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Uncoupling of Initiation and Reinitiation Rates during HeLa RNA Polymerase II Transcription In Vitro

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RNA polymerase II transcription is influenced both by how rapidly a gene is induced and by the rate at which continuous reinitiation occurs after induction. We show here that in vitro the rates of these two critical steps need not be the same. For activator GAL-AH-dependent HeLa transcription, the rate of assembling a preinitiation complex is significantly slower than the rate of reinitiation. Although reinitiation is rapid, it still requires ATP hydrolysis. This unexpected uncoupling of the rates of initiation and reinitiation implies that in regulating mammalian promoter activity, one must consider separately the controls on initiation during induction and the controls on the subsequent reinitiation events.

Transcriptional control of RNA polymerase II genes requires inducing transcription and then maintaining an appropriate level of continued RNA synthesis. The induction process has been studied extensively both in vivo and in vitro. In vitro, the assembly of an initial functional transcription complex follows an ordered pathway involving the sequential addition of many factors to the promoter (2, 18, 19). At TATA-containing promoters, basal factor assembly begins with the recognition of the TATA box. Many factors then assemble sequentially to form various closed complexes in which the start site is not yet open and available for copying into RNA. A series of rapid steps, in which ATP is used to locally denature the start site to form an open complex, follow (22, 23). This open complex uses template-complementary nucleoside triphosphates (NTPs) to initiate transcription rapidly. The start site renatures after the polymerase begins elongation (12, 22, 23).

Much less is known about how transcription is subsequently maintained by reinitiation. It is very important to begin such studies, since the bulk of mRNA is likely produced by this process. An earlier indirect study showed that a template transcribed previously was preferred over an identical DNA that had never been transcribed (9). Apparently, after the first polymerase leaves the promoter, certain factors or modifications remain associated with the active template (21, 24). These results indicate that reinitiation of transcription need not recapitulate all steps that are required during assembly of an initial transcription complex. In principle, the rates and factor requirements could differ substantially. One impediment to studies of these issues is the difficulty in studying reinitiation by using transcription assays, since there are not yet assays that easily distinguish the first and second transcripts that are produced from a promoter.

In this report we focus on three aspects of reinitiation. First, we will develop a protocol to detect the reinitiation transcription complex. This will be done by assaying the complex directly by chemical probing rather than by attempting to distinguish first- and second-round transcripts. Second, we will measure the rate of formation of reinitiation complexes and compare this with the rate of formation of complexes during the first round of initiation. Third, we will determine whether reinitiation also has a requirement for ATP, as has been reported for initiation (1, 6, 12, 20). The data will show that for GAL-AH-activated transcription, reinitiation is unexpectedly rapid but nevertheless still requires ATP. This uncoupling of rates has potentially important implications, since it is the rate of reinitiation that most likely determines how much RNA is made from a transcribed gene.

MATERIALS AND METHODS

High-pressure liquid chromatography-purified ribonucleoside triphosphates and deoxyribonucleoside triphosphates were purchased from Pharmacia. Cordycephin 5′-triphosphate (3′ deoxy-ATP), ATPγS, Sarkosyl, and α-amanitin were purchased from Sigma. The HeLa cell nuclear extract was kindly supplied by Michael Carey and was made as previously described (7). The DNA template containing a truncated E4 promoter with nine upstream GAL4 sites and the activator GAL4-AH were also from Michael Carey.

The in vitro transcription assay was as previously described (3, 4, 22, 23). The reaction mixture contained 25 μl of HeLa nuclear extract (6 mg of protein per ml), 8.25 mM magnesium chloride, 200 ng of pGEM carrier DNA, 10 ng of template DNA, 500 μM (each) the four NTPs as indicated and was incubated at 30°C. For the pseudo one-round transcription assay, nuclear extract, DNA template, carrier DNA, and $MgCl_2$ were incubated in the absence of NTPs for 28 or 30 min to synchronize the formation of preinitiation complexes. NTPs were then added for 2 min. mRNA was assayed by primer extension. In some experiments, the nuclear extract was treated with 4 U of hexokinase and 250 μM glucose during preincubation to deplete endogenous ATP.

The in vitro potassium permanganate assay was as previously described (22). Nuclear extract, DNA template, carrier DNA, and magnesium chloride were incubated for 30 min. A total of 500 μM dATP or 125 μM ATP as indicated was added for 2 min just prior to a 4-min treatment with 6 mM potassium permanganate. Shorter permanganate treatments gave similar experimental results. A total of 10 ng of supercoiled template was used.

Potassium permanganate was also used to assay polymerase II reinitiation open complexes. Nuclear extract,

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DNA template, carrier DNA, and magnesium chloride were incubated for 30 min, and then all four NTPs at 125 μM were added for 2 min. After this 2-min chase, α-amanitin was added to trap any incoming polymerases, and potassium permanganate was used to probe strand reopening.

RESULTS

Development of a template-limited system supporting reinitiation. The first goal is to establish a system in which reinitiation can occur in an environment in which the availability of factors does not limit transcription. Under these circumstances initiation using a free pool of excess factors occurs, and partially assembled transcription complexes do not accumulate for lack of factors. This is important for studies of reinitiation; it avoids potential confusion from partly assembled complexes that begin to transcribe only after factors have been released by transcription of other templates.

The experimental system is identical to the one we used previously to study parallel transcription and open complex formation assays (12, 22, 23). The DNA template contains a truncated adenovirus E4 promoter as a source of TATA sequence and nine GAL-4 DNA binding sites upstream of TATA. General transcription factors are provided by a HeLa cell extract, and the chimeric GAL4-AH protein is added as an activator. Previous experiments have shown that basal transcription of this promoter is very low, and transcription is therefore virtually completely dependent on the added activator (22, 23).

The experimental protocol uses conditions that limit transcription to approximately one round (12) (see below). The assay begins with a lengthy (28-min) preincubation of HeLa transcription extract, promoter DNA, and activator GAL-AH in the absence of NTPs. This preincubation time is sufficient to essentially saturate available templates (see Fig. 4 and 6) (22, 23), but transcription initiation does not occur, since nucleotides are absent. Synchronous mRNA production is then begun with the addition of nucleotides. After a short 2-min nucleotide pulse, the reaction is stopped. As shown below, this pulse is just long enough to allow one round of transcription, since the polymerase initiates with a half-time of 30 s (data not shown), and the RNA detected is only 96 bases long. The 96-base-long extension product of the RNA produced by this protocol is shown in Fig. 1.

This one-round assay was done in the form of a titration in which the amount of extract was held constant and the amount of DNA was varied. Figure 1A shows the results of this titration. As the amount of template increases, the amount of transcript increases approximately proportionally. Thus, over this range of template concentrations, transcription is limited by the amount of DNA present. We chose the very low concentration of 10 ng of plasmid DNA (lane 2) as the standard condition.

The next experiment shows that this system is capable of supporting multiple rounds of transcription. Lane 1 of Fig. 1B shows the RNA produced in the one-round initiation protocol under these standard conditions. Lane 2 shows the RNA produced in a parallel reaction in which nucleotides were present from the very outset, that is, during the entire 30 min. This should allow continuous transcription, including reinitiation, to occur. The comparison shows that significantly more RNA is produced under these latter conditions. Isolation and radioactive counting of the bands show that approximately four times as much RNA is produced in a 30-min free transcription assay (lane 2) compared with that

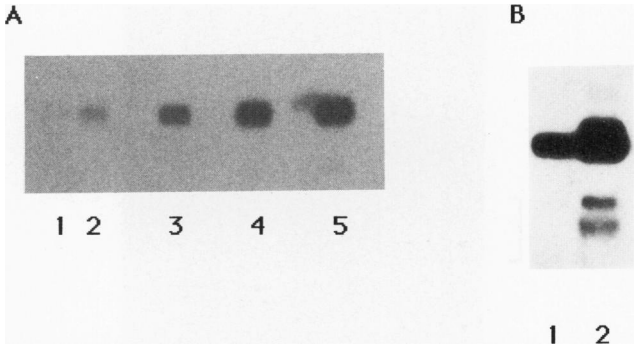

FIG. 1. (A) Template-limiting transcription. Different amounts of promoter plasmid were transcribed with a constant amount of HeLa extract in a pseudo one-round transcription assay. Lane 1, 4 ng; lane 2, 10 ng; lane 3, 20 ng; lane 4, 40 ng; lane 5, 80 ng. (B) With 10 ng of DNA template, the in vitro transcription system is capable of supporting multiple rounds of transcription. Lane 1, pseudo one-round transcription assay with 10 ng of plasmid. Incubation was for 28 min and was followed by a 2-min nucleotide pulse; lane 2, nucleotides were added at the very beginning of the incubation and therefore were present for the entire 30 min, allowing continuous transcription including reinitiation. Panels A and B are from separate experiments.

produced in the single-round assay discussed above (lane 1). In addition, if preinitiation complexes are allowed to accumulate as in a first-round assay and NTPs are then added for 20 min, the amount of RNA produced is about tripled compared with that in the 2-min nucleotide pulse (not shown). Thus, we conclude that the system is capable of reinitiation.

Detection of the reinitiation open complex. Previously we used potassium permanganate to identify the open complex formed during initial assembly of preinitiation complexes at this promoter (22, 23). The reagent reacts selectively with the single-stranded thymines at the start site within this open complex. These are detected as a series of hypersensitive bands in primer extension assays in which the permanganate-modified DNA is copied. Several criteria have been used to conclude that this hypersensitivity is associated with templates involved in productive transcription. The requirements for forming hypersensitive sites parallel those for producing transcript in every regard tested. The requirements include the need for an upstream activation system containing GAL4-VP16, GAL4-AH, or Sp1 and the appropriate DNA binding sites (12, 22); the need for multiple sites when GAL4-VP16 is used (22); and the need for a basal element, either TATA or initiator (12). Hypersensitivity and transcription also have common basal transcription factor requirements (23) and a common ATP-β-γ hydrolysis requirement (12). The hypersensitive complex disappears as the polymerase leaves the promoter during initiation of productive transcription in response to GAL-VP16 (22) or Sp1 (12). Both productive transcription and this loss of hypersensitivity do not occur in the presence of the polymerase II inhibitor α-amanitin (12, 22). If the polymerase is artificially paused during productive transcription, the hypersensitivity appears in the new position of the pausing (22). In the cases of both the permanganate assay and transcription assays, only a minority of templates yield a signal.

These previous experiments demonstrate that the permanganate sensitivity associated with a productive open tran-

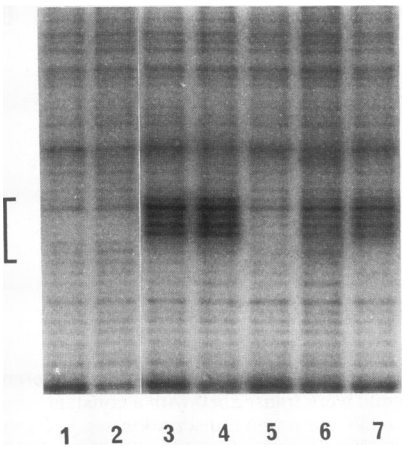

FIG. 2. GAL4-AH-stimulated polymerase II open complex formation and the identification of polymerase II reinitiation complex. Closed complexes were assembled for 28 min; ATP or dATP was added next as indicated below. The complexes were then probed with permanganate. Lane 1, GAL4-AH was omitted; lane 2, dATP was omitted. Lanes 1, 3, and 4 contained dATP, and lane 3 contained 20% extra nuclear extract. The start site potassium permanganate hypersensitivity (open complex signal) is bracketed. Lanes 5 to 7, closed complexes were formed, as described above, in the absence of dATP, and then NTPs were added for 2 min; lane 5, permanganate probing was immediate; lanes 6 and 7, α-amanitin was added, and permanganate probing was either 3 min later (lane 6) or 8 min later (lane 7).

scription complex is lost upon transcription initiation, showing that the start site recloses after the productive polymerase leaves the promoter. Our new protocol is designed to detect the reopening of the start site that should occur during reinitiation by new RNA polymerases.

First, we assayed for permanganate sensitivity of the preinitiation complex in the one-round protocol by adding dATP but no other nucleotides. As discussed above, this should lead to an accumulation of open complexes poised to begin the first round of initiation. The permanganate sensitivity associated with this first-round open complex is shown in Fig. 2, lanes 3 and 4. This is similar to experiments done previously (12, 22, 23), and it shows the typical series of bands that mark the series of thymines within the promoter start site region. Omission of either GAL4-AH (lane 1) or dATP (lane 2) leads to a failure to produce the signal. When all four nucleotides required for initiation are included with activator, the result shown in lane 5 is obtained. As expected, the start site has reclosed, as evidenced by the loss of permanganate hypersensitivity. The nucleotides were added for only 2 min, corresponding to the one-round transcription assay described above. This illustrates that 2 min is a sufficient time for the promoter to be cleared of first-round open complexes. The half-time for this reclosing event is approximately 30 s under the conditions of this experiment (not shown). The rate is expected to vary with nucleotide concentration (5), but we have not investigated this.

In Fig. 2, lanes 6 and 7, the RNA polymerase II transcription inhibitor α-amanitin was added directly after this 2-min nucleotide pulse. We demonstrated previously that α-amanitin can cause accumulation of open complexes in the presence of nucleotides by inhibiting transcription initiation (12, 22). As shown in lanes 6 and 7, open complexes, which

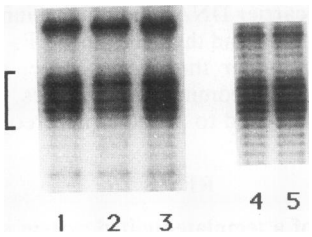

FIG. 3. α-Amanitin does not alter the amount of first-round open complexes in the presence or absence of NTPs. Complexes were assembled for 28 min; a 2-min treatment with dATP followed (lanes 1 and 5). Lane 4 was identical, except that α-amanitin was present from the outset. dATP was omitted in lanes 2 and 3, but either cordycephin (dATP lacking a 3′ hydroxyl and thus incompetent for elongation) (lane 2) or α-amanitin plus the four nucleotides (lane 3) were present from the outset. The reactions were assayed for permanganate sensitivity over the start site (bracketed). Lanes 1 to 3 and lanes 4 and 5 are from separate experiments.

had disappeared upon the addition of nucleotides (lane 5), now begin to reappear. Figure 3 shows that the use of α-amanitin does not seriously perturb the properties of open complexes. α-Amanitin can be added before complex assembly has begun (lane 4) without detectable effect (compare with control lane 5). In addition, α-amanitin traps open complexes without significant loss in the presence of nucleotides (compare lane 3 with lane 1). We conclude that this protocol, which uses α-amanitin after initiation has occurred, is capable of detecting the reinitiation open complex.

Comparing the rates of opening during initiation and reinitiation. Next, we measured the rate at which open complexes assemble during the first round of transcription under these experimental conditions. All components were mixed together at the start of the reaction, except that NTPs were omitted. Samples were removed and probed for DNA opening by the permanganate protocol during a subsequent 30-min time course. Figure 4 shows that open complexes

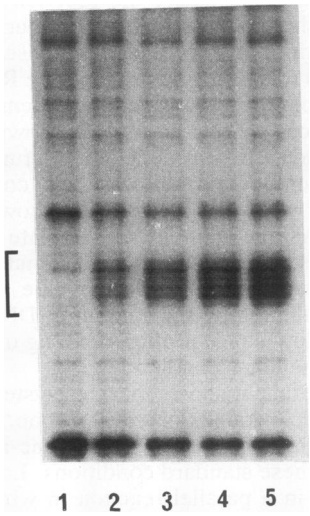

FIG. 4. Kinetics of first-round initiation open complex formation. The template DNA, nuclear extract, and activator were incubated for various times, dATP was added (lanes 2 to 5) for 2 min, and then the open complexes were probed with permanganate at the specified times. The times (including 2 min of dATP treatment) are as follows: lane 1, 30 min without dATP added; lane 2, 5 min; lane 3, 10 min; lane 4, 20 min; lane 5, 30 min.

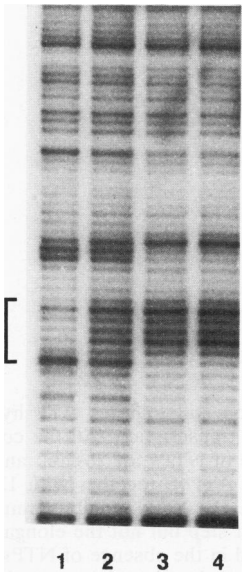

FIG. 5. Rate of reinitiation open complex formation. First-round open complexes were assembled, and after a 2-min treatment with NTPs to clear the first polymerase, α-amanitin was added for the specified times. Samples were then probed with permanganate. The times are as follows: lane 1, 0 min; lane 2, 3 min; lane 3, 8 min; lane 4, 18 min.

accumulate progressively over these 30 min, as evidenced by the increasing permanganate signal (bracketed). The reaction does not approach saturation until near the end of the time assayed (longer times do not yield an increasing signal [data not shown]). Analysis of repetitions of this experiment indicates a reaction half-time of approximately 10 min. This is slightly faster than half-times of 12 to 15 min measured previously by somewhat different experimental systems (23) and compares with times of 8 to 20 min measured in other systems (9, 24).

The next experiment measures the rate at which assembly of reinitiation complexes occurs. The protocol was a simple adaptation of that of Fig. 2, in which the reinitiation open complex was identified. All components except nucleotides were added to assemble nearly saturation levels of open complexes, as confirmed in Fig. 4. NTPs were then added for 2 min, which allows these open complexes to initiate, as shown in Fig. 2. At this time, α-amanitin, which is capable of trapping the reinitiation open complex, as shown in Fig. 2 and 3, was added. Samples were removed at various times and assayed for reopening by using permanganate.

Figure 5 shows the progress of reopening by using this protocol. When probed at 3, 8, or 18 min after the 2-min pulse with nucleotides, the start site is reopened, as evidenced by the sensitivity marked over the bracketed start site region (Fig. 5). However, in this case the reaction is nearly saturated by the very first sampling time, representing a 2-min nucleotide pulse followed by a 3-min α-amanitin treatment. This is in marked contrast to the results of assaying first-round assembly in Fig. 4, in which the reaction did not approach saturation until 20 or 30 min (see also Fig. 6). Analysis of repetitions of this experiment gave a reaction half-time of 3 min or perhaps slightly less. We conclude that opening the start site during reinitiation is at least threefold faster than opening the start site during initial transcription.

Quantitative analysis of the extent of hypersensitivity indicated that reinitiation is not 100% efficient in our in vitro system. Our best estimate is that the melting signals of Fig. 5 are approximately three-fourths those of Fig. 4. We do not know if this reinitiation efficiency applies to the third and subsequent rounds of transcription, but if it does, continuous transcription will progressively decline. Something like this is likely happening, since the data of Fig. 1 demonstrated that only four times as much RNA is made at 30 min as was obtained in a one-round assay. This may actually represent a slow round of initial transcription followed by many rapid rounds of lower-efficiency reinitiation with decreasing amounts of transcript being produced each round.

This slow round of initial transcription involves all available templates, as confirmed by monitoring the properties of Sarkosyl-resistant complexes. We determined an amount of Sarkosyl that does not prevent initiation by assembled complexes at this promoter but does prevent assembly of initiation-competent complexes (unpublished results); this is higher than reported previously for the adenovirus major late promoter (9). We then measured the accumulation of the initiation-competent complexes resistant to this concentration of Sarkosyl (Fig. 6A). The result shows that these Sarkosyl-resistant preinitiation complexes form with the same kinetics as open complexes, with a half-time of approximately 10 min (see above discussion). Thus, the reaction is essentially complete at 30 min, excluding the already unlikely possibility that the 75% signal from second-round open complexes is due to first-round complexes that did not form in 30 min. In addition, we determined that the first-round complexes that formed at 30 min initiated transcription synchronously. This is shown in Fig. 6B; nucleotides were added to those complexes in the presence of Sarkosyl and were shown to complete transcription within two min. Thus, the 30-min incubation leads to saturating amounts of first-round complexes that initiate synchronously and within 2 min. The reappearance of open complexes after nucleotide addition (Fig. 5) then represents new polymerases opening the DNA, as also implied by the results of Fig. 1 to 5.

Reinitiation still requires ATP. ATP hydrolysis is required during the first round of transcription, but it is not known whether reinitiation also requires ATP hydrolysis. To address this issue, we take advantage of the properties of the nucleotide analog ATPγS [adenosine 5′-*O*-(3-thiotriphosphate)]. It has been shown that ATPγS inhibits polymerase II transcription initiation by inhibiting the ATP-β-γ hydrolysis step (6). Such hydrolysis is required for assembly of first-round open complexes and transcription (12, 22). Thus, ATPγS should at least block first-round transcription. We wish to confirm this and learn if it will also interfere with transcription that results from reinitiation.

Figure 7A shows the consequences of adding ATPγS in the first-round transcription protocol used in this study. When 1.75 mM ATPγS is added along with the usual 125 μM nucleotides, transcription is substantially blocked (lane 1 versus lane 2). The large excess of ATPγS appears to inhibit the use of ATP in transcription. To confirm that this is not an elongation block, the experiment of Fig. 7B was done. Lane 1 shows that no transcription occurs if ATPγS replaces ATP in the mix of four nucleotides. However, if dATP is added as a source of hydrolyzable ATP, then transcription occurs (lane 2) even when ATPγS replaces ATP. Since dATP is not an elongation substrate (6, 20) (lane 3 of Fig. 6A), the appearance of transcript in lane 2 confirms that ATPγS is used in elongation, as expected. The lack of a signal in lane 1 is therefore not due to an inability of ATPγS to support

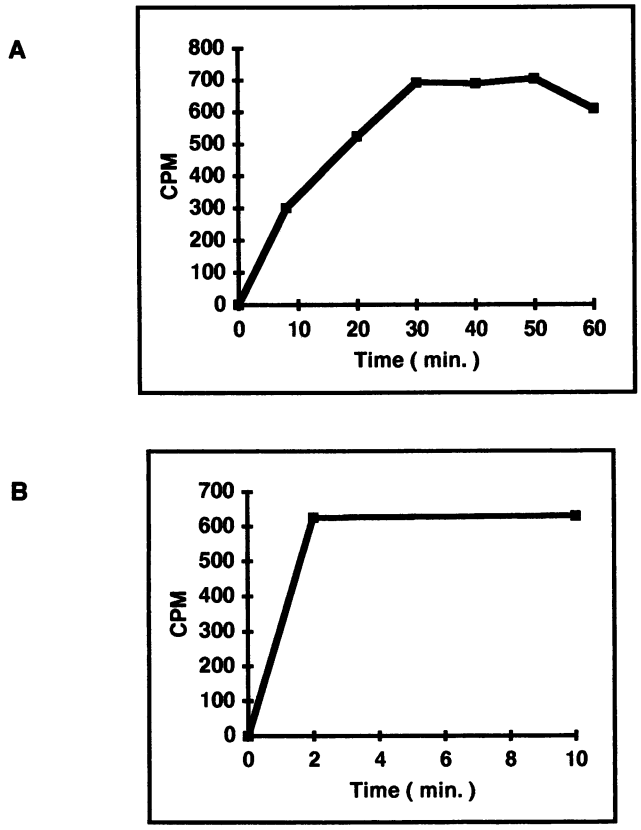

FIG. 6. Rate of formation and synchronous initiation by Sarkosyl-resistant complexes. (A) Free components were mixed for the times indicated, and 0.08% Sarkosyl was then added; 30 s later, 500 μM nucleotides were added and were followed immediately by Sarkosyl to a final concentration of 0.16%. The reaction was stopped 5 min later, and the resulting RNAs were detected by electrophoresis and quantified by Cerenkov counting. It was found that 0.08% Sarkosyl inactivated free components but did not block initiation of assembled closed complexes and that 0.16% Sarkosyl blocked initiation but not elongation (unpublished data). (B) Preinitiation complexes were assembled for 30 min, and then 0.08% Sarkosyl was added for 30 s and was followed by 500 μM nucleotides to begin initiation; 0.16% Sarkosyl was then added to prevent potential reinitiation. RNA was quantified at 2 and 10 min.

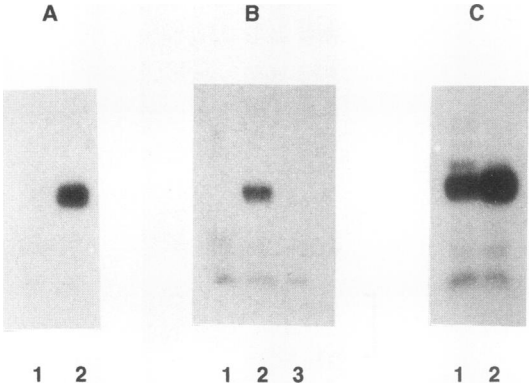

FIG. 7. Reinitiation still requires ATP hydrolysis. (A) ATPγS inhibits initial-round transcription. All the components were incubated in the absence of NTPs for 28 min, and then 125 μM NTPs (lane 2) or 1.75 mM ATPγS together with 125 μM NTPs (lane 1) were added for 2 min. (B) ATPγS inhibits transcription by inhibiting the ATP requirement step but not the elongation step. All components were incubated in the absence of NTPs for 28 min, and then different combinations of nucleotides were added for 2 min to allow at most the initial round of transcription. Lane 1, 125 μM (each) GTP, CTP, UTP, and ATPγS were added; lane 2, 125 μM (each) dATP, ATPγS, GTP, CTP, and UTP were added; lane 3, 125 μM (each) dATP, GTP, CTP, and UTP were added. (C) ATPγS inhibits reinitiation. All components were incubated in the absence of NTPs for 28 min, and then NTPs were added. After 2 min, 1.75 mM ATPγS was added (lane 1) or left out (lane 2) for 28 min.

elongation but is due to its inability to provide an efficient source of hydrolyzable ATP during initiation.

Figure 7C addresses whether ATP hydrolysis is also required during reinitiation. First-round preinitiation complexes are assembled in the absence of nucleotides and then allowed to initiate in the standard protocol with 125 μM (each) ATP, GTP, CTP, and UTP for 2 min. The inclusion of ATP is necessary, since first-round transcription cannot occur in its absence. After this pulse, ATPγS is added to 1.75 mM (lane 1) or not added (lane 2) and transcription reinitiation is then allowed to proceed. Recall that this ratio of ATPγS to ATP substantially inhibited first-round transcription (Fig. 6A). The results show that the addition of ATPγS inhibits transcription in this reinitiation protocol (lane 2 versus lane 1). The inhibition is not complete, since the protocol demands that the efficient first round of transcription be allowed to proceed in both cases. The inhibition in this protocol is therefore restricted to effects at the level of reinitiation. We infer that ATP hydrolysis is required during assembly of reinitiation complexes, since ATPγS blocks initiation but not elongation under these conditions (Fig. 7A and B).

DISCUSSION

These results have shown that the rates of transcription initiation and reinitiation by human RNA polymerase II can differ. In response to the activator GAL4-AH, open transcription complexes, at a truncated adenovirus E4 promoter, first assemble with a half-time of approximately 10 min. In the presence of NTPs, these open complexes support very rapid initiation accompanied by reclosing of the start site. Reassembly and reinitiation then proceed very rapidly, with a half-time of less than 3 min. Thus, this system has the interesting and unexpected property of facilitated reinitiation, wherein subsequent rounds of transcription should be faster than the first.

Several previous studies suggest possible causes of this uncoupling of initiation and reinitiation rates. They suggest that certain factors are modified or left behind on the template after the first round of transcription. For example, both TFIID and activator GAL4-VP16 (21, 24) appear to remain associated with the template after initial transcription. It is not known whether other factors also remain behind. The cause of the more rapid reinitiation may then be that during reinitiation these factors need not be rebound, thus shortening the time required to assemble a second productive transcription complex. The binding of TFIID and GAL4-VP16 appears to be too fast (10, 24) to account for the saving of more than 7 min during reinitiation, although rates may vary with conditions. Since we have suggested recently that the rate-limiting step in this pathway involves the action of the TFIIA fraction (23), it is possible that the change induced by this fraction is fixed on the template and need not

be done again during reinitiation in this system. Interestingly, the TFIIA fraction seems to be a critical target for regulating activated transcription (11, 15, 16).

Reinitiation in this system appears to require the use of ATP during each round of assembly. One formal possibility for the cause of facilitated reinitiation would be that a component was slowly phosphorylated in the first round but did not need to be rephosphorylated during reinitiation. This seems less likely, since ATP is required for each round. Most likely the ATP is used to reopen the DNA start site during each round as it is required to open it during the first round. We cannot rule out, however, that ATP is used as part of a continuing need to phosphorylate polymerase II during each round of initiation (17).

In one previous study of basal *Drosophila* transcription, reinitiation was inferred to be no faster than initiation, and both were rapid, on the order of 3 min (13). In studies of GC box-dependent transcription in a HeLa extract (unpublished), we have found that the rate of initiation can also be very rapid if experimental conditions are optimized. Indeed, it has long been known from bacterial transcription studies that the rate of open complex formation varies predictably with the concentration of essential protein factors (8, 14). However, there is no precedent in the extensive prokaryotic transcription literature for uncoupling of the rates of initiation and reinitiation, as observed here for GAL-AH-dependent polymerase II transcription. Thus, although the rates of initiation and reinitiation could conceivably vary, the difference in rate observed in our experimental system is unprecedented and demonstrates the potential for uncoupling the two processes in mammalian cells.

Slow initiation followed by rapid reinitiation has potentially interesting physiological consequences. In principle, it enlarges the possibilities for transcriptional regulation, since initiation and reinitiation could conceivably be regulated independently. Although induction in mammalian cells can be rapid, in many cases it is more important to control the specificity of induction tightly than to allow induction to proceed very rapidly. This differs substantially from bacterial transcription, which must respond rapidly to allow effective competition for limited nutrients. As just discussed, there is no evidence that the rates of initiation and reinitiation differ in prokaryotes, but they can differ for mammalian transcription, as in the system studied here. Perhaps a slow initial rate in a mammalian system reflects complexities associated with the physiological need to prevent inappropriate induction. The mechanism leading to faster continued transcription would allow rapid expression even for a slowly induced gene once the initial barrier was overcome. Total mRNA production could be regulated separately from induction by use of changes in the reinitiation rate, which accounts for the bulk of transcription. It will be interesting to determine whether the factors that control the rates of initiation and reinitiation differ in a promoter sequence-dependent manner.

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