 2 mM EDTA, 80% formamide, 0.1% bromphenol blue, 0.12% xylene cyanol, 0.06% SDS) and resolved by electrophoresis through 5% acrylamide gels.

Ternary Complex Cleavage Assay—Bio-Gel 30 columns were equilibrated by overlaying each column with 200 μl of chase buffer lacking nucleotides and centrifuging for 2 min in the swinging bucket rotor of an IEC clinical centrifuge at a setting of 4 (approximately 1000 relative centrifugal force); this was done four times. Reactions (25 μl) were set up as described for the read-through assay and were incubated for 1 min at 30 °C. The reactions were then diluted 5-fold into cold stop buffer at 30 °C, and the incubation was continued for 1 additional min. This procedure produces a mixture of complexes stalled at TII, TIB, and TIA as well as run-off transcripts. These complexes were applied to Bio-Gel 30 columns prepared as described above and centrifuged for 2 min 30 s at a setting of 4. This was repeated with a second column, a procedure which almost completely removed unincorporated nucleotides from the isolated ternary complexes.

Isolated complexes were incubated at 30 °C in the presence of either TFIIS protein or TFIIS storage buffer, with or without nucleotides (0.8 mM). When a-amanitin was used, it was added to a final concentration of 100 μg/ml heparin and 0.1 mM CTP) and incubated for 45 s at 30 °C. This procedure labels the 5'-proximal region of all transcripts with equivalent amounts of 32P such that quantitation of cpm in each transcript allows a direct comparison of the number of transcripts of each molecular weight protein standards from Bio-Rad, sizes are in kilodaltons, as indicated.

Quantitation of Transcripts in Polyacrylamide Gels—Quantitation of radioactivity in polyacrylamide gels was performed using the Molecular Dynamics PhosphorImager system. Transcripts with 3' ends at TII, TIB, TIA, and the run-off transcript were identified, and the cpm in each was determined. The total cpm in these four types of transcripts was determined for each time point and is called (Sum). The fraction of complexes stopped at TII was calculated as (TII cpm)/(Sum). The fraction stopped at TIB was calculated as (TIB cpm)/(Sum) - (TII cpm). The fraction stopped at TIA was calculated as (TIA cpm)/(Sum) - (TIB cpm).

RESULTS

TFIIS Activity in S. cerevisiae—A partially purified protein from S. cerevisiae (P37) had been shown to stimulate elongation by both RNA polymerase I and II in nonspecific assays (1-3). This activity was analogous to that of SII, a protein purified from mouse Ehrlich ascites cells, which stimulated the elongation reaction of mouse RNA polymerase II in nonspecific assays (44). The SII protein is a member of the TFIIS family of proteins, cloned from mouse (21), human (20, 26), and Drosophila (24). Each of these proteins promotes transcript read-through as well as nascent transcript cleavage (10). Protein sequences predicted from the gene encoding P37, PPR2 (5, 22), place it into the TFIIS family. To see whether this protein was a functional homolog of TFIIS, the previously reported P37 purification (1) was used to generate a fraction enriched in TFIIS/P37 activity; about 10-15% of the protein in this preparation migrates as expected for the P37 protein (Fig. 1B, lane 3). This preparation stimulates yeast RNA polymerase II 2-5-fold in nonspecific transcription assays (data not shown). Furthermore, the stimulatory activity in this preparation is specific for yeast RNA polymerase II and does not stimulate Drosophila or calf thymus RNA polymerase II (data not shown).

The P37-containing fraction was tested for promoting read-through by RNA polymerase II using a 3'-deoxycytidine-ex- tended template containing the three intrinsic blocks to elongation from the human histone H3.3 gene. Purified yeast RNA polymerase IIa recognizes and stops at these sites (38). Transcripts with 3' ends at TII, TIB, and TIA are observed 45 s after the addition of chase mixture to initiated complexes (Fig. 2, lane 2). The TII and TIB sites stop the polymerase with low efficiency, 10% or less. However, the polymerase recognizes the TIA site with approximately 50% efficiency. During continued incubation, some ternary complexes stalled at the site resume elongation. However, over half of the complexes originally stopped at TIA were still present at 45 s, suggesting that this step might be rate limiting.
stopped at TII do not read through the site even after 30 min of incubation at 30 °C (Fig. 2, lane 12). When TFIIS/P37 protein is added to ternary complexes stalled at these sites, transcript elongation begins within 5 min (Fig. 2, lane 9), and most complexes initially stalled at TIIa elongate transcripts to the runoff within 30 min (Fig. 2, lane 11). Clearly, the P37-containing fraction exhibits a biochemical read-through activity similar to that observed with other members of the TFIIS family.

Read-through by Purified TFIIS—To more definitively assign the read-through activity to the TFIIS/P37 protein, the cloned sequences for this protein were expressed as an oligohistidine fusion in E. coli. Similarly, a protein with a 113-amino-acid amino-terminal deletion relative to the full-length TFIIS was expressed as an oligo-histidine fusion protein. The fusions facilitated purification and did not affect activity (data not shown).

The purified fusion proteins containing the full-length (Fig. 1B, lane 2) or the truncated TFIIS (Fig. 1B, lane 1) were used in combination with several variant forms of yeast RNA polymerase II (Δ4,7, IIa, ΔCTD; Fig. 1A). The ability of each variant to recognize the intrinsic sites TIIa, TIIb, and TII and to elongate through them in the presence or absence of the TFIIS proteins was compared. Data for the Δ4,7 form of the polymerase with both forms of TFIIS are displayed in Fig. 3; the quantitative analyses for all forms of the polymerase and TFIIS are displayed in Fig. 4. There are no major differences in the behavior of these different forms of polymerase. They are essentially identical in recognizing these intrinsic blocks to elongation and in read-through of these sites in response to TFIIS. All three polymerase variants recognize the TII and TIIb sites with approximately 10% efficiency and the TIIa site with about 50% efficiency. When either form of TFIIS is added, the complexes resume elongation, and within 30 min, 80% of the transcripts originally ending at TIIa have been elongated. None of the transcripts which remain at TIIa after 30 min is elongated during continued incubation, and it is likely that these transcripts have been released from the polymerase. In the absence of TFIIS, over half of the transcripts with 3'-ends at TIIa have not been elongated, even after 50 min of incubation (Figs. 3 and 4).

Clearly, neither the COOH-terminal domain of the largest subunit nor subunits four or seven are required for TFIIS to interact with the polymerase and promote read-through of these intrinsic blocks to elongation in vitro. In addition, the amino-terminal 113 amino acids of the yeast TFIIS protein are unnecessary for read-through function in vitro. Proteins with amino-terminal truncations from human (27), mouse (7, 45), and Drosophila (11) also have been shown to be functional in stimulating elongation by the cognate RNA polymerase II.

These results are both qualitative and quantitative. The kinetics of read-through as well as the stoichiometry of TFIIS to polymerase necessary to promote read-through are identical in all tested combinations (Fig. 4 and data not shown). The difference in kinetics observed in Fig. 4 is due to the different ratios of TFIIS to polymerase used in each series of experiments. When comparable ratios are used, the read-through kinetics are identical for the two forms of TFIIS. At a molar ratio of 5:1 TFIIS to RNA polymerase II protein, the maximum rate of read-through is obtained. Additional TFIIS does not accelerate read-through (data not shown).

Cleavage of Nascent Transcripts in Ternary Complexes—Mammalian and Drosophila TFIIS proteins stimulate cleavage of the nascent transcript in stalled ternary complexes containing RNA polymerase II (6–11). A similar cleavage reaction has been observed in ternary complexes formed with vaccinia virus RNA polymerase (46) which contains a subunit with sequence

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\(^5\) G. Ciprés-Palacin and C. Kane, unpublished results.
ternary complexes containing the A4,7 form of RNA polymerase II were divided into two parts, and incubation was continued with the addition of either TFIIS or the storage buffer. Aliquots were stopped at various times. A, reactions containing full-length TFIIS. The molar ratio of full-length TFIIS to RNA polymerase II was approximately 5:1. B, reactions containing truncated TFIIS. The molar ratio of truncated TFIIS to RNA polymerase II was approximately 5:1. Lane 1: RNA from initiated complexes; lane 2, RNA from ternary complexes chased to the Tla, Tlb, and TII sites and the runoff; lanes 3–6, RNA from ternary complexes incubated with TFIIS, stopped after 5, 10, 30, or 50 min of incubation, respectively; lanes 7–10, RNA from ternary complexes incubated in the absence of TFIIS, stopped after 5, 10, 30, or 50 min of incubation, respectively.

Fig. 4. Quantitation of read-through activity by variant forms of RNA polymerase II. Elongation assays with each form of RNA polymerase II were performed as described under "Experimental Procedures." Either full-length (A) or truncated (B) TFIIS was added, and reactions were incubated for the times indicated. RNAs were quantitated as described under "Experimental Procedures." A, percent of RNAs with 3' ends at Tla with or without the full-length TFIIS protein with each form of RNA polymerase II at a molar ratio of 2:1, TFIIS:RNA polymerase II. B, percent of RNAs with 3' ends at Tla with or without the truncated TFIIS protein with each form of RNA polymerase II at a molar ratio of 5:1, TFIIS:RNA polymerase II. Each point represents the average of two experiments. Filled symbols represent time points in the absence of TFIIS; open symbols represent time points in the presence of TFIIS. ▲ = TII, ■ = δA4,7, ● = δCTD.

similarity to the TFIIS family of proteins (47). In all of these cases, the 5' end of the transcript is retained and elongated by the active complex after release of the 3'-terminal fragment (6–11). This cleavage has been observed with complexes stalled by controlling nucleotide levels (8, 46, 48, 49), by a DNA-binding protein (50), or by naturally occurring blocks to elongation (6, 7, 9, 49). In the case of complexes stalled at the Tla site in the human histone H3.3 gene, cleavage of the transcript promoted by mammalian TFIIS appears to occur before read-through of the site is detectable (7). The similarities in read-through activity between yeast TFIIS and the mammalian factor suggested that the yeast protein might also stimulate transcript cleavage.

Fig. 3. Comparison of ability of the full-length and truncated TFIIS proteins to stimulate read-through by RNA polymerase II. Ternary complexes containing the ΔA4,7 form of RNA polymerase II were formed as described under "Experimental Procedures." At 2 min 30 s, reactions containing ternary complexes stalled at intrinsic blocks to elongation were divided into two parts, and incubation was continued with the addition of either TFIIS or the storage buffer. Aliquots were stopped at various times. A, reactions containing full-length TFIIS. The molar ratio of full-length TFIIS to RNA polymerase II was approximately 2:1. B, reactions containing truncated TFIIS. The molar ratio of truncated TFIIS to RNA polymerase II was approximately 5:1. Lane 1, RNA from initiated complexes; lane 2, RNA from ternary complexes chased to the Tla, Tlb, and TII sites and the runoff; lanes 3–6, RNA from ternary complexes incubated with TFIIS, stopped after 5, 10, 30, or 50 min of incubation, respectively; lanes 7–10, RNA from ternary complexes incubated in the absence of TFIIS, stopped after 5, 10, 30, or 50 min of incubation, respectively.

No shortened transcripts resulting from cleavage had been seen in the experiments described above (Figs. 2 and 3), even with samples taken within 5 s after the addition of TFIIS (Fig. 2, lane 3). To test for cleavage in the absence of nucleotides, ternary elongation complexes containing RNA polymerase IIa stalled at the Tla, Tlb, and TII sites were isolated. The incubation of either form of TFIIS with these complexes in the absence of nucleotides produces transcripts shorter than the Tla transcript. Data with truncated TFIIS are shown (Fig. 5, lanes 7–9). Full-length TFIIS promotes cleavage at the same rate to give the same pattern of cleared products. Cleavage of transcripts with 3' ends at TII can also be detected (data not shown). Complexes incubated in the absence of TFIIS produce no shortened transcripts (Fig. 5, lane 10). The shortened transcripts formed in the presence of either form of TFIIS can be elongated upon the addition of nucleotides (Fig. 5, lanes 7–9). These results are consistent with nascent transcript cleavage within active ternary complexes in the presence of TFIIS.

Ternary complexes containing each of the three variants of RNA polymerase II were compared for cleavage activity in response to the P37-containing fraction (data not shown). In this comparison, the kinetics and pattern of cleavage, the inhibition of cleavage by α-amanitin, and the elongation of truncated transcripts upon nucleotide addition were indistinguishable, regardless of the polymerase in the ternary complex. Thus TFIIS stimulates cleavage of the nascent transcript in ternary complexes containing yeast RNA polymerase II, and the first 113 amino acids of the TFIIS protein are not required for this stimulatory activity in vitro. Experiments examining elongation subsequent to the cleavage reaction indicate that the nucleotides are being removed from the 3' end of the transcript (data not shown), a result similar to that seen with the cleavage reaction in ternary complexes of vaccinia virus RNA polymerase (46) or complexes with mammalian or Drosophila RNA polymerase II in response to TFIIS (7–9, 11). The exact sizes of the primary cleavage products removed from the 3' end in yeast ternary complexes are not yet known.

Within 10 s after the addition of TFIIS to stalled isolated ternary complexes, cleavage has occurred, and shortened transcripts can be detected (Fig. 5, lane 2). At a TFIIS to RNA

A

Lane

1

2

3

4

5

6

7

8

9

10

RO

Tla

Tlb

TII

B

Lane

1

2

3

4

5

6

7

8

9

10

RO

Tla

Tlb

TII

Fig. 3. Comparison of ability of the full-length and truncated TFIIS proteins to stimulate read-through by RNA polymerase II. Ternary complexes containing the ΔA4,7 form of RNA polymerase II were formed as described under "Experimental Procedures." At 2 min 30 s, reactions containing ternary complexes stalled at intrinsic blocks to elongation were divided into two parts, and incubation was continued with the addition of either TFIIS or the storage buffer. Aliquots were stopped at various times. A, reactions containing full-length TFIIS. The molar ratio of full-length TFIIS to RNA polymerase II was approximately 2:1. B, reactions containing truncated TFIIS. The molar ratio of truncated TFIIS to RNA polymerase II was approximately 5:1. Lane 1, RNA from initiated complexes; lane 2, RNA from ternary complexes chased to the Tla, Tlb, and TII sites and the runoff; lanes 3–6, RNA from ternary complexes incubated with TFIIS, stopped after 5, 10, 30, or 50 min of incubation, respectively; lanes 7–10, RNA from ternary complexes incubated in the absence of TFIIS, stopped after 5, 10, 30, or 50 min of incubation, respectively.

Fig. 4. Quantitation of read-through activity by variant forms of RNA polymerase II. Elongation assays with each form of RNA polymerase II were performed as described under "Experimental Procedures." Either full-length (A) or truncated (B) TFIIS was added, and reactions were incubated for the times indicated. RNAs were quantitated as described under "Experimental Procedures." A, percent of RNAs with 3' ends at Tla with or without the full-length TFIIS protein with each form of RNA polymerase II at a molar ratio of 2:1, TFIIS:RNA polymerase II. B, percent of RNAs with 3' ends at Tla with or without the truncated TFIIS protein with each form of RNA polymerase II at a molar ratio of 5:1, TFIIS:RNA polymerase II. Each point represents the average of two experiments. Filled symbols represent time points in the absence of TFIIS; open symbols represent time points in the presence of TFIIS. ▲ = TII, ■ = ΔA4,7, ● = ΔCTD.
polymerase II ratio of 5:1, most of the transcripts with 3′ ends at TIA have been shortened within 5 min (Fig. 5, lane 4). As mentioned above, this ratio results in the maximum rate of read-through of this site by the polymerase. Longer incubation in the presence of TFIIS produces further shortening of transcripts within active ternary elongation complexes (Fig. 5, lanes 5 and 6). These shortened transcripts are still associated with ternary complexes, because within 10 s after nucleotide addition, shortened transcripts have been elongated back to the TIA site (Fig. 5, lane 7). However, detectable read-through of the TIA site is somewhat slower, reaching a limit within 10 min of incubation (Fig. 4B).

It remains unclear whether TFIIS itself generates the cleavage of the nascent transcript or if the function of TFIIS might be to stimulate an inherent cleavage activity of RNA polymerase II. To examine this, isolated ternary complexes were treated with 100 μg/ml α-amanitin prior to the addition of TFIIS. RNA polymerase II is the most sensitive of the nuclear polymerases to this toxin which interferes with transcript elongation (51). This α-amanitin treatment significantly decreases, but does not completely prevent, transcript cleavage in response to TFIIS (Fig. 5, lane 11 compared with lane 6), nor does it prevent elongation of the cleaved transcripts back to the TIA site. However, extensive shortening of cleaved transcripts appears to be prevented; the first detectable cleavage intermediate is apparently not shortened further (Fig. 5, compare lane 11 with lanes 2–4). These results could indicate that a fraction of the RNA polymerase II-containing complexes are insensitive to α-amanitin. Alternatively, transcript elongation is a multistep process (52), and α-amanitin may not interfere with the cleavage reaction, although it may block elongation at some other step such as polymerase translocation (51, 53). A more trivial explanation, that the α-amanitin-insensitive cleavage is due to either RNA polymerase I or III, is very unlikely; the polymerases used in these experiments have been purified using immunoaffinity (41), taking advantage of antibody directed against the COOH-terminal repeated sequence unique to the largest subunit of RNA polymerase II (31, 35). In addition, no large subunits coincident with those expected for either RNA polymerase I or III have been visualized upon sensitive silver staining of polymerase preparations (Fig. 1A).

**DISCUSSION**

The conserved COOH-terminal domain of the largest subunit of RNA polymerase II does not have a significant effect on the ability of the polymerase to recognize intrinsic blocks to elongation or to read through them in response to the elongation factor TFIIS. Similarly, polymerase lacking subunits four and seven recognizes these blocks to elongation and reads through them in response to TFIIS. However, *in vivo*, the CTD of the largest subunit is essential (54). Furthermore, deletion of subunit four causes slow growth and temperature sensitivity of the cells (55), whereas deletion of subunit seven is lethal (36). The *in vitro* results would suggest that these *in vivo* phenotypes are unrelated to TFIIS function.

Ternary complexes containing all three variant forms of RNA
polymerase II exhibit the ability to cleave their nascent transcripts and read-through blocks to elongation in the presence of TFIIS. However, none of these experiments details how the cleavage reaction might promote read-through nor what the mechanism of the cleavage reaction might be. α-Amanitin dramatically inhibits the TFIIS-stimulated cleavage (Refs. 6, 8, 9, 11, and 53 and this report). Since this toxin binds to the polymerase, this inhibition suggests that transcript cleavage might be catalyzed by the polymerase protein with TFIIS stimulating an activity intrinsic to the polymerase. Alternatively, the TFIIS protein may be involved more directly in catalyzing the cleavage reaction, and the binding of α-amanitin may interfere with the interaction between TFIIS and the polymerase in the ternary complex.

Cleavage of nascent transcripts seems to be a feature in a general mechanism that allows RNA polymerases to resume elongation after stalling before reaching the end of a transcription unit. Such stalled complexes may terminate transcription, resulting in transcript release, if the reactions promoting read-through are not carried out quickly enough to defeat transcript release. Ternary complexes stopped at the sites described here can cleave nascent transcripts within 10 s after the addition of TFIIS, yet productive read-through of these sites requires an incubation of many minutes in the presence of both TFIIS and nucleotides. Approximately 50% of RNA polymerase II molecules stop at the Tiα site. Perhaps, following cleavage in the presence of TFIIS, 50% of these ternary complexes elongating the shortened transcripts stop once again at the Tiα site, even in the presence of TFIIS and nucleotides, whereas the other 50% pass through to the runoff. This cycle of stopping, cleaving, and elongating would continue until essentially all complexes pass through the block to elongation.

When mammalian RNA polymerase II is stopped at these sites in vitro, transcript release can be detected under physiological salt concentrations, although this release is not quantitative and is likely to be a slow process (39). On the other hand, in the presence of TFIIS cleavage is very rapid (6, 7), and elongation through these sites can be nearly quantitative (7, 16, 17). Such sites may be distinct from some intrinsic termination sites where the polymerase would stop and transcript release would be rapid and from pause sites where the polymerase would stop and quantitatively resume elongation in a "finite" period of time. Alternatively, such sites which cause transcription arrest or a block to elongation might represent sequences on a continuum for which termination or read-through is determined by both the stability of the ternary complex at that specific site and the ability of that complex to respond to accessory elongation regulatory factors. If this were the case, the properties of ternary complexes would be expected to vary at different sites.

Indeed, the structures of ternary elongation complexes for both bacterial RNA polymerase and RNA polymerase II can change during elongation (53, 56–60). Each distinct structure might represent a distinct and specific target for regulation, targets that might terminate transcription or continue elongation in response to changing conditions within the cell.

There are a growing number of characterized eukaryotic genes containing "conditional" blocks to elongation or regulated transcription arrest sites within the transcription unit (12, 13). It is probable that not all of these regions are regulated by TFIIS, and likewise, not all of the regions that have been identified as blocks to elongation in vivo block elongation by purified RNA polymerase II in vitro. More than one mechanism promotes terminator read-through by elongation complexes of bacterial RNA polymerase (61), and several different mechanisms might contribute to the balance between termination and read-through at sites for RNA polymerase II that fall within genes.

Transcript cleavage was first described in ternary complexes of E. coli RNA polymerase (62), and this cleavage reaction is promoted by at least two protein factors, greA and greB (63, 64). Stalled ternary complexes formed with mammalian (6–9), Drosophila (11), and yeast RNA polymerase II (this report) can cleave and then elongate nascent transcripts in response to TFIIS. Cleavage of the nascent transcript has also been observed in stalled ternary complexes formed with vaccinia RNA polymerase which contains a subunit with sequence similarity to TFIIS (46, 47). Cleavage of the nascent transcript has also been seen in isolated ternary complexes containing yeast RNA polymerase III. Furthermore, purified yeast RNA polymerase I stopped by the mouse template binding termination factor TTF-1 produces a transcript shortened from the primary transcription product by about 10 nucleotides (see Fig. 3 in Ref. 65); in this case, this shortened product may be an intermediate in the eventful 3′ end processing of the ribosomal RNA transcript. However, as TTF-1 itself does not generate this cleavage product (66), it is possible that RNA polymerase I also cleaves its nascent transcript when stopped during elongation, although this cleavage may not be part of a read-through mechanism.

Certainly, the diversity of polymerases and organisms in which nascent transcript cleavage within ternary complexes is seen supports the idea that this reaction is physiologically relevant. Identifying the molecular details of this reaction will be an important next step in understanding how such a reaction can modulate the use of regulated blocks to elongation in the cell.

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