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Transcription elongation factor SII

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Summary

RNA chain elongation by RNA polymerase II (pol II) is a complex and regulated process which is coordinated with capping, splicing, and polyadenylation of the primary transcript. Numerous elongation factors that enable pol II to transcribe faster and/or more efficiently have been purified. SII is one such factor. It helps pol II bypass specific blocks to elongation that are encountered during transcript elongation. SII was first identified biochemically on the basis of its ability to enable pol II to synthesize long transcripts.⁽¹⁾ Both the high resolution structure of SII and the details of its novel mechanism of action have been refined through mutagenesis and sophisticated *in vitro* assays. SII engages transcribing pol II and assists it in bypassing blocks to elongation by stimulating a cryptic, nascent RNA cleavage activity intrinsic to RNA polymerase. The nuclease activity can also result in removal of misincorporated bases from RNA. Molecular genetic experiments in yeast suggest that SII is generally involved in mRNA synthesis *in vivo* and that it is one type of a growing collection of elongation factors that regulate pol II. In vertebrates, a family of related SII genes has been identified; some of its members are expressed in a tissue-specific manner. The principal challenge now is to understand the isoform-specific functional differences and the biology of regulation exerted by the SII family of proteins on target genes, particularly in multicellular organisms.

Introduction

Transcription by RNA polymerase II can be regulated at many points ranging from pre-initiation complex formation through transcript termination. The elongation phase of transcription represents a dynamic process that varies depending upon the sequences transcribed. Growing evidence suggests that the composition of the elongation complex changes en route to the end of a gene and that it may include RNA processing machinery such as the capping and splicing enzymes. The complex must also navigate through chromatin and specific sequences that impede elongation.

Numerous eukaryotic transcription elongation factors have been purified and defined by using biochemical assays that monitor the stimulation of RNA chain elongation.⁽²⁾ There are at least two ways that factors stimulate elongation *in vitro*. One is to increase the average elongation rate in a DNA sequence-independent manner. The other is to enable pol II to generate long RNAs from short ones after it has become arrested, a template-engaged but inactive state, or before it terminates transcription and is released from DNA. Two factors, ELL and Elongin, increase elongation rates and have been implicated in certain human genetic diseases. Two other factors, PTEF-b and SII (also known as TFIIS) enable pol II to synthesize long RNAs. PTEF-b is a protein kinase that promotes long transcript synthesis

from the human immunodeficiency virus (HIV) long terminal repeat but may be involved in elongation on cellular genes.⁽³⁾ SII is mechanistically distinct from other elongation factors in its mode of action, in that it facilitates RNA chain elongation by restarting an arrested polymerase via the activation of a nuclease intrinsic to pol II. Here we review SII protein and gene structure and function and discuss potentially fruitful lines of future work on SII regulation and biology.

Biochemical activities

SII is a zinc-containing nuclear protein of 35,000 Da.^(1,4) It contains distinct biochemical activities that can be measured in vitro. These include: stimulation of transcript elongation, binding to pol II, and activation of a nascent RNA cleavage activity in the pol II elongation complex. An interesting, albeit weak, nucleic acid-binding activity is evident in a truncated version of the protein.⁽⁵⁾

Stimulation of elongation

SII was originally shown to stimulate purified pol II's ability to incorporate nucleotides into RNA in a nonspecific transcription assay using DNA of high complexity.⁽¹⁾ SII's role at the elongation phase of transcription had been suspected and was confirmed when promoter-based specific initiation on cloned DNA templates was reconstituted with fractionated general initiation factors.^(6,7) Semi-synthetic DNA duplexes, containing single strand extensions on one 3'-end, have also contributed to our understanding of SII function. These "tailed templates" allow pure pol II to start RNA chains at a single site without general initiation factors, and elongation can be monitored on any DNA sequence.⁽⁸⁾

The development of these in vitro assays made it possible to follow a single round of transcription and a wave of synchronous elongation by pol II. It became clear that specific DNA sequences could arrest polymerase and that SII stimulated pol II's ability to escape from these "arrest" sites. A handful of such sites were identified when pol II was trapped at them in the absence of SII⁽⁹⁻¹¹⁾ or when the SII inhibitor sarkosyl, was used.⁽¹²⁾ A consensus sequence for arrest sites has not been developed. A tract of thymidines in the nontemplate strand is a common, but not invariant, feature of arrest sites. Non B-DNA structure is important for the activity of a well-characterized site in a human histone gene but may not be part of all sites.⁽¹³⁾

In addition to intrinsic DNA sequences, SII enables pol II to transcribe past DNA-bound protein and small DNA-bound drugs.^(14,15) Hence, the blocking agent or mechanism is not an important part of the signal for SII stimulation. Not all blocked polymerases are substrates for SII-mediated stimulation. SII is unable to stimulate readthrough of a cyclobutane pyrimidine dimer that stops elongation virtually completely.⁽¹⁶⁾ SII is also unable to help pol II transcribe through nucleosomal or histone H3/H4-wrapped templates.^(17,18) This limitation to SII activity, discussed below, can be explained, at least in part, by our current understanding of its mechanism of action.

RNA polymerase II binding

Purified SII interacts with purified pol II by a number of assays including immunoprecipitation and centrifugation.^(4,6,19-21) Binding is sensitive to ionic strength and the detergent sarkosyl. Use of native gel electrophoresis allowed the determination of an apparent K_{eq} of 100 nM for yeast SII binding to yeast pol II.⁽²²⁾ SII has also been found in more complex forms of pol II thought to be initiating holoenzymes in vivo.⁽²³⁾ It is believed that a surface of pol II is sufficient to target SII to an elongation complex. A difference in affinity of SII for template-engaged versus free polymerase, however, has not been reported.

It is possible that the cryptic nucleic acid binding activity of the carboxy-terminal domain of SII (see below) contributes to the binding energy of the interaction.⁽⁴⁾

Nascent RNA cleavage and readthrough

The cleavage of nascent RNA, with subsequent readthrough, is a unique feature of SII. It was revealed only after the advent of transcription systems in which elongation complexes could be assembled and then isolated from nucleotide substrates. In these systems, polymerase without SII remains active but is unable to elongate RNA chains.⁽²⁴⁻²⁷⁾ In the presence of SII and magnesium, the nascent transcript is cleaved near its 3'-end. Cleavage can be inhibited by α -amanitin, a pol II inhibitor.⁽²⁴⁻²⁶⁾ A similar activity has been described for bacterial RNA polymerase and two functionally related proteins, GreA and GreB.⁽²⁸⁾ For bacterial and eukaryotic complexes, cleavage leads to transcriptional readthrough of arrest sites.

Nuclease activity is an intrinsic property of RNA polymerase but its rate is greatly stimulated by GreA, GreB, or SII.^(29,30) For arrested complexes, an oligonucleotide product is released from the RNA 3'-end. It has been inferred that the ribonuclease activity is carried out by the polymerization catalytic site itself.⁽³⁰⁾ No chemical or mutational evidence dissecting the cleavage and polymerization activities has yet been reported.

How does RNA cleavage result in readthrough? Cleavage precedes, and is an obligatory intermediate in, SII-mediated readthrough.⁽³¹⁾ After cleavage, pol II remains associated with the template and transcript and the RNA is substrate for renewed elongation.⁽³²⁾ During arrest, polymerase's active site may lose contact with the 3'-end of the RNA.^(24,32,33) An attractive model is that nascent RNA cleavage is carried out by the active site positioned at an internal phosphodiester bond. Hydrolysis presents a fresh 3'-hydroxyl group to the catalytic site, a prerequisite for polymerization. Most evidence is consistent with the idea that repeated rounds of hydrolysis and re-extension serve to increase the number of encounters of polymerase with an arrest site. Each encounter results in fractional readthrough of the site while the probability of arrest per encounter does not change. The efficiency of SII-independent readthrough is an intrinsic property of the particular blockage. After sufficient rounds of cleavage-resynthesis, all pol II eventually reads through the arrest site, as long as the site has a finite probability of readthrough. SII alone is unable to facilitate readthrough of complete blockages to elongation such as thymidine dimers; additional attempts at elongation after cleavage are fruitless when the blocking efficiency is 100% per encounter.

It has also been proposed that SII-activated nascent RNA cleavage plays a role in controlling the fidelity of transcription.⁽²⁴⁻²⁶⁾ This idea was, no doubt, prompted by the extensive research on nuclease activities of DNA polymerases, whose main function is presumed to reverse misincorporation events by excising them as the daughter strand is made.⁽³⁴⁾ Indeed, it has recently been demonstrated that the SII-stimulated nuclease of pol II can remove misincorporated nucleotides in vitro.^(35,36) This function, however, is not necessarily wholly distinct from SII's role as a stimulator of elongation, since misincorporation by pol II leads to a slowed rate of addition of the next nucleotide to the RNA 3'-end.⁽³⁶⁾ Part of the signal for SII activation of the nuclease for proofreading could result from altered elongation rates and/or altered elongation complex conformation following misincorporation.^(35,36)

SII activation of nascent RNA cleavage can be observed in elongation complexes other than those that are arrested or have misincorporated ribonucleotides. Typically, cleavage is not apparent in elongation-competent complexes because polymerization competes with cleavage and the former is the favored reaction at most template positions. RNA cleavage can be observed in vitro in nucleotide-starved complexes located at many template positions

that are not arrest sites. It is, however, a slower reaction than an arrested complex and the oligonucleotide released from the 3'-end is usually a dinucleotide, not the larger oligonucleotides characteristic of the arrested complex.^(37–39) This observation is taken as evidence of a structural distinction that typifies the arrested state and is manifested by a cleavage-ready polymerase.

Surprisingly, duplex DNA is not required for yeast pol II to carry out SII-stimulated nascent RNA cleavage.⁽⁴⁰⁾ This was seen in vitro for binary complexes formed between purified pol II and RNA. The RNA was bound tightly (K_{eq} 40–100 nM) and served not only as a substrate for cleavage but as one for extension when NTPs were provided. The cleavage increment varied somewhat in these experiments, releasing fragments of one to three nucleotides as well as seven to eleven nucleotides. This and other evidence suggest that RNA structure may contribute to the susceptibility and position of cleavage.⁽⁴⁰⁾ It also shows that determinants of increment size are harbored within the protein and RNA, although the presence of a DNA template could certainly modify cleavage site selection.

Nascent RNA cleavage is thought to be the critical step by which SII restores elongation competence to pol II. The activity of mutant RNA polymerase II and SII proteins, however, suggests that SII facilitates a conformational change in addition to activating cleavage.^(40,41) Yeast pol II lacking subunit 9 (pol II- Δ 9) is relatively “arrest-resistant”. Adding recombinant Rpb9 to pol II- Δ 9 restores the arrest phenotype to that of wild-type pol II⁽⁴¹⁾ Those pol II- Δ 9 molecules that do become arrested are more refractory to reactivation by SII than wild type pol II. Nevertheless, the intrinsic cleavage activity of pol II and pol II- Δ 9 are similar, which suggests that SII is required for another step in addition to that of activating nascent RNA hydrolysis. This extra step may be an SII induced conformational change in pol II that is also influenced by Rpb9.⁽⁴¹⁾ Any such conformational change is stable and does not require NTPs, since complexes that have cleaved their RNA can restart chain elongation when SII is removed or inactivated.^(27,32)

Nucleic acid binding

SII may activate the polymerase's nuclease activity allosterically simply by docking with the enzyme. An important unresolved question, however, is whether SII accelerates catalysis through a more intimate interaction with the polymerase active site, the transcript, or the template. Full length SII binds nucleic acids poorly if at all.⁽⁴⁾ A truncated recombinant derivative of human SII, which contains the carboxy-terminal 51 amino acids, a domain called a “zinc ribbon”, binds with micromolar affinity to single- and double-stranded oligodeoxyribonucleotides, single-stranded oligoribonucleotides, and RNA:DNA hybrids^(4,5) (Fig. 1A). This domain is reminiscent of that used by oligosaccharide and oligonucleotide-binding proteins in which solvent-exposed hydrophobic amino acids interact with RNA bases splayed across the sheet's surface.⁽⁴²⁾ Two adjacent amino acid pairs in this region, Arg-Trp and Asp-Glu, are important for SII's cleavage and elongation stimulating activity, suggesting both electrostatic and hydrophobic interactions between protein and nucleic acid.^(4,43) A preferred substrate for binding is polyribouridylylate, a common RNA sequence found at pause and arrest sites.⁽⁵⁾ One model suggests that upon binding the polymerase, a conformational change in SII reveals this otherwise cryptic nucleic acid binding domain. SII contact with nucleic acids in the transcription bubble may be involved in positioning the scissile phosphodiester bond, facilitating product release, or even in contributing chemically active residues to catalysis. Proof of such an active role is lacking but the demonstration, by photocrosslinking, of contact between SII and the 3'-end of the nascent RNA in an arrested elongation complex is consistent with this idea.⁽⁴⁴⁾ A similar finding has been made for GreA in an *E. coli* elongation complex.⁽⁴⁵⁾

The following minimal set of ordered events is suggested for SII-activation of pol II: SII binds to arrested pol II, RNA polymerase hydrolyzes the nascent transcript at an internal phosphodiester bond, a conformational rearrangement follows (but may precede) cleavage, and nucleotide incorporation resumes. Multiple rounds of cleavage and resynthesis may be required for full readthrough, with more rounds expected for stronger arrest sites.

Protein structure

SII structure is best understood in the case of the yeast protein, which is composed of three domains (I, II, and III; Fig. 1A) as determined by limited proteolysis and nuclear magnetic resonance (NMR).⁽⁴⁶⁾ Domain I of yeast SII extends from the N-terminus to residue 130.^(46,47) Domain II comprises residues 131–240 and is tethered to domain III (amino acids 260–309) through a short, unstructured linker.^(4,46,47) A functional requirement for domains II and III and the peptide that links them have been defined. Domain I has interesting features, but no biochemical function has yet been ascribed to it.

Domains II and III are the most highly conserved parts of SII. They are sufficient to carry out all of SII's known in vitro and in vivo activities. Using NMR spectroscopy, the structures of isolated domains I, II, and III, and a polypeptide encompassing domains II and III have been determined for yeast SII^(46,47), V. Olmsted and C. Arrowsmith, personal communication). That of domain III has been solved for the human protein.^(48,49)

Domain II contains a 3-helix bundle with a short N-terminal strand (Fig. 1A⁽⁴⁶⁾). This region is necessary and sufficient for pol II-binding and accounts for virtually all of the affinity of SII for pol II.⁽⁴²⁾ Mutation of a cluster of solvent accessible lysines and arginines, in human and yeast domain II, reduces polymerase binding, which suggests that a basic patch on SII makes contact with pol II.^(42,47,50,51) This is consistent with the salt-sensitivity of the interaction and the identification, by mutation, of acidic residues in the large subunit of pol II that may represent its SII docking site.⁽²²⁾

A linker region of approximately 20 amino acids is located between domains II and III (dashed line in Fig. 1A). A mixture of isolated domains II and III are inactive in transcription in vitro, suggesting they must be connected to stimulate elongation.⁽⁴²⁾ The linker is conformationally flexible, as it does not adopt a single defined position in solution. Nevertheless, it is important for the activity of SII in vitro.⁽⁴⁷⁾ A number of charged amino acid to alanine substitutions in the linker region yield proteins that bind pol II but are inactive for stimulating cleavage or readthrough.⁽⁴²⁾ Similar properties were observed for derivatives with a 5-amino acid insertion or deletion in the linker.⁽⁴²⁾ The picture that emerges is one in which an important specific spatial relationship between domains II and III is achieved upon binding to the elongation complex.^(42,47)

Although mammalian pol II and insect SII (and vice versa) can interact, neither yeast and *Drosophila*, nor yeast and mammalian SII-pol II pairings work together.⁽²⁰⁾ By creating chimeric mouse/yeast SII proteins, the region that confers species-specific pol II interaction was mapped to a polypeptide that encompasses domain II and the linker for the yeast protein.⁽²⁰⁾ Refined analysis suggests that the linker itself confers upon SII the ability to cooperate with its cognate polymerase (C. Kane, personal communication).

The zinc ribbon (domain III) is the most conserved part of SII. Three antiparallel β -sheets are stabilized by a tetrad of cysteines that chelate zinc^(4,47–49) (Fig. 1A). The structure is unlike the α -helix-containing zinc fingers found in many sequence-specific DNA binding proteins.⁽⁴⁸⁾ This domain contains the cryptic nucleic acid binding activity described above. It is indispensable for SII function and is the best candidate for the site of direct contact between SII and RNA in the elongation complex.^(4,5) A flexible loop linking the amino-

terminal two β -strands of the zinc ribbon contains critical acidic residues postulated to be involved in catalysis either by acting as general bases, or through the chelation of magnesium, an essential cofactor in the cleavage reaction.^(4,5,42) Structure of the *E. coli* GreA protein which, like SII, induces cleavage of RNA in arrested transcription complexes, has also been determined⁽⁴⁵⁾ (Fig. 1B). Despite their high degree of functional similarity, including polymerase-binding, nuclease activation, and stimulation of readthrough, the primary and 3-dimensional structures of SII and GreA are quite dissimilar (Fig. 1A vs. 1B).^(45–49) GreA has a long, finger-like coil of α -helices at its amino-terminus that is critical for it to activate nascent RNA cleavage and stimulate elongation by *E. coli* polymerase.^(45,52) A basic patch on the tip of the finger is near residues that crosslink to RNA in an elongation complex.⁽⁴⁵⁾ At its carboxy-terminus it has a β -sheet domain that is responsible, together with the N-terminal domain, for polymerase binding.^(45,52) Like SII, optimal function of GreA requires that both domains be physically linked.⁽⁵²⁾

Domain I is the most mysterious part of SII. Its recently solved structure shows a four β -helix bundle (V. Olmsted and C. Arrowsmith, personal communication). Although it has no known biological or biochemical function, it shares limited homology with the A-subunit of Elongin, and CRSP70, a coactivator for transcription factor SP1.^(53,54) Both SII and Elongin A can bind to a preparation of pol II holoenzyme that contains, amongst other proteins, the general transcription factors TFIIB, TFIID, TFIIE, TFIIIF, and TFIIH.⁽²³⁾ Unlike its binding to core pol II, domain I of SII is involved in an interaction with a pol II holoenzyme.⁽²³⁾ The significance of the SII-holoenzyme interaction is unclear, although it has been suggested that pol II-associated SII may participate in the transcription initiation process.⁽²³⁾

Domain I is also the most phylogenetically divergent portion of the protein and the most variable region of the tissue-specific SII isoforms found in a single organism.⁽⁵⁵⁾ It is possible that this region is involved in the cellular regulation of SII, as it contains several phosphorylation sites.⁽⁵⁶⁾ Since early reports that phosphorylated SII is inactive in stimulating pol II, little has been done on the functional consequence or biological significance of post-translational modifications of SII.^(56,57) Understanding the role of domain I will be an important goal of future work.

Tissue specific isoforms and gene family

The SII gene family

SII is phylogenetically ubiquitous. A single gene has been identified in *S. pombe*, *S. cerevisiae*, *D. melanogaster*, and *C. elegans* through cloning or sequencing projects.^(58–61) Multiple genes have been identified in vertebrate genomes, including amphibian, rodent, and human.^(19,62–67) The *Xenopus*, rat, mouse, and human SII genes represent a family whose members show distinct expression patterns across tissues.⁽⁵⁵⁾ One form is expressed in essentially all tissues and has been called “general SII”; another is found predominantly in testis and ovary; and transcripts from the third are found predominantly in heart, liver, skeletal muscle, and kidney.^(68–70) These three genes have been named *TCEA1*, *TCEA2*, and *TCEA3*, respectively, by the Human Genome Database and *Tcea1*, *Tcea2*, and *Tcea3* by the Mouse Genome Database. Mouse *Tcea2* has been studied in detail to identify the cell type of expression. Transcripts were found specifically in spermatocytes as well as postmeiotic haploid cells such as round and elongated spermatids.^(71,72) In *Xenopus*, this form of SII has been shown to be expressed in additional adult tissues by using an isoform-specific and sensitive transcript protection assay.⁽⁵⁵⁾ It has been suggested that the high sensitivity afforded by the reverse transcriptase-polymerase chain reaction could reveal a broader, albeit low level, of expression of the tissue restricted forms.⁽⁵⁵⁾ The presence of the protein variants will need to be analyzed more thoroughly to better define the cell types of

expression. Recombinant versions of all three SII isoforms display activity in vitro with no differences yet identified between isoforms.

SII genomics

A gene for general SII in humans (*TCEA1*) was mapped to chromosome 3p22 → p21.3.⁽⁷³⁾ Mouse *Tcea2*, has been mapped to a segment of chromosome 2 homologous to human chromosome 20q13.⁽⁷⁴⁾ Pseudogenes have also been identified in *Xenopus* and humans.^(55,63)

The intron-exon structure has been defined for many of the SII genes. *TCEA1*, a human general SII gene is intronless.⁽⁶³⁾ The human testis-specific isoform (*TCEA2*) is intron-containing.⁽⁷⁵⁾ Thus far, all other cloned SII genes contain introns, except that of *S. cerevisiae*. It is possible that there is an intron-containing general SII gene but, if so, it has yet to be found in the human genome. Curiously, all three isoforms of the *Xenopus* and mouse genes use rare splicing signals at intron 6 (AT/AC instead of GT/AG at the 5'/3' intron boundaries) ⁽⁶⁶⁾; G. Morgan, personal communication). Other non-canonical splicing and polyadenylation signals are found in the *Xenopus* gene as well.⁽⁶⁶⁾ Nascent RNA processing is physically coupled to transcription elongation through pol II itself.⁽⁷⁶⁾ Recent evidence also suggests that, in yeast, the rates of transcript elongation and mRNA precursor processing are coupled in vivo (K. Howe, Ph.D. thesis; K. Howe and M. Ares, personal communication). One attractive but unproven possibility is that this specialty contributes to an autoregulatory mechanism in which SII controls its own expression. When SII is abundant, elongation through the SII gene is efficient and production of its mRNA is reduced due to the inefficient use of the nascent transcript's non-canonical processing signals.⁽⁶⁶⁾ Conversely, when there is an SII deficit, elongation through the gene may be delayed and the relatively poorly processed nascent RNA has more time to be acted upon by the processing machinery. Elongation through SII genes, however, has not been studied in vitro or in vivo.

In vivo role of SII

While it is clear that SII can activate nascent RNA cleavage, stimulate elongation, and facilitate removal of misincorporated bases during transcription by pol II in vitro, there is little direct evidence that any of these are the function of SII in vivo. Elongation is notoriously difficult to study in vivo since nascent transcripts are heterogeneous in size and processed rapidly, making them rare, especially against a background of the corresponding mature mRNA. Misincorporation of bases into RNA, or deleterious sequelae therefrom, have also been difficult to document in vivo. Neither intermediates nor products of the nascent RNA cleavage activity have been identified in vivo. Nevertheless, biochemical studies of transcription elongation in mammalian and yeast systems, combined with *S. cerevisiae*'s fully sequenced genome and ease of molecular genetics, have facilitated the in vivo analysis of SII's function in transcription elongation. Progress has also been facilitated through use of a phenotype of yeast elongation factor mutants which is thought to report an elongation defect. This phenotype is sensitivity to the drug 6-azauracil (6AU).

The gene encoding yeast SII (*PPR2*) was first identified in a screen for genes that regulate pyrimidine biosynthesis because a mutation in it caused sensitivity to 6-azauracil (6AU^s).⁽⁵⁹⁾ 6AU is a drug that inhibits enzymes of de novo nucleotide biosynthesis and reduces cellular GTP and UTP levels.⁽⁷⁷⁾ The gene was subsequently rediscovered based on identification of an in vitro activity carried out by DNA strand transfer protein α , and called *DSTI*.⁽⁷⁸⁾ The Saccharomyces Genome Database has chosen *DSTI* as the preferred name for the SII gene. This single copy gene is not essential for viability in *S. cerevisiae* or *S. pombe*.^(60,79) SII null mutants in both yeasts are 6AU^s, however.^(60,79) As a result, 6AU^s has been considered a

phenotypic marker for mutations in genes thought to represent components of the transcription elongation machinery. SII mutants in yeast are also sensitive to mycophenolic acid (MA), another drug that reduces cellular GTP levels.⁽⁷⁷⁾ Addition of guanine to drug-containing medium rescues the sensitivity to either of these drugs.⁽⁷⁷⁾ The basis of these drug sensitivities can be understood in the context of in vitro experiments showing that a reduction in nucleotide substrate concentration causes pol II to elongate slowly, which in turn results in a higher propensity for pol II to become arrested.^(24,25,31) The 6AU^s phenotype is thought to result from in vivo nucleotide-depletion, compromised elongation efficiency, and an increased requirement of mRNA synthesis upon SII.^(80,81) It should be mentioned, however, that not all 6AU^s mutations fall within genes encoding transcription elongation machinery. For example, a mutant allele of the transcriptional activator for genes in the pyrimidine biosynthetic pathway, *PPR1*, was identified through a 6AU^s phenotype.⁽⁵⁹⁾ Nor is it the case that all mutations in elongation machinery components necessarily confer 6AU^s upon cells. The idea that 6AU^s is frequently a marker for altered elongation properties, however, is supported by the finding that mutations in pol II subunit genes also confer 6AU^s upon yeast. These observations can be explained at the molecular level for two types of mutations in pol II. A 6AU^s mutation in the largest subunit (*RPB1*) results in a 50-fold reduction in the affinity of SII for pol II in vitro.⁽²²⁾ A 6AU^s point mutation in a region of the second largest subunit (*RPB2*) thought to reside near the pol II catalytic pocket, reduces the average elongation rate of pol II. This in turn makes the enzyme arrest-prone in vitro and in vivo.⁽⁸²⁾ Consistent with this idea, the former mutations are suppressed by over-expression of SII whereas the latter are not, since raising the SII concentration through over-expression in the *RPB1* mutant would be expected to drive an otherwise weak SII-pol II association.^(80,81) In the cells bearing the “slow” polymerase mutation, the defect is an intrinsic property of the enzyme and SII is not limiting, hence higher levels of SII, whether pol II-bound or free are ineffectual.

Recently, a number of yeast genes and proteins and their human homologues, have been identified as elongation factors. These include the *SPT4*, *SPT5*, and *SPT6* genes that were previously implicated in chromatin and transcription.⁽⁸³⁾ Genetic and biochemical evidence implicate these genes in transcription elongation. An *spt4* null mutation and some mutant alleles of the essential *SPT5* and *SPT6* genes, confer 6AU^s upon *S. cerevisiae*.⁽⁸⁴⁾ Combining mutations in *SPT4*, *SPT5*, or *SPT6* with a deletion of *DST1* results in conditional synthetic lethality, suggesting that when the Spt4/5/6 machinery is made defective by mutation, SII function is required.⁽⁸⁴⁾ The large subunit of FACT, a protein complex that stimulates elongation on chromatin templates in vitro, is related to the yeast *SPT16* gene which shares mutant phenotypes with *SPT4*, *SPT5*, and *SPT6*.^(83,85,86) This suggests that a complicated kinetic coupling of elongation rate and chromatin modification exists and that under some circumstances slow elongation may be protective.

A complex called Elongator, which is associated with the elongating form of pol II, has also been identified in yeast.⁽⁸⁷⁾ Ablation of two genes that encode subunits of Elongator, *ELP1*, and *ELP3*, is neither lethal nor 6AU-sensitizing. Double mutants with a deletion of *DST1* and a deletion of either *ELP1* or *ELP3*, however, display a 6AU^s phenotype that is stronger than that of a *DST1* single mutant.^(87,88) In a similar set of experiments, mutant alleles of *SPT16* which do not confer 6AU^s, were found to suppress the 6AU^s phenotype of an *DST1* and *SPT4* deletion mutations.⁽⁸⁶⁾ The authors suggest that impaired elongation in *SPT16* mutants reduces the frequency of arrest and the reliance of transcription upon SII or Spt4.

A recent study has enlarged our appreciation of the spectrum of in vivo elongation defects that can be assayed by 6AU^s.⁽⁸¹⁾ In this work, a disruption of *DST1* was combined with the aforementioned *RPB2* mutation (*rpb2-10*) that generates a hyper-arresting polymerase in vitro.⁽⁸¹⁾ Cells with either mutation show a moderately reduced (twofold) growth rate in the

presence of 6AU. Yeast that contain both mutations show a synergistic 6AU super-sensitive phenotype manifested by a severe reduction in doubling time (34-fold). This allele-specific interaction suggests that when SII is available, the crippled polymerases in *rpb2-10* cells employ it to improve elongation, consistent with the ability of this hyper-arresting enzyme to use SII for readthrough in vitro.⁽⁸²⁾ Depriving the hyper-arresting enzyme of SII adds an insult to the elongation machinery and synergistically impacts growth rate. The effects of the combined mutations upon growth rate correlates with effects on transcript levels. These double mutant yeast have about one-half the normal amount of poly(A)⁺ RNA which is severely depressed upon 6AU treatment.⁽⁸¹⁾ These data suggest that SII acts generally for optimal mRNA synthesis and provides evidence in eukaryotes that mRNA levels can be altered by a transcription elongation-targeting drug.⁽⁸¹⁾ Combining any two 6AU^s mutations does not necessarily result in a synthetic drug sensitivity. When a 6AU^s mutation in *RPB1* that compromises SII-binding is combined with a disruption of SII, the resulting sensitivity is comparable to either alone.^(80,81) This is to be expected since a reduction in SII-binding by pol II should be inconsequential in the absence of SII. Although the 6AU^s phenotype has become popular, and virtually synonymous with an elongation defect, a more complete molecular understanding of the set of genes, physiological conditions, and identity of the elongation factors involved, will be needed to confirm models derived from genetic experiments.

It is interesting to note that SII, which can be thought to function through the pol II catalytic site via nascent RNA cleavage, has a genetic, biochemical, or structural relationship with three proteins of the transcription machinery that, when mutated, alter the transcription start site in vivo. These are TFIIB, Rpb1, and Rpb9 and the implication is that they participate in setting the register of the template “reading head” of pol II.^(89–91) Recently, a synthetic lethal screen was carried out in yeast deleted for *DST1* in order to identify which genes, when mutated, make SII required for cell viability (J. Davie and C. Kane, personal communication). The resulting genes were sorted into nine complementation groups, one of which is *SNF2*, the catalytic subunit of the Swi/Snf chromatin remodeling complex. Mutations in genes encoding other subunits of the Swi/Snf complex also display synthetic lethality with a *DST1* deletion (J. Davie and C. Kane, personal communication). These findings, the work on *SPT4*, *SPT5*, *SPT6*, and *SPT16*, and the recent finding that *ELP3* is a histone acetyltransferase,⁽⁸⁸⁾ represent a growing body of evidence that part of SII's function in vivo is involved with chromatin remodeling.

These recently described genetic interactions between yeast elongation factors and pol II subunits are shown schematically in Figure 2.

Conclusion

Biochemical analysis has revealed a fascinating activity of SII, activation of nascent RNA cleavage, that enables pol II to be reactivated from transcriptional arrest and proofread its transcripts. High resolution structure of SII has elucidated an interesting structural motif, the zinc ribbon, which is found in a growing class of proteins that interact with nucleic acids. As for other elongation factors defined through biochemical assays in vitro, the biological role of SII in gene regulation is a pressing question. Molecular genetic work in yeast supports the role of SII as a general transcription factor in vivo. Identifying the DNA sequences or other characteristics that make certain genes more or less dependent upon SII, will be important in determining its overall role in transcription and how its activity is integrated with other elongation factors that target pol II. Recent and continued advances in SII comparative genomics will be important in understanding SII function in metazoans and particularly in mammals. The existence of this gene family prompts two key questions: 1) Are SII isoforms

functionally distinct? and 2) Does derangement of SII in humans and other vertebrates result in dysfunction or disease?

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References

1. Sekimizu K, Kobayashi N, Mizuno D, Natori S. Purification of a factor from *Ehrlich ascites* tumor cells specifically stimulating RNA polymerase II. *Biochemistry*. 1976; 15:5064–5070. [PubMed: 990265]
2. Reines D, Conaway JC, Conaway RC. The RNA polymerase II general elongation factors. *Trends Biochem Sci*. 1996; 21:351–355. [PubMed: 8870500]
3. Jones KA. Taking a new TAK on tat transactivation. *Genes Dev*. 1997; 11:2593–2599. [PubMed: 9334323]
4. Agarwal K, Baek KH, Jeon CJ, Miyamoto K, Ueno A, Yoon H. Stimulation of transcript elongation requires both the zinc finger and RNA polymerase II binding domains of human TFIIS. *Biochemistry*. 1991; 30:7842–7851. [PubMed: 1868060]
5. Yoon HS, Sitikov AS, Jeon CJ, Agarwal K. Preferential interaction of the mRNA proofreading factor TFIIS zinc ribbon with rU-dA base pairs correlates with its function. *Biochemistry*. 1998; 37:12104–12112. [PubMed: 9724522]
6. Reinberg D, Roeder RG. Factors involved in specific transcription by mammalian RNA polymerase II. *J Biol Chem*. 1987; 262:3331–3337. [PubMed: 3818644]
7. Reines D, Chamberlin MJ, Kane CM. Transcription elongation factor SII (TFIIS) enables RNA polymerase II to elongate through a block to transcription in a human gene in vitro. *J Biol Chem*. 1989; 264:10799–10809. [PubMed: 2471707]
8. Kadesch TR, Chamberlin MJ. Studies of in vitro transcription by calf thymus RNA polymerase II using a novel duplex DNA template. *J Biol Chem*. 1982; 257:5286–5295. [PubMed: 7068686]
9. Bengal E, Flores O, Krauskopf A, Reinberg D, Aloni Y. Role of the mammalian transcription factors IIF, IIS, and IIX during elongation by RNA polymerase II. *Mol Cell Biol*. 1991; 11:1195–1206. [PubMed: 1996086]
10. Kash SF, Innis JW, Jackson AU, Kellems RE. Functional analysis of a stable transcription arrest site in the first intron of the murine adenosine deaminase gene. *Mol Cell Biol*. 1993; 13:2718–2729. [PubMed: 8474437]
11. Sato K, Ito R, Baek KH, Agarwal K. A specific DNA sequence controls termination of transcription in the gastrin gene. *Mol Cell Biol*. 1986; 6:1032–1043. [PubMed: 3097503]
12. Hawley DK, Roeder RG. Separation and partial characterization of three functional steps in transcription initiation by human RNA polymerase II. *J Biol Chem*. 1985; 260:8163–8172. [PubMed: 2409080]
13. Kerppola TK, Kane CM. Analysis of the signals for transcription termination by purified RNA polymerase II. *Biochemistry*. 1990; 29:269–278. [PubMed: 2157481]
14. Reines D, Mote J Jr. Elongation factor SII-dependent transcription by RNA polymerase II through a sequence-specific DNA-binding protein. *Proc Natl Acad Sci USA*. 1993; 90:1917–1921. [PubMed: 8446609]
15. Mote J Jr, Ghanouni P, Reines D. A DNA minor groove-binding ligand both potentiates and arrests transcription by RNA polymerase II. *J Mol Biol*. 1994; 236:725–737. [PubMed: 8114090]
16. Donahue BA, Yin S, Taylor JS, Reines D, Hanawalt PC. Transcript cleavage by RNA polymerase II arrested by a cyclobutane pyrimidine dimer in the DNA template. *Proc Natl Acad Sci USA*. 1994; 91:8502–8506. [PubMed: 8078911]

17. Izbán MG, Luse DS. Factor-stimulated RNA polymerase II transcribes at physiological elongation rates on naked DNA but very poorly on chromatin templates. *J Biol Chem.* 1992; 267:13647–13655. [PubMed: 1618865]
18. Chang CH, Luse DS. The H3/H4 tetramer blocks transcript elongation by RNA polymerase II in vitro. *J Biol Chem.* 1997; 272:23427–23434. [PubMed: 9287358]
19. Hirashima S, Hirai H, Nakanishi Y, Natori S. Molecular cloning and characterization of cDNA for eukaryotic transcription factor S-II. *J Biol Chem.* 1988; 263:3858–3863. [PubMed: 3346229]
20. Shimoaraiso M, Nakanishi T, Kubo T, Natori S. Identification of the region in yeast S-II that defines species specificity in its interaction with RNA polymerase II. *J Biol Chem.* 1997; 272:26550–26554. [PubMed: 9334234]
21. Rappaport J, Cho K, Saltzman A, Prenger J, Golomb M, Weinmann R. Transcription elongation factor SII interacts with a domain of the large subunit of human RNA polymerase II. *Mol Cell Biol.* 1988; 8:3136–3142. [PubMed: 3145407]
22. Wu J, Awrey DE, Edwards AM, Archambault J, Friesen JD. In vitro characterization of mutant yeast RNA polymerase II with reduced binding for elongation factor TFIIS. *Proc Natl Acad Sci USA.* 1996; 93:11552–11557. [PubMed: 8876173]
23. Pan G, Aso T, Greenblatt J. Interaction of elongation factors TFIIS and Elongin A with a human RNA polymerase II holoenzyme capable of promoter-specific initiation and responsive to transcriptional activators. *J Biol Chem.* 1997; 272:24563–24571. [PubMed: 9305922]
24. Izbán MG, Luse DS. The RNA polymerase II ternary complex cleaves the nascent transcript in a 3' → 5' direction in the presence of elongation factor SII. *Genes Dev.* 1992; 6:1342–1356. [PubMed: 1378419]
25. Reines D. Elongation factor-dependent transcript shortening by template-engaged RNA polymerase II. *J Biol Chem.* 1992; 267:3795–3800. [PubMed: 1371280]
26. Wang D, Hawley DK. Identification of a 3' → 5' exonuclease activity associated with human RNA polymerase II. *Proc Natl Acad Sci USA.* 1993; 90:843–847. [PubMed: 8381534]
27. Guo H, Price DH. Mechanism of DmS-II-mediated pause suppression by *Drosophila* RNA polymerase II. *J Biol Chem.* 1993; 268:18762–18770. [PubMed: 7689559]
28. Borukhov S, Sagitov V, Goldfarb A. Transcript cleavage factors from *E. coli*. *Cell.* 1993; 72:459–466. [PubMed: 8431948]
29. Orlova M, Newlands J, Das A, Goldfarb A, Borukhov S. Intrinsic transcript cleavage activity of RNA polymerase. *Proc Natl Acad Sci USA.* 1995; 92:4596–4600. [PubMed: 7538676]
30. Rudd MD, Izbán MG, Luse DS. The active site of RNA polymerase II participates in transcript cleavage within arrested ternary complexes. *Proc Natl Acad Sci USA.* 1994; 91:8057–8061. [PubMed: 8058756]
31. Reines D, Ghanouni P, Li QQ, Mote J Jr. The RNA polymerase II elongation complex. *J Biol Chem.* 1992; 267:15516–15522. [PubMed: 1379232]
32. Gu W, Powell W, Mote J Jr, Reines D. Nascent RNA cleavage by arrested RNA polymerase II does not require upstream translocation of the elongation complex on DNA. *J Biol Chem.* 1993; 268:25604–25616. [PubMed: 7503982]
33. Chamberlin M. New models for the mechanism of transcription elongation and its regulation. *Harvey Lectures.* 1995; 88:1–21. [PubMed: 1285418]
34. Kornberg, A.; Baker, TA. DNA replication. W.H. Freeman and Company; New York: 1992. p. 116-121.
35. Jeon CJ, Agarwal K. Fidelity of RNA polymerase II transcription controlled by elongation factor TFIIS. *Proc Natl Acad Sci USA.* 1996; 93:13677–13682. [PubMed: 8942993]
36. Thomas MJ, Plata AA, Hawley DK. Transcriptional fidelity and proofreading by RNA polymerase II. *Cell.* 1998; 93:627–637. [PubMed: 9604937]
37. Izbán MG, Luse DS. SII-facilitated transcript cleavage in RNA polymerase II complexes stalled early after initiation occurs in primarily dinucleotide increments. *J Biol Chem.* 1993; 268:12864–12873. [PubMed: 8509420]
38. Izbán MG, Luse DS. The increment of SII-facilitated transcript cleavage varies dramatically between elongation competent and incompetent RNA polymerase II ternary complexes. *J Biol Chem.* 1993; 268:12674–12885.

39. Gu W, Reines D. Identification of a decay in transcription potential that results in elongation factor dependence of RNA polymerase II. *J Biol Chem*. 1995; 270:11238–11244. [PubMed: 7744757]
40. Johnson TL, Chamberlin MJ. Complexes of yeast RNA polymerase II and RNA are substrates for TFIIS-induced RNA cleavage. *Cell*. 1994; 77:217–224. [PubMed: 7513257]
41. Awrey DE, Weilbaecher RG, Hemming SA, Orlicky SM, Kane CM, Edwards AM. Transcription elongation through DNA arrest sites. *J Biol Chem*. 1997; 272:14747–14754. [PubMed: 9169440]
42. Awrey DE, Shimasaki N, Koth C, Weilbaecher R, Olmsted V, Kazanis S, Shan X, Arellano J, Arrowsmith CH, Kane CM, Edwards AM. Yeast transcript elongation factor (TFIIS), structure and function. *J Biol Chem*. 1998; 273:22595–22605. [PubMed: 9712888]
43. Jeon CJ, Yoon HS, Agarwal K. The transcription factor TFIIS zinc ribbon dipeptide Asp-Glu is critical for stimulation of elongation and RNA cleavage by RNA polymerase II. *Proc Natl Acad Sci USA*. 1994; 91:9106–9110. [PubMed: 8090778]
44. Powell W, Bartholomew B, Reines D. Elongation factor SII contacts the 3'-end of RNA in the RNA polymerase II elongation complex. *J Biol Chem*. 1996; 271:22301–22304. [PubMed: 8798387]
45. Stebbins CE, Borukhov S, Orlova M, Polyakov A, Goldfarb A, Darst SA. Crystal structure of the GreA transcript cleavage factor from *Escherichia coli*. *Nature*. 1995; 373:636–640. [PubMed: 7854424]
46. Morin PE, Awrey DE, Edwards AM, Arrowsmith CH. Elongation factor TFIIS contains three structural domains: solution structure of domain II. *Proc Natl Acad Sci USA*. 1996; 93:10604–10608. [PubMed: 8855225]
47. Olmsted VK, Awrey DE, Koth C, Shan X, Morin PE, Kazanis S, Edwards AM, Arrowsmith CH. Yeast transcript elongation factor (TFIIS), structure and function. *J Biol Chem*. 1998; 273:22589–22594. [PubMed: 9712887]
48. Qian X, Jeon CJ, Yoon HS, Agarwal K, Weiss MA. Structure of a new nucleic-acid-binding motif in eukaryotic transcriptional elongation factor TFIIS. *Nature*. 1993; 365:277–279. [PubMed: 7626141]
49. Qian X, Gozani SN, Yoon HS, Jeon CJ, Agarwal K, Weiss MA. Novel zinc finger motif in the basal transcriptional machinery: three-dimensional NMR studies of the nucleic acid binding domain of transcriptional elongation factor TFIIS. *Biochemistry*. 1993; 32:9944–9959. [PubMed: 8399164]
50. Cipres-Palacin G, Kane CM. Cleavage of the nascent transcript induced by TFIIS is insufficient to promote read-through of intrinsic blocks to elongation by RNA polymerase II. *Proc Natl Acad Sci USA*. 1994; 91:8087–8091. [PubMed: 8058762]
51. Cipres-Palacin G, Kane CM. Alanine-scanning mutagenesis of human transcript elongation factor TFIIS. *Biochemistry*. 1995; 34:15375–15380. [PubMed: 7578153]
52. Koulich D, Orlova M, Malhotra A, Sali A, Darst SA, Borukhov S. Domain organization of *Escherichia coli* transcript cleavage factors GreA and GreB. *J Biol Chem*. 1997; 272:7201–7210. [PubMed: 9054416]
53. Aso T, Lane WS, Conaway JW, Conaway RC. Elongin (SIII): a multi-subunit regulator of elongation by RNA polymerase II. *Science*. 1995; 269:1439–1443. [PubMed: 7660129]
54. Ryu S, Zhou S, Ladurner AG, Tjian R. The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. *Nature*. 1999; 397:446–450. [PubMed: 9989412]
55. Labhart P, Morgan GT. Identification of novel genes encoding transcription elongation factor TFIIS (TCEA) in vertebrates: conservation of three distinct TFIIS isoforms in frog, mouse, and human. *Genomics*. 1998; 52:278–288. [PubMed: 9790746]
56. Horikoshi M, Sekimizu K, Hirashima S, Mitsuhashi Y, Natori S. Structural relationships of the three stimulatory factors of RNA polymerase II from *Ehrlich ascites* tumor cells. *J Biol Chem*. 1985; 260:5739–5744. [PubMed: 4039323]
57. Hirai H, Sekimizu K, Horikoshi M, Nakanishi Y, Natori S. Stimulation of transcription from accurate initiation sites by purified S-II. *FEBS Lett*. 1988; 238:119–122. [PubMed: 3169244]
58. Oh Y, Yoon J, Baek KH. Isolation and characterization of the gene encoding the *Drosophila melanogaster* transcriptional elongation factor, TFIIS. *Biochim Biophys Acta*. 1995; 1262:99–103. [PubMed: 7772609]

59. Hubert JC, Guyonvarch A, Kammerer B, Exinger F, Liljelund P, Lacroute F. Complete sequence of a eukaryotic regulatory gene. *EMBO J.* 1983; 2:2071–2073. [PubMed: 6139279]
60. Williams LA, Kane CM. Isolation and characterization of the *Schizosaccharomyces pombe* gene encoding transcript elongation factor TFIIS. *Yeast.* 1996; 12:227–236. [PubMed: 8904334]
61. Wilson R, Ainscough R, Anderson K, Baynes C, Berks M, Bonfield J, Burton J, Connell M, Copsey T, Cooper J, Coulson A, Craxton M, Dear S, Du Z, Durbin R, Favello A, Fulton L, Gardner A, Green P, Hawkins T, Hillier L, Jier M, Johnston L, Jones M, Kershaw J, Kirsten J, Laister N, Latreille P, Lightning J, Lloyd C, McMurray A, Mortimore B, O'Callaghan M, Parsons J, Percy C, Rifken L, Roopra A, Saunders D, Shownkeen R, Smaldon N, Smith A, Sonnhammer E, Staden R, Sulston J, Thierry-Mieg J, Thomas K, Vaudin M, Vaughan K, Waterston R, Watson A, Weinstock L, Wilkinson-Sproat J, Wohldman P. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature.* 1994; 368:32–38. [PubMed: 7906398]
62. Chen HC, England L, Kane CM. Characterization of a HeLa cDNA clone encoding the human SII protein, an elongation factor for RNA polymerase II. *Gene.* 1992; 116:253–258. [PubMed: 1378807]
63. Park H, Baek KH, Jeon CJ, Agarwal K, Yoo OJ. Characterization of the gene encoding the human transcriptional elongation factor TFIIS. *Gene.* 1994; 139:263–267. [PubMed: 8112616]
64. Yoo OJ, Yoon HS, Baek KH, Jeon CJ, Miyamoto K, Ueno A, Agarwal K. Cloning, expression and characterization of the human transcription elongation factor, TFIIS. *Nucleic Acids Res.* 1991; 19:1073–1079. [PubMed: 1708494]
65. Kanai A, Kuzuhara T, Sekimizu K, Natori S. Heterogeneity and tissue-specific expression of eukaryotic transcription factor S-II-related protein mRNA. *J Biochem.* 1991; 109:674–677. [PubMed: 1917889]
66. Plant KE, Hair A, Morgan GT. Genes encoding isoforms of transcription elongation factor TFIIS in *Xenopus* and the use of multiple unusual RNA processing signals. *Nucleic Acids Res.* 1996; 24:3514–3521. [PubMed: 8836176]
67. Kugawa F, Shinga J, Imagawa M, Arae K, Nagano M, Shibata M, Shiokawa K, Aoki M. Cloning of *Xenopus* TFIIS and its expression in oocytes and early embryos. *Biochem Biophys Res Commun.* 1996; 222:541–546. [PubMed: 8670241]
68. Xu Q, Nakanishi T, Sekimizu K, Natori S. Cloning and identification of testis-specific transcription elongation factor S-II. *J Biol Chem.* 1994; 269:3100–3103. [PubMed: 8300645]
69. Umehara T, Kida S, Yamamoto T, Horikoshi M. Isolation and characterization of a cDNA encoding a new type of human transcription elongation factor S-II. *Gene.* 1995; 167:297–302. [PubMed: 8566795]
70. Taira Y, Kubo T, Natori S. Molecular cloning of cDNA and tissue-specific expression of the gene for SII-K1, a novel transcription elongation factor SII. *Genes to Cells.* 1998; 3:289–296. [PubMed: 9685180]
71. Ito T, Xu Q, Takeuchi H, Kubo T, Natori S. Spermatocyte-specific expression of the gene for mouse testis-specific transcription elongation factor S-II. *FEBS Lett.* 1996; 385:21–24. [PubMed: 8641458]
72. Umehara T, Kida S, Hasegawa S, Fujimoto H, Horikoshi M. Restricted expression of a member of the transcription elongation factor S-II family in testicular germ cells during and after meiosis. *J Biochem.* 1997; 121:598–603. [PubMed: 9133631]
73. DiMarco SP, Glover TW, Miller DE, Reines D, Warren ST. Transcription elongation factor SII (TCEA) maps to human chromosome 3p22 → p21.3. *Genomics.* 1996; 36:185–188. [PubMed: 8812434]
74. Ito T, Seldin MF, Taketo MM, Kubo T, Natori S. Gene organization and chromosome mapping of the testis-specific S-II. *Mammalian Genome.* 1998; 9:915–917. [PubMed: 9799847]
75. Weaver Z, Kane CM. Genomic characterization of a testis-specific TFIIS (TCEA2) gene. *Genomics.* 1997; 46:516–519. [PubMed: 9441762]
76. Bentley D. Coupling RNA polymerase II transcription with pre-mRNA processing. *Curr Opin Cell Biol.* 1999; 11:347–351. [PubMed: 10395561]
77. Exinger F, Lacroute F. 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. *Curr Genet.* 1992; 22:9–11. [PubMed: 1611672]

78. Clark AB, Dykstra CD, Sugino A. Isolation, DNA sequence, and regulation of a *Saccharomyces cerevisiae* gene that encodes DNA strand transfer protein. *Mol Cell Biol.* 1991; 11:2576–2582. [PubMed: 1850099]
79. Nakanishi T, Nakano A, Nomura K, Sekimizu K, Natori S. Purification, gene cloning, and gene disruption of the transcription elongation factor S-II in *Saccharomyces cerevisiae*. *J Biol Chem.* 1992; 267:13200–13204. [PubMed: 1618824]
80. Archambault J, Lacroute F, Ruet A, Friesen JD. Genetic interaction between transcription elongation factor TFIIS and RNA polymerase II. *Mol Cell Biol.* 1992; 12:4142–4152. [PubMed: 1508210]
81. Lennon JC III, Wind M, Saunders L, Hock MB, Reines D. Mutations in RNA polymerase II and elongation factor SII severely reduce mRNA levels in *Saccharomyces cerevisiae*. *Mol Cell Biol.* 1998; 18:5771–5779. [PubMed: 9742094]
82. Powell W, Reines D. Mutations in the second largest subunit of RNA polymerase II cause 6-azauracil sensitivity in yeast and increased transcriptional arrest in vitro. *J Biol Chem.* 1996; 271:6866–6873. [PubMed: 8636112]
83. Winston F, Chaleff DT, Valent B, Fink GR. Mutations affecting Ty-mediated expression of the *HIS4* gene of *Saccharomyces cerevisiae*. *Genetics.* 1984; 107:179–197. [PubMed: 6329902]
84. Hartzog GA, Wada T, Handa H, Winston F. Evidence that Spt4, Spt5, and Spt6 control transcription elongation by RNA polymerase II in *Saccharomyces cerevisiae*. *Genes Dev.* 1998; 12:357–369. [PubMed: 9450930]
85. Orphanides G, LeRoy G, Chang CH, Luse DS, Reinberg D. FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell.* 1998; 92:105–116. [PubMed: 9489704]
86. Orphanides G, Wu WH, Lane WS, Hampsey M, Reinberg D. The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. *Nature.* 1999; 400:284–288. [PubMed: 10421373]
87. Otero G, Fellows J, Li Y, deBizemont T, Dirac AMG, Gustafsson CM, Erdjument-Bromage H, Tempst P, Svejstrup JQ. Elongator, a multi-subunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation. *Mol Cell.* 1999; 3:125–129. [PubMed: 10024886]
88. Wittschieben BO, Otero G, de Bizemont T, Fellows J, Erdjument-Bromage H, Ohba R, Li Y, Allis CD, Tempst P, Svejstrup JQ. A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. *Mol Cell.* 1999; 4:123–128. [PubMed: 10445034]
89. Archambault J, Jansma DB, Kawasoe JH, Arndt DT, Greenblatt J, Friesen JD. Stimulation of transcription by mutations affecting conserved regions of RNA polymerase II. *J Bacteriol.* 1998; 180:2590–2598. [PubMed: 9573141]
90. Berroteran RW, Ware DE, Hampsey M. The *sua8* suppressors of *Saccharomyces cerevisiae* encode replacements of conserved residues within the largest subunit of RNA polymerase II and affect transcription start site selection similarly to *sua7* (TFIIB) mutations. *Mol Cell Biol.* 1994; 14:226–237. [PubMed: 8264591]
91. Sun ZW, Tessmer A, Hampsey M. Functional interaction between TFIIB and the Rpb9 (Ssu73) subunit of RNA polymerase II in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 1996; 24:2560–2566. [PubMed: 8692696]

Figure 1.

Structure of transcript cleavage and elongation factors SII and GreA. A) Schematic montage of the tripartite SII domain structure based upon the NMR derived models of domain II of yeast SII (modified from ref. 46) and the domain III zinc ribbon of human SII (modified from ref. 48). Domain I (yellow line) NMR structure will be presented elsewhere (V. Olmsted and C. Arrowsmith, personal communication). The flexible linker between domains II and III is represented as a dashed yellow line. Amino acid side chains that constitute a hydrophobic core of domain II are shown in green, yellow, red, and fuchsia. The approximate positions of critical aspartic (D) and glutamic acid (E) residues in the loop of the zinc ribbon are indicated. The position of four cysteine residues (C) shown chelating

(dotted lines) a zinc ion (sphere) were sketched onto the zinc ribbon structure. The relative sizes of the domains are not to scale. B) Structure of *E. coli* GreA protein as determined by X-ray crystallography (modified from ref. 45). RNA in an elongation complex crosslinks to the tip of the extended α -helical “finger” domain.⁽⁴⁵⁾ The aminoterminal (N) helical finger is responsible for activating transcript cleavage and elongation. The carboxy-terminal (C) globular domain is largely responsible for GreA binding to *E. coli* RNA polymerase.⁽⁴⁵⁾

Figure 2.

Summary of a network of genetic interactions (arrows) between the SII gene *DST1* (triangle), and genes encoding RNA polymerase II subunits (rectangles), chromatin related proteins (circles and ellipse), and subunits of the Elongator complex (squares).