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The active site of RNA polymerase II participates in transcript cleavage within arrested ternary complexes

(transcript elongation/pyrophosphorolysis)

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ABSTRACT **RNA polymerase II may become arrested during transcript elongation, in which case the ternary complex remains intact but further RNA synthesis is blocked. To relieve arrest, the nascent transcript must be cleaved from the 3′ end. RNAs of 7–17 nt are liberated and transcription continues from the newly exposed 3′ end. Factor SII increases elongation efficiency by strongly stimulating the transcript cleavage reaction. We show here that arrest relief can also occur by the addition of pyrophosphate. This generates the same set of cleavage products as factor SII, but the fragments produced with pyrophosphate have 5′-triphosphate termini. Thus, the active site of RNA polymerase II, in the presence of pyrophosphate, appears to be capable of cleaving phosphodiester linkages as far as 17 nt upstream of the original site of polymerization, leaving the ternary complex intact and transcriptionally active.**

RNA polymerase II may become arrested during transcript elongation, in which case the polymerase remains in ternary complex but cannot continue RNA synthesis. To recover from this condition, cleavage of the transcript from the 3′ end is necessary (1, 2). Elongation factor SII facilitates this cleavage reaction, which occurs 7–17 nt from the transcript 3′ end (1, 2). The 3′-OH generated by transcript cleavage is accessible to the catalytic site of the RNA polymerase and elongation resumes from the point of cleavage (1–7). Elongation-competent ternary complexes that have stopped transcription because an NTP is missing from the reaction mixture (which we refer to as stalled complexes) can also undergo SII-facilitated transcript cleavage, but the products in this case are predominantly dinucleotides (5).

The necessity for transcript cleavage in recovery from arrest suggested to us that in the arrested state the catalytic site of the polymerase has lost contact with the 3′ end of the transcript. If this were true, then the cleavage reaction can be understood as a means of producing a new substrate for chain elongation. Pyrophosphorolysis is the reversal of normal RNA synthesis; NTPs are regenerated by successively rejoining NMPs from the 3′ end of the RNA to pyrophosphate (PP_i; see refs. 8 and 9). Such a reaction requires access of the catalytic site to the 3′ end of the nascent RNA. Thus, we predicted from our initial model that arrested complexes would be unable to undergo pyrophosphorolytic cleavage. However, two groups have recently reported that arrested RNA polymerase II ternary complexes do cleave their nascent transcripts in the presence of PP_i (1, 6). As the initial cleavage products were not identified in these experiments, it remained unclear whether PP_i mediates transcript cleavage in arrested complexes from the 3′ end of the transcript or from internal sites as is the case with SII-facilitated cleavage. To address this important issue, we have treated arrested complexes with PP_i and directly measured the increment of transcript cleavage by resolution of the released RNA fragments on high-percentage polyacrylamide gels. Surprisingly, within any given arrested complex, the cleavage sites utilized during pyrophosphorolysis and in SII-facilitated transcript truncation are essentially the same. The PP_i-mediated cleavage products contain 5′-triphosphate residues as one would predict for pyrophosphorolysis. These results demonstrate that the active site of RNA polymerase II can participate in the breaking of internal phosphodiester bonds in the nascent transcript. They strongly suggest that the catalytic site of RNA polymerase II participates in SII-facilitated transcript truncation.

MATERIALS AND METHODS

Templates and Ternary Complexes. The pUC18-based plasmids pML5-4NR and pML20 have been described (see refs. 4 and 10). Both constructs contain adenovirus 2 major late promoters slightly modified within their initial transcribed regions to allow transcription complexes to be conveniently stalled predominantly at +15 (pML5-4NR) or at +20 (pML20). The procedures for generating either ternary complexes stalled at particular positions early in elongation or relatively homogenous preparations of ternary complexes arrested within the intrinsic arrest site (+194) of pML5-4NR have been described (2, 4, 10). For all complexes used in this study, a final gel filtration step was included to remove unincorporated NTPs and Mg^{2+} before transcript cleavage was carried out.

Transcript Cleavage Reactions. Human recombinant SII (rSII) purified as described (11) was either kindly provided by Robert Landick (Washington University, St. Louis) or prepared by our laboratory using the expression vector pET11d-RAP38 generously supplied by Zachery Burton (Michigan State University, East Lansing). SII-facilitated and PP_i-mediated transcript cleavage reactions were performed in the absence or presence of chase NTPs essentially as described (2, 5). PP_i concentrations and times of incubation are given in the figure legends.

Modification and Digestion of Cleavage Products. Calf intestinal phosphatase (CIP; Boehringer Mannheim) treatment of cleavage reaction products and UTP and CTP standards was performed as described (5) except that the 8- to 17-nt cleavage products were purified and concentrated by ethanol precipitation after CIP treatment. For nuclease P1 digestion, cleavage products generated with either SII or PP_i were purified from 90-μl reaction mixtures, concentrated by eth-

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Abbreviations: CIP, calf intestine phosphatase; r, recombinant.

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anol precipitation, resolved on a 28% sequencing gel, and recovered by the crush-soak method. The RNAs were purified, supplemented with 100 ng of tRNA, concentrated by ethanol precipitation, and resuspended in 50 μl of 50 mM sodium acetate prior to the addition of 4 μl (144 units) of nuclease P1 (GIBCO/BRL). After a 1-hr incubation at 37°C, each reaction was treated with 10 μg of proteinase K at 25°C for 35 min and then extracted with phenol/$CHCl_3$ and $CHCl_3$. Samples were lyophilized, resuspended, and desalted using DE-52 anion-exchange resin (Whatman) and a volatile running buffer (triethylammonium acetate) essentially as described (5) and then resolved by chromatography on polyethyleneimine-cellulose plates (Polygram Cell 300, PEI/UV_{254}, Macherey & Nagel) developed in 1 M LiCl. The plates were exposed to a PhosphorImager screen (Molecular Dynamics) for 3 days or to preflashed Kodak X-AR film at −70°C with a Lightning Plus intensifying screen for 30 days.

RESULTS

RNA polymerase II and transcription factors were assembled into preinitiation complexes by incubating circular plasmid DNA templates bearing the adenovirus 2 major late promoter in HeLa cell nuclear extract (4, 10). These promoters contain slightly modified initial transcribed regions such that polymerases initiate and advance to either +15 or +20 upon the addition of an appropriate subset of the NTPs. The stalled ternary complexes generated by elongation with an NTP subset are extremely stable and are further purified by the addition of Sarkosyl, followed by a gel filtration step that removes the detergent. Sarkosyl rinsing also removes the Mg^{2+} and NTPs required for initiation, most nonspecific DNA binding proteins from the DNA template, and most, if not all, of the known elongation factors from the ternary complex. Sarkosyl-rinsed ternary complexes will resume elongation if supplied with Mg^{2+} and NTPs. However, efficient transcription at physiological rates is only achieved when reactions are supplemented with elongation factors TFIIF and SII (12). A portion of transcribing complexes also recognizes intrinsic arrest sites, such as those found within the pML5-4NR template (10), even in the presence of saturating elongation factors and NTPs (12). These transcriptional blockades can be subsequently overcome by SII-facilitated transcript cleavage (1, 2).

It was important to demonstrate initially that pyrophosphorolysis occurs in the expected manner on stalled complexes. As the starting material we used Sarkosyl-rinsed, CTP-labeled U20 complex, prepared with the pML20 template (Fig. 1, lane 5; ternary complexes are named according to the length and 3′ end residue of their transcript). U20 complexes are also unavoidably contaminated with trace amounts of CTP and CDP (5). As expected, supplementation of these complexes with PP_i and Mg^{2+} resulted in transcript truncation with the release of NTPs (lane 2). By contrast, SII-mediated transcript shortening (lane 3) yielded 5′-monophosphate dinucleotides (pNpNs). These RNA fragments have previously been identified as pNpNs by comigration and other studies (5). Also as previously reported (4), stalled complexes are capable of transcript truncation, albeit at greatly reduced rates, in the absence of exogenously added factor (lane 4).

PP_i Also Mediates Transcript Truncation by Arrested Complexes. To investigate the effect of PP_i on arrested complexes, we used the pML5-4NR template, which has been shown to have an intrinsic arrest site at +194 (10, 12). The sequence of the RNA-like strand surrounding the pML5-4NR arrest site is 5′-GTATATCCAGTGATTTTTTCTCCATTTTAGCTTCCTTAGCTCCTG-3′. Arrest occurs during transcription through the poly-T segment; the last base transcribed is the A residue paired to the underlined T. We have previously

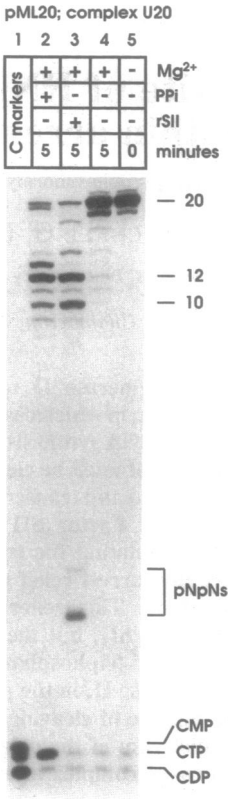

FIG. 1. Pyrophosphorolysis and SII-facilitated transcript truncation by stalled complex U20. Sarkosyl-rinsed U20 complex (lane 5) was supplemented to 2 mM PP_i (lane 2), 5 μg of human rSII per ml (lane 3), and 7.8 mM Mg^{2+} (lanes 2–4) followed by a 5-min incubation at 37°C. Truncated transcripts and cleavage products were resolved on a 28% (25:3 acrylamide/bisacrylamide) 14 × 24 cm sequencing gel; the bromophenol blue marker was run to 12.5 cm. The CTP, CDP, and CMP markers (lane 1) were generated by partial CIP treatment of [α-^{32}P]CTP.

described conditions that allow the generation of essentially homogenous populations of complexes labeled during synthesis of the initial 20 nt of the transcript and arrested at the site shown above (4). The U194 complexes are truly arrested as the addition of 1 mM ATP, CTP, and UTP allowed almost none of the complexes to continue transcription (Fig. 2, compare lane 8 with lane 1). Consistent with prior observations (2), incubation of U194 complexes with SII and Mg^{2+} generated a substantial amount of 180-nt transcript with a corresponding reduction in the amount of 194-nt transcript (lanes 6 and 7). There was very little cleavage activity in the absence of added factor (Mg^{2+}-only reactions, lanes 2 and 3). To demonstrate that complexes bearing the shortened transcripts were elongation competent, U194 complex was simultaneously incubated with Mg^{2+}, SII, ATP, CTP, and UTP. This treatment generated 186-nt transcripts (lanes 12 and 13), presumably produced by complexes elongating from +180 up to the first position at which GTP is required (see the arrest site sequence above). When we treated U194 complexes with PP_i instead of SII, we were surprised to observe very similar results. A significant level of 180-nt transcript was generated in response to incubation with PP_i and Mg^{2+} (lanes 4 and 5); several minor products longer than 180 nt were also observed. Importantly, when U194 complex was treated with Mg^{2+}, PP_i, ATP, CTP, and UTP, 186-nt RNA was generated (lanes 10 and 11), exactly as seen in the SII case. Thus, the 180-nt RNAs produced by PP_i treatment could not have been generated by a series of single nucleotide cleavages from the 3′ end of the nascent RNAs. The results in lanes 10 and 11 strongly suggest that pyrophosphorolysis

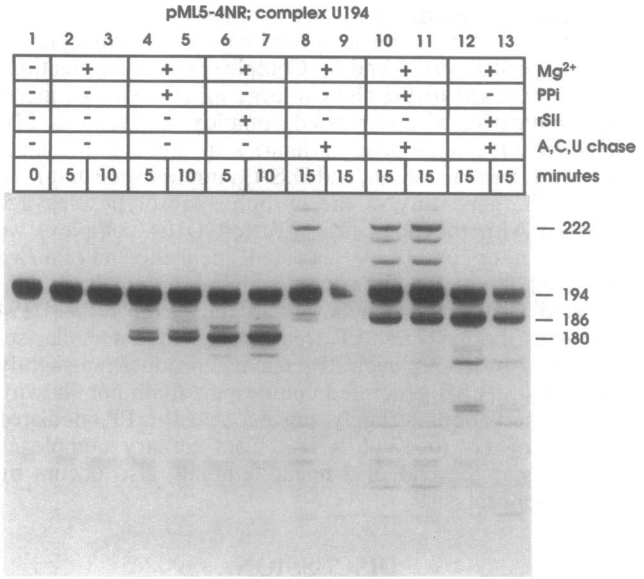

FIG. 2. Transcript truncation by arrested U194 complexes occurs in the presence of either SII or PP_i. Transcriptionally arrested U194 complex (lane 1) was supplemented with 2 mM PP_i, 1.6 μg of rSII per ml, 1 mM ATP, CTP, and UTP, and 8 mM Mg^{2+} as indicated. After the indicated times at 37°C, each reaction was processed and resolved on a 10% (29:1 acrylamide/bisacrylamide) sequencing gel.

can support endonucleolytic cleavages at positions relatively far upstream of the transcript 3′ end, as is the case for SII-facilitated transcript cleavage.

It is important to note that some of the original U194 complexes escaped arrest during PP_i treatment by the cleavage of <6 nt from the nascent RNA, since incubation of the complexes with PP_i and ATP, CTP, and UTP also led to elongation to the first three G stops downstream of the arrest site (lanes 10 and 11). This was expected since longer exposures of the autoradiogram in Fig. 2 showed a nearly continuous ladder of faint bands extending down from 194 after PP_i incubation (lanes 4 and 5), in addition to the major band at 180. The fact that many of these PP_i-treated complexes passed through one or two G stops in the presence of ATP, CTP, and UTP presumably resulted from GTP contamination in our ATP, CTP, and UTP, and not a change in the properties of the complexes resulting from exposure to PP_i, since complexes that escaped arrest during the Mg^{2+}-only incubation chased to the same positions as the PP_i-treated complexes (compare lanes 8 and 10). Detectable 180-nt cleavage product could be generated from arrested U194 complexes with PP_i levels as low as 0.1 mM in a 20-min incubation (data not shown), but in contrast to the case with stalled complexes (Fig. 1) efficient pyrophosphorolysis in arrested complexes required millimolar levels of PP_i. Also, the PP_i-mediated reaction in arrested complexes was considerably slower than that seen with stalled complexes; complete cleavage with U194 was not obtained in 20 min, whereas all of the stalled U20 complexes had cleaved at least once in 5 min.

PP_i Mediates Transcript Truncation in Large (up to 17 nt) Increments. To determine more definitively the PP_i-mediated cleavage increment(s), we uniformly labeled the RNA within the arrested U194 complexes with [α-^{32}P]UTP (U194-U complexes), treated the complexes with SII or PP_i, and resolved the cleavage products on a 28% sequencing gel. As shown in Fig. 3, lanes 3 and 4, each cleavage activity produced a set of RNAs roughly 8–17 nt long. The most abundant RNA migrated just ahead of a 15-nt marker (markers not shown), as expected from the predominance of the 180-nt RNA among the U194 complex truncation products (Fig. 2, lanes 4–7).

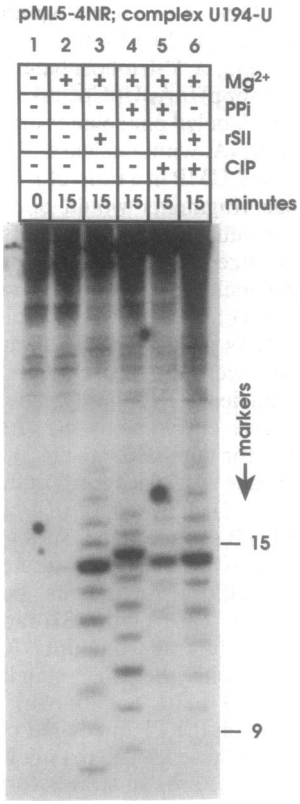

FIG. 3. Large increment transcript cleavage by arrested U194 complex occurs in the presence of either SII or PP_i. Arrested U194 complexes containing uniformly labeled RNAs were generated by chasing C15/U18 complexes with 1 mM ATP, CTP, and GTP and 20 μM [α-^{32}P]UTP. After purification by gel filtration, equal aliquots of U194 complex (lane 1) received 1.6 μg of rSII per ml (lanes 3 and 6), 2 mM PP_i (lanes 4 and 5), and 8 mM Mg^{2+} (lanes 2–6). After a 15-min incubation at 37°C, the reactions were stopped and the RNAs were purified and concentrated by ethanol precipitation. The RNAs in lanes 5 and 6 were further treated with CIP. RNAs were resolved as described in the legend to Fig. 1 except that the bromophenol blue dye was run to 22 cm.

None of the 8- to 17-nt RNAs was present in the nontreated sample (lane 1) and only trace amounts were created by incubation of the complexes with Mg^{2+} alone (lane 2). While the distribution of fragments generated by SII and PP_i appeared comparable, the RNAs did not comigrate. This was not unexpected, since fragments generated with SII contain a 5′-monophosphate (2, 5), whereas the pyrophosphorolytic products would be expected to bear 5′-triphosphate termini. Short RNAs that have identical sequences but differing numbers of 5′-phosphates are easily resolved using this gel system (2, 5). The cleavage products generated by either PP_i or SII incubations changed mobility after CIP treatment (compare lanes 3 with 6 and 4 with 5). Moreover, the SII-facilitated and PP_i-mediated cleavage products comigrated after CIP treatment and the product distributions appeared nearly identical (compare lanes 5 and 6). Therefore, both SII-facilitated and PP_i-mediated cleavages occurred at the same locations and to essentially the same relative extent within the nascent transcript.

The results from Fig. 3 strongly suggest that the PP_i-generated cleavage products contain triphosphate termini. To demonstrate unequivocally the presence of 5′-triphosphate termini in the PP_i-generated cleavage products we treated these 8- to 17-nt RNAs with nuclease P1, which cleaves 3′-5′ phosphodiester linkages to generate nucleoside 5′-monophosphates. Thus, P1 digestion of the PP_i-mediated cleavage products would give NMPs except for the 5′-most

residue, which would be released as the corresponding NTP. The major PP_i cleavage product from U194 RNA appeared to be a 14-mer; assuming a 5′-triphosphate terminus, the sequence would be 5′-**ppp**UpA**p**UpCpCpAp**G**p**U**pGpA**p**U**p**U-**p**U**p**U-3′, where the labeled phosphates are in boldface type. Digestion of this RNA with nuclease P1 would generate radiolabeled UTP and UMP in a ratio of 1:6. Both the 12- and 16-nt PP_i cleavage fragments would also be expected to contain 5′ end U residues. Based on the distribution of both cleavage fragment size and U content, we calculated that ≈7.5% of the radiolabeled U would be liberated as UTP after nuclease P1 digestion of the 8- to 17-nt PP_i cleavage products. The 8- to 17-nt RNAs generated by PP_i-mediated and SII-facilitated reactions were eluted from 28% sequencing gels and treated with nuclease P1; the digestion products were resolved by thin-layer chromatography. As expected, UTP was not a substrate for nuclease P1 (Fig. 4, compare lanes 3 and 5). The nuclease P1 digestion products of the PP_i-generated RNA (lane 2) show a spot that clearly comigrates with a bona fide UTP sample (lane 3); 8.4% of the labeled nucleoside phosphates in lane 2 comigrated with the UTP standard and essentially all of the rest comigrated with a UMP standard. In the case of the SII-facilitated cleavage products (lane 1), only trace amounts (0.7%) of labeled material comigrated with the UTP standard. Finally, we independently verified (data not shown) the presence of 5′-triphosphate ends on the PP_i-mediated cleavage products by demonstrating that these RNAs, but not RNAs released by SII-facilitated cleavage, are substrates for the capping enzyme guanylyltransferase, which will only add GTP to RNAs with di- or triphosphate 5′ ends (13). We conclude that the cleavage activity exhibited by arrested U194 complex in the presence of PP_i is indeed pyrophosphorolysis.

Recently, it has been suggested that RNA polymerase II ternary complexes may become arrested for reasons other than template sequence. We have discovered that RNA

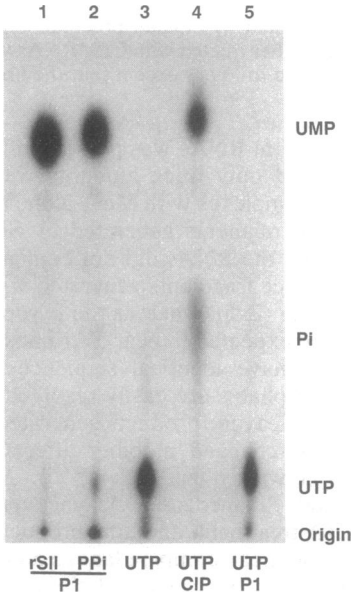

FIG. 4. Nuclease P1 digestion of PP_i-mediated and SII-facilitated large cleavage fragments from U194 complex. SII-facilitated and PP_i-mediated transcript cleavage fragments from arrested U194 complex were prepared as described in the legend to Fig. 3. After fractionation on a 28% sequencing gel, the 8- to 17-nt cleavage products were harvested from the gel, purified, and digested with nuclease P1. The nucleotides generated by P1 cleavage (lanes 1 and 2) were resolved by thin-layer chromatography developed with 1 M LiCl. As controls, [α-^{32}P]UTP (0.47 fmol) was treated with either CIP (lane 4) or nuclease P1 (lane 5).

polymerase II often halts RNA synthesis 5–8 nt upstream from the end of the template strand when transcribing linear DNAs (see also refs. 1 and 6). Complexes at these positions that are exposed to SII show a large initial cleavage increment, indicative of an arrested complex. Subsequent SII-facilitated cleavages occur primarily in dinucleotide units (M.G.I., I. Samkurashvili, and D.S.L., unpublished data). To explore the generality of the pyrophosphorolytic cleavage observed with the bona fide arrested U194 complex, we tested whether complexes "arrested" near the end of a *Pvu* II-cleaved linear template would also generate large PP_i-mediated cleavage products. As in the case of the U194 complexes, both SII and PP_i treatment yielded a similar set of 11- to 17-nt RNAs; each PP_i cleavage product ran slightly slower than its SII-generated counterpart (data not shown). These observations strongly suggest that the PP_i-mediated cleavage of 11- to 17-nt RNAs from ternary complexes arrested near the end of a linear template also occurs by pyrophosphorolysis.

DISCUSSION

Several laboratories have shown that in the presence of elongation factor SII, RNA polymerase II ternary complexes can cleave their nascent RNAs in a 3′→5′ direction, retaining the 5′ portion of the transcript in active complex (3, 4, 6, 7). For stalled complexes SII-facilitated cleavage occurs primarily in successive dinucleotide increments (ref. 5; see Fig. 1), whereas arrested complexes truncate their transcripts 7–17 nt from the transcript 3′ end (ref. 2; see Fig. 3). In both cases transcription resumes at the appropriate location with respect to the underlying template. The latter observation in particular led us to propose that the nuclease activity and the catalytic site of RNA polymerase II may be linked (2). However, data obtained from initial studies of the cleavage reaction did not conclusively identify any of the constituents of the nuclease. Highly purified prokaryotic and eukaryotic RNA polymerase ternary complexes exhibit residual transcript cleavage activity in the absence of added stimulatory factors GreA/GreB (14, 15) and SII, respectively (3, 4, 6). This residual activity could result from either a low level of factor contamination or the intrinsic ability of the RNA polymerase to serve as an endonuclease. RNA polymerase II must participate in transcript cleavage since this reaction is inhibited by α-amanitin (3, 4, 6). However, some ternary complexes are partially resistant to the effects of the toxin with respect to both SII-facilitated (4) and PP_i-mediated (M.D.R. and D.S.L., unpublished data) transcript cleavage. RNA polymerase/RNA binary complexes will also cleave RNA; this activity requires SII and at least the first truncation cycle appears resistant to α-amanitin (16). Moreover, RNA polymerase II is capable of making one or more phosphodiester bonds in the presence of α-amanitin (refs. 17 and 18; unpublished data). Thus, α-amanitin inhibition of cleavage does not necessarily indicate the involvement of the catalytic site during the truncation reaction.

We show here that pyrophosphorolytic cleavage by arrested RNA polymerase II complexes occurs as far as 17 bases upstream of the original site of bond formation. Since the fragments produced by PP_i-mediated cleavage have 5′-triphosphate ends, cleavage cannot be attributed to contaminating SII or an SII-like factor, which would give fragments with 5′-monophosphate ends (2, 5). We cannot absolutely exclude the possibility that the pyrophosphorolysis we observe is carried out by an unknown site. However, we feel that the evidence very strongly favors the active site of the polymerase as the agent of endopyrophosphorolysis. Pyrophosphorolytic removal of single nucleotides from the transcript 3′ end is a known property of the active site of the RNA polymerase. Also, positioning the active site at the point of

cleavage is an attractive concept since the catalytic center would automatically be in the correct location to begin chain elongation once a new 3'-OH has been generated. We have not proven that the polymerase II active site performs the cleavage reaction in the presence of SII, but this idea is strongly supported by the near-identity of fragment patterns produced in the SII and PP_i cleavage reactions with arrested complexes, in terms of both location and relative frequency of cleavage (see Fig. 3). Given that the catalytic site is capable of efficient transcript cleavage in the presence of PP_i, it is reasonable to suppose that the much lower level of cleavage seen with highly purified ternary complexes in the absence of PP_i or SII reflects an intrinsic ability of the catalytic site to serve as an endonuclease. This idea is supported by the recent finding that ternary complexes containing RNA polymerase prepared from an *Escherichia coli* strain that lacks both of the known prokaryotic transcript cleavage factors (GreA and GreB) retain residual transcript cleavage activity (19).

If we assume that the RNA polymerase catalytic site is involved in both pyrophosphorolytic and SII-facilitated transcript cleavage, then it may seem surprising that the distribution of chase products was not identical when cleavage was performed with these two reagents on arrested U194 complexes in the presence of ATP, CTP, and UTP (Fig. 2). The SII-facilitated reaction resulted in all of the cleavage products stalling at the upstream G stop at position +186, while a significant portion of the chase products from the PP_i-mediated reaction were stalled at downstream G stops (Fig. 2, compare lanes 10 and 11 with lanes 12 and 13). We attribute this result to a difference in the rate of transcript cleavage in the presence of SII versus PP_i, coupled with a tendency of the arrested complexes to reacquire elongation competence upon extended residence at the arrest site. In the experiment shown in Fig. 2, a small but easily detectable portion of the arrested U194 complexes did chase after a 15-min incubation with 1 mM ATP, CTP, and UTP, in the absence of PP_i or SII. If arrested U194 complexes have a low probability of resuming transcription, such that elongation activity can be detected only with extended chase times (>10 min), then the results in Fig. 2 are not surprising. It is important to emphasize that SII-facilitated cleavage under the conditions used here is detectable after 15 sec and nearly complete within a few minutes (2, 5), whereas pyrophosphorolysis, as noted above, is much slower, requiring a 10- to 15-min incubation to see substantial cleavage (data not shown). Thus, we would expect in SII-facilitated reactions containing CTP, UTP, and ATP that cleavage would nearly always precede spontaneous reactivation, thereby generating complexes stalled at a G stop upstream of the original arrest site. However, the longer time needed for PP_i-mediated cleavage should permit some complexes to escape arrest spontaneously, leading to a partitioning of complexes between G stops upstream and downstream of the initial site of arrest. This kinetic explanation must be somewhat oversimplified, since more complexes accumulate at the downstream G stops in the presence of PP_i than in its absence, given equivalent incubation times (Fig. 2, compare lanes 8 and 10). Thus, it is possible that PP_i may influence the equilibrium between arrest and elongation competency.

Finally, it should be noted that unlike arrested U194 complex, stalled U20 complex generated different cleavage products in the presence of SII and PP_i. SII-facilitated cleavage by stalled complexes occurred in primarily dinucleotide increments with trace levels of NMPs, whereas pyrophosphorolysis occurred in the expected single nucleotide increment (Fig. 1; see also ref. 5). Also, in contrast to arrested U194 complexes, stalled complexes exhibited roughly comparable rates of PP_i-mediated and SII-facilitated cleavage (Fig. 1 and data not shown). If stalled complexes are capable of PP_i-mediated endonucleolytic cleavage but this reaction requires many minutes, as observed with arrested complexes, one would then expect the much more rapid exonucleolytic cleavage to dominate the reaction. The favored dinucleotide increment in SII-facilitated reactions may reflect steric considerations imposed by SII upon binding to the ternary complex via protein–protein and/or protein–nucleic acid interactions (20).

In summary, we have shown that the active site of RNA polymerase II can serve as an endonuclease in the presence of PP_i, and we provide evidence that the active site participates directly in SII-facilitated transcript cleavage as well. Among the many questions raised by these findings, one of the most compelling concerns the process by which the active site is relocated far upstream of the original site of chain elongation in the case of transcriptional arrest. One way to envision this is to suppose that the active site is associated with an RNA binding domain that can slip upstream on the transcript at the onset of arrest (16, 21). Regardless of the exact mechanism, RNA polymerase must be capable of extraordinary internal flexibility in order to accommodate the movement of the active site by as many as 17 nt upstream without disruption of the ternary complex. A major challenge for the future will be determining the signals in the template and transcript which force RNA polymerase into the arrested state.

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