Alanine-Scanning Mutagenesis of Human Transcript Elongation Factor TFIIS†

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ABSTRACT: TFIIS is a transcription elongation factor that binds to RNA polymerase II and allows it to transcribe through a variety of transcriptional blockages by inducing cleavage near the 3′ end of the nascent transcript. Although this cleavage reaction plays a key role in the process of reactivation of transcription by TFIIS, the exact mechanism by which TFIIS promotes readthrough by RNA polymerase II is not completely understood. We therefore undertook a systematic mutagenesis of the C-terminal half of TFIIS (ΔTFIIS) to evaluate the contribution of charged residues in this region to induce transcript cleavage and promote readthrough *in vitro*. Twenty-two ΔTFIIS alanine-scanning mutants were constructed by substitution of alanine for each amino acid in clusters of charged residues in the C-terminal half of HeLa TFIIS. The ability to induce transcript cleavage and readthrough of these mutants was tested *in vitro* using RNA polymerase II ternary elongation complexes arrested at a block to elongation. This alanine-scanning mutagenesis analysis allowed the identification of regions or residues important for the activity of TFIIS. Many of the mutants were reduced alike in both cleavage and readthrough activities. However, in several cases there was no simple correlation between these activity reductions.

Eukaryotic gene expression can be regulated by the action of transcription factors that control the elongation activity of RNA polymerase II during the process of mRNA synthesis (Kane, 1994). These transcription elongation factors can be classified into at least two functional classes depending on their ability to increase the overall rate of RNA chain elongation as do TFIIF, TFIIX (Bengal et al., 1991), and the recently cloned SIII/elongin (Garrett et al., 1994), or on their capacity to promote readthrough by RNA polymerase II through a variety of transcriptional impediments as does TFIIS (Izban & Luse, 1993b; Mote et al., 1994; Reines, 1992; Reines & Mote, 1993).

When RNA polymerase II elongation complexes become stalled at certain sequences within transcription units such that they can neither elongate nor dissociate, the elongation factor TFIIS, by stimulating a nucleolytic cleavage reaction of the nascent transcript, promotes RNA polymerase II readthrough at these sites (Reines, 1992). This cleavage reaction seems to be part of a general mechanism by which TFIIS releases various types of arrested ternary elongation complexes (Christie et al., 1994; Izban & Luse, 1993a,b; Kerppola & Kane, 1991; Mote et al., 1994; Reines, 1992; Reines & Mote, 1993). It has been postulated that this hydrolytic cleavage reaction facilitates readthrough by providing a mechanism to resolve different types of blocks to elongation (Reines, 1992). Initial studies of the cleavage reaction did not identify the proteins responsible for this ribonuclease activity. Recently it has been shown that RNA polymerase II cleavage sites utilized during pyrophosphorolysis and in TFIIS-facilitated transcript truncation are the same (Rudd et al., 1994), providing strong evidence that the catalytic site of RNA polymerase II participates in this factor-dependent transcript cleavage reaction. In addition, in prokaryotes, where this cleavage reaction was first demonstrated (Surratt et al., 1991), there is also similarity between reverse translocation caused by either pyrophosphate addition or elongation factor GreA (Feng et al., 1994), a result supporting the idea that cleavage is catalyzed by the active site of the polymerase. In addition, at elevated pH, cleavage can be easily observed in ternary complexes containing either purified *Escherichia coli* RNA polymerase (Orlova et al., 1995) or purified RNA polymerase II (R. Weilbaecher and C. M. Kane, unpublished results) in the absence of any accessory factor. Although TFIIS greatly stimulates this cleavage reaction for RNA polymerase II at physiological pH, TFIIS also may be needed in other steps necessary for factor-mediated readthrough (Cipres-Palacin & Kane, 1994).

Here we report the characterization of 22 alanine-scanning mutants of human TFIIS. Each has been quantitatively examined for its ability to stimulate readthrough and cleavage. Specific residues were identified within the last 171 amino acids of the human TFIIS which are important for the activity of the factor. Many of these alanine-scanning mutants have decreased cleavage and readthrough stimulation. However, like the previously reported mutant TFIIS7 (Cipres-Palacin & Kane, 1994), some of these mutants stimulate efficient cleavage but are almost inactive for promoting readthrough. It has been hypothesized that factors like TFIIS may stimulate cleavage by stabilizing the positioning of the active site of RNA polymerase II over a particular internal phosphodiester bond (Chamberlin, 1995). The data presented here suggest that TFIIS is also inducing or sustaining a structural change in the ternary elongation complex necessary for the process of readthrough.

EXPERIMENTAL PROCEDURES

Materials. The RNase inhibitor Inhibit-ACE was obtained from 5′ → 3′, Inc. Nucleotides were obtained from Pharmacia. Sequenase was from U.S. Biochemical Corp. [α-^{32}P]-

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CTP (>400 Ci/mmol; 1 Ci = 37 GBq) and [α-^{35}S] dATP were obtained from Amersham. Bio-Gel 30 columns were obtained from Bio-Rad. His-Bind metal chelation resin was from Novagen. RNA polymerase II was purified from calf thymus as described (Kerppola & Kane, 1988).

Strains and Plasmids. *E. coli* strain CJ236 (*dut*$^-$, *ung*$^-$) was used to prepare uracil-containing template DNA for mutagenesis (Kunkel et al., 1987). A fragment containing the last 171 amino acids of the open reading frame of HeLa TFIIS (Chen et al., 1992), fused to 6 histidines at its N terminus, was prepared using the polymerase chain reaction (PCR) (Mullis & Faloona, 1987). The PCR product was digested with *Nde*I and *Bam*HI and then ligated into the 5.4-kb *Nde*I-*Bam*HI-digested pET22b(+) (Novagen). This plasmid is referred to as pET22b(+)/ΔTFIIS, and the protein produced from this plasmid is referred to as ΔTFIIS.

Oligonucleotide-Directed Mutagenesis. Twenty-two synthetic oligonucleotides were constructed to mutate combinations of charged amino acids (Asp, Glu, Arg, Lys, and His) in the C-terminal half of the cDNA of HeLa transcript elongation factor TFIIS (Figure 1). The heteroduplex region of each mutagenic oligonucleotide was flanked by regions complementary to the TFIIS HeLa cDNA coding sequence (Chen et al., 1992). At the 5′ end of the oligonucleotide, the complementary region corresponded to a T_m of at least 35 °C (approximately 12 nucleotides), whereas on the 3′ side the complementary region corresponded to a T_m of at least 25 °C (approximately 9 nucleotides). The synthetic oligonucleotides were used to direct synthesis by T4 DNA polymerase against a single-stranded, uracil-containing template of pET22b(+)/ΔTFIIS. Mutant plamids were selected in a *dut*$^+$, *ung*$^+$ host, *E. coli* TG1 (Kunkel et al., 1987), and the nature of the mutation was confirmed by dideoxy chain termination DNA sequencing (Sanger et al., 1977).

Purification of Alanine Mutant Proteins. The ΔTFIIS alainine mutants were purified as previously described (Cipres-Palacin & Kane, 1994) using a His-Bind metal chelation resin.

Readthrough and Cleavage Assays. The ability of different ΔTFIIS alanine mutants to promote readthrough and induce transcript cleavage was assayed as described (Cipres-Palacin & Kane, 1994). Ratios of TFIIS to RNA polymerase II were maintained at 400:1 in the experiments presented in order to facilitate quantitative comparisons across fixed timepoints. Lower ratios were also tested, but the lengthy incubation times required with some of the mutants raised concerns about polymerase inactivation.

Quantitation of Transcripts in Polyacrylamide Gels. Quantitation of radioactivity in transcripts resolved by denaturing gel electrophoresis was performed using the Molecular Dynamics PhosphorImager System. Values were determined from at least three replicate experiments per timepoint.

Readthrough. Transcripts with 3′ ends at TII, TIb, TIa, and the run-off transcripts were identified, and the counts per minute (cpm) in each was determined. The total cpm in these four types of transcripts was determined for each timepoint and is called (Sum). The fraction of complexes stopped at TIa was calculated as (TIa cpm)/[(Sum) − (TIb cpm) − (TII cpm)]. Readthrough activity was calculated as the difference between the fraction of transcripts with 3′ ends at TIa in the presence and absence of TFIIS for each timepoint. Relative readthrough activity induced by the mutant proteins shown in Figure 4 was expressed as (% readthrough induced by the mutant TFIIS)/(% readthrough induced by wild-type protein). Thus, in the figure, the relative wild-type value is 100%.

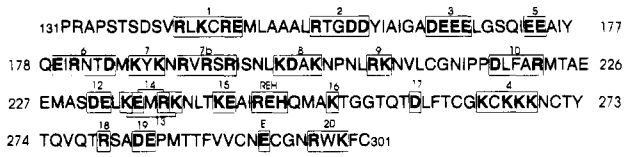

FIGURE 1: Alanine-scanning mutants of the C-terminal half of HeLa TFIIS. The last 171 amino acids of the human TFIIS (ΔTFIIS) are presented, and the location of the charged to alanine mutants are boxed and identified by numbers. Charged residues (bold) within the boxes were changed to alanines in a single mutant.

Cleavage. The efficiency of the cleavage reaction relies on quantitating the amount of transcript with 3′ ends at the TIa site. As the cleavage reaction continues, the amount of transcript at this position decreases, and the rate and extent of this disappearance are monitored in the presence and absence of TFIIS and mutants of TFIIS. The disappearance of TIa rather than the appearance of intermediate cleavage products is followed. The amount of intermediate cleavage product at any one time depends on its appearance from cleavage of TIa and its rate of disappearance to smaller products. In contrast, TIa provides a stable initial product whose disappearance directly reflects a single event, the cleavage reaction.

RESULTS

Alanine Scanning Mutagenesis of the C-Terminus of the Human Transcription Elongation Factor TFIIS. A "charged-to-alanine" scanning strategy was used to identify functional residues in human TFIIS. A deletion mutant expressing the last 171 amino acids (ΔTFIIS) encoded by the Hela TFIIS cDNA is able to promote cleavage and readthrough *in vitro* (Cipres-Palacin & Kane, 1994), and a similar truncated version of the human kidney protein functions in readthrough nearly identically to that of the full-length protein (Agarwal et al., 1991). Similarly truncated proteins from rodents (Horikoshi et al., 1985: Reines et al., 1992), *Drosophila* (Guo & Price, 1993), and *Saccharomyces cerevisiae* (Christie et al., 1994; Nakanishi et al., 1995) have also been shown to be fully functional. Thus, we selected this C-terminal portion of the protein as the target for alanine-scanning mutagenesis (Gibbs & Zoller, 1991).

Sets of charged amino acids (Asp, Glu, Arg, Lys, and His) in the C-terminal region of the HeLa cDNA of TFIIS were systematically mutated to alanine by oligonucleotide-directed mutagenesis of a single-stranded U-containing DNA template of pET22b(+)/ΔTFIIS (Cipres-Palacin & Kane, 1994). To reduce the number of mutants required to scan this region, up to four charged residues were mutated simultaneously when they were within a segment of six amino acids. Since GCT is the preferred codon for alanine in *E. coli*, it was used for all substitutions. Twenty-two charged to alanine mutants were constructed (Figure 1). The mutant proteins were overexpressed in *E. coli* and purified to >90% homogeneity using a nickel-chelating column (Cipres-Palacin & Kane, 1994). All but one of the overexpressed mutant proteins were soluble and recovered in equivalent amounts. Mutant TFIIS1 was soluble but was recovered as a smaller fraction of total protein.

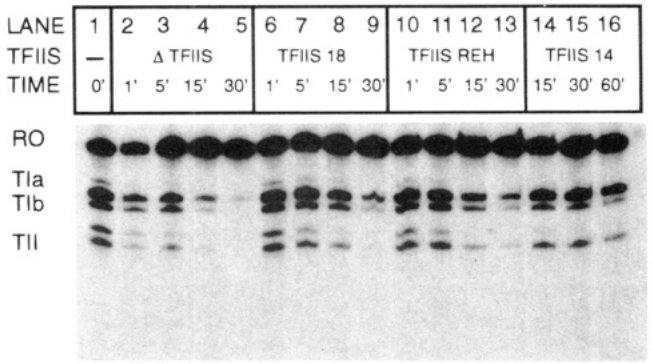

FIGURE 2: Comparison of ability of ΔTFIIS and mutant proteins TFIIS18, TFIISREH, and TFIIS14 to stimulate readthrough by RNA polymerase II. Ternary complexes containing purified calf thymus RNA polymerase II were formed as previously described (Cipres-Palacin & Kane, 1994). After 4.5 min of incubation, TFIIS protein or storage buffer was added to reaction mixtures containing ternary complexes stalled at intrinsic blocks to elongation. The molar ratio of each TFIIS protein to RNA polymerase II was ≈400:1. Lane 1: RNA from ternary complexes chased to the TIa, TIb, and TII sites and the runoff (RO). ΔTFIIS (lanes 2−5), TFIIS18 (lanes 10−13), TFIISREH (lanes 10−13), and TFIIS14 (lanes 14−16), were added to ternary complexes, and aliquots of the reaction mixture were stopped following incubation at 37 °C for 1, 5, 15, and 30 min after TFIIS addition. Readthrough was quantitated by examining the ratio of transcripts in each lane.

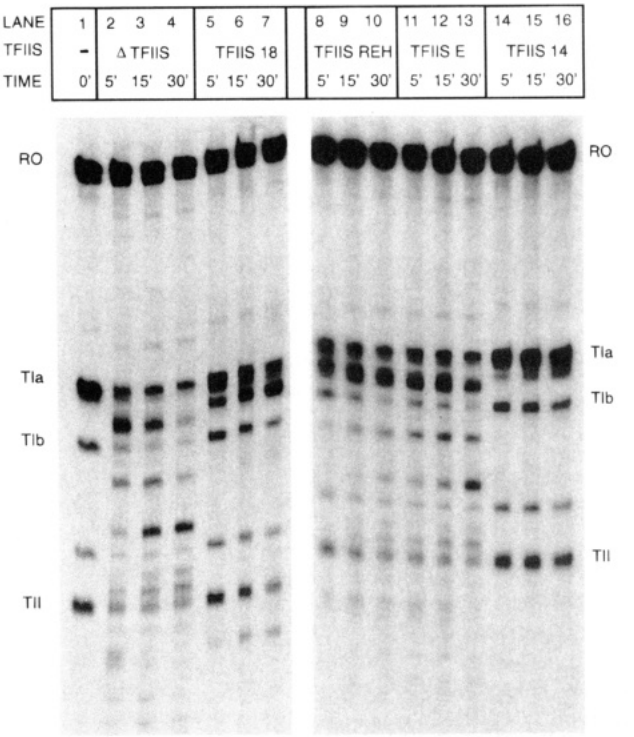

FIGURE 3: Cleavage of nascent transcripts in ternary complexes mediated by ΔTFIIS or mutants TFIIS18, TFIISREH, TFIISE, and TFIIS14. Ternary complexes were formed with RNA polymerase II and were isolated as described previously (Cipres-Palacin & Kane, 1994). Lane 1: RNAs from isolated complexes without further incubation. ΔTFIIS (lanes 2−4), TFIIS18 (lanes 5−7), TFIISREH (lanes 8−10), TFIISE (lanes 11−13), and TFIIS14 (lanes 14−16) were added to isolated ternary complexes, and aliquots of the reaction mixture were stopped following incubation for 5, 15, and 30 min after TFIIS addition. The molar ratio of each TFIIS protein to RNA polymerase II was ≈400:1. Cleavage of transcripts ending in TIa was quantitated by monitoring the disappearance of radioactivity at the TIa position in the gel.

All the mutants were tested for their ability to promote readthrough and cleavage of the nascent transcript in stalled ternary elongation complexes. The properties of two additional mutants, TFIIS5 and TFIIS7, have been reported elsewhere (Cipres-Palacin & Kane, 1994).

Analysis of the Readthrough and Cleavage Activities Promoted by Alanine-Scanning Mutants of Human ΔTFIIS. TFIIS promotes readthrough by purified RNA polymerase II at specific blocks to elongation, called TIa, TIb, and TII, in the human histone H3.3 gene (Reines et al., 1989; SivaRaman et al., 1990), in part by inducing cleavage of the nascent transcripts in ternary elongation complexes stalled at these sites (Reines, 1992). We used a 3′-extended template containing the human histone H3.3 gene (Reines et al., 1989) to analyze the ability of this collection of mutants to promote readthrough and to induce transcript cleavage. In the absence of TFIIS, RNA polymerase II stops at the TIa, TIb, and TII sites (Reines et al., 1989; SivaRaman et al., 1990) (Figure 2, lane 1). The TIa site stops the polymerase more efficiently than either TIb or TII (SivaRaman et al., 1990; Cipres-Palacin & Kane, 1994). Thus, the differences in the ability to promote (a) readthrough at the efficient TIa site and (b) transcript cleavage of complexes stalled at TIa were compared for all the alanine-scanning mutants.

Ternary elongation complexes stalled at the TIa, TIb, and TII sites were incubated in the presence of ΔTFIIS (Figure 2, lanes 2−5) or one of the mutants. The results for three representative mutants are shown (Figure 2, lanes 6−16), and readthrough was quantitated using PhosphorImager analysis. After 30 min of incubation with ΔTFIIS, only 10% of the ternary complexes initially formed remained stalled at the TIa site (Figure 2, compare lanes 1 and 5), whereas 38% and 31% of these complexes were still at the TIa site when they were incubated with either TFIIS18 (Figure 2, lane 9) or TFIIS REH (Figure 2, lane 13), respectively. In the same time period, 90% of the complexes remained stalled at the TIa site in the presence of TFIIS14 (Figure 2, lane 15). Ten percent readthrough can be detected with confidence in this assay, and thus this value of 90% stalled complexes reflects the lower limit of detection for this assay. Upon extended incubation with TFIIS14 (Figure 2, lane 16), a very low level of increased readthrough was observed (less than an additional 10%).

The mutant proteins were also tested for stimulation of transcript cleavage using isolated ternary elongation complexes containing RNA polymerase II stopped at the TIa, TIb, and TII sites. As described previously, incubation of these complexes with the ΔTFIIS protein results in cleavage of transcripts with 3′ ends that originally were located at the TIa, TIb, and TII sites [Cipres-Palacin and Kane (1994) and Figure 3, lanes 2−4]. No intrinsic cleavage was detected in the absence of ΔTFIIS even after 30 min of incubation (data not shown). After 30 min of incubation in the presence of either TFIIS REH (Figure 3, lane 10) or TFIIS E (Figure 3, lane 13), 54% and 58% of the transcripts in complexes stopped at the TIa site were cleaved, whereas ΔTFIIS (Figure 3, lane 4) promoted cleavage of 68% of the transcripts halted at the TIa site in the same period of time. Mutant TFIIS18 promoted cleavage of 46% of the transcripts at the TIa site after 30 min of incubation. This extent of cleavage is similar to that induced by ΔTFIIS after 1 min (data not shown), and clearly the kinetics of cleavage are slower for each of these three mutants than for ΔTFIIS. Nonetheless, readthrough was easily detected for each of these three mutants (Figures

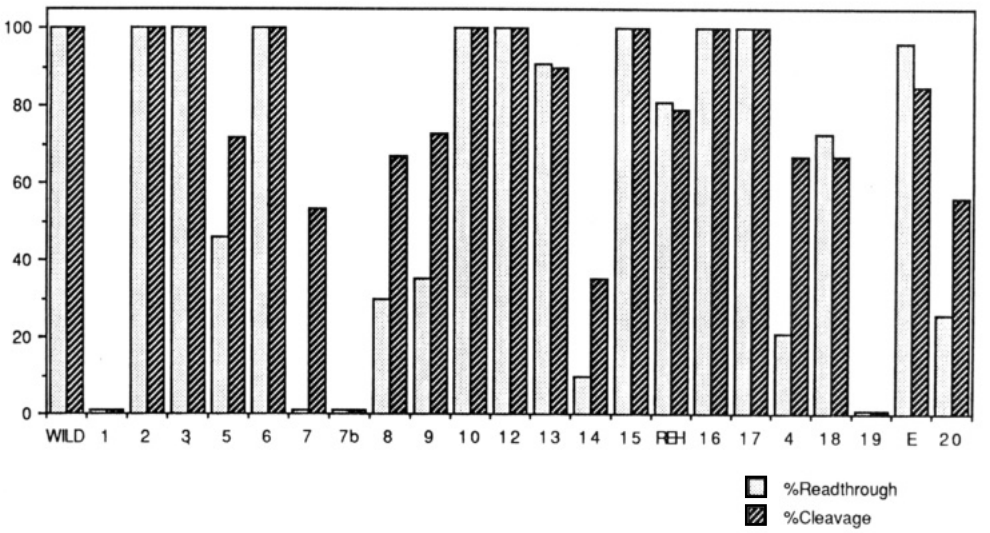

FIGURE 4: Quantification of the readthrough and cleavage activities of the alanine-scanning TFIIS mutants. Activities of alanine-scanning TFIIS mutants are expressed as a percentage of the unmutated ΔTFIIS (wild-type) activities. The readthrough values were determined as in Figure 2 following a 30-min incubation. The cleavage values were determined as in Figure 3 following a 30-min incubation. Each value represents the average of at least three experiments. See Figure 1 for the residues substituted in each mutant.

2 and 4). For TFIIS14, only 25% of the TIa transcript was cleaved after 30 min, and a low but detectable amount of readthrough was observed with this mutant in the same time period. In contrast, for TFIIS7, over 50% of the TIa transcript was cleaved in this time period, although no readthrough was detected in 30 min (Cipres-Palacin & Kane, 1994).

In addition to quantitative differences in rate and extent of cleavage induced by the TFIIS mutants, qualitative differences were also observed in the cleavage patterns (compare lanes 2 and 7, Figure 3) indicating that the polymerase itself was responding distinctly to different TFIIS proteins. A summary of both readthrough and cleavage activities for each of the 22 alanine-scanning mutants is presented relative to the wild-type ΔTFIIS (Figure 4). The properties of mutants TFIIS5 and TFIIS7 have been reported previously (Cipres-Palacin & Kane, 1994), and they are included for purposes of comparison.

Twelve of the ΔTFIIS alanine-scanning mutants displayed activities equal to (mutants: 2, 3, 6, 10, 12, 15, 16, and 17) or approaching (mutants: 13, REH, 18, and E) that displayed by wild-type ΔTFIIS (Figure 4). These results indicate that the residues mutated in these constructs were not essential for stimulating readthrough at intrinsic blocks to elongation nor inducing transcript cleavage in stalled ternary elongation complexes containing RNA polymerase II (Figure 4). The remaining mutants displayed a range of quantitative defects relative to wild-type. Only three of the mutants had no detectable readthrough or cleavage activities.

One of these inactive mutants, TFIIS7b, has three arginine residues mutated within a 10-amino acid region suggested to be part of a RNA polymerase II binding domain (Agarwal et al., 1991). Thus the lack of activity observed with this mutant might be explained by a perturbation in the binding of the factor to the enzyme. Curiously, mutation of adjacent residues (TFIIS 7) results in the elimination of readthrough but not cleavage activity, and mutation of residues just upstream (TFIIS6) has no effect on activity. A second inactive mutant, TFIIS19, has two residues mutated in a disordered loop flanked by an antiparallel β-sheet within a "Zn-ribbon domain" of TFIIS (Qian et al., 1993). The results with this mutant are in agreement with recently reported results demonstrating that any change in these residues eliminates stimulation of both elongation and RNA cleavage activities of RNA polymerase II (Jeon et al., 1994), emphasizing the crucial importance of these specific amino acids for TFIIS activity.

To examine the relationship between cleavage and readthrough more quantitatively, the two activities were directly compared between mutant proteins. If cleavage was a simple precedent for readthrough, then the mutants with similar reductions in cleavage rate and extent should show similar reductions in readthrough rate and extent. Indeed, this relationship did hold in several instances (mutants 13, 18, REH, and E). However, in other instances, this relationship was less apparent. In the presence of mutant TFIIS 20 and TFIIS 7, 38% and 36%, respectively, of the transcripts stalled at the TIa site were cleaved after 30 min of incubation (data not shown). Although these two mutants promoted similar levels of cleavage, no detectable readthrough was observed with TFIIS 7 in a 30-min incubation (Cipres-Palacin & Kane, 1994), whereas TFIIS 20 was able to promote readthrough of 25% of the complexes stalled at the TIa site. With mutant TFIIS14, 25% of the transcripts stalled at TIa were cleaved in 30 min, although only 10% of the complexes could readthrough the TIa site in the same time period. In addition, TFIIS 4 induced the same amount of cleavage as TFIIS18 in 30 min although readthrough promoted by TFIIS 4 was less than a third as efficient as the TFIIS18 in the same time period. For these mutants, while both activities are indeed reduced, there is no simple, direct correlation between the reduction of cleavage and the reduction of readthrough. All the mutants are acting upon purified RNA polymerase II, which itself carries out both the cleavage and the readthrough reactions. Clearly the ternary complex responds differently to the different TFIIS proteins.

DISCUSSION

The elongation stage of mRNA synthesis is controlled by the action of transcription factors that regulate the activity of RNA polymerase II. One of these factors, TFIIS, has

been shown to promote the ability of RNA polymerase II to read through a variety of transcriptional blocks to mRNA elongation [reviewed in Reines (1994)]. In the presence of TFIIS, stalled ternary elongation complexes containing RNA polymerase II can cleave their nascent transcripts in a 3′ → 5′ direction, allowing the enzyme to resume elongation (Reines, 1992).

The contribution of charged amino acids (located in the C-terminal half of human TFIIS) to the activity of TFIIS was analyzed by systematic substitution with alanine. Although we cannot eliminate the possibility that these mutations are affecting the overall structure of the protein, several features from our study reinforce previous observations (Gibbs & Zoller, 1991) indicating that the structural perturbations caused by the alanine substitutions are localized. For instance, in mutant TFIIS13, Lys-234 and Lys-238 are changed, and the mutant retains almost full activity. In contrast, mutant TFIIS14 changes residues that lie between those changed in TFIIS13 (Glu-235 and Arg-237), and TFIIS14 activity is severely compromised (Figure 4). In addition, although changing four lysines at positions 265, 267, 268, and 269 in mutant TFIIS4 only reduced activity, changing Cys-266 inactivated the protein (data not shown).

Only three other mutants were completely inactive—TFIIS1, TFIIS7b, and TFIIS19. Each of these contained changes to residues highly conserved between TFIIS proteins of different species (Chen et al., 1992). In contrast, although the amino acid mutated in TFIIS17 is completely conserved among predicted TFIIS protein sequences, this residue can be replaced with alanine, and the protein retains full activity (Figure 4).

Two different regions of TFIIS have been implicated directly in binding to RNA polymerase II (Agarwal et al., 1991; Horikoshi et al., 1990). One of them corresponds to amino acids Arg-246, Glu-247, and His-248 (Horikoshi et al., 1990). When these three amino acids were all replaced by leucine, the resulting mutant lost its ability to interact with the polymerase and was inactive at stimulating transcription by RNA polymerase II (Horikoshi et al., 1990). However, mutation of the same three residues to alanine in TFIIS REH only reduced readthrough and cleavage activities by 20% (Figure 4), indicating that these amino acids are not crucial for activity of TFIIS and that the perturbation caused by the leucine replacement may be more disruptive than with alanine. By deletion analysis (Agarwal et al., 1991), Agarwal and co-workers identified a second region of TFIIS (amino acids 186−195, Figure 1) that was required for RNA polymerase II binding. Deletion of this sequence created a protein that was unable to bind polymerase, to stimulate transcription or to promote readthrough (Agarwal et al., 1991). We constructed two alanine-scanning mutants in this region, TFIIS7 and TFIIS7b. The latter is completely inactive, whereas the former has the unusual property of stimulating cleavage but not readthrough (Cipres-Palacin & Kane, 1994). It is not yet known whether these mutants have an altered interaction with the polymerase relative to wild-type ΔTFIIS.

Three-dimensional NMR studies of the last 50 amino acids of TFIIS indicate that they are involved in a novel zinc-binding motif designated the Zn ribbon (Qian et al., 1993). This Zn ribbon structure contains a three-stranded antiparallel β-sheet. Alanine-scanning mutants made within this structure had a variety of effects on TFIIS activity. The most dramatic effect was the complete loss of activity in TFIIS19 which changes Asp-282 and Glu-283, two residues conserved in all the TFIIS related sequences. These residues have also been changed to a variety of other amino acids (Jeon et al., 1994), and any change completely inactivates the protein. These residues are within a disordered loop between β-sheets 1 and 2 (Qian et al., 1993), and thus their function cannot be predicted. Mutant TFIIS18, in contrast, is only three residues away from this site and shows only a modest decrease in activity. These results suggest that the specific amino acid residues Asp-282 and Glu-283 are essential for TFIIS activity (Jeon et al., 1994).

The mutants with the most severe reductions in TFIIS activity were located all throughout the C-terminal region of the protein. Likewise, mutants with partially disrupted functions did not cluster in a particular location defined by the linear representation of the amino acid sequences. Thus, no distinct separable functional "domains" were identified by this alanine-scanning mutagenesis analysis, and only by changing residues Lys-186 and Lys-188 (TFIIS 7) was transcript cleavage operationally separated from detectable readthrough activity (Cipres-Palacin & Kane, 1994). The individual contributions of each of these residues is under investigation.

Previous structural−functional studies revealed that a region necessary for the interaction with RNA polymerase II (amino acids 186−195) as well as the Zn-ribbon structure (amino acids 256−301) is essential for the activity of the factor (Agarwal et al., 1991). However, there are clearly residues outside these regions also necessary for TFIIS activity. Purification recoveries as well as the close proximity of mutated residues with dramatically different effects on activity, suggest that these mutants do not have dramatically altered structures. Indeed, the charged residues would be expected to reside on the surface of the protein (Gibbs & Zoller, 1991). However, until direct structural studies have been completed, the possibility remains that many of these residues could be important for folding or maintaining structure rather than for specific catalytic activities.

Recent studies strongly suggest that the catalytic site of RNA polymerase II participates in TFIIS-facilitated transcript cleavage (Rudd et al., 1994). The TFIIS protein may act to reposition the catalytic site of the polymerase (Chamberlin, 1994; Rudd et al., 1994). If TFIIS is needed actively to sustain a repositioned catalytic site, then one might expect that its continued presence would be necessary to effect readthrough following cleavage. However, with wild-type *Drosophila* and rat TFIIS, sarkosyl can be added to interfere with TFIIS following the cleavage event but before addition of nucleotides, and the polymerase still can readthrough efficiently (Gu et al., 1993; Guo & Price, 1993). These results would predict that readthrough occurs passively following the cleavage event and would predict a rather direct correlation between readthrough and cleavage. Many mutants described here do show a direct correlation between rates or extents of cleavage and readthrough [see also Cipres-Palacin and Kane (1994)]. However, for others, the relationship is less obvious. Thus, the mechanism by which TFIIS exerts its effects may be more complex. Perhaps this factor induces other conformational changes in the polymerase necessary in the process of readthrough. A similar role for TFIIS by which it induces a conformational change in the polymerase has been suggested in the mechanism of tran-

scription-coupled DNA repair (Donahue et al., 1994). Such a conformational change might allow the cellular repair machinery more effective access to the damaged DNA. Identifying residues in the polymerase important for cleavage and readthrough in response to TFIIS will be an important next step in evaluating TFIIS function.

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