RNA Polymerase: Correlation between Transcript Length, Abortive Product Synthesis, and Formation of a Stable Ternary Complex†

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ABSTRACT: In order to investigate the relationship between the stability of the ternary complex RNA polymerase·T_7 D_{111} DNA·RNA product and the length of the bound RNA product, we have developed a protocol for the production of stable ternary complexes of known length and composition. The assembly of the ternary complex is achieved by utilizing a dinucleotide tetraphosphate (pppApU) as a selective primer, which is augmented by one or more appropriate nucleotides. The labeled products were characterized by autoradiography of gel electrophoresis patterns, which were then quantified. The criterion for stability is the protection from perturbations (a salt-jump or a rifampicin challenge), which effectively inhibit initiation. The formation of a bound ribotetranucleotide ternary complex confers stability and terminates abortive product synthesis.

The formation of a stable ternary complex [enzyme·template–pppN$(pN)_x$] is considered to occur when short abortive products cease to be produced and elongation proceeds to the normal termination point. The length of product at which this stable ternary complex is achieved has been suggested by Sienbenlist et al. (1980) to be related to the number of bases made available as the DNA is unfolded to yield a single-strand region upon formation of the initial binary complex between the enzyme and the promoter region of the template. The formation of a set of short products occurs repetitively as the bound enzyme recycles over this domain until it is finally committed to elongation and breaks out of this cycle.

Hansen & McClure (1980) have shown that abortive oligonucleotide products of length three to nine bases are formed and released from the template $[d(A\text{-}T)]_n$.[1] When the transcript is eight to nine bases long, sigma (σ) factor is released and the existing stable ternary complex is committed to elongate until termination occurs. Carpousis & Gralla (1980) and Gralla et al. (1980), using *lac* UV 5 DNA as a template, have shown that abortive products of length two to six bases are released and that a stable ternary complex is achieved at a product length of four to six bases, from which elongation can continue until termination occurs. Grachev (1980), using a T_7 restriction nuclease fragment containing the A_0, A_1, A_2, and A_3 promoters as a template, has shown that abortive products of a length up to 9–11 bases are released and that a stable ternary complex is achieved at a product length of 10 bases. Nierman & Chamberlin (1980), using T_7 DNA as a template and a low concentration of all NTP substrates, have shown the production and release of only an abortive dimer product. Taylor & Burgess (1979) using λrif^d 18 DNA have shown that an initiated complex that is resistant to a high-salt wash can form only in the presence of the specific combination of NTPs encoded in the promoters–5′-mRNA base sequence; but maximum stability is attained only after the mRNA elongation has proceeded beyond five bases.

In this report, we describe a technique for the formation of short products of defined length and sequence. The technique employs pppApU as a dimer primer for the major promoter (A_1) of the T_7 D_{111} DNA template to which one to three additional ultrapure NTPs are added to form either the 3-mer (pppApUpC), the 4-mer (pppApUpCpG), or the 20-mer (pppApUpCpGpA...). The stability of these ternary complexes was assessed by a rifampicin or high-salt challenge prior to the addition of all NTPs required for elongation to the termination point. The product formed in each case was detected by TLC or urea–polyacrylamide gel electrophoresis. A stable ternary complex resistant to rifampicin or high-salt challenge was achieved coincident with the stoichiometric formation of a bound ribotetranucleotide product.

Experimental Procedures

Materials

Ultrapure HPLC ATP, UTP, CTP, and GTP were purchased from ICN; rifampicin was from Boehringer; ultrapure urea was from Schwarz/Mann; acrylamide and *N,N′*-methylenebis(acrylamide) were from Bio-Rad Laboratories. The [α-^{32}P]CTP was obtained from New England Nuclear. The deletion mutant of T_7 phage D_{111} was the gift of J. Dunn and was the source of the template DNA. The DNA was isolated from purified phage according to the method of Thomas & Abelson (1966). The DNA-dependent RNA polymerase was purified from *Escherichia coli* strain K^{12} according to the procedure of Zillig et al. (1970) and was approximately 98% pure as judged by $NaDodSO_4$ gel electrophoresis. The purified enzyme had a σ/E ratio of 0.8. The dinucleotide tetraphosphate pppApU was produced by incubating RNA polymerase, T_7 ΔD_{111} DNA, ATP, and UTP in the presence of rifampicin for 6 h [a variation of the method of Smagowicz & Scheit (1978)]. The product was purified twice by column chromatography on DEAE with a TBic buffer system at pH 7.8 and a concentration gradient elution over the range 0.01–0.5 M with respect to buffer. The product purity was assessed by TLC with the WASP and IBAW solvent systems.

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[1] Abbreviations: NTP, nucleotide 5′-triphosphate; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; $[d(A\text{-}T)]_n$, poly[d(A-T)]·poly[d(A-T)]; Eσ, RNA polymerase holoenzyme; WASP solvent, water–saturated $(NH_4)_2SO_4$–isopropyl alcohol (18:80:2); IBAW solvent, isobutyric acid–concentrated NH_4OH–water (66:1:33); SA, specific activity (cpm/pmol); TBic, triethylamine bicarbonate buffer; TLC, thin-layer chromatography; $NaDodSO_4$, sodium dodecyl sulfate; DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; HPLC, high-pressure liquid chromatography; ATP, adenosine 5′-triphosphate; GTP, guanosine 5′-triphosphate; CTP, cytidine 5′-triphosphate; UTP, uridine 5′-triphosphate; Cl_3CCOOH, trichloroacetic acid; $\mu\uparrow$, salt-jump challenge.

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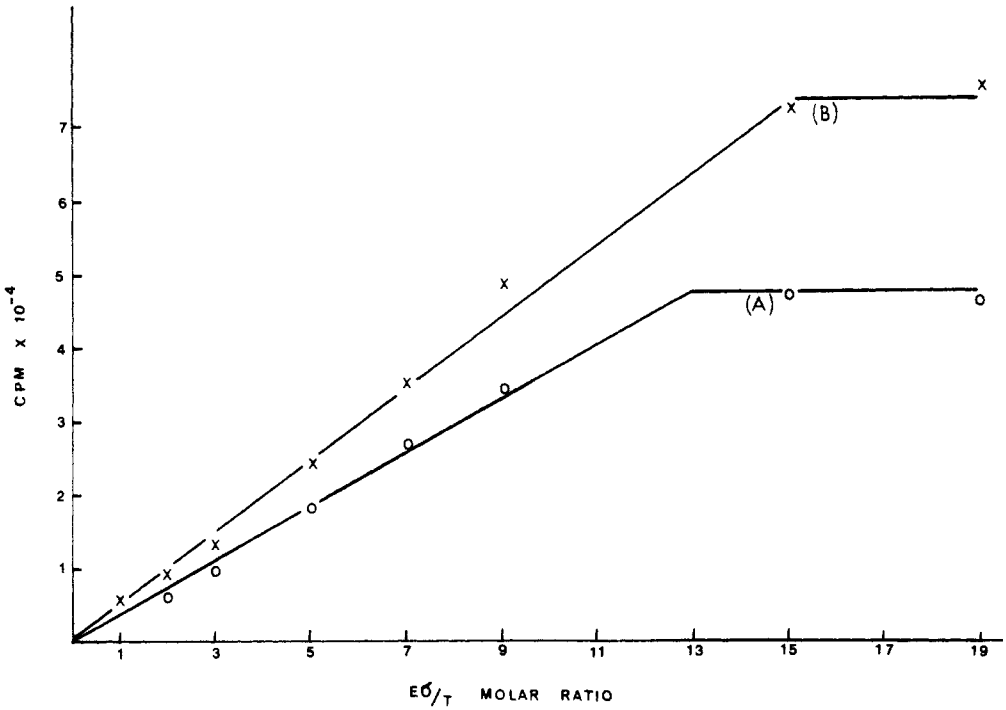

FIGURE 1: Activity as a function of $E\sigma/T_7$ D_{111} DNA. (A) $(E\sigma$ + template$)_5(NTP)_2$. Each reaction containing 0.166 pmol of T_7 D_{111} DNA and variable amounts of RNA polymerase ($E\sigma$) was incubated for 5 min at 37 °C in 40 mM Tris, pH 7.9, 80 mM KCl, 1 mM DTT, and 10 mM $MgCl_2$. NTP = 400 μM ATP, CTP, and GTP + 200 μM [^{3}H]UTP. The subscripts denote minutes of incubation at 37 °C for each phase of the reaction. (B) $(E\sigma$ + template$)_5$ (pppApU + CTP + GTP$)_5(\mu\uparrow)_2(NTP)_2$. To the binary complex as described in (A) were added the initiating substrates to final concentrations of 0.1 mM pppApU and 8 μM CTP and GTP. $\mu\uparrow$ symbolizes a salt-jump challenge (90 → 270 mM KCl).

Methods

Polymerase Assay. The standard assay system in a total volume of 0.1 mL was 40 mM Tris-HCl, pH 7.9, 80 mM KCl, 1 mM DTT, 10 mM $MgCl_2$, and 0.1 mM pppApU (3′–5′) and contained 0.166 pmol of T_7 ΔD_{111} DNA and 0.8 pmol of holoenzyme. Following a 5-min preincubation of the enzyme and DNA at 37 °C, the appropriate HPLC nucleotide triphosphates (8 μM final concentration) were added to initiate the reaction. After 5 min, these mixtures were then challenged, by the addition of either rifampicin (2 μM final concentration) or KCl (270 mM final concentration) for 2 min prior to the addition of all four NTPs (ATP, CTP, and GTP, 400 μM final concentration; [^{3}H]UTP, 200 μM final concentration; SA = 200 cpm/pmol). The reaction was terminated 2 min later by the addition of 1 mL of cold 5% Cl_3CCOOH, then filtered onto glass filter disks, dried, and then counted in a toluene-base cocktail with a Beckman L-100 scintillation counter.

Short-Production Analysis. In a total volume of 0.06 mL, the typical reaction was 40 mM Tris-HCl, pH 7.9, 80 mM KCl, 1 mM DTT, 10 mM $MgCl_2$, and 0.5 mM pppApU when used. The reaction contained 0.39 pmol of T_7 D_{111} DNA and 2.9 pmol of enzyme. After a 5-min preincubation of the binary complex at 37 °C, various combinations of three or less NTPs (one of which was [α-^{32}P]CTP, 1.7×10^6 cpm/pmol) were added and reacted for 3, 6, 15, 30, or 60 min. For termination of the reaction, the mixtures were plunged in ice, and EDTA (0.055 M final concentration) was added.

Reaction samples (15 μL) were spotted onto cellulose TLC sheets, developed in WASP solvent, dried, and then assayed by autoradiography. The radioactive spots were subsequently recovered from the TLC plate and counted.

Alternatively, reaction products were analyzed by electrophoresis on 24% polyacrylamide [acrylamide:bis(acrylamide) = 29:1], 50 mM Tris–borate, pH 8, 1 mM EDTA, and 7 M urea gels. A 10-μL aliquot of (0.25%) bromophenol blue was added and the total volume loaded onto the gel. Electrophoresis was conducted at 200 V (~5 mA) for 12–14 h. The product bands were visualized by autoradiography; those of interest were excised from the gel and counted.

Results

$E\sigma$/Template Ratio. The titration of the T_7 D_{111} template (0.166 pmol) with increasing amounts of RNA polymerase holoenzyme exhibited a saturation level at an $E\sigma$/template mole ratio of 12–15. The binary complex was incubated at 37 °C for 5 min, and then all four NTPs were added (Figure 1A). Elongation was terminated after 2 min.

With the same range of $E\sigma$/template ratios, the plateau effect was investigated for the binary complex incubated with pppApU + CTP + GTP for 5 min at 37 °C, followed by a salt jump. Upon addition of the four NTPs, elongation was allowed to proceed for 2 min. As shown in Figure 1, both the binary complex and the ternary complex curves exhibit about the same saturation plateau. However, in the presence of the ternary complex, the activity was increased about 10%.

Kinetic Studies of Protection from Rifampicin or a Salt Jump. Results of the kinetic study are presented in Table I. The stability of the ternary complexes was measured as a function of elongation activity following a rifampicin or salt-jump challenge.

In the absence of a preincubation with appropriate NTPs, the binary complex exhibits minimal activity when subjected to either a salt or rifampicin challenge (Table IA). Preincubation of the binary complex with the dimer primer (pppApU) prior to the challenge did not offer any additional protection. Slight protection of the complex was observed upon formation of the 3-mer transcript. However, maximal protection coincided with the addition of pppApU + CTP + GTP. Lowering the GTP concentration from 8 to 2 μM during the preincubations decreased the protection level by approximately 15%

Table I: Rifampicin or Salt-Jump Kinetic Protection Studies. Reaction Protocol: $(E\sigma + \text{template})_5(N')_5$ ($\mu\uparrow$ or rifampicin)$_2$(NTP)$_2$[a]

	N′	% activity: neither	$\mu\uparrow$ (90–270 mM)	rifampicin (2 μM)
A			3.9	5.4
	pppApU		5.7	5.4
	pppApU, C		14.8	19.6
	pppApU, C, G		100.0	100.0
	pppApU, C, G, A		82.6	85.8
B	pppApU, G		9.9	9.9
	pppApU, A		5.2	8.2
	pppApU, G, A		7.8	9.6
	pppApU, C, A			8.2
C	pppApU, C, G	100	90.1	53.9
	pppApU, C	92.3	7.2	6.8
		90.9	4.2	0.8

[a] In the experimental protocol at the top of the table, N′ represents the addition of one or more of the following initiating substrates to the binary complex ($E\sigma$·T_7 D_{111} DNA): pppApU, CTP, GTP, or ATP. Each 0.1-mL reaction containing 0.166 pmol of T_7 D_{111} DNA and 0.83 pmol of RNA polymerase ($E\sigma$) was incubated for 5 min at 37 °C in 40 mM Tris, pH 7.9, 80 mM KCl, 1.0 mM DTT, and 10 mM $MgCl_2$. The preincubation substrates (N′) were added to final concentrations of 0.1 mM for pppApU and 8.0 μM for CTP, GTP, and ATP. The subscripts denote minutes of incubation at 37 °C for each phase of reaction. The activity values for each reaction in (A) and (B) were compared to N′ = pppApU, CTP, and GTP, which was taken as 100% activity. In section C the activity values for each reaction were compared to N′ = pppApU, CTP, and GTP in the absence of a salt or rifampicin challenge.

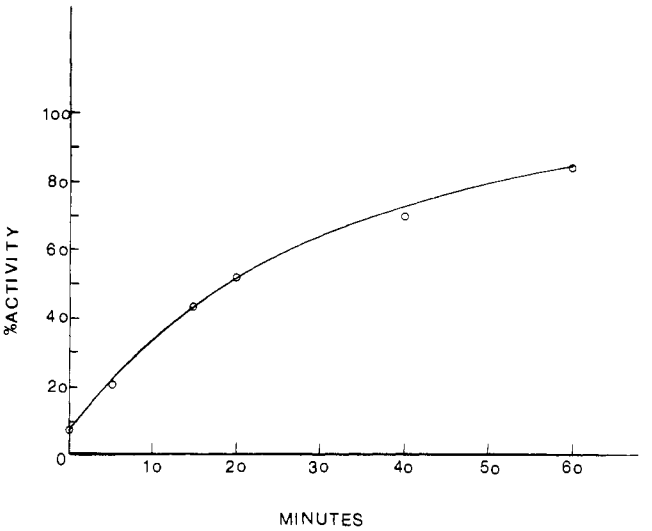

FIGURE 2: Time course of protection provided against a salt-jump challenge by addition of NTP components complementary to the minor promotor C of T_7 D_{111} DNA (ACAGGU...): $(E\sigma + \text{template})_5(N')_t(\mu\uparrow)_2(NTP)_2$. Initiating substrates (N′) at a final concentration of 0.4 mM ATP + 0.15 mM GTP + 8 μM CTP were added to the binary complex as described in Figure 1. The salt-jump challenge and elongation steps are described as in Figure 1. The activity values were compared to N′ = pppApU, CTP, and GTP for a 5-min incubation, which was assigned a value of 100%.

Table II: Product Ratio Analysis of Gel Electrophoresis. Reaction Protocol: $([^{32}P]CTP)(E\sigma + \text{template})_5(N')_t$[a]

N′	time (min)	2-mer	3-mer	4-mer	5-mer
pppApU, C	2	0.24	1.6	0.29	
	6	0.29	3.8	0.61	0.18
	60	0.66	4.6	0.68	0.19
pppApU, C, G	2		0.64	0.18	
	6	0.1	0.86	0.37	0.08
	60	0.29	1.2	0.42	0.14
pppApU, C, G, A	2		0.64	0.14	
	6	0.1	0.88	0.56	0.07
	60	0.28	1.30	0.41	0.11
A, U, C	2		3.6	0.57	
	6	0.7	4.5	0.88	0.09
	60	0.09	0.5	0.35	0.10
A, C, G	2	0.34	0.17	0.10	0.10
	6	0.59	0.17	0.11	0.22
	60	1.7	0.72	0.34	0.93
pppApU, C, A	2	0.2	1.7	0.34	
	6	0.39	3.3	0.54	0.17
	60	0.66	4.1	0.82	0.19
A, C	2		0.20	0.06	
	6	1.6	1.5	0.46	
	60	2.3	2.3	0.37	0.11

[a] In the experimental protocol at the top of the table, N′ represents the additions of initiating substrates to the binary complex as in Table I. Each reaction contained 0.39 pmol of T_7 D_{111} DNA and 2.9 pmol of holoenzyme, and the final concentrations of the N′ additions were (a) 0.5 mM pppApU + 0.13 μM [α-^{32}P]CTP, (b) 0.5 mM pppApU + 0.13 μM [α-^{32}P]CTP + 8 μM GTP, (c) 0.5 mM pppApU + 0.13 μM [α-^{32}P]CTP + 8 μM GTP + 8 μM ATP, (d) 0.4 mM ATP + 0.13 μM [α-^{32}P]CTP + 0.15 mM UTP, (e) 0.4 mM ATP + 0.13 μM [α-^{32}P]CTP + 8 μM GTP, (f) 0.5 mM pppApU + 0.13 μM [α-^{32}P]CTP + 8 μM ATP, and (g) 0.4 mM ATP + 0.13 μM [α-^{32}P]CTP. Product ratios are expressed as the mole ratio of product/T_7 D_{111} DNA.

(data not shown). The ternary complex formed in the presence of pppApU + CTP + GTP + ATP protected against the challenges somewhat less effectively than the tetrameric ternary complex. In this case, GTP and ATP at a concentration of 8 μM and/or 2 μM did not substantially alter the observed activity (data not shown).

Combinations of the dimer primer (pppApU) and NTPs, which did not allow the formation of the A_1 short transcripts (pppApUpCpG), provided only base-line protection against each challenge (Table IB). As a control set (Table IC), the activity of the binary complex and two ternary complexes was measured with and without a challenge test. The 4-mer ternary complex gave essentially complete protection (90%) against a salt-jump challenge, whereas only 53% protection was provided against a rifampicin challenge. Neither the 3-mer ternary complex nor the binary complex provided more than 10% protection against either a rifampicin or a salt challenge. It is of interest to note that the activity of the 4-mer ternary complex is consistently greater than that of the binary complex or 3-mer ternary complex even *in the absence* of a challenge.

Figure 2 depicts the time-dependent increase in activity for the ternary complexes formed at the minor C promoter. The preformed binary complex was incubated with ATP + CTP + GTP for intervals ranging between 5 and 60 min. After a salt-jump challenge, long-chain synthesis was monitored for 2 min. Formation of the minor ternary complex (pppApCpApGpG) required a 60-min incubation period in order to achieve an 80% protection level.

Short-Product Analysis. Table II summarizes a series of short-product studies using [α-^{32}P]CTP. In most cases, as the reaction time was increased, the production of transcripts also increased. Under conditions where abortive initiation dominates, product template ratios greater than 1 are observed. For the pppApU–C, A–U–C, pppApU–C–A, and A–C incubation mixtures, a greater than stoichiometric accumulation was evident for the abortive 3-mer transcripts. In addition, the A–C incubation mixture produced the 2-mer transcript from the C promoter in excess.

Upon the formation of the ternary complex in the presence of pppAU, CTP, and GTP *or* pppApU, CTP, GTP, and ATP

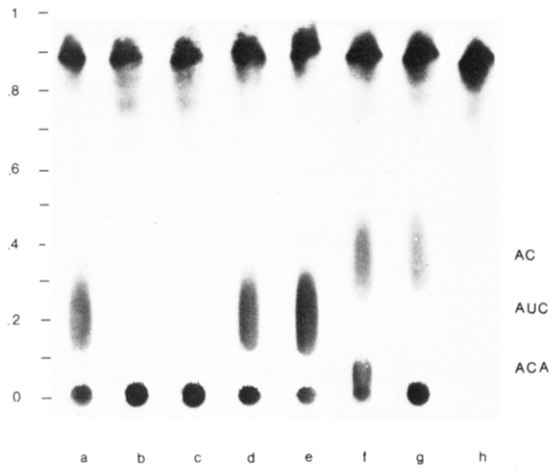

FIGURE 3: TLC autoradiogram of short products. Reaction protocol was $(E\sigma + \text{template})_5(N')_{15}$. The N′ additions to the binary complex (as in Table II) are (a) 0.5 mM pppApU + 0.13 μM [α-^{32}P]CTP, (b) 0.5 mM pppApU + 0.13 μM [α-^{32}P]CTP + 8 μM GTP, (c) 0.5 mM pppApU + 0.13 μM [α-^{32}P]CTP + 8 μM GTP + 8 μM ATP, (d) 0.5 mM pppApU + 0.13 μM [α-^{32}P] CTP + 8 μM ATP, (e) 0.4 mM ATP + 0.13 μM [α-^{32}P]CTP + 0.15 mM UTP, (f) 0.4 mM ATP + 0.13 μM [α-^{32}P]CTP, (g) 0.4 mM ATP + 0.13 μM [α-^{32}P]CTP + 8 μM GTP, and (h) 8 μM [α-^{32}P]CTP.

or ATP, CTP, and GTP, the yield of the 3-mer approached stoichiometric amounts. The production of the 4-mer in the pppApU–C–G and pppApU–C–G–A assays gave less than stoichiometric ratios, and the presence of longer transcripts was apparent. A stoichiometric quantity of the 5-mer was recorded for the A–C–G assay (presumably from the C promoter; pppApCpApGpG). This assay displayed the largest increase in transcript production with time. A 4-fold increase of the 3-mer and a 9-fold increase in the 5-mer were observed, over the 60-min incubation.

In the TLC WASP system, the 2-mer and 3-mer transcripts can be separated from the longer products, which do not migrate from the origin (Figure 3). Synthesis of the trinucleotide pppApUpC was abortively produced in the pppApU–C, pppApU–C–A, and A–U–C reaction mixtures while diminished quantities appeared in the pppApU–C–G and pppApU–C–G–A reaction mixtures. The pppApC dimer was mainly observed in the A–C and A–C–G reaction mixtures. Only the pppApU–C–G, pppApU–C–G–A, and A–C–G reaction mixtures retained essentially all the radioactivity at the origin. Qualitatively, these data are in good agreement with the short-product analysis by gel electrophoresis.

Discussion

Transcription of the DNA template by RNA polymerase can be divided into four individual processes: (a) binding of the holoenzyme to the DNA promotor site, (b) initiation, (c) elongation, and (d) termination of RNA synthesis (Chamberlin, 1976). The steps associated with the completion of the initiation event and the commitment to productive elongation are not clearly understood. The completion of initiation can be operationally defined as cessation of the production of abortive short products and the formation of a stable ternary complex committed to elongation. The formation of a stable ternary complex has been suggested to be either a discrete event (Hansen & McClure, 1980) or one generated over a wide range of ribonucleotide transcript lengths (Gralla et al., 1980; Carpousis & Gralla, 1980). Abortive synthesis of oligonucleotides ranging from 2 to 11 bases has been reported in different systems with different templates. In this paper, for the T_7 D_{111} A_1 promoter we observed the abortive synthesis of the 2-mer and 3-mer transcripts and maximum protection against either a rifampicin or salt challenge, commencing with the formation of a ribotetranucleotide ternary complex.

Many laboratories (Sippel & Hartmann, 1968; Wehrli & Staehlin, 1971; Wehrli et al., 1976; Baehr et al., 1976; Yarbrough et al., 1976; Rhodes & Chamberlin, 1975) have studied the complex inhibitory mechanism of rifampicin on RNA chain synthesis. Johnston & McClure (1976), McClure & Cech (1978), and McClure et al. (1978) have shown that the formation of the first phosphodiester bond is not inhibited by rifampicin, whereas formation of the second phosphodiester bond is inhibited. However, Carpousis & Gralla (1980) have reported the reiterative synthesis of the 2-mer and 3-mer transcripts for the UV *lac* 5 template in the presence of rifampicin. Our kinetic protocol permitted the investigation of the synthesis of several different ternary complexes containing the bound 2-mer, 3-mer, 4-mer, and 20-mer transcripts. Substantial protection against a rifampicin challenge was observed with a preformed ternary complex with at least a 4-mer ribonucleotide transcript. As the 54% protection level suggests, rifampicin's inhibition must extend beyond the initiation event.

The preformed ternary complex with at least a tetrameric transcript, however, provided complete protection against a salt challenge. A salt challenge, therefore, inhibited only the initiation event. From our data with T_7 D_{111} A_1 promoter, we conclude that a stable ternary complex is achieved concomitant with the formation of the 4-mer transcript.

An enhanced maintenance of activity accompanying the formation of a stable ternary complex is suggested in Figure 1. For each Eσ/template ratio examined, the ribotetranucleotide ternary complex curve (B) exhibited an increased activity over that of the control. Quantitatively, the difference in activity between the tetrameric ternary complex and the binary complex or the trimeric ternary complex is approximately 10%. This apparent augmented stability may be a selective kinetic feature.

The mutant T_7 D_{111} template contains the major A_1 promoter (pppApUpCpGpA...) and several minor promoter sites. The minor C promoter sequence begins with pppApCpApGpG. Stahl & Chamberlin (1977) have shown that preferential initiation occurs at the A_1 promoter of the D_{111} template at low Eσ/template ratios. The reduced protection level observed with the ternary complex formed in the presence of pppApU + CTP + GTP + ATP (Table IA) was unexpected. The use of a suitable dinucleotide primer complemenary to the promoter site effectively induces selective initiation (So & Downey, 1970). RNA chain initiation still occurs at the A_1 promoter with low ATP concentrations (Nierman & Chamberlin, 1980). Consequently, ATP and pppApU may compete for the initial substrate binding site. Once ATP (K_m = 150 μM) does bind, initiation could pause since the second nucleotide triphosphate (UTP) is not present in the assay, and the dissociation of ATP from the binary complex is probably a rate-determining factor. These factors could explain the observed decrease in protection. Abortive initiation at the minor C promoter might also account for the diminished protection, since the effective concentration of NTPs available at the A_1 promoter would be reduced; this is unlikely since the product formation is slow for this promoter.

An important limitation of our kinetic study is its inability to measure the direct production of specific abortive oligonucleotides. Working at an effective Eσ/template ratio of less than 1 (Figure 1) limits productive elongation to a single round, and any dissociation and reassociation of the RNA polymerase

is abolished upon formation of a stable ternary complex. Productive initiation is the result of the enzyme finally escaping the cyclic nature of abortive initiation (Carpousis & Gralla, 1980). According to our kinetic data, productive initiation occurs after the formation of the bound 4-mer product. In other words, the probability of dissociation of the 4-mer transcript from the ternary complex may approach zero. We quantitated all abortive synthesis using the short-product assay.

A stoichiometric release of the 3-mer transcript is observed concomitant with the formation of a stable tetrameric ternary complex. In those reactions where the combination of NTPs could not form a stable ternary complex, the 3-mer transcript was produced in ratios greater than 1. Obviously, the capability of productive elongation reduced the degree of abortive initiation. For the T_7 D_{111} A_1 promoter, abortive initiation continued up to and including the 3-mer transcript. Using a different method (TLC), Neirman & Chamberlin (1980) have reported the abortive synthesis of only the dinucleoside tetraphosphate.

The noncomplementary 4-mer and 5-mer transcripts observed in the abortive short-product assays (such as A, U, and C) may result from "forced" elongation. The rate of misincorporation may increase as the primed enzyme pauses during initiation due to (1) the absence of specific required NTPs or (2) transcription initiating from other sites. The addition of a noncomplementary base may lead ultimately to a reduced binding interaction with the enzyme and stimulate the abortive release of such complexes.

The observation that rifampicin inhibits the formation of pppApUpC from pppApU + CTP is interesting (data not shown) in that Johnston & McClure (1976) have shown that rifampicin does not inhibit the formation of the first phosphodiester bond. The dimer primer pppApU must therefore occupy a site on the enzyme that mimics the occupancy of the normal translocated dimer product after the formation of the first phosphodiester bound by the enzyme. Thus, the formation of the phosphodiester bond between pppApU and CTP resembles the formation of the second phosphodiester bond on the enzyme surface.

The use of a dimer primer pppApU augmented by CTP and GTP allows the formation of a bound ribotetranucleotide ternary complex, which terminates abortive product synthesis and confers resistance to a rifampicin or a salt-jump challenge. Since the length of abortive products releasing during initiation appears to vary among different templates (natural or synthetic), the formation of a stable ternary complex committed to elongation may require a different discrete length of bound oligoribonucleotide product for each different promoter.

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