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In vitro characterization of mutant yeast RNA polymerase II with reduced binding for elongation factor TFIIS

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ABSTRACT **We have reported previously the isolation and genetic characterization of mutations in the gene encoding the largest subunit of yeast RNA polymerase II (RNAPII), which lead to 6-azauracil (6AU)-sensitive growth. It was suggested that these mutations affect the functional interaction between RNAPII and transcription-elongation factor TFIIS because the 6AU-sensitive phenotype of the mutant strains was similar to that of a strain defective in the production of TFIIS and can be suppressed by increasing the dosage of the yeast TFIIS-encoding gene, *PPR2*. RNAPIIs were purified and characterized from two independent 6AU-sensitive yeast mutants and from wild-type (wt) cells. *In vitro*, in the absence of TFIIS, the purified wt polymerase and the two mutant polymerases showed similar specific activity in polymerization, readthrough at intrinsic transcriptional arrest sites and nascent RNA cleavage. In contrast to the wt polymerase, both mutant polymerases were not stimulated by the addition of a 3-fold molar excess of TFIIS in assays of promoter-independent transcription, readthrough or cleavage. However, stimulation of the ability of the mutant RNAPIIs to cleave nascent RNA and to read through intrinsic arrest sites was observed at TFIIS:RNAPII molar ratios greater than 600:1. Consistent with these findings, the binding affinity of the mutant polymerases for TFIIS was found to be reduced by more than 50-fold compared with that of the wt enzyme. These studies demonstrate that TFIIS has an important role in the regulation of transcription by yeast RNAPII and identify a possible binding site for TFIIS on RNAPII.**

RNA polymerase II (RNAPII) can be regulated at the transcription-elongation step as well as at the initiation step (reviewed in refs. 1 and 2). Four general transcription-elongation factors, TFIIS, TFIIF, elongin (SIII), and ELL, have been shown *in vitro* to increase the overall elongation rate of transcribing RNAPII. TFIIS (SII, RAP38) stimulates incorporation of nucleotides into RNA in promoter-independent transcription (3) and facilitates the passage of RNAPII through several kinds of transcriptional blocks such as intrinsic arrest sites (4) and certain DNA-bound proteins (5). TFIIS exerts its function by binding directly to RNAPII (6, 7) and stimulating an intrinsic RNAPII endoribonuclease activity (8, 9). The other three elongation factors, TFIIF (RAP30/74), elongin, and ELL, function differently from TFIIS in that they cannot relieve RNAPII molecules that are already arrested. Instead, these factors increase the overall elongation rate of transcribing RNAPII (7, 10–14), probably by preventing arrest of RNAPII at transcriptional blocks.

Little is known about regions of RNAPII that are involved in transcript elongation and in interaction with general elongation factors. In the yeast *Saccharomyces cerevisiae*, deletion of the TFIIS-encoding gene, *PPR2*, renders cells viable but sensitive to the uracil analog, 6-azauracil (6AU) (15). Addition of 6AU to a yeast culture results in a significant depletion of intracellular GTP and UTP pools (15). Since reducing NTP concentrations *in vitro* decreases the elongation rate and increases pause and arrest of RNA polymerase (16–19), it has been hypothesized (15, 20) that a similar effect may be obtained *in vivo* by growth of yeast cells in the presence of 6AU. Thus, the 6AU-sensitivity of the PPR2 deletion mutant (ppr2Δ) cells may be related directly to the well-characterized TFIIS-mediated readthrough of intrinsic blocks to elongation by RNAPII *in vitro*. This reasoning led to the speculation that mutations resulting in 6AU-sensitive growth might also exist in regions of the subunits of RNAPII that are involved in the TFIIS-mediated function.

A genetic search of the gene, *RPO21*, which encodes the largest subunit of RNAPII, revealed seven mutations leading to 6AU-sensitive growth (20). All of them affected amino-acid residues that were located between the highly-conserved regions G and H of Rpo21p (20–22) and all were suppressed by elevated dosage of wild-type (wt) *PPR2* (20), implicating a perturbation of TFIIS-dependent elongation. Two models were proposed to explain this result (20). First, the mutant polymerases themselves might have aberrant elongation properties that result in an increase in the number of mutant polymerase molecules stopped on the DNA template and therefore require a greater number of TFIIS molecules. Second, the alterations in the mutant polymerases may weaken the interaction between RNAPII and TFIIS, demanding more TFIIS to drive the interaction toward the formation of a normal number of active RNAPII-TFIIS complexes.

To distinguish between these models, we have purified two of the seven mutant polymerases and have performed a series of *in vitro* assays. We report here that the alterations in the mutant RNAPIIs weaken the interaction between the enzymes and TFIIS.

MATERIALS AND METHODS

Purification of Proteins. Recombinant yeast TFIIS protein, tagged at the N terminus with six histidine residues (to facilitate purification) and a five-amino acid recognition element for bovine-heart-muscle kinase (to facilitate labeling; ref. 23), was purified to virtual homogeneity from *Escherichia coli* cells essentially as described (24). No significant differences were detected in the stimulation of readthrough and cleavage activities of RNAPII between this preparation of recombinant TFIIS protein and a variety of yeast TFIIS proteins described previously (24). Wt and mutant RNAPIIs were purified to near homogeneity from yeast strains expressing wt RNAPII, *rpo21-18* (I1237TRARV), or *rpo21-24* (E1230K) mutant RNAPII (20) as described (25). Protein concentrations were determined by the method of Bradford (26).

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Abbreviations: RNAPII, eukaryotic RNA polymerase II; 6AU, 6-azauracil; ts, temperature sensitive; wt, wild type.

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***In Vitro* Transcription Assays and RNase-Cleavage Assays.** Promoter-independent transcription assays using poly(rC) or denatured calf-thymus DNA as a template were performed as described (7, 25). TFIIS-stimulated readthrough assays and ternary-complex cleavage assays using poly(dC)-tailed human histone H3.3 DNA as a template were performed as described (24). Briefly, RNA transcripts were pulse-labeled at the 5′ ends by incubating in the presence of 33.3 nM [α-^{32}P]-CTP [10.0 μCi, 3000 Ci/mmol (1 Ci = 37 GBq), DuPont] and 0.8 mM each ATP, GTP, and UTP for 75 sec, followed by elongating to the three intrinsic blocks in the presence of 0.1 mM unlabeled CTP and 100 μg/ml heparin (to prevent reinitiation) for 75 sec. Most of the nascent transcripts derived from this template were displaced from the template DNA strand (27). Ternary complexes stopped at the intrinsic arrest sites were treated in two different ways. The readthrough assay was done as follows. Yeast TFIIS was added, transcription was allowed to proceed for the designated intervals, and the resulting transcripts were collected by ethanol precipitation and were resolved by electrophoresis on a 7% polyacrylamide gel (19:1 acrylamide/bisacrylamide) containing 8.3 M urea, 2 mM EDTA, and buffered with 89 mM Tris·borate. The transcripts were quantified by using PhosphorImager (Molecular Dynamics). The fraction of transcripts arrested was determined as [(TIa cpm) + (TIb cpm) + (TII cpm)]/[(TIa cpm) + (TIb cpm) + (TII cpm) + (run-off cpm)]. The cleavage reaction was done as follows. The stopped ternary complexes were purified from unincorporated nucleotides by two sequential Bio-gel 30 spin columns (Bio-Rad). TFIIS was then added to the purified complexes and at designated times aliquots were removed and the reaction was stopped by the addition of a buffer containing 80% formamide and 0.1% SDS. The transcripts were resolved as described above.

Native PAGE. Labeling of a truncated derivative of TFIIS protein (lacking the first 130-amino acid residues) with [γ-^{32}P]ATP was achieved by following a described procedure (23). This truncated TFIIS was fully active both *in vitro* (24) and *in vivo* (28) and was used because it is more stable than the full-length TFIIS following labeling with [γ-^{32}P]ATP. RNAPII-TFIIS complexes were formed in binding buffer (20 mM Tris·OAc, pH 7.5/10 μM $ZnCl_2$/10 mM DTT/10% glycerol) in a final volume of 10 μl. Following incubation on ice for 30 min, the complexes were resolved on 5% native polyacrylamide gels (37.5:1 acrylamide/bisacrylamide) run at a constant voltage of 100 volts at 4°C for 3 hr in a buffer that contained 50 mM Tris·Cl, 50 mM boric acid, 10 μM ZnSO4, and 1% glycerol (pH 8.3), at 4°C. The amount of TFIIS–RNAPII binary complexes was determined using the Molecular Dynamics PhophorImager with ^{32}P-labeled TFIIS blotted on to filter paper as a standard. The apparent dissociation constant (k_d) was determined using Scatchard analysis.

RESULTS

Specific Polymerization Activities of wt and Mutant Polymerases on a Poly(rC) Template Are Comparable. The polymerization activities of the wt and mutant polymerases were compared on a poly(rC) template. On this template, purified RNAPII incorporates GMP in a template-dependent manner (29) under various conditions and produces high radioactive signals (at least 10-fold higher than background) that represent newly synthesized RNAs. Under normal conditions (1 mM GTP and 30°C), specific polymerization activities of wt and mutant polymerases were comparable (Fig. 1 *A* and *B*). The activities were estimated to be in the range of 500–620 nmol of nucleotide incorporated per min per mg of protein at 30°C. Since the mutant strains used in this study are 6AU-sensitive (which is probably related to the depletion of intracellular GTP pool by 6AU; ref. 15) as well as temperature-sensitive for growth, the purified polymerases were also assayed at reduced GTP concentration (20 μM; Fig. 1*C*) and at elevated reaction temperatures (42°C; Fig. 1*D*). Under these two sets of conditions, the polymerization activities of both wt and mutant

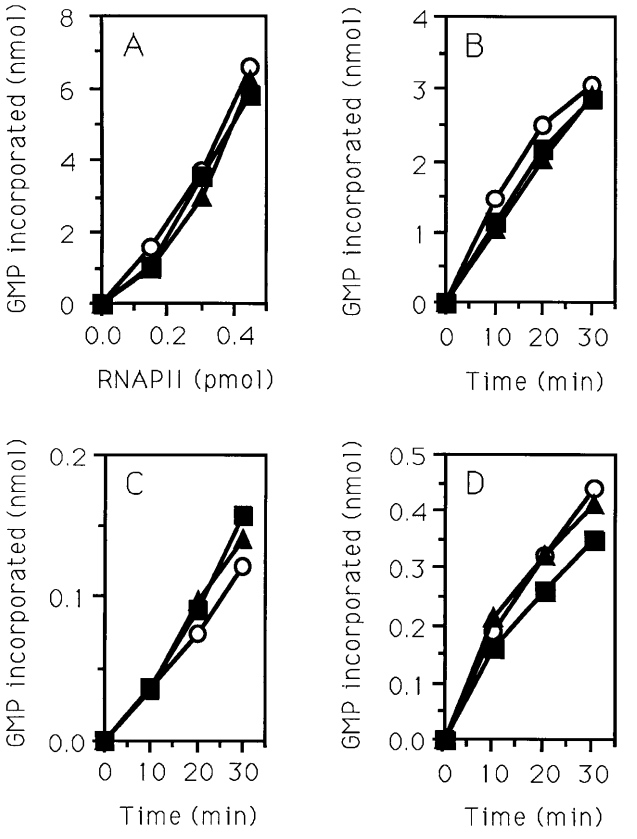

FIG. 1. Promoter-independent transcription assays of polymerases from wt (○), *rpo21-18* (I1237TRARV) (■), and *rpo21-24* (E1230K) (▲) strains using poly(rC) as a template. The polymerization activities of the wt and mutant enzymes were compared under normal reaction conditions (1 mM GTP and incubation at 30°C) (*A* and *B*) and under conditions of reduced GTP concentration (20 μM) (*C*) or elevated temperature (42°C) (*D*). The reactions in *A* were incubated for 30 min; 0.30 pmol of each polymerase was used in *B–D*.

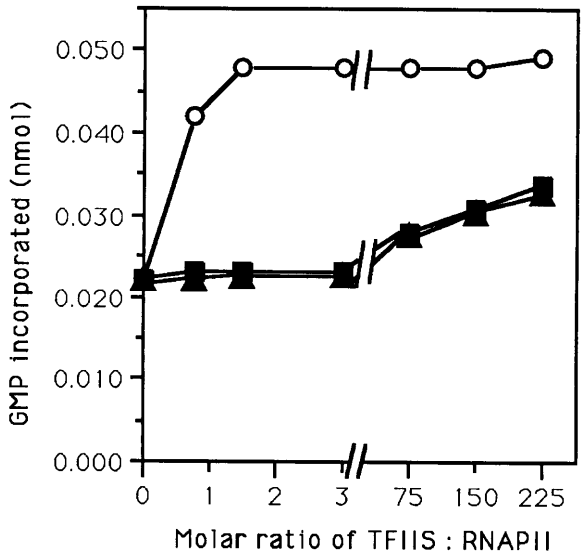

FIG. 2. Stimulation by TFIIS of RNAPII from wt (○), *rpo21-18* (I1237TRARV) (■), and *rpo21-24* (E1230K) (▲) strains. Denatured calf-thymus DNA was used as a template to assay 0.30 pmol of each polymerase. Reactions were incubated at 30°C for 20 min.

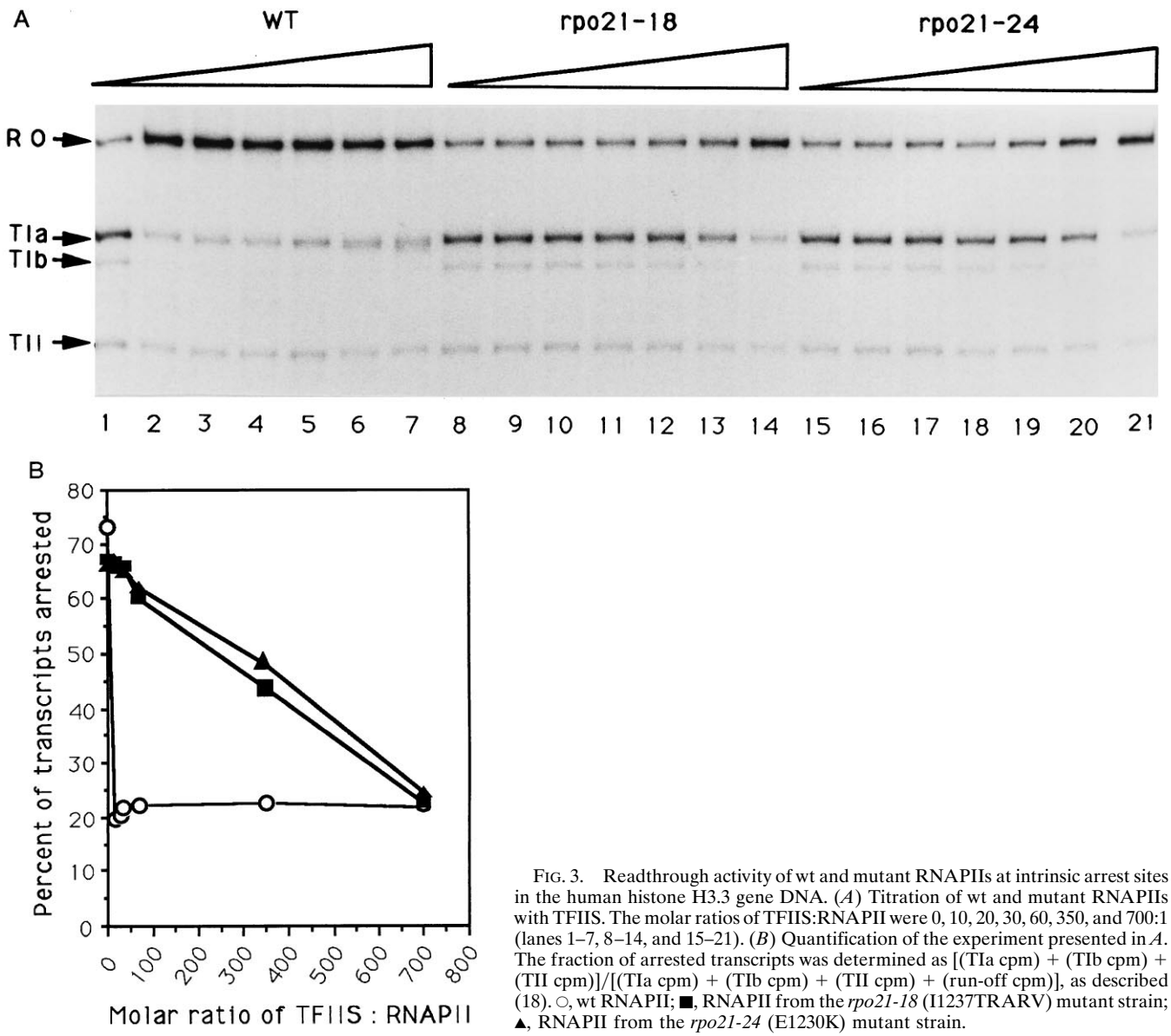

FIG. 3. Readthrough activity of wt and mutant RNAPIIs at intrinsic arrest sites in the human histone H3.3 gene DNA. (*A*) Titration of wt and mutant RNAPIIs with TFIIS. The molar ratios of TFIIS:RNAPII were 0, 10, 20, 30, 60, 350, and 700:1 (lanes 1–7, 8–14, and 15–21). (*B*) Quantification of the experiment presented in *A*. The fraction of arrested transcripts was determined as [(TIa cpm) + (TIb cpm) + (TII cpm)]/[(TIa cpm) + (TIb cpm) + (TII cpm) + (run-off cpm)], as described (18). ○, wt RNAPII; ■, RNAPII from the *rpo21-18* (I1237TRARV) mutant strain; ▲, RNAPII from the *rpo21-24* (E1230K) mutant strain.

polymerases were decreased to a similar extent. Therefore, the two *rpo21* mutations (I1237TRARV and E1230K) do not exacerbate the negative effect of nucleotide-substrate limitation or heat on the polymerization activity of RNAPII.

Polymerization Activity of Mutant RNA Polymerases Is Insensitive to Stimulation by TFIIS. Purified RNAPII transcribes denatured duplex DNA at a low efficiency, which can be stimulated 2- to 10-fold by TFIIS (6). Wt and mutant RNAPII preparations were tested for this property by using denatured calf thymus DNA as a template (Fig. 2). In the absence of TFIIS, the activities of wt and mutant polymerases were indistinguishable. Maximum stimulation of wt RNAPII by TFIIS was obtained at a TFIIS/RNAPII molar ratio of ≈1.5:1. In contrast, at the same TFIIS/RNAPII molar ratio, or slightly higher, no significant stimulation of the mutant enzymes was detected. At a molar ratio of at least 75:1, stimulation by TFIIS was observed. Even at the highest molar ratio tested (225:1), only ≈45% of the maximal stimulation of wt polymerase was achieved for the mutant enzymes.

High Concentrations of TFIIS Are Required by the Mutant RNA Polymerases to Approach Levels of wt Readthrough of Intrinsic Arrest Sites on Human Histone H3.3 DNA. Readthrough activity of wt and mutant polymerases at intrinsic blocks to elongation were compared in an assay using poly(dC)-tailed human histone H3.3 gene DNA as a template. It has been shown that purified yeast RNAPII recognizes and stops at the three intrinsic transcriptional arrest sites on the human histone H3.3 DNA but does not terminate (24, 25), and that yeast TFIIS can stimulate RNAPII to read through these sites (24).

Two observations were noteworthy at the outset. First, wt and mutant polymerases stop at the identical intrinsic arrest sites in the human histone H3.3 gene (Fig. 3*A*, lanes 1, 8, and 15); no additional arrested complexes were observed in the case of the two mutant polymerases. Second, comparable percentages of wt and mutant polymerase molecules were arrested at each of the three sites. For example, the proportion of molecules arrested at the TIa site following a 30-min incubation was 64% for the wt enzyme and 58% for both mutant enzymes (Fig. 3*A*, lanes 1, 8, and 15). The time course of the readthrough activity of the wt and mutant enzymes at the three intrinsic arrest sites was examined. In the absence of TFIIS, wt and mutant enzymes behaved indistinguishably following reaction times of 5, 10, and 30 min (data not shown). These observations, as well as those presented above, indicate that the two *rpo21* mutations do not affect the polymerization activity of RNAPII on different templates. Thus, the suggestion that the mutant enzymes are more prone to arrest *in vivo* (see above and ref. 20) is not likely to be correct.

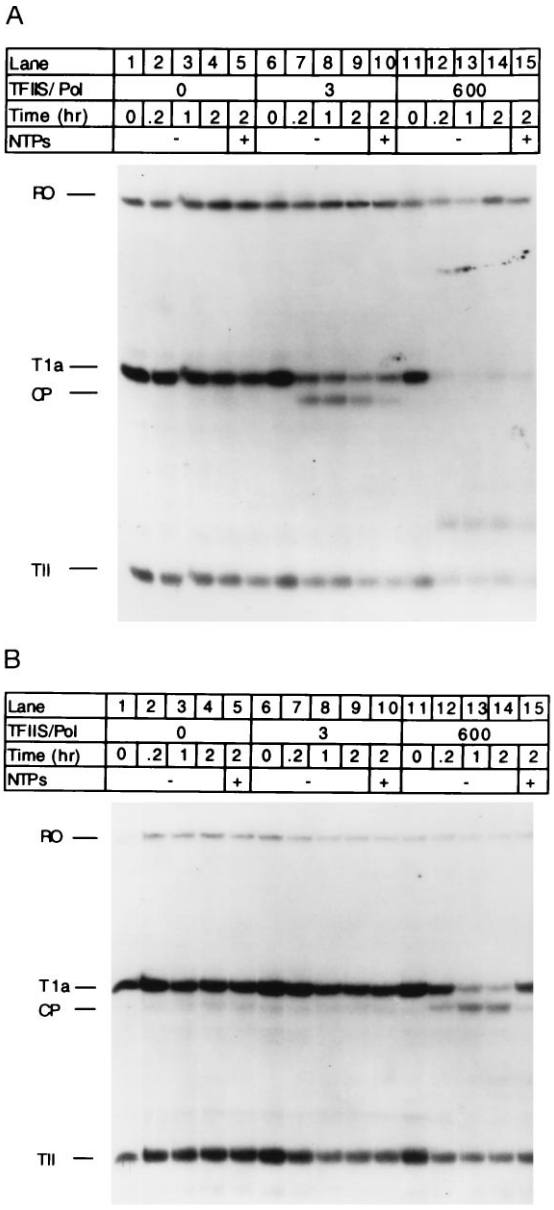

FIG. 4. Transcript cleavage induced by TFIIS. Ternary elongation complexes containing wt (*A*) or rpo21-24 RNAPII (*B*) were purified free of nucleotides and incubated for various times with no TFIIS (*A* and *B*, lanes 1–5), a 3:1 molar ratio of TFIIS/RNAPII (*A* and *B*, lanes 6–10) or a 600:1 molar ratio of TFIIS/RNAPII (*A* and *B*, lanes 11–15). Following a 2-hr incubation period, nucleotides were added to the complexes to allow for RNA-chain extension. The resulting transcripts were resolved by gel electrophoresis and visualized by autoradiography. The mobilities of the run off (RO), the T1a, the TII, and the cleaved product (CP) are indicated on the left.

A striking difference was observed between the wt and the two mutant polymerases in the presence of a 10-fold molar excess of TFIIS (Fig. 3*A*, lanes 2, 9, and 16). The fraction of arrested wt RNAPII was reduced to ≈20%. In contrast, at this molar ratio, the fraction of arrested mutant RNAPIIs remained unchanged, suggesting that at this level TFIIS does not stimulate mutant RNAPIIs to read through the arrest sites.

In an effort to mimic the *in vivo* observation that the *rpo21* mutants can be suppressed by overproduction of TFIIS (20), TFIIS-stimulated readthrough activity of the two mutant polymerases was tested at greatly elevated TFIIS/RNAPII molar ratios. As shown in Fig. 3, only massive addition of TFIIS to the mutant polymerases (over 600:1, TFIIS/RNAPII) resulted in readthrough comparable to that of the wt polymerase.

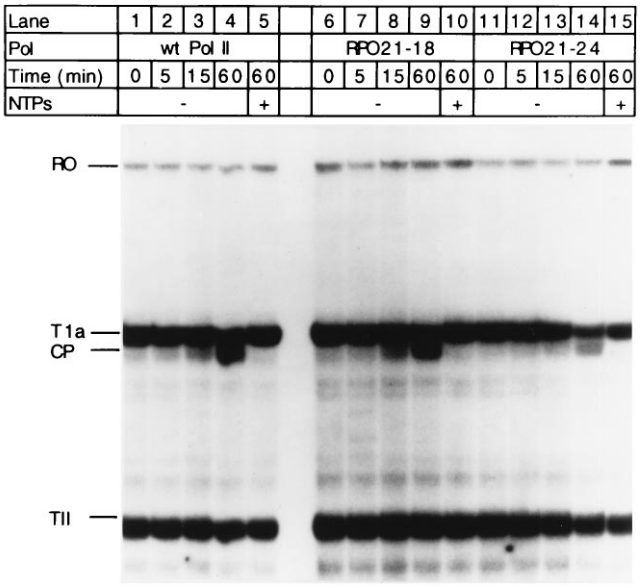

FIG. 5. Intrinsic cleavage properties of wt and mutant polymerases. Ternary elongation complexes containing wt (lanes 1–5), rpo21-18 (I1237TRARV) (lanes 6–10), or rpo21-24 (E1230K) (lanes 11–15) RNAPII were purified free of nucleotides and incubated at pH 9.5 for 0, 5, 15, and 60 min to reveal the intrinsic cleavage activity of polymerase. Following 60 min, nucleotides were added to the complexes to allow for RNA-chain extension. The transcripts were resolved by gel electrophoresis and visualized by autoradiography. The mobilities of the run off (RO), the T1a, the TII and the cleaved product (CP) are indicated on the left.

Stimulation of Intrinsic RNase Activity in the Mutant RNA Polymerases Requires High Concentrations of TFIIS. The RNase activity (both intrinsic and TFIIS-stimulated) of wt and mutant RNAPII preparations was compared. Ternary elongation complexes containing RNA polymerase, DNA template and nascent RNA transcript arrested at the TIa, TIb, and TII sites and run-off transcripts were isolated free of nucleotides by centrifugal gel filtration (24). In the absence of TFIIS, incubation of the ternary complexes for as long as 2 hr revealed only very low levels of intrinsic RNase activity (Fig. 4 *A* and *B*, lanes 2–4). In this assay and under these conditions, the two mutant polymerases behaved identically to each other, thus only the data obtained from the Rpo21-24 (E1230K) enzyme are shown in Fig. 4. In the presence of a 3-fold molar excess of TFIIS, incubation for 0.2, 1, and 2 hr of ternary complexes containing wt RNAPII resulted in cleavage of a majority of the transcripts with 3′-ends at the TIa site (Fig. 4*A*, lanes 7–9). In contrast, incubation of ternary complexes containing the Rpo21-24 (E1230K) mutant polymerase (at 3:1, TFIIS/RNAPII) resulted in no more cleavage than was seen in the absence of TFIIS (Fig. 4*B*, compare lanes 7–9 to lanes 2–4). Therefore, the intrinsic RNase activity of the two mutant enzyme complexes is not stimulated by addition of this amount of TFIIS protein.

When the mutant enzymes were incubated in the presence of a *600*-fold molar excess of TFIIS (Fig. 4*B*, lanes 12–14), the RNA-cleavage ability was stimulated to a level that was similar to that shown by the wt enzyme in the presence of a 3-fold molar excess of TFIIS (compare Fig. 4*B*, lanes 12–14 to Fig. 4*A*, lanes 7–9). Therefore, provided sufficient TFIIS protein is present, the stimulated cleavage activity of the two mutant polymerases can approach normal levels.

Under the above readthrough and cleavage assay conditions (pH 8.0), only low levels of intrinsic cleavage activity of the polymerases were observed. Nevertheless, when the isolated ternary complexes were incubated in buffer at pH 9.5, the intrinsic RNase activity of the polymerase was clearly observ-

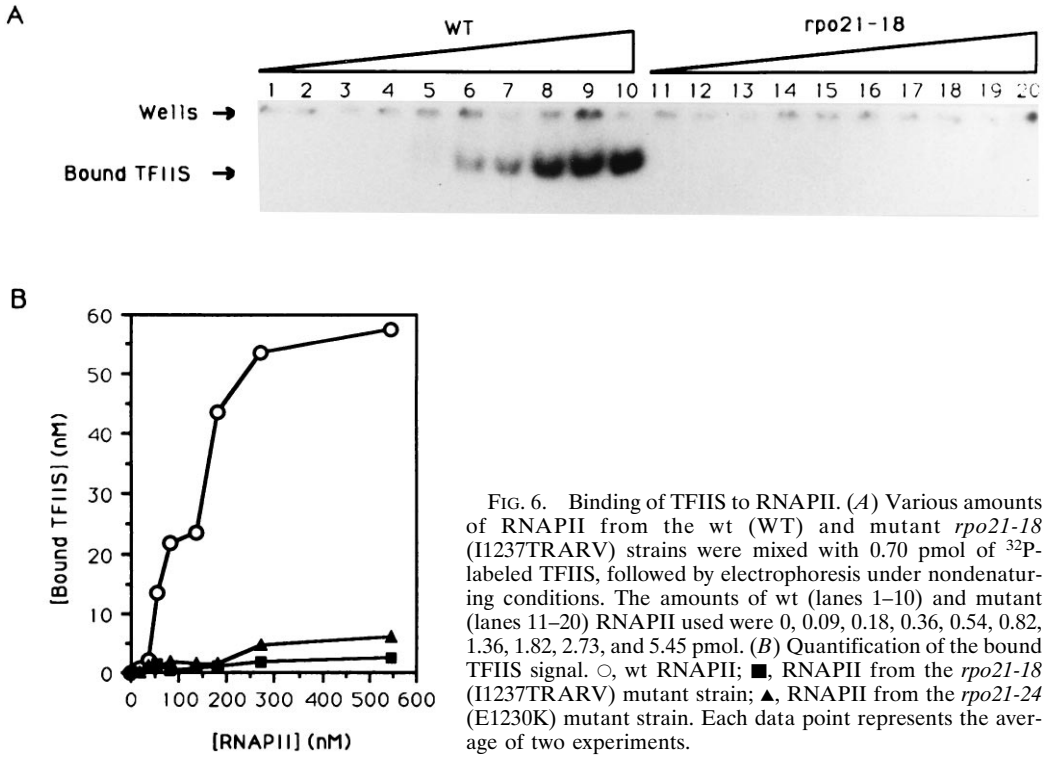

FIG. 6. Binding of TFIIS to RNAPII. (*A*) Various amounts of RNAPII from the wt (WT) and mutant *rpo21-18* (I1237TRARV) strains were mixed with 0.70 pmol of ^{32}P-labeled TFIIS, followed by electrophoresis under nondenaturing conditions. The amounts of wt (lanes 1–10) and mutant (lanes 11–20) RNAPII used were 0, 0.09, 0.18, 0.36, 0.54, 0.82, 1.36, 1.82, 2.73, and 5.45 pmol. (*B*) Quantification of the bound TFIIS signal. ○, wt RNAPII; ■, RNAPII from the *rpo21-18* (I1237TRARV) mutant strain; ▲, RNAPII from the *rpo21-24* (E1230K) mutant strain. Each data point represents the average of two experiments.

able. As shown in Fig. 5, in the absence of TFIIS, incubation for 5, 15, or 60 min of the ternary complexes (at pH 9.5) revealed that the intrinsic RNA-cleavage ability of the Rpo21-18 (I1237TRARV) mutant polymerase was comparable to that of the wt enzyme (Fig. 5, lanes 2–4 and lanes 7–9). The Rpo21-24 (E1230K) mutant polymerase showed a slightly diminished cleavage efficiency (Fig. 5, lanes 12–14). Therefore, we suggest that the *rpo21* mutations do not affect the intrinsic RNA-cleavage ability of RNAPII ternary complexes.

Mutant RNA Polymerases Have Reduced Binding Affinity for TFIIS. The behavior of the mutant enzymes in the *in vitro* readthrough and cleavage assays suggests that the *rpo21* mutations may affect the interaction between RNAPII and TFIIS. Therefore, the physical interaction between TFIIS and wt or mutant RNAPIIs was examined by electrophoresis under nondenaturing conditions (Fig. 6). Various amounts of RNAPII were incubated with ^{32}P-labeled TFIIS to form RNAPII/TFIIS complexes. The complexes were then resolved on a 5% native polyacrylamide gel. Under the electrophoretic conditions used in this experiment (pH 8.3), free TFIIS migrates out of the wells toward the cathode and the RNAPII/TFIIS complex toward the anode (Fig. 6*A*, lanes 1–10). Quantification of the signal corresponding to the bound TFIIS indicated that more TFIIS bound to wt RNAPII than to the two mutant enzymes (Fig. 6*B*), suggesting that the interaction between TFIIS and wt RNAPII was stronger than that between TFIIS and mutant RNAPIIs. The apparent dissociation constant (k_d) for the interaction between TFIIS and wt RNA polymerase was estimated to be in the range of 0.8–1.5 $\times$ 10^{-7} M. The apparent dissociation constant for the binding of TFIIS to the two mutant enzymes was estimated to be in the range of 1.0–5.0 $\times$ 10^{-5} M. Binding of TFIIS to the mutant polymerases was observed only at high levels of the mutant RNAPs (data not shown). We conclude that the *rpo21* mutations result in a reduction in the binding affinity of the mutant enzymes for TFIIS by more than 50-fold. Thus, they identify a site on RNAPII that is important for TFIIS binding.

DISCUSSION

We have observed that two mutations, which lead to 6AU-sensitive growth and affect amino-acid residues that are located between the highly conserved regions G and H of the largest subunit of RNAPII (Rpo21p; ref. 20), reduce the binding affinity of the mutant enzymes for TFIIS by more than 50-fold. As a consequence, very large amounts of TFIIS protein are required to stimulate the arrested mutant enzymes in cleavage of nascent RNAs and readthrough of intrinsic arrest sites.

These *in vitro* observations offer a plausible explanation for the 6AU-sensitive phenotype of the *rpo21* mutants and the suppression of the 6AU sensitivity by increased gene dosage of *PPR2*. Addition of 6AU to a yeast culture results in more than a 10-fold depletion of the intracellular GTP pool and a 2- to 3-fold depletion of the UTP pool (15). Several investigators have observed that the efficiency with which elongating RNAP becomes paused at intrinsic pause sites is related directly to the elongation rate of transcribing RNA polymerase and to NTP substrate concentration; a reduction in the concentration of one or all of the four NTPs enhances arrest of RNA polymerase (16–19). Therefore, the ultimate effect of 6AU on growing cells might be an increase in the number of transcribing RNAPII molecules that arrest or pause on the DNA template. When this occurs, cell growth becomes dependent on TFIIS. With the help of TFIIS, wt RNAPII might be able to complete a sufficient number of transcripts to permit growth in the presence of 6AU, whereas the mutant enzymes might not because the interactions between TFIIS and the mutant enzymes are weakened. An increase in the gene dosage of wt *PPR2* leads to increased production of yeast TFIIS protein and an elevated intracellular concentration of TFIIS. By driving the assembly of a sufficient number of active RNAPII–TFIIS complexes, an elevated *PPR2* gene dosage compensates for the reduced binding affinity of mutant RNAPII for TFIIS and allows growth.

Six of the seven 6AU-sensitive *rpo21* mutants (including the two described in this study, E1230K and I1237TRARV) are also temperature-sensitive (ts) for growth (20), which might also be related to reduced binding of the mutant enzymes for TFIIS. We have observed that the stopping efficiency of wt RNAPII at intrinsic arrest sites is increased substantially at elevated temperature (unpublished data). For example, at 38°C, the fraction of arrested wt RNAPII at the first and

weaker arrest site in the human histone H3.3 gene, TII, increases to ≈50% from the 15–25% seen at 30°C (data not shown). Therefore, raising the temperature may achieve a similar effect as that generated by adding 6AU to yeast growth medium—i.e., more transcribing RNAPII molecules may stop on the DNA template, rendering cell growth dependent on TFIIS function. However, since the ts phenotype of *rpo21* mutants cannot be suppressed by overproduction of TFIIS (20), one would have to assume that at a high temperature, the interactions between TFIIS and the mutant enzymes are so weak that they cannot be restored. Alternatively, alterations in the G–H region of Rpo21p that confer 6AU-sensitivity could also affect other RNAPII functions, such as promoter-dependent initiation, termination, or assembly of the enzyme. Two observations support this possibility. First, the *ppr2*Δ mutant strain has a near wt growth rate at the permissive temperature of 30°C, whereas one of the 6AU-sensitive *rpo21* strains, *rpo21-7* (T1141TSSSS), has a very slow growth rate at permissive temperature (20). Second, at least one ts mutation in the G–H region of Rpo21p (C1240Y) has been shown to affect the assembly of RNAPII at a nonpermissive temperature (30).

All seven of the *rpo21* mutations leading to 6AU-sensitive growth affect amino acid residues that are clustered between highly conserved regions G and H of Rpo21p (20–22). We suggest that our *rpo21* mutations might weaken the interaction between TFIIS and RNAPII by altering a site on RNAPII that is involved in direct interaction between TFIIS and RNAPII. The nature of the rpo21-24 (E1230K), which changes a glutamic acid to a lysine, suggests that a portion of this TFIIS binding site may be negatively charged. TFIIS might be expected to bind to a negatively charged surface because TFIIS is very basic. The RNAPII/TFIIS interaction would thus be predicted to be partly ionic in nature, which is consistent with the observations that the RNAPII/TFIIS interaction is known to be disrupted by low to moderate salt concentrations. However, we cannot rule out the possibility that the mutations induce conformational changes that reduce indirectly the binding of TFIIS to RNAPII.

Conserved region B of Rpo21p has also been implicated in TFIIS binding (31); a fusion protein containing a fragment of region B, as well as a monoclonal antibody thought to be directed to region B, when added at high concentrations partially inhibit TFIIS-stimulated transcription. However, the interpretation of those experiments is not entirely clear, since no direct interaction between the polymerase domain and TFIIS was measured and the inhibition of TFIIS activity was not reported to be overcome by the addition of excess TFIIS. Nevertheless, if both conserved regions B and G–H do play a role in TFIIS interaction, these two domains might be in close proximity in the RNA polymerase II structure.

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