Transcription Elongation Factor SII (TFIIS) Enables RNA Polymerase II to Elongate through a Block to Transcription in a Human Gene in Vitro*

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Elongation and termination by RNA polymerase II are important regulatory steps for eukaryotic gene expression. We have previously studied the transcription of linear DNA templates where specific initiation of transcription by highly purified RNA polymerase II can be achieved in the absence of promoters and promoter-specific factors. Using these templates we have shown that a human histone gene, H3.3, contains sequences (intrinsic terminators) within which purified RNA polymerase II will efficiently terminate transcription (Reines, D., Wells, D., Chamberlin, M. J., and Kane, C. M. (1987) J. Mol. Biol. 196, 299–312). Curiously, these signals were found within an intron, 3'-untranslated, and protein-encoding regions of the gene suggesting that they might act to attenuate transcription of H3.3 in vivo.

Here we show that intrinsic terminator sequences from an H3.3 gene intron also block in vitro transcript elongation by RNA polymerase II when the enzyme has initiated transcription from a promoter using highly purified transcription initiation factors. However, under the conditions used for promoter-specific transcription there is little transcript release. Instead the polymerase can pause at these sites for periods exceeding 60 min. We have identified and partially purified an activity from HeLa cells that causes the transcription complex to read through this block to transcription elongation. This readthrough activity fractionates with a previously characterized elongation factor (SII) over three chromatographic columns. A homogeneous preparation of calf thymus SII can also provide this activity in trans. This factor may facilitate passage of the RNA polymerase II transcription complex through such intragenic sites in cellular genes in vivo.

The completion of a primary transcript by RNA polymerase II is essential for gene expression. A growing number of genes transcribed by RNA polymerase II are regulated, at least in part, at the level of transcript elongation (Bentley and Groudine, 1986; Eck and Bornkamm, 1986; Nepveu and Marcé, 1986; McGeady et al., 1986; Mechti et al., 1986; Bender et al., 1987; Fort et al., 1987; Kao et al., 1987; McCachren et al., 1988; Watson, 1988; Bhat and Padmanaban, 1988). RNA polymerase II stops transcription within these genes before completing the primary transcript; in many cases the ability of polymerase to read through the elongation block is conditional. The biochemical basis for this type of regulation is not understood.

One valuable approach to understanding the regulatory mechanisms that govern gene expression involves in vitro reconstitution of specific transcription using purified proteins. This approach has been used successfully for dissecting prokaryotic gene regulation (reviewed in Platt, 1986; Hoopes and McClure, 1987; and Yager and von Hippel, 1987). Advances in identifying protein factors required for transcription initiation at promoter sites have been an important step toward this goal in eukaryotes (Samuels and Sharp, 1986; Reinberg and Roeder, 1987a; Reinberg et al., 1987; Conaway et al., 1987; Zheng et al., 1987; Conaway and Conaway, 1989; Flores et al., 1988; Buratowski et al., 1988; Cavallini et al., 1988). However, it seems likely that, as is the case for prokaryotic transcription, multiple factors will also be needed for elongation and termination.

Factors involved in eukaryotic transcription elongation and termination have also been described (Lenifer and Lezius, 1972; Seiffert et al., 1973; Sekimizu et al., 1976; Spindler, 1979; Revie and Dohmus, 1979; Sawadogo et al., 1981; Reinberg and Roeder, 1987b; Rappaport et al., 1987; Hirashima et al., 1987; Shuman et al., 1987; Bartsch et al., 1988; Gottlieb and Steitz, 1989). One protein in particular, SII, influences the elongation reaction carried out by RNA polymerase II (Natori et al., 1973) and has been shown to be important for nuclear RNA synthesis (Ueno et al., 1979). This factor can also influence the ability of RNA polymerase II to read through a transcriptional pause site within the adenovirus genome (Reinberg and Roeder, 1987b; Rappaport et al., 1987).

In our previous studies of transcription elongation and termination we have used an alternative template system that allows highly purified RNA polymerase II to initiate transcription in the absence of any other cellular proteins (Kadesch and Chamberlin, 1982; Dedrick and Chamberlin, 1985; Kane and Chamberlin, 1985; Dedrick et al., 1987; Reines et al., 1987; Kane, 1988; Kerppola and Kane, 1988). Such transcription elongation must involve only the polypeptides present in the RNA polymerase itself. With this system we have identified a number of sites recognized by the purified polymerase as potential intrinsic transcription terminators. At these terminators the purified enzyme stops RNA chain elongation and releases the newly synthesized RNA products. We

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were initially surprised to find that these sites occurred within
the body of a human histone gene (H3.3; Reines et al., 1987).
The strength and location of these intrinsic termination sites
suggested that RNA polymerase II required accessory elonga-
tion factors in transcribing full-length transcripts or that
most transcribing RNA polymerase II molecules fail to com-
plete a full-length transcript. Several observations favored the
notion of an accessory elongation factor. Transient transcrip-
tion experiments suggested to us that these sequences can be
read through in vivo. Furthermore, transcription of this, as
well as other genes, in isolated nuclei also implies that such
factors exist and are important for regulating eukaryotic gene
expression (Bentley and Groudine, 1986; Eick and Born-
kamm, 1986; Nepveu and Marcu, 1988; McGeady et al., 1986;
Mechti et al., 1986; Bender et al., 1987; Fort et al., 1987; Kao
et al., 1987; McCachren et al., 1988; Watson 1988; Bhat and
Padmanabhan, 1988).
We report here on the properties of a promoter-initiated RNA
polymerase II transcription complex that efficiently stops RNA
chain elongation in response to an intrinsic signal in a human
histone gene. Furthermore, we can promote readthrough by this
complex when the reactions are supplemented with fractions from
human cells or with a homogenous preparation of an elongation
factor (SII) from calf thymus.

**EXPERIMENTAL PROCEDURES**

**Materials**

- 3P-Labeled nucleotides were purchased from Amersham Corp.
- Ultrapure (fast protein liquid chromatography-purified) unlabeled
  nucleotides were purchased from Pharmacia LKB Biotechnology Inc.
- a-Amanitin, sodium {\(\alpha\)}-lauroylsarcosine (Sarkosyl), heparin, torula
  yeast RNA, and polyvinyl alcohol were purchased from Sigma.
- Phosphocellulose (P11) and DEAE-cellulose (DE52) were purchased from
  Whatman.
- Single-stranded DNA-agarose was purchased from Bethesda Research
  Laboratories.

**Plasmids and Templates**

The plasmid pAdTerm-2 contains the histone H3.3-intron intronic
terminators T\(_{\alpha}\), T\(_{\beta}\), and T\(_{\gamma}\) (Reines et al., 1987) subcloned
downstream of the adenovirus major late promoter. These histone
sequences reside on a 285-bp T\(_{\alpha}\)q restriction fragment removed from
the genomic subclone pHuH3-640 (Reines et al., 1987). This DNA
fragment contains sufficient sequence information for termination
by highly purified RNA polymerase II at sites Ia, Ib, and I\(_{\beta}\). The
T\(_{\alpha}\)q restriction fragment was inserted into the unique Accl site of the
plasmid pDNAadML (Conaway and Conaway, 1988) carrying a synthetic oligonucleotide containing the
adenovirus-2 major late promoter sequence from -50 to +10 inserted into
the KpnI and XbaI sites of pUC18. A runoff template was
generated from this plasmid by cleaving it with the restriction enzyme
NdeI which cuts the plasmid once (see also Fig. 1A).

Transcription by purified calf thymus RNA polymerase II was
accomplished using a 2'-extended ("tailed") DNA template. This template
was prepared as described by Reines et al. (1987) from the plasmid
pUC18-EF2 provided to us by E. Falck-Pederson (Cornell University
Medical College, New York). This plasmid contains mouse &major
intron sequence and an oligonucleotide containing the
adenovirus-2 major late promoter sequence from -50 to +10 inserted into
the KpnI and XbaI sites of pUC18. A runoff template was
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the KpnI and XbaI sites of pUC18. A runoff template was
generated from this plasmid by cleaving it with the restriction enzyme
NdeI which cuts the plasmid once (see also Fig. 1A).

**In vitro Transcription Reactions**

**Transcription on 3'-Extended Templates**—The labeled template
described above (pUC18-EF2) was transcribed with purified calf thymus
RNA polymerase II as described previously (Reines et al., 1987). Two
25-µl transcription reactions were assembled in transcription buffer
(70 mM Tris-HCl (pH 8.0), 150 mM NaCl, 20% (v/v) glycerol, 6 mM
MgCl\(_2\), 5 mM spermidine, 0.15 mM dithiothreitol) containing 800 µM
each of ATP, UTP, and GTP, 20 µM [\(\alpha\)\(^32\)P]CTP (60,000 cpm/pm),
0.5 µg of labeled pUC18-EF2 prepared as described above, and 12.5
milliunits of calf thymus RNA polymerase II (1 unit is the amount of
enzyme needed to incorporate 1 nmol of CMP into acid-insoluble
form in a 10-minute reaction under the standard conditions described
by Hodo and Blatti (1977) using sheep cerebral cortex RNA as a
template). The reaction was started by incubation at 37°C for 5
minutes and samples were withdrawn from each reaction after 1 minute
and made 220 µg/ml in yeast RNA and 100 µM in EDTA (final volume of 260 µl)
on ice. Forty-five minutes later the remainder of each reaction was
incubated for 45 minutes at 37°C. 50-µl aliquots were withdrawn at various
times and placed into the EDTA/ RNA solution on ice as described
above for the 1-min samples. Nucleic acids were extracted with
phenol:chloroform, precipitated with ethanol and analyzed by elec-
trophoresis and autoradiography as described below.

**Transcription in HeLa Nuclear Extracts**—HeLa cells were grown
in spinner culture at 37°C to a density of 4·6·10\(^5\) cells/ml in
Joklik’s modified Eagle’s medium supplemented with 2 mM glutamine
and 5% calf serum. Extracts from HeLa cell nuclei were prepared,
dialyzed into buffer "D" (20 mM Tris-HCl, pH 7.9, 20% glycerol (v/v),
0.1 M KCl, 0.2 mM EDTA, 1 mM dithiothreitol), and used for
in vitro transcription essentially as described by Dignam et al. (1983).
 Fifty microliter reactions contained NdeI-cleaved pAdTerm-2 and
50% (v/v) nuclear extract protein (250 µg). RNA was synthesized in
vitro for 50 min at 30°C and was labeled with [\(\alpha\)\(^32\)P]GTP at 25 µM
in vitro transcription reaction buffer containing 800 µM each of
ATP, UTP, and GTP, 100 µM unlabeled CTP, and 100 µg/ml heparin.
Radioactive RNA was isolated using phenokchloroform extraction and
ethanol precipitation, washed twice, and dissolved in H\(_2\)O.

**Plasmid pAdTerm-2** contains the histone H3.3-intron intronic
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the KpnI and XbaI sites of pUC18. A runoff template was
generated from this plasmid by cleaving it with the restriction enzyme
NdeI which cuts the plasmid once (see also Fig. 1A).
Factor-mediated Elongation by RNA Polymerase II

A standard reaction included: a 20-μl preincubation step containing 0.4 milligrams of rat liver RNA polymerase II, 100–200 ng of pADTerm-2 DNA (cut with NdeI), fraction D (2–4 μg), and 5 units of placentatic ribonuclease inhibitor in 20 mM Hepes-NaOH, pH 7.9, 20 mM Tris-HCl, pH 7.9, 2% polyvinyl alcohol (w/v), 0.4 mg/ml bovine serum albumin, 0.15 M KCl, 2 mM dithiothreitol, and 7% (v/v) glycerol. The mixture was incubated at 28 °C for 30 min. The reaction was then diluted with 33 μl of a solution containing fraction B’ (1 μl) and 1 ng of α-polypeptide in the same buffer lacking KCl and incubated for another 20 min. Magnesium chloride, ATP, UTP, and CTP were added in a volume of 6 μl to final concentrations of 7 mM, 20 μM, and 20 μM, respectively. Incubation proceeded under these limiting nucleotide conditions for another 20 min. This procedure resulted in the formation of elongation complexes containing a 14-nucleotide transcript (the first GTP in the transcript is at position 15). HeLa protein or purified calf thymus RNA polymerase II was added at the start of the elongation phase of the reaction. Reactions were stopped with an equal volume of proteinase K buffer (2% sodium dodecyl sulfate (w/v), 0.25 M Tris, pH 7.5, 25 mM EDTA, 300 mM NaCl), 5 μg of proteinase K, and 10 μl of RNA and incubated at room temperature for 5 min. Nucleic acids were precipitated with ethanol and prepared for electrophoresis and autoradiography as described below.

Fractionation of HeLa Nuclear Extract Proteins

HeLa nuclear extract protein was prepared from 30 liters of cells as described by Dignam et al. (1983). The protein was chromatographed over F11, DE22, and single-stranded DNA-agarose as described by Reimberg and Roeder (1987b) for the purification of TFIIIS.

Electrophoresis and Autoradiography

Generally, electrophoresis was carried out on 5% polyacrylamide gels (38:2, acrylamide:bisacrylamide) in TBE (89 mM Tris, 89 mM boric acid, pH 8.0, 1 mM EDTA) containing 3.3 M urea. Ethanol precipitates of nucleic acids were dissolved in TBE, 80% formamide, 0.025% xylene cyanol, and 0.025% bromphenol blue, heated at 95 °C for 5 min, and chilled on ice. Twenty-centimeter gels were run in TBE until the xylene cyanol dye was approximately 15 cm from the origin. Gels were dried onto Whatman No. 3MM paper. In some cases, discontinuous polyacrylamide/urea gels (gels containing a 5% polyacrylamide gel poured on top of a 15% polyacrylamide gel) were used to resolve both small (14 nucleotides) and large (530 nucleotides) transcripts. These gels were not dried. All gels were exposed to X-Omat film (Kodak) at –80 °C with an intensifying screen (DuPont). Marker RNAs of known sizes were prepared by in vitro transcription of plasmids pKK5-1 (+15) and pKK4-121 (+15) (lanes M) by B. Broissius, T. Thayer, and B. Broissius, 1985 with Escherichia coli RNA polymerase holoenzyme purified by the method of Gonzalez et al. (1977).

RESULTS

Transcription Elongation In Vitro Studied with a Purified Promoter-initiating System—In prior studies we have demonstrated that purified RNA polymerase II can cease transcription elongation and release RNA chains at discrete sites within a human histone gene (Reines et al., 1987). Because the polymerase stops transcription and releases its transcripts, we have termed such sites intrinsic termination sites. Such studies of transcription using extended templates assume that the properties of elongating RNA polymerase II are similar regardless of whether the enzyme has initiated transcription from a promoter with initiation factors or from a 3′-extended template in the absence of initiation factors. To test this directly, we have analyzed transcription through three sites in an intron of the human histone H3.3 gene (Ia, Ib, and Ii; Reines et al., 1987) by RNA polymerase II which had initiated transcription from the adenovirus major late promoter in the presence of additional factors (Fig. 1A). This promoter is well characterized and relatively strong in vitro.

Accurate transcription initiation by RNA polymerase II can be reconstituted from purified chromatographic fractions derived from animal cells (Matsui et al., 1980; Tsai et al., 1981; Dynan and Tjian, 1983; Samuels et al., 1982; Davison et al., 1983; Parker and Topol, 1984; Conaway et al., 1987). However, in order to distinguish the activity of initiation factors from that of potential elongation factors it was important to obtain the initiation factors in highly purified form. As well, relatively large amounts of initiation factors were needed for biochemical studies of termination.

Recently, a highly purified specific transcription system for RNA polymerase II has been described (Conaway et al., 1987; Conaway and Conaway, 1988, 1989). These workers exploited rat liver as an inexpensive and abundant source of material from which large amounts of transcription factors could be extensively purified. With these proteins, RNA polymerase II can initiate transcription from the adenovirus major late promoter and transcribe through the histone intron termination sites (Fig. 1A). Elongation by RNA polymerase II was studied using an experimental protocol limiting transcription to a single round of initiation events. This was achieved by forming defined ternary complexes (DNA, RNA, and protein) arrested at a unique position on the template (+15) and preventing subsequent reinitiation by RNA polymerase II (see Fig. 1B and "Experimental Procedures").

An analysis of transcription elongation from the adenovirus major late promoter demonstrated that RNA polymerase II stops elongation at the same sites previously characterized as intrinsic termination sites (Fig. 2A). The precise location of the 3′ termini of these transcripts was confirmed using S1-nuclease protection experiments. Highly purified calf thymus RNA polymerase II also stopped at these sites when it was substituted for the partially purified rat liver RNA polymerase II used in the reconstituted transcription reaction.

These initial studies were done using relatively low concentrations of NTPs, the conditions employed in earlier studies of transcription initiation in the rat liver system (Conaway et al., 1987). However, it is known that pausing in vitro by RNA polymerase II is sensitive to NTP concentrations. This led us to test the effect of increasing concentrations of NTPs on elongation in this system.

Under these conditions the pattern of transcription elongation and termination was significantly changed (Fig. 2B). RNA polymerase II elongated efficiently through sites Ib and Ii although site Ia still halted transcription elongation. This raised the question of whether the cessation of elongation in the earlier experiments using low levels of NTPs was due to true termination or to long pausing at these sites. In prior studies we have followed transcript release by nitrocellulose filter binding. However, this method is not applicable to the current experiments since the high levels of protein in the reactions interfere with this assay.

To attempt to distinguish between long pausing and termination/release, transcription was allowed to proceed at a low concentration of nucleotides until RNA polymerase II had reached sites Ib, II, and Ia. The nucleotide concentration was then increased (Fig. 2C). The results show clearly that transcripts associated with sites II and Ib were chased to larger sizes. Transcripts in band Ia were not chased under these conditions; however, we show below that these transcripts can also be further elongated in the presence of a protein factor. Hence all of the transcripts with 3′ ends at sites Ib, II, and Ia are components of paused ternary com-
pAdTerm-2 DNA as described under “Experimental Procedures.”

Promoter molecules. Elongation phase: ATP, UTP, CTP, and GTP were added to 800 μM unless otherwise indicated, and incubation continued at 28°C as described under “Experimental Procedures.”

Note that there is no ability that transcript Ia had been synthesized in the presence of HeLa protein but was exceptionally sensitive to degradation. To test for RNA degradation, transcript Ia was synthesized by RNA polymerase II in a reconstituted rat liver transcription reaction in the absence of the HeLa nuclear proteins; α-amanitin and the nuclear extract were added and incubation was continued. Although some transcript breakdown occurs during this prolonged incubation, no preferential degradation of transcript Ia was observed (Fig. 4, lane 5). This makes it unlikely that polymerase had stopped transcription at site Ia but that the transcripts with 3'-ends at site Ia were completely degraded. We concluded, therefore, that the HeLa extract contained a “readthrough” factor for RNA polymerase II.

A Complementation Assay for Readthrough Activity—Using these observations we developed a complementation assay for “readthrough” activity, since RNA polymerase II, which initiated transcription using purified rat liver initiation factors, efficiently stopped transcription at histone site Ia (Fig. 2) and HeLa cell extracts contained a trans-acting factor which enabled RNA polymerase II to read through this block to elongation (Fig. 3). Increasing amounts of HeLa cell nuclear extract were added to transcription reactions containing ternary complexes formed with RNA polymerase II and rat liver initiation factors (see Fig. 1B). Elongation was continued in the presence of the HeLa protein and all four nucleotides. Essentially all polymerase molecules were able to elongate past site Ia when 5 μg or more of HeLa protein were added to the elongation phase of the reaction (Fig. 4, lanes 1–4). These results show that HeLa nuclear extracts contain an activity that can allow RNA polymerase II to read through a block to elongation in a human gene and that this activity could be titrated. Further, actively elongating RNA polymerase II was required to obtain readthrough activity as amanitin-treated ternary complexes was continued for 30 min at 28°C. In some experiments HeLa cell extract protein (Figs. 4 and 8), chromatographically fractionated HeLa protein (Fig. 5), or pure SII protein (Fig. 6) was added during the elongation phase.
FIG. 2. Transcription of intrinsic termination sites by RNA polymerase II using purified rat liver transcription initiation factors. A, elongation at "low" nucleotide concentrations. Transcription of the plasmid pAdTerm-2 (Fig. 1A) was carried out with purified transcription initiation factors from rat liver (Conaway et al., 1987). A 300-μl (5 standard reaction volumes) reaction was assembled as described (Fig. 1B and "Experimental Procedures"). After ATP, UTP, and [α-32P]CTP were added, incubation proceeded until ternary complexes had been formed (see legend to Fig. 1B). A 60-μl aliquot was removed and diluted into proteinase K buffer as described under "Experimental Procedures" (0'). Sarkosyl (0.24%) was added to prevent the reinitiation of transcription by additional RNA polymerase II enzymes. Unlabeled GTP and CTP were added to final concentrations of 200 and 620 μM, respectively, while the final concentrations of ATP and UTP remained at 20 μM during elongation. The reaction was incubated at 28 °C. At the indicated times, 60-μl samples were withdrawn, and each was diluted into proteinase K buffer as described under "Experimental Procedures." Nucleic acids were isolated from the sample taken at each time point and electrophoresed on a discontinuous gel system consisting of a 5% polyacrylamide gel stacked on a 15% polyacrylamide gel. The interface between the 5 and 15% gels is indicated to the left of the figure. The migration positions of radiolabeled RNAs of known size that were also run on this gel are indicated. The RNA species with 3'-ends at intrinsic termination sites Tm, Tb, and Tn are so indicated. B, elongation at "high" nucleotide concentration. This experiment was carried out identically to that described in part A above, except that heparin (10 μg/ml) was added to prevent reinitiation. The final concentration of each NTP during the elongation phase was 780 μM each for ATP and UTP and 770 μM each for GTP and CTP. The 14-nucleotide transcript synthesized by ternary complexes before the elongation phase of the reaction was started is indicated (14mer). The discontinuous polyacrylamide gel (5%/15%) system was also used here. C, ternary complexes at sites Ib and II can continue elongation after increasing the nucleotide concentration. Ternary complexes were formed as described in part A. The reaction was then brought to 200 μM in GTP and 620 μM in unlabeled CTP; the concentration of ATP and UTP remained unchanged. Elongation under these conditions took place for 30 min. Half of the reaction was then quenched (lane marked LO). The remainder of the reaction was brought to 760 μM each in ATP and UTP, 1 mM in GTP, and 1.4 mM in CTP, and incubation proceeded for another 20 min (lane marked HI). RNA was isolated from both samples and analyzed on a 5% polyacrylamide gel as described under "Experimental Procedures." Lane M', RNA markers of 260, 380, 420, and 540 nucleotides.
Factor-mediated Elongation by RNA Polymerase II

HeLa

DNA (µg/ml): 8 16 24 24

runoff

FIG. 3. RNA polymerase II in a HeLa nuclear extract does not stop transcription at the intrinsic termination sites. An extract from HeLa cell nuclei was prepared essentially as described by Dignam et al. (1983). Transcription reactions (Dignam et al., 1983) contained the concentrations of DNA (NdeI-cleaved pAdTerm-2) indicated at the top of the figure. In this experiment RNA was synthesized in a single incubation reaction with all four NTPs. The resulting RNA was uniformly labeled with 25 µM [α-32P]GTP. ATP, UTP, and CTP were present at 600 µM each. α-Amanitin (1 µg/ml) was included from the start of the reaction in the lane labeled +α. Labeled RNA was isolated after 50 min of transcription and analyzed by electrophoresis on a 5% polyacrylamide gel and autoradiography. The runoff transcript and sizes of reference RNAs that were run on this gel are indicated.

stopped at site Ia did not produce longer transcripts (Fig. 4, lane 5).

It is interesting to note that HeLa readthrough activity was also effective in allowing RNA polymerase to efficiently elongate past the site defined by the 325-nucleotide transcript with a 3’-end in vector sequences (see above and Fig. 4).

Identification of Readthrough Activity—Natori and co-workers first reported the purification and characterization of a protein, SII, that stimulates transcription by RNA polymerase II (reviewed in Natori, 1982). This factor also enables

RNA polymerase II to efficiently read through a well characterized pause site within the adenovirus major late transcription unit (Maderius and Chen-Kiang, 1984; Mok et al., 1984; Hawley and Roeder, 1985; Reinberg and Roeder, 1987b; Rappaport et al., 1987). Therefore, we wanted to determine whether the readthrough activity identified here for histone

FIG. 4. Complementation assay for readthrough activity. A 540-µl reaction (9 standard-reaction volumes) was prepared as described under “Experimental Procedures,” and ternary complexes were formed as described in the legend to Fig. 1B under “Experimental Procedures.” The reaction was separated into 60-µl aliquots on ice and made 400 µM in all 4 NTPs and 10 µg/ml in heparin. Sixty microliters of buffer D (see “Experimental Procedures”) containing the indicated amounts of HeLa nuclear extract protein were added to each reaction aliquot (lanes 1–4). Incubation was continued for 30 min. The RNA was extracted with phenolchloroform and precipitated with ethanol. Labeled RNA was analyzed by gel electrophoresis and autoradiography as described under “Experimental Procedures.” In lane 5 one aliquot of “ternary” complexes was incubated for 30 min with 400 µM NTPs without HeLa protein. α-Amanitin (1 µg/ml) was added followed by the HeLa protein, and the incubation was continued for an additional 30 min before processing for electrophoresis. The runoff transcript and transcript ending at site Ia are indicated to the right of the figure. RNA transcripts of 260, 380, 420, and 540 nucleotides were run in lane M’.
site Ia was similar to elongation factor SII.1

To this end we fractionated the HeLa nuclear extract as described by Reinberg and Roeder (1987b) for the purification of SII. On phosphocellulose, DEAE-cellulose, and single-stranded DNA-agarose, readthrough activity partitioned with fractions reported by Reinberg and Roeder (1978b) to contain SII (Fig. 5).5

These results suggested that at least part of the readthrough activity detected in HeLa cell nuclear extracts was due to elongation factor SII. In fact, the addition of NTPs and a homogeneous preparation of calf thymus SII (Rappaport et al., 1987) to preformed rat liver ternary complexes resulted in virtually quantitative readthrough of site Ia (Fig. 6A, compare lanes 2 and 3).

This result demonstrated that SII, added after 13 phospho-

FIG. 6. Readthrough activity of pure calf thymus SII protein. A, elongation in the presence and absence of SII. Ternary complexes were formed as described under “Experimental Procedures” and the legend to Fig. 1B. No protein (−), SII buffer (buf., see below), or 50 ng of SII protein (SII) were added to aliquots of ternary complexes. Each reaction was also made 800 μM in ATP, CTP, UTP, and GTP, and incubation was continued for 30 min. RNA was isolated and analyzed by electrophoresis as described under “Experimental Procedures.” In lanes 1–3, heparin (10 μg/ml) was added to the ternary complexes to prevent reinitiation. In lanes 4–6 Sarkosyl (0.25%) was added to the ternary complexes to prevent reinitiation. Runoff transcripts and transcript Ia are designated on the left of the figure. Marker RNAs were run in lanes M and M′ as described above. B, histone site Ia is a pause site for RNA polymerase II. Ternary complexes treated with heparin (10 μg/ml) were added to the ternary complexes to prevent reinitiation. Runoff transcripts and transcripts with 3′ termini at sites Ia are indicated. RNAs of known sizes (505, 625, 665, 785, and 1620 nucleotides) appear in lane M.

FIG. 5. Fractionation of HeLa readthrough activity. HeLa nuclear extract was chromatographed on phosphocellulose and DEAE-cellulose as described by Reinberg and Roeder (1987b). No protein (−) or 1 μg of protein from the most concentrated fraction of each step-eluted protein peak was assayed for readthrough activity in the elongation phase of a standard rat liver transcription reaction (as described for the unfractionated nuclear extract in Fig. 4). Fractions from phosphocellulose chromatography (column output, flow-through, 0.3 M, 0.5 M, and 1 M KCl eluates) were designated ON, P.1, P.3, P.5, and P.7, respectively. Fractions from DEAE-cellulose chromatography (column output, flow-through, and 0.3 M KCl eluate) were designated ON, DE.1, and DE.3, respectively. Runoff transcripts and transcripts with 3′ termini at sites Ia are indicated. RNAs of known sizes (505, 625, 665, 785, and 1620 nucleotides) were run in lane M.
whether an active elongation complex was arrested at site Ia, termination at sites Ib and I1 was instead due to formation of that transcript Ia was still associated with an active enzyme reaction to exert its effect during elongation. Therefore, this factor can exert its effect on the stalled complex. This raised the question of whether stallI was added to the reaction after transcript Ia had been synthesized (Fig. 6B). Since SII enabled RNA polymerase II to continue chain elongation beyond site Ia, we can conclude that transcript Ia was still associated with an active enzyme complex. Therefore, this factor can exert its effect on the stalled complex.

**The Effect of Sarkosyl on the Readthrough Activity of SII**—Sarkosyl (N-lauroylsarcosine) is an anionic detergent that has been extensively used to study transcription initiation and elongation (Gariglio et al., 1974, 1981; McKnight and Paltmier, 1979; Ackerman et al., 1983; Coppola and Luse, 1984; Tolunay et al., 1984; Hawley and Roeder, 1985, 1987; Conaway and Conaway, 1988; Zhang-Keck and Stallcup, 1988). Initiated RNA polymerase II molecules are resistant to relatively high levels of this detergent. Uninitiated or “free” RNA polymerase molecules are sensitive to inactivation by Sarkosyl (Gariglio et al., 1974). In addition, Sarkosyl has been reported to cause enhanced pausing or premature termination at a specific site within the adenovirus major late transcription unit (Hawley and Roeder, 1985). This could result from an effect of Sarkosyl on either RNA polymerase or an associated elongation factor. Our use of purified transcription systems, one that uses only RNA polymerase II protein (3′-extended templates; Reines et al., 1987) and one that utilizes the polymerase and accessory initiation factors (this report), provided an opportunity to identify the target of Sarkosyl action.

To see if Sarkosyl affects the polymerase itself, we first transcribed 3′-extended templates with highly purified calf thymus RNA polymerase II in the presence and absence of Sarkosyl. When added before nucleotides, Sarkosyl (0.25%) serves to inhibit the initiation of transcription by RNA polymerase II on 3′-extended templates as has been shown for initiation at promoter sites (Hawley and Roeder, 1985). Conversely, the addition of Sarkosyl to the elongation phase of the transcription reaction (after the addition of nucleotides) did not result in a significant change in the average elongation rate of RNA chains (Fig. 7). Sarkosyl-induced pausing was not observed either. Therefore, Sarkosyl did not serve to generally disable the elongation capacity of purified RNA polymerase II. This suggested that the exaggerated transcriptional pausing brought about by Sarkosyl (Hawley and Roeder, 1985) resulted from an interaction of Sarkosyl with a component of the transcription machinery other than RNA polymerase II.

We wanted to test directly whether Sarkosyl inhibited the readthrough function of SII. This seemed likely given the following observations. First, in the absence of SII, RNA polymerase II pauses at specific sites within the adenovirus major late transcription unit (Reinberg and Roeder, 1987b). The addition of SII (Rappaport et al., 1987; Reinberg and Roeder, 1987b) has been shown to provide RNA polymerase II with the ability to read through these pause sites. Second, Sarkosyl dramatically enhanced the pausing at these sites when transcription was reconstituted from chromatographic fractions containing partially purified HeLa initiation factors and SII (Hawley and Roeder, 1985). When Sarkosyl was added to the elongation phase of a reconstituted rat liver transcription reaction (Fig. 6A, lanes 4 and 5), polymerase molecules accumulated at site Ia to the same extent as the control (Fig. 6A, lanes 1 and 2). Therefore, Sarkosyl does not influence stopping at site Ia in this purified system. However, when the reconstituted rat liver transcription reaction was supplemented with SII, Sarkosyl inhibited the ability of this protein to provide readthrough activity (Fig. 6A, compare lanes 3 and 6). This same level (0.25%) of Sarkosyl also inactivated the readthrough activity present in a HeLa nuclear extract (Fig. 8). Thus Sarkosyl, at levels which have been shown to cause the accumulation of prematurely terminated (or paused) transcripts in the adenovirus major late transcription unit (Hawley and Roeder, 1985), prevented SII from promoting readthrough at site Ia. It is reasonable to conclude that the inhibitory effect of Sarkosyl on SII function is responsible for the apparent enhancement.

**FIG. 7.** Sarkosyl does not affect transcription elongation by purified RNA polymerase II on a “tailed” template. Transcription using purified RNA polymerase II on a tailed template (plasmid pUC18EF-2, a subclone of the mouse a-major globin gene) was carried out as described under “Experimental Procedures.” RNA was synthesized in vitro in two separate (+ and −) two-part reactions such that ternary complexes could be formed and chased. During the “chase” phase of transcription (after dilution, see “Experimental Procedures”) one reaction was made 0.23% (w/v) in Sarkosyl (+), and the other was not (−). At the indicated times after the start of the reactions, samples were withdrawn and RNA was isolated and analyzed by electrophoresis on a 5% polyacrylamide gel as described under “Experimental Procedures.” RNAs of known sizes were run in lanes M and M′.
of pausing observed in the adenovirus transcription unit (Hawley and Roeder, 1985).

**DISCUSSION**

Using 3'-extended templates we and others have studied important aspects of transcription elongation and termination by purified RNA polymerase II (Kadesch and Chamberlin, 1982; Price and Parker, 1984; Dedrick and Chamberlin, 1985; Kane and Chamberlin, 1985; Coulter and Greenleaf, 1985; Dedrick et al., 1987; Reines et al., 1987; Reinberg and Roeder, 1987b; Lorch et al., 1987; Kane, 1988; Kerppola and Kane, 1988; Sluder et al., 1988; Mougey and Dennis, 1988). These studies have been useful in understanding the activities carried out by purified RNA polymerase II. For example, we have obtained provocative evidence that sequences within eukaryotic genes block transcript elongation and appear to have physiological significance (Reines et al., 1987; Kerppola and Kane, 1988).

This approach is predicated on the assumption that the elongating enzyme faithfully responds to template signals regardless of the means by which it has initiated transcription (i.e. from a promoter or 3'-extended template). In this report we have explicitly tested this assumption. We have found that intragenic signals originally defined on 3'-extended templates which block transcription through a human histone gene also serve as a block to elongation for RNA polymerase II which had initiated from a promoter. These findings support the validity of using 3'-extended templates to study transcription by purified RNA polymerase.

Surprisingly, although sites Ia, Ib, and II stop RNA polymerase II, there is an unexpected difference in the transcript release reaction at these sites with the promoter-dependent system as compared to our previous studies with 3'-extended templates. In particular, transcription using the promoter-initiated system leads to extended pausing at the same sites at which we demonstrated termination/transcript release with 3'-extended templates. Further analysis has confirmed that release does occur on several different 3'-extended templates.

There are at least three differences between the two transcription systems that could influence transcript release. First, the initiation reaction may influence the recognition by RNA polymerase of a site as a terminator or a pause. The accessory factors required for promoter-specific initiation may alter the conformation or subunit composition of the elongating polymerase in a way that influences the release reaction at sites which stop the enzyme. Whereas SII can allow read-through of such sites, there may be proteins involved in the initiation reaction which associate with the elongating polymerase to stabilize the ternary complex when it stops at such sites.

Second, the reaction conditions differ rather dramatically between transcription on the 3'-extended and promoter-containing templates. For example, the ionic strength of the rat liver reconstituted transcription reaction is lower (60 mM KCl) than that previously used with 3'-extended templates (150 mM NaCl). The termination reaction of purified E. coli RNA polymerase can show extremely large changes when the ionic conditions are altered (Neff and Chamberlin, 1980). In addition, changes in ionic strength can specifically affect the transcript release reaction of the bacterial enzyme. The effect of ionic strength on the elongation reaction of purified RNA polymerase II is currently being examined in detail.

Third, the tetrapetide constructions studied here differ from those used previously. Thus the transcripts synthesized in the two different in vitro experiments contain different nucleotide sequences. For purified E. coli RNA polymerase, the first 20-25 nucleotides in the transcription unit can alter the termination properties of the enzyme at sites hundreds of nucleotides farther downstream (Telesnitsky and Chamberlin, 1989; Goliger et al., 1989). The mechanism of this effect is not known. Further, it is possible that transcript release may be a function of primary sequence or specific secondary structures in the nascent transcript as has been shown for E. coli RNA polymerase. While we know that specific RNA secondary structures are not required to stop RNA polymerase II (Dedrick et al., 1987; Reines et al., 1987; Kerppola and Kane, 1988; Mougey and Dennis, 1988). These
1988), we do not yet know if particular features of the template or nascent transcript may affect the transcript release reaction. All three of these possible explanations are being investigated.

Despite the fact that we have not yet defined exactly which parameters control transcript release, site Ia clearly serves as a strong block to elongation through this histone gene in both purified transcription systems. While RNA polymerase II pauses briefly at many sites during transcription, the very long pausing at sites Ia, Ib, and II is unusual. Indeed site Ia serves to block transcription in vitro for hours while RNA polymerase remains in a potentially active ternary complex that can respond to elongation factors. Experiments analyzing transcription of H3.3 in isolated nuclei from HeLa cells also suggest that transcript elongation is blocked within this H3.3 intron. In vivo, a paused complex may serve as an intermediate to a termination event, i.e., as a target for the influence of regulatory factors. A polymerase elongation complex poised within a transcription unit could likely respond to changing cellular conditions more rapidly than one which must reassociate with other proteins at the promoter prior to initiation. Thus, a cell might carefully control whether to prematurely terminate within a gene or to pause during the course of transcription across a gene.

We have shown here that extracts from mammalian cells of numerous species serve as a source of factors that permit RNA polymerase II to read through histone site Ia. Rat liver appears to contain an SII-like readthrough activity as do human and bovine cells. SII-like proteins have been identified in yeast, insect, and mammalian cells by immunological or chromatographic criteria (Sekimizu et al., 1979; Sawadogo et al., 1981; Egyhazi et al., 1984; Rappaport et al., 1987; Reinberg and Roeder, 1987b). Since readthrough activity (SII) from various species can interact with RNA polymerase II from heterologous species (this report, Rappaport et al., 1987), it appears that this feature of the transcription machinery is conserved at least between mammals. These results further define transcriptional readthrough as an important process in controlling eukaryotic gene expression.

Transcriptional pause sites for promoter-initiated RNA polymerase II have previously been detected in viral sequences through the use of purified HeLa transcription initiation factors (Hawley and Roeder, 1985; Reinberg and Roeder, 1987b; Rappaport et al., 1987). In particular, a site in the adenovirus major late transcription unit stops the enzyme, and SII is effective in allowing RNA polymerase II to read through this site (Hawley and Roeder, 1985; Reinberg and Roeder, 1987b, Rappaport et al., 1987). It is plausible that sites such as that described in adenovirus and H3.3 occur more generally in other genes. However, they may have gone undetected since many investigators use unfractionated cell extracts containing readthrough activity, truncated (run-off) templates, and indirect assays (primer extensions, nuclease analysis) to study transcription in vitro; all of these may preclude the detection of polymerase stop sites.

Transcription in isolated nuclei also suggests that the readthrough process is important in regulating the synthesis of transcripts from adenovirus (Maderios and Chen-Kiang, 1984; Mok et al., 1984) and histone H3.3* as well as other genes (Bentley and Groudie, 1986; Eick and Bornkamn, 1986; Nepveu and Marcu, 1986; McGeady et al., 1986; Mecht et al., 1986; Bender et al., 1987; Fort et al., 1987; McCaethen et al., 1988; Watson, 1988; Bhat and Padmanaban, 1988). It will be important to test whether SII, or SII-like proteins, are involved in the regulation of transcription in vivo and if this type of mechanism may be more generally utilized in cells. It is possible that the ability of a cell to properly express genes is determined by the abundance, or post-translational modification, of SII. Although SII can be phosphorylated in vivo (Egyhazi et al., 1984; Hirashima et al., 1985), the effect of this modification on readthrough activity remains to be determined.

How does SII operate to promote transcriptional readthrough? Previous work has shown that SII can bind to purified RNA polymerase II (Horikoshiet al., 1984; Reinberg and Roeder, 1987b; Rappaport et al., 1987) and RNA polymerase II in transcription complexes (Horikoshi et al., 1984). Perhaps the interaction of SII with RNA polymerase II converts the enzyme into a readthrough conformation which would be immune to the signal for transcriptional arrest. A similar function has been shown for the λ phage-encoded N and Q proteins which interact with an elongating bacterial RNA polymerase transcription complex to render it resistant to termination downstream. Whereas N protein requires at least four other bacterial proteins for its function, Q can act on E. coli RNA polymerase alone although full activity requires the E. coli nusA protein (reviewed by Roberts, 1988). The SII protein can also suppress pausing by purified E. coli RNA polymerase. In fact, purified RNA polymerase II transcribing 3'-extended templates can respond to SII in the absence of any other protein (Sluder et al., 1989).

We have also shown that Sarkosyl, a reagent commonly used to study transcription in vitro, can inhibit the activity of SII but has little, if any, effect on elongation by highly purified RNA polymerase II. Previous studies (Hawley and Roeder, 1985) have described the apparent induction of pausing by Sarkosyl. This observation can now be readily explained since Sarkosyl inhibits the pause-suppressing activity of SII and can therefore unmask latent (SII-suppressed) pause sites for RNA polymerase II. The interpretation of experiments using Sarkosyl should take this into consideration.

A second transcription inhibitor, heparin, has been used routinely for studying transcription in vitro. Heparin inhibits initiation by RNA polymerase II (Reinberg and Roeder, 1987b) while having no observable effect on the elongation properties of purified RNA polymerase II, as we show here. The SII protein also functions to promote readthrough in the presence of heparin. In fact, SII is active on purified RNA polymerase II in the presence of heparin concentrations 10-fold greater than those used in the studies described here. Thus, we think it likely that SII can function efficiently in the presence of this drug. However, SII does bind to heparin-Sepharose columns (Rappaport et al., 1987), and we have not excluded the possibility that heparin may interact with SII in solution to alter its reaction mechanism in some quantitative manner. Further studies will resolve this question.

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REFERENCES

Ackerman, S., Bunick, D., Zandomeni, R., and Weinmann, R. (1983) Nucleic Acids Res. 11, 6041-6064

Bartsch, I., Schonberg, C., and Grummt, I. (1988) Mol. Cell Biol. 8, 3891-3897

Bender, T. P., Thompson, C. B., and Kuehl, W. M. (1987) Science 237, 1473-1476

Bentley, D. L., and Groudie, M. (1986) Nature 321, 702-706

Bhat, G. J., and Padmanaban, G. (1988) Biochem. Biophys. Res. Commun. 151, 737-742

* J. W. Roberts, personal communication.
