Effect of Sarkosyl and heparin on single-step addition reactions catalysed by wheat-germ RNA polymerase II–poly[d(A–T)]transcription complexes

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Incubation of purified wheat-germ RNA polymerase II with poly[d(A–T)] template, Mn^{2+} , U–A dinucleoside monophosphate primer and UTP substrate resulted in catalytic formation of the trinucleoside diphosphate U–A–U, in accordance with the results of previous studies. Both Sarkosyl and heparin inhibited completely and immediately (within less than 1 min) U–A–U synthesis, if either of these compounds was added to the assays during the progress of the reaction. This behaviour is in marked contrast to that reported for single-step addition reactions catalysed by *Escherichia coli* RNA polymerase on the same template [Sylvester & Cashel (1980) *Biochemistry* 19, 1069–1074]. However, treatment of the transcription complexes with Sarkosyl or heparin for periods sufficient to abolish U–A–U formation completely did not suppress completely the ability of such complexes to elongate RNA chains. Hence, the effect of Sarkosyl or heparin on the rate of U–A–U synthesis was predominantly due to change in the rate (or in the mechanism) of trinucleotide product release by the transcription complexes. Furthermore, once U–A–U synthesis has begun on the poly[d(A–T)] template, the transcription complexes became resistant to the action of a competitor DNA such as poly[d(G–C)]. The results are consistent with a model where at least a sizeable fraction of the enzyme molecules remains associated with the DNA template upon formation of a single phosphodiester bond.

INTRODUCTION

It is now well established that when *Escherichia coli* RNA polymerase initiates transcription, the enzyme will form and release short oligonucleotides, rather than continue to elongate RNA chains (reviewed in von Hippel *et al.*, 1984). During this process, termed abortive initiation, the RNA polymerase is continuously recycled at initiation and productive synthesis of a long RNA chain is essentially an escape from the cycling reaction (Carpousis & Gralla, 1980). Under experimental conditions allowing only the synthesis of a single phosphodiester bond with a short primer, such as a dinucleotide and a single ribonucleoside triphosphate substrate, the behaviour of the prokaryotic transcription complex is reminiscent of that encountered in abortive initiation in that the enzyme will repeatedly release the nascent RNA and reinitiate transcription, without release of the polymerase from the promoter. Using poly[d(A–T)] as template, U–A as primer and UTP as substrate, Sylvester & Cashel (1980) demonstrated that *E. coli* RNA polymerase will synthesize the trinucleotide U–A–U in a catalytic fashion, i.e. in larger amounts than that of the enzyme in the transcription assay. However, the absence of inhibition by heparin, a polyanion known to bind free RNA polymerase but not the enzyme engaged on a DNA template, led these authors to propose that the enzyme remains tightly bound to the template during formation of U–A–U. Hence, with this model system *E. coli* RNA

polymerase exhibits some of the characteristic features that have been described for reactions conducted with natural DNA templates. Studies on specific transcription *in vitro* of class II genes by mammalian systems such as nuclear extracts have suggested that RNA polymerases II behave similarly to prokaryotic RNA polymerase in that, before being committed to the elongation mode, the enzymes pass through a stage in which transcripts are produced abortively (Luse & Jacob, 1987; Luse *et al.*, 1987). These studies also showed that RNA synthesis by RNA polymerase II could be separated into several functional steps, which can be defined on the basis of successive changes in the sensitivity to Sarkosyl [an anionic detergent presumed to behave similarly to heparin (Gariglio *et al.*, 1974; Dynan & Burgess, 1979; Ackerman *et al.*, 1983)]: (i) the initiation and synthesis of a short oligonucleotide that can be inhibited by Sarkosyl, and (ii) the completion of the full-length transcript in a step that is resistant to inhibition by Sarkosyl (Coppola & Luse, 1984; Hawley & Roeder, 1985, 1987; Luse & Jacob, 1987; Luse *et al.*, 1987; Conaway & Conaway, 1988).

In previous studies, we have shown that the behaviour exhibited by purified wheat-germ RNA polymerase II in the first catalytic steps of transcription compares well to that described for *E. coli* RNA polymerase and for the more complex transcription systems derived from animal cells (Job *et al.*, 1988*a,c,d*). To analyse this system further, we sought to characterize the effects of Sarkosyl

Abbreviations used: dinucleoside monophosphate primers and trinucleoside diphosphate products are referred to as dinucleotides and trinucleotides, respectively.

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and heparin on single-step addition reactions and productive elongation catalysed by wheat-germ RNA polymerase II on a poly[d(A-T)] template. In this study, we found that Sarkosyl and heparin were very effective in blocking the synthesis of U-A-U primed by U-A, even if these compounds were introduced in the assay during the progress of the reaction. However, under such experimental conditions, the transcription complexes supported RNA chain elongation, following addition of the missing triphosphate substrate ATP dictated by the template sequence. Thus, Sarkosyl and heparin were found to affect mostly the step of trinucleotide product release rather than preventing reinitiations by inactivation of free RNA polymerase molecules.

MATERIALS AND METHODS

Reagents

Ribonucleoside triphosphates and the dinucleoside monophosphates were purchased from Sigma Chemical Co. and Boehringer (Mannheim). [α - 32 P]UTP (410 Ci/mmol; 1 Ci = 3.7×10^{10} Bq) was from Amersham. Poly[d(A-T)] and poly[d(G-C)] were from P-L Biochemicals. Sarkosyl (sodium *N*-lauroylsarcosine) was from Fluka; heparin (sodium salt) was from Sigma. All other chemicals and buffer components were of reagent grade. Wheat-germ RNA polymerase II (essentially in the IIA form) was purified by the method of Jendrisak & Burgess (1975), as previously described (Job *et al.*, 1984). The *E. coli* RNA polymerase holoenzyme was purchased from Boehringer (Mannheim).

Reaction assays

Unless otherwise noted in the Figure legends, the reaction mixtures leading to U-A-U synthesis contained 18 nM-enzyme, 1.8 μ M-poly[d(A-T)] (in terms of nucleotide units), 0.9 mM-U-A, 1.5 mM-MnCl₂ and 5 μ M-[α - 32 P]-UTP, in transcription buffer as described in Job *et al.* (1987). The protocol used for all reactions included a 10 min incubation at 35 °C of all components, except the triphosphate substrates, followed by addition of 5 μ M-[α - 32 P]UTP (synthesis of U-A-U) or 5 μ M-[α - 32 P]UTP + 20 μ M-ATP (synthesis of poly[r(A-U)]), and a second incubation at 35 °C. Sarkosyl or heparin, at final concentration as indicated in the Figure legends, was added to the assays at different stages: (i) simultaneously with poly[d(A-T)] template at the start of the first 10 min incubation at 35 °C; (ii) during the course of the synthesis of U-A-U or that of poly[r(A-U)]; or (iii) 7 min after the addition of [α - 32 P]UTP and prior to that of ATP. For this latter protocol, the periods of incubation between additions of Sarkosyl or heparin and ATP varied and are indicated in the Figure legends. Final volumes were 10 μ l and assays were incubated for 30 min at 35 °C. For kinetic experiments, final volumes were 450 μ l, and incubations were effected in 1.5 ml stoppered Eppendorf tubes at 35 °C. Portions (5 μ l) were withdrawn at timed intervals. Reactions were stopped by adding 2 vol. of stop solution containing 1 mM-EDTA, 80 % formamide, and 0.1 % Xylene Cyanol.

Activity measurements

RNA synthesis was measured by the use of trichloroacetic acid precipitation of 5 μ l (reaction mixtures + stop solution) spotted on Whatman GF/C filters (Job

et al., 1987). Synthesis of U-A-U was quantified after t.l.c. on poly(ethyleneimine)-cellulose sheets (obtained from Merck) of 2 μ l (reaction mixture + stop solution) (Randerath & Randerath, 1967; Job *et al.*, 1987) with 1 M-formic acid/0.1 M-LiCl as the solvent system, until the front had migrated 10 cm. Under these conditions, U-A-U migrated with an R_F value of 0.64, whereas unreacted UTP remained bound at the origin. Following radioautography for 18 h at -80 °C, all spots containing radioactivity were cut out and counted for radioactivity.

Gel chromatography

To investigate whether U-A-U was released by the transcription complexes, reaction assays were subjected to separation by rapid chromatography on Sephadex G-25 (Pharmacia) (Luse & Jacob, 1987) using a Jouan centrifuge, at 1000 rev./min. G-25 (1 ml) columns were prepared in 1 ml disposable syringes plugged with a Teflon disk, using transcription buffer/1.5 mM-MnCl₂ as running buffer. After packing the columns by centrifugation for 1 min, 50 μ l transcription assays were loaded, and the columns were centrifuged for 1 min. Then 50 μ l of running buffer was loaded, followed by 1 min centrifugation. The two initial fractions (each of 50 μ l) were collected as the excluded fractions. The included fractions were obtained by successive 100 μ l rinses.

RESULTS

Effect of Sarkosyl

The effect of Sarkosyl on U-A-U formation was investigated according to two protocols. In the first, enzyme and Sarkosyl were incubated together in transcription buffer for 10 min at 35 °C, in the presence of poly[d(A-T)], MnCl₂ and U-A primer (Fig. 1a). Subsequently, UTP was added and incubation was continued for 30 min. Fig. 1(a) shows that U-A-U synthesis was abolished with Sarkosyl at 0.12 %. The second protocol was to carry out the first 10 min incubation as described above, but in the absence of Sarkosyl; then to initiate U-A-U synthesis by adding UTP. Sarkosyl at 0.12 % was introduced 5 min later, and incubation was continued. Fig. 1(b) shows that if Sarkosyl was added once U-A-U synthesis had begun, there was an immediate and complete shut off of the reaction.

It is known that Sarkosyl does not inhibit RNA chain elongation by RNA polymerases II (Gariglio *et al.*, 1974). This property was also detected for synthesis of poly[r(A-U)] catalysed by wheat-germ RNA polymerase II on the poly[d(A-T)] template (Fig. 1c).

In the absence of Sarkosyl, the reaction of single-step addition proceeded catalytically (Fig. 1b), indicating that reinitiation was occurring during steady-state accumulation of U-A-U and that the trinucleotide product was released by the transcription complex. This was assessed by rapid chromatography of reaction assays leading to U-A-U synthesis on Sephadex G-25. RNA polymerase activity was recovered in the second excluded fraction, whereas U-A-U and UTP eluted mostly with the second, third and fourth included fractions, thereby demonstrating that U-A-U was released by the ternary transcription complexes (not shown).

One possible explanation for the inhibition pattern in Fig. 1(b) could therefore be that during reinitiation the

Fig. 1. Effect of Sarkosyl on U-A-primed U-A-U synthesis and on poly[r(A-U)] synthesis catalysed by wheat-germ RNA polymerase II

Reactions were performed as described under 'Materials and methods' in the presence of 18 nM-wheat-germ RNA polymerase II, 1.8 μM -poly[d(A-T)], 0.9 mM-U-A and 1.5 mM-MnCl₂. After 10 min at 35 °C, the reaction of U-A-U synthesis was initiated by adding 5 μM -[α -³²P]UTP (1 pmol = 5700 c.p.m.); that of poly[r(A-U)] by adding 5 μM -[α -³²P]UTP + 20 μM -ATP. (a) Effect of Sarkosyl concentration on U-A-U synthesis. Sarkosyl was added at the start of the first 10 min incubation at 35 °C. U-A-U synthesis was measured after 30 min at 35 °C. (b) Progress curves for U-A-U synthesis: □, reaction in the absence of Sarkosyl; ■, reaction in the presence of Sarkosyl (to 0.12%) introduced at the start of the first 10 min incubation at 35 °C; ▲, Sarkosyl (to 0.12%) addition 5 min (arrow) after initiation of U-A-U synthesis. (c) Progress curves for poly[r(A-U)] synthesis; symbols as in (b).

enzyme did not remain bound to the template and was trapped by Sarkosyl. In order to investigate this possibility, reaction assays were prepared, containing enzyme, template, MnCl₂, and U-A primer. After 10 min at 35 °C, U-A-U synthesis was initiated by adding [α -³²P]UTP; 7 min later, Sarkosyl at 0.12% was added and incubation was continued for various periods. Then ATP was added, and poly[r(A-U)] synthesis was quantified by the use of trichloroacetic acid precipitation. Fig. 2 shows that even though U-A-U synthesis was arrested upon incubation for 10 min with Sarkosyl (see Fig. 1b), the transcription complexes having formed a single phosphodiester bond could still support RNA synthesis, if they were supplemented with ATP. This finding is in excellent agreement with previous observations that the addition of a specific dinucleotide and a single ribonucleoside triphosphate permits the formation of a highly stable complex between wheat-germ RNA polymerase II and SV40 DNA (Sara-gosti *et al.*, 1979).

Effect of heparin

One of the objectives of the present study was to compare the properties of wheat-germ RNA polymerase II in the process of recycling to those reported with the well documented prokaryotic RNA polymerase. Since for this latter enzyme heparin, but not Sarkosyl, had been used to define the stability of the ternary transcription complexes (Sylvester & Cashel, 1980), we wished to characterize the effect of this polyanion on the reactions of single-step addition catalysed by the plant enzyme. If the polyanion was present in the assays during the first 10 min incubation in the absence of the triphosphate substrate, it was found that heparin at 0.5 $\mu\text{g}/\text{ml}$ completely inhibited polymerase activity (Fig. 3a), in agreement with the amount of heparin needed to inactivate wheat-germ RNA polymerase II determined earlier by Dynan & Burgess (1979). As observed with Sarkosyl, the addition of heparin to 5 $\mu\text{g}/\text{ml}$ in transcription assays 2 min or

Fig. 2. Sarkosyl-resistant poly[r(A-U)] synthesis

Reactions were performed as described under 'Materials and methods' in the presence of 18 nM-enzyme, 1.8 μ M-poly[d(A-T)], 0.9 mM-U-A and 1.5 mM-MnCl₂. After 10 min at 35 °C, the reaction of U-A-U synthesis was initiated by adding 5 μ M-[α -³²P]UTP (1 pmol = 4834 c.p.m.); 7 min later Sarkosyl (to 0.12%) was added and incubation was continued for 1 min (a) or 10 min (b). Poly[r(A-U)] synthesis was measured at 35 °C following addition of 20 μ M-ATP (arrow). □, Reactions conducted in the absence of Sarkosyl; ■, reactions in the presence of Sarkosyl.

10 min after initiation of U-A-U synthesis was associated with an immediate and complete block in the formation of the product (Fig. 3b). This behaviour is in marked contrast to that reported for *E. coli* RNA polymerase, since with the prokaryotic enzyme catalytic synthesis of U-A-U primed by U-A on poly[d(A-T)] was resistant to the action of heparin at 80 μ g/ml (Sylvester & Cashel, 1980).

Fig. 3(c) shows that, with wheat-germ RNA polymerase II, transcription complexes engaged in productive elongation were resistant to the inhibition by heparin at 5 μ g/ml, in agreement with the results of Dynan & Burgess (1979). However, impediment of productive elongation was more pronounced than when Sarkosyl was employed, especially if heparin was introduced to the assay shortly after the start of poly[r(A-U)] synthesis (e.g. at 2 min instead of 10 min; compare Figs. 1c and 3c).

We further characterized the effect of heparin, employing the experimental protocol described for Sarkosyl in Fig. 2. U-A-U synthesis was allowed to proceed for 7 min. Then, heparin to 5 μ g/ml, followed 1 min later by

ATP to 20 μ M, were added and incubation was continued. Unlike Sarkosyl (Fig. 2), heparin reduced notably the rate of poly[r(A-U)] synthesis (Fig. 4a). However, these data indicate that a fraction of the polymerase molecules engaged in the synthesis of U-A-U became resistant to heparin inactivation. Fig. 4(a) also shows that the progress curve obtained in the presence of heparin can be divided into two main phases: an initial 5 min stage which was unaffected by heparin, followed by a linear stage during which poly[r(A-U)] synthesis was more severely curtailed. In Fig. 4(b), the same protocol as described above was used throughout, but the period of incubation between additions of heparin and ATP was varied up to 6 min. For all reactions carried out in the presence of heparin, biphasic curves such as that presented in Fig. 4(a) were obtained. The longer the incubation time between additions of heparin and ATP, the stronger the inhibition was observed. However, even upon incubation for 6 min (which definitely blocked U-A-U synthesis; see Fig. 3b), heparin allowed some transcription to continue, even 15 min after it was added.

Effect of a template challenge

In order to further characterize the stability of transcription complexes involved in formation of a single phosphodiester bond, similar experiments to those presented in Figs. 1(b) and 3(b) were performed, but employing poly[d(G-C)] as a competitor DNA. It was found that the rate of U-A-U synthesis on the poly[d(A-T)] template was almost unaffected upon addition of a 10-fold molar excess of poly[d(G-C)] over poly[d(A-T)], if the competitor DNA was added 5 min after U-A-U synthesis had begun. Control experiments, where both DNA species were added simultaneously to the assay, demonstrated that this amount of poly[d(G-C)] provided an efficient competition from transcription of the poly[d(A-T)] template (not shown).

DISCUSSION

Sarkosyl and heparin have been widely employed to characterize the stability of ternary transcription complexes *in vitro* with prokaryotic and eukaryotic RNA polymerases. For instance, using a HeLa nuclear extract, Luse & Jacob (1987) showed that Sarkosyl at 0.05% completely eliminated the synthesis of A-C-U by RNA polymerase II (Sarkosyl at 0.2% does not inhibit transcription elongation with this system) with A-C primer, UTP substrate, and a template bearing the adenovirus 2 major late promoter. However, A-C-U synthesis was still detected at 0.025% Sarkosyl, although the reaction without Sarkosyl yielded somewhat more trinucleotide product than did the reaction with Sarkosyl (Luse & Jacob, 1987). From these results, it was deduced that the initiation-competent complex remained intact during multiple rounds of abortive initiation as is the case with *E. coli* RNA polymerase (Sylvester & Cashel, 1980; Carpousis & Gralla, 1985). We have used this methodology to investigate the mechanism of recycling of wheat-germ RNA polymerase II in the process of U-A-U synthesis. From the results presented, it appears that one may draw different conclusions about the stability of transcription complexes whether one measures directly the appearance of the trinucleotide product or the ability of transcription complexes having formed a single phosphodiester bond to yield elongation-

Fig. 3. Effect of heparin on U-A-primed U-A-U synthesis and on poly[r(A-U)] synthesis catalysed by wheat-germ RNA polymerase II

Reactions were performed as described under 'Materials and methods' in the presence of 18 nM-enzyme, 1.8 μ M-poly[d(A-T)], 0.9 mM-U-A and 1.5 mM-MnCl₂. After 10 min at 35 °C, the reaction of U-A-U synthesis was initiated by adding 5 μ M-[α -³²P]UTP (1 pmol = 8114 c.p.m.); that of poly[r(A-U)] by adding 5 μ M-[α -³²P]UTP + 20 μ M-ATP. (a) Effect of heparin concentration on U-A-U synthesis. Heparin was added at the start of the first 10 min incubation at 35 °C. U-A-U synthesis was measured after 30 min at 35 °C. Results (*v*) are normalized to the rates obtained in the absence of heparin (*V*₀). (b) Progress curves for U-A-U synthesis: □, reaction in the absence of heparin; ■, reaction in the presence of heparin (5 μ g/ml) added at the start of the first 10 min incubation at 35 °C; ▲, heparin (5 μ g/ml) addition 2 min (arrow) after initiation of U-A-U synthesis; +, heparin (5 μ g/ml) addition 10 min (arrow) after initiation of U-A-U synthesis. (c) Progress curves for poly[r(A-U)] synthesis. Symbols as in (b).

competent complexes. Firstly, both Sarkosyl and heparin were found to inhibit strongly U-A-U formation, if either of these compounds was added to the assays during the progress of the reaction. The effect occurred within less than 1 min after Sarkosyl or heparin was added, suggesting that the free enzyme generated through abortive cycling was trapped by Sarkosyl or heparin.

Secondly, treatment of the transcription complexes with Sarkosyl or heparin for periods sufficient to completely abolish U-A-U formation did not suppress completely the ability of such complexes to elongate RNA chains. Thus, the competence for elongation of complexes engaged in the synthesis of U-A-U was almost unimpeded by Sarkosyl, even upon incubation for 10 min with an amount of the detergent sufficient to block trinucleotide synthesis. Under the same experimental conditions, heparin reduced the competence for elongation of the complexes involved in the synthesis of a

single phosphodiester bond. However, RNA synthesis was not fully abolished and the kinetic data show that the inactivation of the transcription complexes was rather a slow process in comparison to the very short time needed to stop U-A-U synthesis in the single-step addition experiments. It appears that in these experiments the process of attack of the transcription complexes by heparin resembles more closely that observed if heparin was added to the assays shortly after poly[r(A-U)] had begun (compare Figs. 4a and 3c), rather than that observed when measuring directly the effect of heparin on the extent of U-A-U formation (compare Figs. 4a and 3b). It is also important to note that heparin at 5 μ g/ml prevented U-A-U and poly[r(A-U)] syntheses, when provided to the assays simultaneously with the poly[d(A-T)] template. It follows that the polymerase should have been irretrievably trapped by heparin, if the enzyme dissociated from the template during reinitiation in

Fig. 4. Heparin-resistant poly[r(A-U)] synthesis

Reactions were performed as described under 'Materials and methods' in the presence of 18 nM-enzyme, 1.8 μ M-poly[d(A-T)], 0.9 mM-U-A and 1.5 mM-MnCl₂. After 10 min at 35 °C, the reaction of U-A-U synthesis was initiated by adding 5 μ M-[α -³²P]UTP (1 pmol = 4834 c.p.m.); 7 min later, heparin (to 5 μ g/ml) was added and incubation was continued at 35 °C. (a) Poly[r(A-U)] synthesis was measured at 35 °C following addition of 20 μ M-ATP (arrow) 1 min after heparin: □, reaction conducted in the absence of heparin; ■, reactions in the presence of heparin. (b) Same experiments as in (a) but the period at 35 °C between additions of heparin and ATP was varied from 0 to 6 min. Results (*v*) are normalized to the rates of poly[r(A-U)] synthesis measured under identical conditions but in the absence of heparin (*V*₀), for instance curve (□) in (a). ■, Initial rate measurements; □, steady-state rate measurements.

U-A-U synthesis. With heparin (Fig. 3b) or Sarkosyl (Fig. 1b), we therefore conclude that the immediate shut-off in synthesis of U-A-U was not caused primarily by inactivation of free RNA polymerase molecules during reinitiation. The present results are consistent with a model where at least a sizeable fraction of the enzyme molecules remains associated with the DNA template upon formation of a single phosphodiester bond. In this respect wheat-germ RNA polymerase II behaved similarly to both *E. coli* RNA polymerase and RNA polymerase II in the mammalian extracts. This contention is further circumstantiated by the observation that once U-A-U synthesis had begun on the poly[d(A-T)] template, the transcription complexes became resistant to the action of a competitor DNA such as poly[d(G-C)]. Therefore, it would appear reasonable to proceed on the assumption that the effect of Sarkosyl or heparin on the rate of U-A-U synthesis is predominantly due to change in the rate (or in the mechanism) of trinucleotide product release by the transcription complexes. Several possibilities may exist. (1) The RNA polymerase could react with Sarkosyl or heparin, even when it is bound to DNA, because the binding sites on the enzyme molecule would not overlap exactly. This would modify the catalytic properties of the transcription complexes. In this context, we note that anionic detergents such as sodium dodecyl sulphate and Sarkosyl alter α -amanitin binding to wheat-germ RNA polymerase II (Brown & Garrity, 1980). Alternatively, there might be a component in the preparation of wheat-germ RNA polymerase II which forces trinucleotide synthesis to proceed catalytically and can be inactivated by Sarkosyl or heparin. (2) Catalytic synthesis of U-A-U would proceed through the occurrence of different forms of the enzyme-poly[d(A-

T)]-U-A-U complex, with distinct reactivities towards Sarkosyl or heparin. In favour of this mechanism, it is worth pointing out that rate-limiting steps assigned to conformational rearrangements of the polymerization complex have been invoked to account for the kinetics of formation of the first phosphodiester bond with both *E. coli* RNA polymerase (Shimamoto & Wu, 1980) and *E. coli* DNA polymerase I (Kuchta *et al.*, 1987). It may also be worth noting that poly[r(A-U)] synthesis catalysed by wheat-germ RNA polymerase II on poly[d(A-T)] as template proceeds through a slow transient burst of activity (see Figs. 1c, 2, 3c and 4a), which has been assigned to a slow transition of the transcription complex in the commitment to productive elongation (Job *et al.*, 1988b).

Models involving several substeps have been proposed to describe the process of commitment to elongation *in vitro*, from template competition assays or by studying the resistance to inactivation by Sarkosyl, with more complex class II genes transcription systems which, in addition to RNA polymerase II, contain transcription factors (Hawley & Roeder, 1985, 1987; Luse *et al.*, 1987; Luse & Jacob, 1987; Conaway & Conaway, 1988). It is possible that the intermediates we postulate in formation of a transcription complex having elongation competence with the purified plant enzyme correspond to some of those that have been characterized with the systems cited above, which should aid in the understanding of the mechanisms by which elongation competence is achieved in RNA polymerase II-mediated transcription.

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