Many proteins involved in eukaryotic transcription are similar in function and in sequence between organisms. Despite the sequence similarities, there are many factors that do not function across species. For example, transcript elongation factor TFII S is highly conserved among eukaryotes, and yet the TFII S protein from Saccharomyces cerevisiae cannot function with mammalian RNA polymerase II and vice versa. To determine the reason for this species specificity, chimeras were constructed linking three structurally independent regions of the TFII S proteins from yeast and human cells. Two independently folding domains, II and III, have been examined previously using NMR (1–3). Yeast domain II alone is able to bind yeast RNA polymerase II with the same affinity as the full-length TFII S protein, and this domain was expected to confer the species selectivity. Domain III has previously been shown to be readily exchanged between mammalian and yeast factors. However, the results presented here indicate that domain II is insufficient to confer species selectivity, and a primary determinant lies in a 30-amino acid highly conserved linker region connecting domain II with domain III. These 30 amino acids may physically orient domains II and III to support functional interactions between TFII S and RNA polymerase II.

In vivo, RNA polymerase II transcription units can vary from several hundreds to millions of base pairs, and these transcribed sequences contain information that regulates the elongation reaction for RNA polymerase II. Several types of blocks to elongation have been identified (4), and these include nucleosomes, DNA lesions, DNA binding proteins, and specific DNA sequences themselves. Thus, modulating the ability of RNA polymerase to recognize and overcome these blocks can regulate gene expression in the cell (4–7). Several proteins that regulate RNA polymerase to recognize and overcome these blocks can regulate gene expression in the cell (4–7). Several proteins have been identified in vitro that affect transcript elongation by RNA polymerase II. One regulatory protein is TFII S, a factor that enables RNA polymerase II to transcribe through a variety of blocks to elongation in vitro (4, 5, 8–11). In mammalian cells, there is a family of TFII S genes, and these are expressed in a tissue- or development-specific pattern (12–17). In Saccharomyces cerevisiae the gene encoding TFII S, PPR2 (18, 19), is single copy but not essential. Disruption strains have several moderate phenotypes but are quite sensitive to the drug 6-azauracil (20).

Across species, TFII S proteins share a high degree of sequence conservation (21). Despite this similarity, S. cerevisiae does not function with metazoan polymerases and vice versa (22, 23). Indeed, even Schizosaccharomyces pombe and S. cerevisiae do not stimulate each other’s polymerases (21). This species specificity is somewhat surprising given the striking similarity of amino acid sequences across these species in the functionally sufficient carboxyl-terminal half of the protein (11). This region of TFII S carries out all known functions in vitro, and these functions include stimulating an arrested RNA polymerase II to reach through blocks to elongation by inducing the polymerase to hydrolyze mononucleotides or oligonucleotides from the 3’ end of the nascent transcript (11). The carboxyl-terminal half of TFII S also is sufficient to complement the drug sensitivity of ppr2Δ strains in vivo (11, 22).

Based on protease digestion studies and NMR structure analysis, yeast TFII S consists of three major structural domains (1–3). Domain I, the amino-terminal portion of TFII S (amino acids 1–130 in yeast), shows the least relatedness across species. This region also is most divergent among the mammalian gene family members. Its function remains unclear because it is unnecessary for any known activities of the TFII S protein (19, 24, 25), although it can interact with at least one RNA polymerase II-containing protein complex (26). This region of the yeast protein is also needed for complementing synthetic lethality observed between ppr2Δ and tfs3Δ, although the reason for this requirement is not known.1 The four helix bundle structure of domain I from yeast likely occurs in both Elongin A and CRISP30 (27). This conservation suggests that each of these proteins may interact with the same target in the transcription complex.

The second domain of TFII S, residues 131–240 in yeast, is α-helical (1, 2). Deletion analysis as well as site-directed mutagenesis have shown that this region of the protein binds to the polymerase (27, 28, 30). Indeed, domain II from yeast binds to RNA polymerase II with the same affinity as the entire carboxyl-terminal fully functional portion of TFII S (28). Alanine scanning mutagenesis with human TFII S characterized several positions, including K187A/K189A, in the domain II region (24) that stimulated an arrested mammalian polymerase to cleave the nascent transcript but not to read through a block to elongation (24). This result indicated that although cleavage is necessary for readthrough (31), it is not sufficient (24). This result also suggested that TFII S may impart at least two functional changes on the polymerase.

The most highly conserved region of TFII S lies within domain III (residues 279–309) (2, 3). Mutagenesis has determined that domain III is critical for TFII S function (29, 32–34). This region possesses a zinc ribbon motif that shares similarity to several small subunits of nuclear polymerases: RPB9 of RNA polymerase II, C11 of RNA polymerase III, and A12.2 of RNA polymerase I (3, 35–37), as well as TFII B (38). This region is able to interact with nucleic acids in vitro, although very weakly, and the functional significance of this interaction is not known.

1 J. Davie and C. M. Kane, unpublished results.
Domains II and III are connected with a linker region of about 30 amino acids (2). In yeast TFIIS, this linker region had no defined conformation based on NMR analysis, but also it was not fully flexible (2). Furthermore, mutational studies with both human and yeast proteins demonstrated that residues in this region as well as their spacing were key to the function of TFIIS (28, 29, 33).

The sequence and structural relatedness of human and yeast TFIIS proteins focused attention on the species-specific interactions necessary for activity. The likelihood that domain II was the determinant was supported by its ability to bind RNA polymerase II. Further, a yeast-murine chimera containing this region had been shown to function with the cognate polymerase (22). However, these chimeras included both the linker region and domain II, and thus a more complete series of chimeric constructions was made to evaluate the linker itself. The results highlight the functional importance of the linker amino acids between domains II and III. Further, the results provide an explanation for the deleterious effect of mutations made in this region of the protein, and they reinforce the suggestion that the physical arrangement of domains II and III when TFIIS encounters the ternary complex are critical for the readthrough stimulation by TFIIS.

MATERIALS AND METHODS

Construction of Human/Yeast TFIIS Mutants—The chimeric human/yeast TFIIS constructs were made by using standard polymerase chain reaction methods to amplify the desired fragments (39). The polymerase chain reaction products were phosphorylated with polyvinylpyrrolidone kinase, digested with NdeI and BamHI, and ligated into pKC16 that had also been digested with NdeI and BamHI. Plasmid pKC16 contains the upstream and downstream sequences flanking the genomic TFIIS open reading frame inserted into pRS 315 (40, 41). Nomenclature and amino acids included in each TFIIS construction are listed in Table I.

Purification of Mutant Proteins—Chimeric TFIIS open reading frames were excised from the pKC16 based plasmids with BamHI and NdeI and ligated into pET15b-HMK (30) that had been similarly digested. Expression of these new open reading frames included both a His6 tag and heart myosin kinase site at the amino terminus. Overexpressed recombinant proteins were purified by the manufacturer (Novagen) with the following modifications. BL21 cells were transformed with an expression plasmid. LB with 100 μg/ml ampicillin was inoculated with a transformant. The culture was grown at 37 °C with shaking to an A660 of 0.6–0.8. Overexpression was induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside for 3 h at 30 °C with shaking.

Domains I and II were then sonicated with a microtip at settings 4–6 (Sonifier Cell Disruptor 300). The supernatant was passed through a column under conditions recommended by Bio-Rad. The columns were equilibrated with 20 mM HEPES, pH 7.5, 10% glycerol, 50 mM NaCl, 0.1% Nonidet P-40, 1 mM DTT, and centrifuged for 2 min in an IEC clinical centrifuge at a setting of 4,000 × g.

The yeast strains used were CH1305 (MATa ade2 ade3 leu2 ura3 lys2 ura3 lys2 pep2::hisG trp1::his3 URA3 hisG) (44). Yeast Transformations—In vitro transcription was performed as described (25) to allow the polymerase to elongate along the template and form an arrested complex at one of the histone H3.3 arrest sites (45). After each reaction was incubated at 30 °C with TFIIS storage buffer (50 mM Tris, pH 7.9, 10% glycerol, 50 mM NaCl, 1 mM DTT) or TFIIS wild type or chimeric proteins at 5:1, 100:1, and 500:1 TFIIS/polymerase molar ratios. Aliquots were taken at 5, 10, and 30 min. Each aliquot was described (25) and resolved on a 6% acrylamide, 7 m urea gel. Quantitation of bands was done with a Molecular Dynamics PhosphorImager system. The fraction of readthrough of the T1A site was calculated as (cpm run-off RNA)/(cpm run-off RNA + cpm T1A RNA).

Relative activity of chimeric to wild type TFIIS proteins was calculated as (fraction readthroughhuman/ fraction readthroughchimeric) × (fraction readthroughchimeric)/(fraction readthroughwild type) = (fraction readthroughhuman)/(fraction readthroughwild type).

Cleavage Assay—The cleavage assay was performed as described (25). Purified, arrested transcription complexes were incubated at 30 °C with either TFIIS storage buffer (50 mM Tris, pH 7.9, 10% glycerol, 50 mM NaCl, 1 mM DTT) or with wild type or chimeric TFIIS proteins at 100:1 or 500:1 TFIIS/polymerase molar ratios. Aliquots were taken at 1, 5, 10, and 30 min. An additional aliquot was incubated with 1 mM ATP, UTP, GTP, and CTP for 10 min at 30 °C and denoted as the “chase.” All reactions were stopped as described (25) and resolved on 6% acrylamide, 7 m urea gels.

Competition Assay—TFIIS was labeled with 35S-p as described (29) with the following modifications. After the kinase reaction to label TFIIS, free [γ-35S]ATP was removed from the reaction by passing the mixture over Bio-Rad columns under conditions recommended by Bio-Rad. The columns were equilibrated with 20 mM HEPES, pH 7.5, 10% glycerol, 100 mM NaCl, 0.01% Nonidet P-40, 1 mM DTT, and centrifuged for 2 min in an IEC clinical centrifuge at a setting of 4,000 × g.

The competition assay was performed as follows. Yeast RNA polymerase II was desalted with a BioSpin 30 micro column (Bio-Rad) equilibrated with 50 mM Tris, pH 8, 10% glycerol, 50 mM NaCl, 1 mM DTT. To form the labeled TFIIS (TFIIS*)/polymerase complex, 0.17 μM TFIIS*, and 17 mM DTT in 20 mM TrisOAc, pH 7.5, 10% glycerol were incubated on ice for 15 min. Then 0.3 μl of this binding reaction was added to 2 μl of 12.5 pmol/μl chimeric TFIIS protein (about 50-fold molar excess over labeled TFIIS*) or simply 2 μl of TFIIS storage buffer with an equivalent amount of bovine serum albumin as a negative control (50 mM Tris, pH 7.9, 10% glycerol, 50 mM NaCl, 1 mM DTT). This mixture was incubated for an additional 20 min on ice. The complexes were resolved on 5% acrylamide, 1% glycerol, 50 mM Tris borate, pH 8.3 gels (at 4 °C) with 50 mM Tris borate, pH 8.3 (at 4 °C), 1 mM DTT running buffer. Gels were electrophoresed at 100 V for 2 h, and the position of the radiolabeled TFIIS was determined by PhosphorImager analysis.

RESULTS

Rationale—The goal of these studies was to derive a structural explanation for the functional species specificity of TFIIS to provide more information on the molecular mechanism by which TFIIS stimulates elongation by RNA polymerase II. The minimal active region of TFIIS was used as the baseline for activity comparisons (24, 34). This region consists of structural domain II, the flexible linker, and domain III (Fig. 1 and Ref. 2). Each of these regions was exchanged between the human and
yeast TFIIS proteins to create a series of six chimeric proteins that were used in comparisons with proteins derived entirely from either the yeast or human TFIIS sequences (Table I). The chimeras were tested for their ability to suppress the drug sensitivity of a ppr2Δ strain \textit{in vivo} and to stimulate either yeast or mammalian RNA polymerase II to cleave the nascent transcript and read through a block to elongation \textit{in vitro}.

This work made the initial assumption that the individual domains from human and yeast TFIIS proteins would fold independently. This assumption was based upon the structural studies of TFIIS (1–3). That is, the structure of yeast TFIIS domain II is the same either as a fragment (amino acids 131–238) or in the context of the minimally active form of TFIIS (amino acids 131–309) (1, 2). Also, the NMR structure of a portion of domain III from human TFIIS was virtually superimposable upon yeast TFIIS domain III (2, 3).

Testing Domain II as the Discriminator for Species Specificity—Previous mutagenesis indicated that domain II of yeast TFIIS interacted with RNA polymerase II as well as did the fully active carboxyl-terminal half of TFIIS (amino acids 131–309). (29). A separate study also had concluded that TFIIS bound to RNA polymerase II through domain II and that this region of the TFIIS protein could distinguish polymerases from different organisms, a result explaining the species-specific activity of the TFIIS protein (22).

To test this hypothesis directly, two human/yeast TFIIS chimeras were constructed that exchanged only domain II (Table II). One chimera, yII-hL-hIII, consisted of residues 131–240 of yeast TFIIS (encompassing structural domain II and denoted “yII”) fused to human sequences (residues 233–301, denoted “hL-hIII”) encompassing the human linker and domain III. As controls, ΔyTFIIS (yII-yL-yIII), residues 131–309. (Fig. 2, compare lanes 3, 4; 24, 25). Curiously, chimera hII-yL-yIII, that contained entirely human TFIIS sequences except for the yeast TFIIS linker region, also was able to facilitate detectable readthrough of the yeast polymerase in the same incubation period, but it required a 500:1 molar excess over polymerase (Fig. 2B, lanes 7–9). In contrast, human/yeast chimeric TFIIS

| TABLE I |
| Definition of chimeric constructions |
| yII-yL-yIII = ΔyTFIIS | Yeast amino acids 131–309 |
| yII-yL-hIII | Yeast amino acids 131–269 |
| yII-hL-hIII | Human amino acids 262–301 |
| yII-hL-yIII | Human amino acids 241–301 |
| hII-hL-hIII = ΔhTFIIS | Human amino acids 131–309 |
| hII-hL-yIII | Human amino acids 131–261 |
| hII-hL-yIII | Yeast amino acids 270–309 |
| hII-yL-yIII | Yeast amino acids 233–301 |
| hII-yL-hIII | Yeast amino acids 241–301 |

Fig. 1. Sequence comparisons of domains for yeast and HeLa TFIIS used to make chimeric proteins. Amino acid residues in domain II, the linker region, and domain III are shown for \textit{S. cerevisiae} and HeLa TFIIS proteins. Shaded letters indicate residues that are either identical or similar between the two organisms.
proteins, yII-hL-yIII, yII-hL-hIII, and hII-hL-yIII were not able to stimulate detectable readthrough by yeast RNA polymerase II even at the 500:1 molar ratios (Fig. 2B, lanes 10–18 and data not shown). These results indicate that the linker region of yeast TFIIS is necessary to stimulate elongation by yeast RNA polymerase II, and the linker region contributes an essential part to the species specificity of TFIIS. Clearly, domain II is not sufficient for conferring species-specific activity on TFIIS.

Nascent transcript cleavage by RNA polymerase II is a necessary prerequisite for readthrough of blocks to elongation, and this cleavage reaction is stimulated by TFIIS (31, 46). In addition, although cleavage is necessary for readthrough, it is not always sufficient (24, 47). It was expected that the chimeric proteins would stimulate cleavage with yeast polymerase in parallel with effects observed during readthrough assays. Thus, wild type ΔyTFIIS, yII-hL-yIII, hII-hL-yIII, and hII-yL-hIII were predicted to stimulate transcript cleavage by yeast RNA polymerase II. The cleavage assays were performed under the same conditions as the readthrough assays. The positive control, ΔyTFIIS, efficiently promoted transcript cleavage within the arrested complex (Fig. 3A, compare lanes 1–4 with lanes 6–13) as did yII-hL-yIII (Fig. 3B, lanes 19–26). Both chimera hII-yL-yIII and chimera hII-hL-hIII could stimulate transcript cleavage by the yeast polymerase, although neither was as effective as the wild type protein (Fig. 3, C and D). The transcript cleavage pattern induced by the chimeric TFIIS proteins is the same as the pattern seen with ΔyTFIIS acting upon elongation complexes; this observation suggests that the mechanism is the same and that only the efficiencies differ. Those TFIIS proteins that did not have the yeast linker (ΔhTFIIS, yII-hL-hIII, yII-hL-yIII, and hII-hL-yIII) did not stimulate transcript cleavage by yeast RNA polymerase II even at the 500:1 molar ratios (data not shown). Note that for all the active chimeras, transcripts in ternary complexes that had undergone 3′-terminal transcript cleavage chased completely, regardless of cleavage efficiency (Fig. 3, lanes C).

In Vivo Complementation—Next, each chimera was tested for complementation of the 6-azauracil drug sensitivity of the TFIIS disruption strain in yeast. If the yeast TFIIS linker region confers species specificity, then ΔyTFIIS, yII-yL-hIII, hII-yL-yIII, and hII-yL-hIII would be expected to function in vivo. Yeast strains deleted for the gene encoding TFIIS (ppr2Δ) are more sensitive than wild type strains to the drug, 6-azauracil (20). Separate ppr2Δ strains containing a plasmid encoding one of the eight TFIIS proteins were patched onto synthetic medium with or without 6-azauracil and then grown for 3 days at 30 °C (Fig. 4). Like ΔyTFIIS, chimera yII-yL-hIII was able to suppress the 6-azauracil sensitivity phenotype of the ppr2Δ yeast strain (Fig. 4). This result was consistent with the in vitro experiments and has also been reported previously with a similar murine/yeast chimera (22). Chimera hII-yL-yIII also complemented the ppr2Δ yeast strain (Fig. 4). The TFIIS proteins ΔhTFIIS, yII-hL-hIII, yII-hL-yIII, and hII-hL-yIII, inactive with yeast RNA polymerase II in vitro, also were unable to complement in vivo (Fig. 4). None of these proteins contained the yeast TFIIS linker region. However, although hI-hL-hIII could stimulate yeast RNA polymerase II in vitro, it did not complement the disruption strain in vivo. This result was obtained when the chimera was expressed from either a low copy number or a high copy number plasmid. However, the question of protein levels in vivo remains to be resolved because sensitive and highly specific antibodies for the human protein are not available. All of these results indicate that the linker region is necessary but clearly not sufficient to confer full activity across species for TFIIS.

Binding to Yeast RNA Polymerase II—The original expectation was that exchange of domain II alone would confer species specificity because of differences in binding to RNA polymerase II from the divergent species. Because the linker had been implicated in species specificity as well, the binding of the various chimeras to yeast RNA polymerase II was examined using a competition binding assay to determine which proteins were able to interact physically with yeast RNA polymerase II. Thus, radiolabeled yTFIIS and yeast RNA polymerase II were mixed in the presence of increasing amounts of unlabeled TFIIS chimeric proteins (Fig. 5). The ability of any chimera to interfere with binding was taken as evidence for its ability to interact directly with RNA polymerase II. As expected, TFIIS proteins containing yeast domain II were effective competitors for binding to yeast RNA polymerase II. The most effective competition was observed for the two proteins containing yeast domain II and the yeast linker, regardless of the source of domain III (Fig. 5, lanes 3 and 9).

None of the proteins with human domain II could compete detectably with yeast TFIIS for binding to the yeast polymerase at the 50:1 ratio (Fig. 5, lanes 4, 7, 8, and 10). When hII-yL-yIII was present in a 100:1 molar excess over ΔyTFIIS (Fig. 5, lane 11), a small but detectable amount of competition was observed. That all the human domain II-containing proteins had such a limited ability to compete with yeast TFIIS for binding was not expected given the functional results of Table II, in particular for hII-yL-yIII and hII-yL-hIII. This finding is further discussed below.

Readthrough and Cleavage Assays with Mammalian RNA Polymerase II—The results of the in vitro transcription assays with yeast RNA polymerase II indicate that residues 240–270 of yeast TFIIS are involved in determining species specificity (summarized in Table II). Reciprocal assays were also carried out in vitro with mammalian RNA polymerase II, and the results suggest that the species specificity determinants for

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Species-specific Activity of TFIIS

<table>
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<th>TABLE II</th>
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<tr>
<td><strong>Summary of TFIIS mutant activities</strong></td>
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<tr>
<td>+, 100–75% wild type activity; +, 50% wild type activity; +/−, 25% wild type activity; −/+, 20% wild type activity; −, less than 20% wild type activity.</td>
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<tr>
<th>Construction</th>
<th>Growth on 6-azauracil</th>
<th>Yeast RNA polymerase II</th>
<th>Calf thymus RNA polymerase II</th>
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<tr>
<td></td>
<td>Readthrough</td>
<td>Cleavage</td>
<td>Binding</td>
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<tr>
<td>ΔyTFIIS</td>
<td>+</td>
<td>++</td>
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<td>yII-hL-hIII</td>
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<td>hII-yL-yIII</td>
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<td>ΔhTFIIS</td>
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*TFIIS: polII or polymerase II molar ratio.*
TFII S proteins may be less flexible with the mammalian polymerase. Based on results with yeast RNA polymerase II, TFII S chimeras containing the human linker region would be expected to function with mammalian RNA polymerase II, regardless of the source of domain II or III. However, in vitro readthrough and cleavage assays with the chimeric proteins and calf thymus RNA polymerase II did not confirm this prediction (Fig. 6, and summarized in Table II). The ΔyTFII S protein promoted readthrough by mammalian RNA polymerase II, as expected (Fig. 6, lanes 3–5, and Refs. 24, 33, 48, and 49). Also as expected no stimulation of readthrough was detected when calf thymus polymerase was incubated with ΔhTFII S, even when this protein was present at a 500-fold molar excess over the polymerase (Fig. 6, lanes 6–8).

Two chimeras that contained the human linker region (yII-hL-hIII and yII-hL-yIII) had no detectable transcription stimulatory activity even at a 500-fold molar excess over the mammalian polymerase (data not shown). Even chimera hII-hL-yIII had barely detectable activity and only at the highest tested molar excess of factor over polymerase, 500:1 (Fig. 6A, lanes 9–11). With the more sensitive cleavage assay, the same results were observed; that is, of all the chimeras, only hII-hL-yIII had detectable cleavage stimulatory activity (Fig. 6B; summarized in Table II). These results would indicate that requirements of the mammalian polymerase may be more delimited such that only the highly conserved zinc ribbon region can be swapped between the human and yeast TFII S proteins. Certainly the mammalian linker cannot promote TFII S function with its cognate polymerase as readily as the yeast linker with yeast RNA polymerase II.

DISCUSSION

It was the purpose of this study to identify the portion of the TFII S protein that was the determinant of species specificity. Not only the RNA polymerase II binding domain but also a rather unstructured region of about 30 amino acids is necessary for the species-specific activity of yeast TFII S. TFII S is not the only transcription factor that binds to RNA polymerase II

FIG. 2. Stimulation of readthrough with yeast RNA polymerase II. A, arrested transcription complexes were formed (lane 1) and then incubated for 30 min under transcription conditions with TFII S storage buffer (buffer) or ΔyTFII S or ΔhTFII S at 5:1, 100:1, or 500:1 TFII S:polymerase molar ratios. The positions of the TIA, TIB, and TII transcripts arising from the human histone H3.3 template (48) are indicated as is the position of the run-off (RO) transcript. B, complexes were formed as in A and were incubated for 30 min under transcription conditions following the addition of the indicated, purified chimeric protein at 5:1, 100:1, or 500:1 TFII S:polymerase ratios. The positions of the TIA, TIB, and TII transcripts arising from the human histone H3.3 template are indicated as is the position of the run-off (RO) transcript. The small black squares indicate the positions of the TIA transcript in the 500:1 lanes.
and distinguishes between species. Human TFIIF can stimulate transcription by *Drosophila* RNA polymerase II, but yeast TFIIF cannot substitute for mammalian TFIIF in an *in vitro* mammalian transcription system. Also, the RNA polymerase II CTD phosphatase from yeast cannot dephosphorylate the CTD on mammalian RNA polymerase II and vice versa. Further, there is a stimulatory effect of TFIIF on the phosphatase activity that is also species-specific. The general transcription factor TFIIB that recruits RNA polymerase II to the promoter has been shown to be species-specific. Although the ability to interact with the polymerase might be expected to be the central determinant in such species-specific activity, the results reported here suggest that the entire explanation is more complex.

Domain swaps between human and yeast TFIIS proteins indicate that the linker region from residues 240–270 in yeast TFIIS is involved in species-specific activity. This 30-amino acid region likely affects the functional arrangement between domain II and domain III of TFIIS and RNA polymerase II. Mutagenesis of residues within this linker region in both yeast and human TFIIS proteins suggests that many amino acids can be changed to alanine with little effect on activity. Some mutations in this region of the yeast protein actually confer tighter binding to RNA polymerase II while having reduced stimulation of cleavage and readthrough. How much sequence flexibility is tolerated can be tested by further mutagenesis, especially of amino acids that are not conserved between the human and yeast TFIIS proteins.

Chimera hII-YL-hIII that consisted of human TFIIS sequence except for the linker region of yeast TFIIS most strikingly emphasizes the linker contribution to species specificity. This protein at high concentration was able to stimulate both cleavage and readthrough by yeast RNA polymerase II. Chimera hII-YL-YIII was even more effective in this stimulation and also complemented the yeast TFIIS disruption strain *in vivo*. Although domain II is responsible for binding to polymerase, it could not on its own confer species specificity because chimeras yII-hL-hIII and yII-hL-YIII were unable to stimulate

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3 J. Conaway, personal communication.
Species-specific Activity of TFIIS

Chimeric proteins with either yeast or human domain II could not complement a disruption strain for TFIIS in vivo.

Chimeric proteins with either yeast or human domain II could compete with wild type TFIIS for binding to the polymerase. This competition occurred whether or not the chimera was actually able to stimulate the polymerase in cleavage and readthrough. However, these competition experiments also clearly showed that human domain II was not as effective as yeast domain II in binding yeast RNA polymerase II. Thus, although the linker region (residues 240–270 of yeast TFIIS) is necessary to confer species specificity, full functional interaction requires other determinants of the TFIIS protein.

Both mammalian domain II and the mammalian linker are required for any detectable functional interaction with mammalian RNA polymerase II (this work and Ref. 22). In contrast, yeast RNA polymerase II can functionally interact with any chima tested that contains the yeast linker region. Perhaps yeast RNA polymerase is less stringent than the mammalian polymerase in requirements for a functional interaction with TFIIS. Although the TFIIS proteins from S. cerevisiae and HeLa are well conserved, TFIIS proteins from metazoans are related more closely to each other than to TFIIS from yeast (11, 21), and the proteins from Drosophila melanogaster, mouse, and human are interchangeable in their ability to stimulate the RNA polymerase II of each species (22, 23). Domain III is the most highly conserved (Figs. 1 and 11), followed closely by that portion of domain II directly implicated in contacting the largest subunit of the polymerase (40). The linker is also very conserved between yeast and metazoans in both sequence and length (Fig. 1), and thus there is no obvious sequence explanation for its contribution to the species specificity.

Comparative RNA polymerase II binding studies have not been done with metazoan TFIIS wild type and mutant proteins. Perhaps there are specific residues in TFIIS human domain II (and Drosophila and mouse domain II) needed for the interaction with mammalian polymerase that do not exist in TFIIS yeast domain II. Alternatively, TFIIS may dock onto the polymerase through domain II, and a subsequent positioning of domain III conferred by the flexible linker may result in TFIIS stimulatory activities, possibly by communicating through Rpb9p (47) or Rpb6p (23, 52).

The interactions of TFIIS functional homologs, GreA and GreB, with bacterial RNA polymerase (53–55) also may shed some light on TFIIS interactions with eukaryotic RNA polymerase II. There are several parallels between TFIIS and the Gre factors: (i) Activity: All can promote transcript cleavage and readthrough with the cognate polymerase (4, 9, 10). (ii) Surface charged patches: The crystal structure of GreA reveals an exposed basic patch (53); domain II of yeast TFIIS has an exposed basic patch (1). The analogous basic patch of the GreB protein contributes to function but not to polymerase binding (55), whereas this patch on TFIIS is thought to contact the largest subunit of the polymerase (30). (iii) Cross-linking: A point near this basic region of GreA cross-links close to the 3′ end of the transcript (56). Cross-linking studies also have placed TFIIS close to the 3′ end of the transcript (57), although the region of TFIIS involved in this cross-linking is not yet known. (iv) Domain structure: GreA consists of two independently folding domains linked by a 12-amino acid loop (55). The domains are incapable of facilitating readthrough when added as two individual peptides in trans (58), and it was hypothesized that the orientation of the two domains in the native protein was critical for function (58). In the case of TFIIS, when domains II and III are added in trans or when the linker is replaced with an 8-amino acid random coil, no activity can be detected in vitro (28, 29).

The possibility that the TFIIS stimulatory mechanism consists of sequential steps has been proposed previously (2, 24, 47). If upon TFIIS binding to polymerase, the linker allows a conformational reorientation of domain III, then mutants that alter binding geometry or reduce conformational flexibility should provide useful information about the way TFIIS functions. The stimulation of polymerase readthrough by TFIIS would require more than simply binding to an arrested polymerase. Additionally, work with a rpb9Δ polymerase showed that this mutant polymerase could bind TFIIS as effectively as the wild type polymerase, but the rpb9Δ polymerase responded very poorly to TFIIS (47). Perhaps Rpb9p is a target for the action of TFIIS domain III, or it may communicate the signal from domain II to the catalytic center of the polymerase. Alternatively, there may be critical nucleic acid contacts that occur between domain III and the arrested ternary complex that not
only stimulate the polymerase to cleave its nascent transcript but also to undergo a conformational change necessary for efficient readthrough. Understanding how the linker region of TFIIS contributes to its activity will provide insight into the elongation mechanism itself and will help in targeting subunits and residues of RNA polymerase II for studies on their particular functions in the cleavage and readthrough reactions that promote elongation.

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REFERENCES
6. Kane, C. M. (1994) Transcription: Mechanisms and Regulation (Conaway,
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