Purified Elongation Factor SII Is Sufficient to Promote Read-through by Purified RNA Polymerase II at Specific Termination Sites in the Human Histone H3.3 Gene*

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Purified RNA polymerase II terminates transcription in vitro at sites within genes which also block transcript elongation in vivo. Studies on a termination site within the first intron of the human histone H3.3 gene have shown that transcription elongation factor SII can promote read-through at this site when the polymerase initiates transcription from a promoter in the absence of the accessory initiation factors. Using 3'-extended templates to direct specific initiation by purified RNA polymerase II, we show here that purified SII is sufficient to effect read-through of this terminator by the purified polymerase alone. Thus, the interaction of purified SII with an elongation complex containing only the polymerase, the template, and the nascent transcript can change the termination properties of RNA polymerase II and can effect read-through of a region that blocks elongation in the cell.

The mechanism controlling the conditional use of such terminators is not known. In some cases, viral proteins have been proposed to act on specific sites in viral genes to modulate transcription read-through (Hay and Aloni, 1985; Kao et al., 1987; Selby et al., 1989; Seiberg et al., 1989). Although the indirect evidence for read-through activity of a human immunodeficiency virus transactivating protein is highly suggestive (Kao et al., 1987; Selby et al., 1989), no direct biochemical evidence supports such a function for this or other eukaryotic viral proteins. There is a purified viral protein that has been shown to cause termination in vitro in response to a specific signal in the early region of the vaccinia genome (Shuman et al., 1987; Shuman and Moss, 1988). Thus it seems likely that proteins responsible for read-through of specific cellular sites may also exist.

For cellular genes, no termination factors specific to particular sites have been reported. However, one protein, SII (transcription factor IIS) (Natori, 1982), can promote read-through at specific sites in vitro within viral (Reinberg and Roeder, 1987; Rappaport et al., 1987) and cellular (Reines et al., 1989) genes when the polymerase has initiated from a general stimulatory initiation factors. Drosophila protein DmS-II also can promote read-through of several sites that cause purified Drosophila RNA polymerase II to pause during transcription (Sluder et al., 1989) although it is not known whether these sites are used by the polymerase in vivo. The mammalian SII protein and its Drosophil analogue DmS-II can also have general stimulatory effects on the elongation reaction of the homologous RNA polymerase II (Natori, 1982; Reinberg and Roeder, 1987; Rappaport et al., 1987; Sluder et al., 1989), as does a yeast protein (P37) (Sawadogo et al., 1980b). In addition, several other activites have been described which also can affect the overall elongation reaction of RNA polymerase II (Lentfer and Lezius, 1972; Seifart et al., 1973; Spindler, 1979; Revie and Dahmus, 1979).

Even though the SII protein has been studied quite extensively, the molecular mechanism by which it exerts its effect is not clear. Proteins containing the general stimulatory activity of SII have been purified from a number of sources (Sekimizu et al., 1976, 1979; Sawadogo et al., 1980b; Reinberg and Roeder, 1987; Rappaport et al., 1987), and a cDNA for mouse SII has been cloned (Hirashima et al., 1988). The yeast and the mouse proteins are able to bind DNA in the presence of very low salt (Sawadogo et al., 1981; Sekimizu et al., 1984), but as each is a basic protein (Sawadogo et al., 1980b; Sekimizu et al., 1981), the significance of this binding is not clear. The mammalian SII proteins can bind directly to purified RNA polymerase II (Horikoshi et al., 1984; Sopita et al., 1985; Reinberg and Roeder, 1987) and to a fusion protein containing a fragment from the largest subunit of that enzyme (Rappaport et al., 1988). The analogous protein from yeast also

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interacts with the largest subunit of yeast RNA polymerase II (Sawadogo et al., 1980b), but unlike the mammalian protein, PS7 also stimulates yeast RNA polymerase I (Sawadogo et al., 1980a, 1981).

Since SII protein can promote read-through of specific sites by promoter-initiated RNA polymerase II in the presence of the accessory initiation factors (Reinberg and Roeder, 1987; Rappaport et al., 1987; Reines et al., 1989), it seemed possible that the initiation factors might be involved in mediating the effect of SII. Alternatively, it was also possible that SII could act directly on purified RNA polymerase II to promote read-through at specific sites used by the cell. We have tested these alternatives directly and have found that purified RNA polymerase II will read through specific sites when purified SII is added to the reaction. The possibility that SII itself controls the utilization of such sites in the cell is discussed.

**EXPERIMENTAL PROCEDURES**

**Materials**

Ribonucleoside triphosphates were purchased from Pharmacia LKB Biosciences, Uppsala, Sweden. [α-32P]CTP (400 Ci/mmol) was obtained from Amersham, Arlington Heights, IL. calf thymus DNA; and 4-5 units (defined below) of purified calf thymus RNA polymerase II. Reactions were incubated for 30 min at 37 °C. Acid-precipitable counts were quantitated as detailed by Dedrick and Chamberlin (1985). One unit of stimulatory activity of the unit defined for the purification of RNA polymerase II (Hodo and Blatti, 1977), and the assay conditions used for the purification of RNA polymerase II.

**Methods**

Purification of SII SII was purified from calf thymus as described (Rappaport et al., 1987). Transcription stimulatory activity was assayed in a nonspecific transcription reaction using sheared calf thymus DNA as template. Assays were incubated in 50-μl reactions containing 10 mM Hepes (pH 7.9), 4 mM MgCl2; 2 mM MnCl2; 50 μM EDTA; 100 μM each of GTP and ATP, GTP, and ATP; and [α-32P]CTP (1000 cpm/pmol); 20 μg/ml calf thymus DNA; and 0.5-4 units (defined below) of purified RNA polymerase II. Reactions were incubated for 30 min at 37 °C. Acid-precipitable counts were quantitated as detailed by Dedrick and Chamberlin (1985). One unit of stimulatory activity of SII was defined as the amount required to stimulate 1 unit of RNA polymerase II to 2 units of activity under the assay conditions described above.

RNA polymerase II was purified by a modification (Kerppola and Kane, 1988) of the procedure described by Hodo and Blatti (1977), which resulted in 50-60% active molecules (determined as described by Kadesch and Chamberlin, 1982). The unit definition used in this manuscript refers to the amount of RNA polymerase II required to incorporate 1 pmol of CMP in 30 min at 37 °C under the conditions used to assay the stimulatory activity of SII. This unit differs from the unit defined for the purification of RNA polymerase II (Hodo and Blatti, 1977), and the assay conditions used for transcription during the SII purification differ from those used for the purification of RNA polymerase II.

Protein concentrations were determined using the Bio-Rad protein assay reagent with bovine serum albumin as the standard. Proteins were analyzed by electrophoresis on SDS-polyacrylamide gels (10%) according to the procedure of Laemmli and Favre (1973). Proteins were visualized by staining with Coomassie Blue or with India ink after the protein was transferred to nitrocellulose (Rappaport et al., 1987).

**Extended Templates**—Plasmid pUCH3Sma contains sequences from the 5′ half of the human histone H3.3 gene inserted into pUC18 such that the intrinsic terminators Tia, Tih, and TII (Reines et al., 1987) are positioned downstream of a unique SmaI site. This plasmid was constructed by deleting the small SmaI fragment from plasmid pUCH3. Plasmid pUCH3 is a construct derived by inserting the EcoRI/SstI fragment of pJU13-149 (Wells et al., 1987) into EcoRI- and SstI-digested pUC18. The template pCpUCH3Sma was prepared essentially as described (Kadesch and Chamberlin, 1982) by linearizing pUCH3Sma with SmaI, adding oligodeoxynucleotide-tydlate residues with terminal deoxynucleotidyl transferase, removing one of the 3′ extensions with XhoI, and creating a run-off template by further digestion with XhoI.

To prepare the template pcyTK243B, the plasmid pTK243B was linearized by digestion with AvaI, and oligodeoxynucleotide extensions were added as described above. The DNA was further cleaved with MluI and SstI to generate a run-off template for unidirectional transcription.

Although transcription from some 3′-extended templates can produce an RNA-DNA hybrid between the nascent transcript and the template strand (Dedrick and Chamberlin, 1985; Kane and Chamberlin, 1986), transcripts produced from these templates in these experiments are displaced from the template during transcription.

**Transcription Reactions**—Transcription was carried out as described previously (Dedrick et al., 1987; Reines et al., 1987) except that the reaction buffer contained 80 mM KCl instead of 150 mM NaCl. In one set of reactions, a ternary complex containing a 16-nucleotide nascent transcript was formed by incorporating the pcvUCH3Sma template with RNA polymerase II in the presence of 20 μM GTP and ATP, 1.5 μM [α-32P]CTP (400 Ci/mmol) for 15 min at 37 °C. Heparin was then added to 100 μg/ml, and aliquots were diluted into a solution containing 0.8 mM ATP, GTP, and UDP; 0.1 mM CTP; and either SII protein or SII storage buffer. The reactions were stopped after 60 min of additional incubation at 37 °C by the addition of 2 volumes of stop buffer containing 20 mM EDTA, 0.5 mM ammonium acetate, and 25 μg/ml carrier RNA. Nucleic acids were resolved by electrophoresis on 6% (w/v) polyacrylamide, 8.3 M urea gels, and visualized by autoradiography. In transcription reactions using pcvTK243B as template, a “chase” protocol described previously (Reines et al., 1987) was followed. After a 90-s incubation at 37 °C, 9 volumes of chase buffer were added which either did or did not contain SII protein. For the time course, samples were withdrawn at various times after the addition of the chase mix and were diluted into ice-cold stop buffer. For the titration study different amounts of SII were included in the chase mix, and reactions were then incubated for 30 min at 37 °C before being diluted into cold stop buffer. Salts effects were examined by including different salts in the chase mix at the indicated concentrations and incubating at 37 °C for 30 min. Reactions were stopped as described above, and nucleic acids were purified by extraction with phenol/chloroform/isoamyl alcohol (25:24:1), precipitated with ethanol, denatured in 80% (v/v) formamide at 90 °C for 5 min, and analyzed on 5% (w/v) polyacrylamide, 8.3 M urea gels. The specific amounts of RNA polymerase II and SII protein included in each experiment are given in the figure legends.

Transcripts were quantitated using the radiolabeled transcription system from AMBIS (San Diego, CA), which determines the cpm present in each transcript on the gel. Termination efficiencies were calculated as detailed previously (Reines et al., 1987) after correcting for the number of CMP residues in each transcript.

**RESULTS**

We have defined previously a number of intrinsic termination sites for purified RNA polymerase II in eukaryotic DNA sequences (Reineco et al., 1987; Kerppola and Kane, 1988). We define an intrinsic termination site as one recognized by RNA polymerase II in the absence of other protein factors. The identification of these sites employed 3′-extended DNA templates with which purified RNA polymerase II can initiate transcription at a specific site in the absence of accessory initiation factors; hence, the properties of the catalytic element of RNA polymerase II can be studied separately from other cellular transcription factors. One set of these intrinsic termination sites falls within the first intron of the human histone H3.3 gene (Reines et al., 1987) in a region that also blocks elongation in Xenopus oocytes and in HeLa cells. The most efficient of these intrinsic termination sites has been designated TIA (Reines et al., 1987, 1989; Kerppola and Kane, 1990). We have shown that this site also blocks elongation

1 The abbreviations used are: Heps, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; SDS, sodium dodecyl sulfate.
2 T. Kerppola and C. Kane, manuscript in preparation.
3 D. Wells, personal communication.
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Read-through at the Tla Site by the promoter-initiated enzyme (Reines et al., 1989). Thus, the pure enzyme and the promoter-initiated enzyme stop at Tla. Further, SII protein can promote read-through at this site by the promoter-initiated polymerase (Reines et al., 1989). Thus, we were in a position to determine unequivocally whether or not SII protein alone could promote read-through at this site or whether accessory initiation factors were also required for read-through activity.

RNA Polymerase II Stops but Does Not Release All Its Transcript at the Tla Site in Vitro—In the course of work testing the effectiveness of the Tla termination site on RNA polymerase II initiated from a promoter in the presence of the general transcription factors, we observed that the enzyme stopped with high efficiency but remained paused at this site in an active stable ternary complex (Reines et al., 1989); that is, the enzyme did not release its transcript to complete the termination process. Previous work testing this termination site with polymerase initiated on 3'-extended templates showed clearly that the enzyme not only stopped but also released its nascent transcript (Reines et al., 1987; Kerppola and Kane, 1990). We suggested several possibilities that could explain the differences in terminator recognition on the two types of templates (Reines et al., 1989).

Although we do not yet understand all the parameters that influence release, we have shown that the reaction conditions themselves determine in part the differential use of the Tla site as a terminator or as a pause site. The enzyme stops efficiently at this site under a wide variety of salt conditions (see below). However, both the rate and the extent of the release reaction can be increased by increasing salt concentrations (Kerppola and Kane, 1990). Since under certain conditions the enzyme stops elongating but does not release all its transcript at this site, the effect of the SII protein on this complex can be tested directly.

Purification of SII Protein—The molecular mass of bovine thymus SII protein has been reported as 38 kilodaltons (Rappaport et al., 1987), and at least two species of SII protein of molecular masses between 34 and 41.5 kilodaltons have been purified from mouse cells (Sekimizu et al., 1976, 1979). The analogous protein from yeast is 37 kDa (Sawadogo et al., 1980a) and from Drosophila, 36 kDa (Sluder et al., 1989). Our final preparation of SII protein contained three polypeptides, with the predominant species having an apparent molecular mass of 33–35 kDa based on its mobility in SDS-polyacrylamide gels (Fig. 1). This polypeptide co-migrates on these gels with calf thymus SII protein provided by Jay Rappaport and Roberto Weinmann (The Wistar Institute, Philadelphia, PA) (data not shown), suggesting that both preparations contain the same purified polypeptide. Mammalian SII is a phosphoprotein, and species of differing mobilities on denaturing gels apparently differ in their phosphorylation patterns (Sekimizu et al., 1981). Other proteins also vary in their mobility on SDS gels depending upon their phosphorylation state (see, e.g. Cadena and Dahmus, 1987). Thus, part of the heterogeneity we observe on this gel could be explained if our preparation of SII contained differentially phosphorylated polypeptides. No other post-translational modifications of this protein have been reported. Our preparation of SII protein, like preparations received from Weinmann and co-workers, is effective in promoting read-through at the histone H3.3 terminator when polymerase has initiated from a promoter (Reines et al., 1989; and data not shown). Thus, we tested it for read-through activity during transcription with highly purified RNA polymerase II.

Purified SII Protein Causes Purified RNA Polymerase II to Read Through the Histone H3.3 Intron Terminator—Transcription was initiated on a 3'-extended template that contained sequences from the first intron of the human histone H3.3 gene. Low concentrations of three of the four nucleotides were used during the first phase of the reaction in order to form a ternary complex containing a nascent transcript 16 nucleotides in length. The reaction was divided in half, and one sample received purified SII protein. Heparin was added to prevent reinitiation by the polymerase. High concentrations of all four nucleotide substrates were also added to permit efficient elongation during the subsequent incubation. The inclusion of SII protein reduced the efficiency with which RNA polymerase II stopped at site Tla (Fig. 2). In this experiment, the stopping efficiency was reduced from 41 to 21% in the presence of SII. In addition, SII could exert its effect even if added after the polymerase had stopped at site Tla (as seen previously with promoter-initiated polymerase (Reines et al., 1989) (Fig. 3A). The same results have been seen with more than one preparation of purified calf thymus protein.

FIG. 1. SDS-polyacrylamide gel electrophoresis of SII and RNA polymerase II. One microgram of protein was applied to each lane of a 10% SDS-polyacrylamide gel. Lane M, molecular mass markers; lane 1, RNA polymerase II; lane 2, SII protein from the final purification step (see below). High concentration of SII protein caused polymerase II to stop efficiently at this site under a wide variety of salt conditions (see below). However, both the rate and the extent of the release reaction can be increased by increasing salt concentrations (Kerppola and Kane, 1990). Since under certain conditions the enzyme stops elongating but does not release all its transcript at this site, the effect of the SII protein on this complex can be tested directly.

FIG. 2. Effect of SII on read-through by highly purified RNA polymerase II. Terminal complexes containing a 16-nucleotide nascent transcript were formed by transcription of template pCpUCH3ASma with purified RNA polymerase II for 15 min at 37 °C as described under “Experimental Procedures.” Elongation in the presence of 0.8 mM ATP, GTP, UTP, and 0.1 mM CTP occurred for 60 min at 37 °C in the presence (+) or absence (−) of 50 ng of SII added to an aliquot containing 46 ng of RNA polymerase II (0.3 units/ml). Transcripts were resolved on a 6% polyacrylamide, 8.3 M urea gel and were detected by autoradiography of dried gels. Transcripts were quantitated using an AMBIS radioanalytic imaging system. The positions of the run-off (RO) and Tla transcripts are indicated.
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FIG. 3. Time course of the effect of SII on RNA polymerase II pausing at the T1a site. A, transcripts were synthesized with RNA polymerase II (5 ng/reaction, 0.35 units/ng) on pCpTK243B using the chase protocol described under "Experimental Procedures." SII protein (30 ng) was (+) or was not (−) added during the chase. The reactions were sampled at various times (times are indicated above the lanes), and transcripts were analyzed by electrophoresis on a 5% acrylamide, 8.3 M urea gel followed by autoradiography. The run-off (RO) RNAs and the transcripts paused at sites T1a, T1b, and T1I (Reines et al., 1987) are indicated. Initiation of chase with the radioanalytical imaging system from AMBIS. To obtain the relative zero time point, H, the gel depicted in A was quantitated using the AMBIS imaging system. Stopping efficiencies were calculated as detailed under "Experimental Procedures" and in the legend to Fig. 3. × refers to reactions containing SII from the first heparin-Sepharose column in the purification; □ refers to reactions containing SII from the second heparin-Sepharose column, the final step of the purification.

FIG. 4. Analysis of the proportionality of read-through activity with the amount of added SII. Transcripts were synthesized using a constant amount of calf thymus RNA polymerase II (5 ng of protein, 0.35 units/ng). Individual reactions contained the indicated amounts of SII protein added along with the chase after 90 s of transcription at 37 °C as described under "Experimental Procedures." Reactions were incubated for 50 min at 37 °C after the addition of the chase. Transcripts were resolved on a 5% polyacrylamide, 8.3 M urea gel and were quantitated using the AMBIS imaging system. Stopping efficiencies were calculated as detailed under "Experimental Procedures" and in the legend to Fig. 3. × refers to reactions containing SII from the first heparin-Sepharose column in the purification; □ refers to reactions containing SII from the second heparin-Sepharose column, the final step of the purification.

SII protein, which supports the idea that read-through activity is a general feature of the purified SII protein. Thus, purified SII protein can promote transcription read-through by purified RNA polymerase II at this site, and factors required for promoter-specific initiation are not required to detect read-through activity promoted by SII protein.

Time Course of Read-through by SII Protein—Ternary complexes were formed with purified RNA polymerase II on template pCpTK243B. The reaction was divided into two parts, and one part received SII protein. Aliquots were removed at various times, and transcripts were resolved on gels. The amount of polymerase stopped at T1a was quantitated as described under "Experimental Procedures." Transcriptional read-through was time dependent and increased for at least 60 min after addition of SII to the reactions (Fig. 3).

Proportionality of SII with Read-through Activity—The read-through activity of SII is proportional to the amount of protein added to the reaction (Fig. 4). However, even at the highest concentration of SII present in these reactions, 15–20% of the transcripts ended at the T1a site. Filter binding assays (Dedrick et al., 1987) were used to show that about half of these remaining transcripts had been released from the polymerase, completing the termination process (data not shown).

If all the protein in our SII fractions were active in the read-through assay, we could estimate the number of SII molecules needed to promote read-through of a fixed number of RNA polymerase molecules in a ternary complex. If all the SII protein added to the reactions were active and if all the RNA polymerase II molecules, active or not, were able to interact with SII, the ratio of SII to RNA polymerase II necessary to effect maximal read-through would be 3:1 (Fig. 4). If SII could only interact with RNA polymerase II engaged in transcription, the ratio could be as high as 10:1 since 30–50% of the polymerase molecules are active. Since SII can bind to RNA polymerase II in the absence of template (Horikoshi et al., 1984; Sopeta et al., 1985; Reinberg and Roeder, 1987), it seems reasonable that the 3:1 ratio would be the more likely; however, without more information on the mechanism by which SII promotes read-through, we cannot estimate accurately the number of active SII molecules. We assume that not all the molecules are active, as there is a gradual loss of activity over time upon freezing and thawing of the protein.

Effect of Ionic Strength on SII Read-through Activity—It has been reported that the nonspecific stimulatory effect of SII on RNA polymerase II is reduced by increasing salt concentrations (Nakanishi et al., 1981a). In addition, salt concentrations of 0.1 M (NH₄)₂SO₄ or 0.2 M NaCl can disrupt the binding of mammalian SII protein to purified RNA polymerase II (Horikoshi et al., 1984; Sopeta et al., 1985). Since the monovalent cation concentration in the mammalian cell is equivalent to approximately 150 mM (Alberts et al., 1989), we tested the effects of various salts on the read-through activity of SII. Although the efficiency with which the polymerase stops at T1a is nearly unaffected by the salt concentrations used in these experiments, both the type of salt and the ionic strength influence the specific read-through activity of SII.
Read-through activity is dramatically reduced with increased concentrations of KCl, NH₄Cl, or (NH₄)₂SO₄. Part of the inhibition of SII activity at higher salt concentrations may be due to its lowered association with the polymerase protein in vitro. However, increased concentrations of potassium glutamate have very little effect on this activity. This result suggests that ionic strength alone is not responsible for inhibition of read-through activity but that particular anions might interfere with the polymerase-SII interaction. Anion-specific effects have been observed for protein-nucleic acid interactions as well (Leirmo et al., 1987).

The primary counteranions in the cell are contributed by carbonates and phosphates as well as acidic residues present on macromolecules (Alberts et al., 1989). Thus, the inhibition of SII read-through activity seen in vitro with increasing concentrations of other counteranions may be distinct from what occurs in the cell. Alternatively, since SII protein presumably functions well in the cell at physiological ionic strength, the in vitro system may lack some accessory component that stabilizes the interaction of SII and RNA polymerase II.

In addition, since increasing ionic strength facilitates the release of the nascent transcript from this site (Kerppola and Kane, 1990), no effect of SII would be expected if a complete termination event had occurred. However, during transcription in 150 mM NaCl or NH₄Cl, little transcript release was observed (data not shown). Therefore, SII did not promote significant read-through at the TJa site in 150 mM concentrations of several salts even though a ternary complex was paused at this site. Thus, the in vitro effect of particular ions or ionic strength seems to be either directly on the SII protein or upon its interaction with the polymerase.

**DISCUSSION**

We have shown that purified mammalian SII protein alone can promote read-through by purified mammalian RNA polymerase II at specific termination sites that also block transcription in the cell. Thus, this type of effect or activity can be communicated directly between RNA polymerase II and the SII protein. These results address a specific, previously unanswered question about whether purified SII protein could function with purified RNA polymerase II to promote read-through of a site used by the cell.

It has been shown previously that pure SII can stimulate transcription by pure RNA polymerase II on nicked and sheared DNA templates (Natori, 1982) and that this effect was conferred upon the elongation reaction. However, transcription upon such templates includes “nicking transcription” (displacement of nontemplate strands to make an RNA-DNA hybrid) (Lavialle et al., 1982), potential RNA-templated transcription in the presence of Mn²⁺ (Zasloff and Felsenfeld, 1977), as well as transcription to produce a displaced nascent transcript. It has also been shown that SII can promote read-through of specific sites when RNA polymerase II has initiated from a promoter in the presence of the collection of initiation factors (Reinberg and Roeder, 1987; Rappaport et al., 1985; Reines et al., 1989). Whether or not such factors were necessary for the read-through activity of SII was the focus of the current study. Recently, purified Drosophila SII was shown to promote read-through by purified Drosophila RNA polymerase II at a number of pause sites in vitro (Sluder et al., 1989). Our results with the purified mammalian proteins acting upon a site used by the cell complement and extend these results with the Drosophila proteins.

Since RNA polymerase II and SII from mammalian cells have been shown to interact (Horikoshi et al., 1984; Supta et al., 1985; Reinberg and Roeder, 1987), our results suggest that SII protein may control directly the use of certain termination sites located within transcription units. The level of transcriptional read-through in vivo may be controlled in part by the abundance of SII protein. The number of molecules of the mammalian protein has been estimated at 120,000 in HeLa cells (Burton et al., 1986) and 360,000 in mouse cells (Tamura et al., 1980). However, the level of mouse SII changes with the differentiation state of the cell in at least two tissues (Tamura et al., 1980). An analysis of the effect of changes in the abundance of SII protein on the amount of read-through of the TJa site in the histone gene in vivo will address this question.

Alternatively, SII activity may be controlled through phosphorylation. SII protein purified from mouse is phosphorylated in the amino-terminal half of the protein (Hirashima et al., 1988). The amino-terminal portion is nearly dispensable for stimulatory activity on nonspecific templates (Nakanishi et al., 1981b). However, the intact protein has been reported to stimulate transcription from several promoters in nuclear extracts only in its nonphosphorylated form (Hirai et al., 1988); the phosphorylated form did not exert this effect. The SII protein we tested here contains predominantly a 33–35-kDa peptide. This protein is likely the nonphosphorylated form based on both SDS-gel mobility and lack of sensitivity to phosphatase treatment (data not shown). Reversible phosphorylation has ample precedent in the control of many biological processes including, perhaps, the functioning of RNA polymerase II itself (Cadena and Dahmus, 1987; Lee and Greenleaf, 1989; Cisek and Corden, 1989; Payne et al., 1988).
Thus, SII phosphorylation might modulate the specific read-through activities intrinsic to this protein.

In addition, other elongation factors or even accessory initiation factors might control SII-mediated read-through activity in the cell. In some cases, the specific promoter from which RNA polymerase II initiates transcription can affect utilization of pause or termination signals downstream (Neuman de Veyvar et al., 1986; Hernandez and Weiner, 1986; Ciliberto et al., 1986; Bentley and Groudine, 1988; Miller et al., 1989; Spencer et al., 1990). Although no other cellular proteins are required to effect read-through at the histone H3.3 T3a site in vitro, such proteins acting at or near the promoter may exert a quantitative effect in the cell.

Additional factors may act more generally, such as human transcription factor IIF (Flores et al., 1989, 1990) and Drosophila factor 5 (Price et al., 1989). Each can bind to RNA polymerase II, and each influences elongation by the polymerase in vitro (Flores et al., 1989; Price et al., 1989). These proteins also are reported to be necessary for promoter-specific initiation (Flores et al., 1989, 1990; Price et al., 1989). A similar or identical factor (RAP30/74) isolated by its affinity for RNA polymerase II contains a DNA helicase activity (Sopha et al., 1989). However, highly purified human transcription factor IIF (Flores et al., 1990) has no detectable helicase activity. The possible effect of a transcription factor IIF-associated helicase on elongation is thus unclear.

Some prokaryotic antitermination proteins require either cis-acting DNA or RNA sequences in the affected transcription units (Friedman and Gottesman, 1983; Platt, 1988; Grayhack et al., 1985; Yang et al., 1987; Roberts, 1988). Whether or not specific nucleic acid sequences or structures are required for SII read-through activity is not known. Results from several laboratorues have shown that SII protein can promote read-through of some sites that stop the polymerase in vitro (Reinberg and Roeder, 1987; Rappaport et al., 1987; Sluder et al., 1989; Reines et al., 1989). However, SII also seems to function much more generally to stimulate transcription by RNA polymerase II on sheared, random sequence DNA templates (Sekimizu et al., 1979; Sawadogo et al., 1980).

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