Effect of low nucleotide concentrations on abortive elongation catalysed by wheat-germ RNA polymerase II

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A kinetic study of the effect of elongating nucleotide concentration on the reactions of abortive elongation catalysed by wheat-germ RNA polymerase II on a poly[d(A-T)] template suggests that the shift from abortive to productive elongation may involve the participation of at least two nucleotides, according to a mechanism very similar to that reported for *Escherichia coli* RNA polymerase. Experiments performed with non-complementary nucleotides with respect to the DNA template, and with substrate derivatives, allow an analysis of the substrate specificity during these reactions. Similar experiments performed with poly[d(A-A-T)]·poly[d(T-T-A)] as template provide a starting point for a better understanding of the effect of DNA sequence on the rates of abortive and productive elongation catalysed by the plant enzyme.

INTRODUCTION

Progress has now been made in the study of the enzymic properties of eukaryotic RNA polymerases II in the catalysis of the abortive elongation reaction, allowing some comparison with the well-documented bacterial enzyme (Kumar, 1982; Chamberlin, 1982; von Hippel *et al.*, 1984). For instance, it was shown that wheat-germ RNA polymerase II is able to catalyse efficiently primer-independent abortive initiation (Mosig *et al.*, 1985). In contrast with previous attempts (Yarbrough, 1982), we have shown that the enzyme can catalyse the reaction of condensation of ribonucleotides to dinucleotide primers, leading to trinucleotide-product formation (Dietrich *et al.*, 1985). Yeast (Lescure *et al.*, 1981) and calf thymus (Vaisius & Wieland, 1982) RNA polymerases II are also active in such reactions, although the rate of abortive elongation differs markedly, depending on the source of the enzyme. Using poly[d(A-T)] as template, we found that experimental conditions can be selected under which catalytic production of trinucleotides occurs (Dietrich *et al.*, 1985), a behaviour reminiscent of that of *Escherichia coli* core RNA polymerase (Sylvester & Cashel, 1981). We have also shown that the extent of abortive elongation, as well as the non-processive behaviour exhibited by the wheat-germ enzyme is, at least in part, dependent on the stability of transcription complexes (Dietrich *et al.*, 1985). In particular, using synthetic DNA templates, we found that the stability of ternary transcription complexes is markedly affected by DNA sequence, bivalent cations and ionic strength (Durand *et al.*, 1982; Dietrich *et al.*, 1985, 1986). Such information on the enzymic properties of the class II RNA polymerases could have important implications in the optimization of experiments *in vitro* to look for factors playing a role in the different steps of the transcription cycle (Weil *et al.*, 1979; Manley *et al.*, 1980).

Using a rapid kinetic technique, Shimamoto & Wu (1980a,b) showed that the mechanism of incorporation

of at least the first nucleotides in reactions catalysed by *E. coli* RNA polymerase is more complicated than was expected: the results suggested binding of more than one substrate molecule to the enzyme–DNA complex during the initiation process, which led to the proposal that the additional nucleotide-binding step may be crucial in driving the initiation reaction or in discriminating between the abortive or the productive elongation pathway. By studying the effect of low substrate concentrations on the extent of productive RNA chain initiation from T7 promoters A1 and A2 by *E. coli* RNA polymerase, Nierman & Chamberlin (1980) also noted that, at 1 μ M substrate concentration, productive initiation from the A1 promoter does not occur, although the dinucleoside tetraphosphate pppA-U is produced at a significant rate under these conditions. Again, the possibility has been raised that there are additional nucleoside-triphosphate-binding sites on *E. coli* RNA polymerase, which may play some role in the process of productive RNA synthesis.

These observations form the basis of the present investigation, where we performed a detailed kinetic study of the effect of elongating nucleotide concentration on the reactions of abortive elongation catalysed by wheat-germ RNA polymerase II.

MATERIALS AND METHODS

Reagents

Nucleotides and the dinucleoside monophosphates were purchased from Sigma and Boehringer. Nucleotide concentrations were calculated from absorbance measurements (Dietrich *et al.*, 1985, 1986). [α^{32} P]UTP (410 Ci/mmol) was from Amersham. Poly[d(A-T)] was from P-L Biochemicals. Poly[d(A-A-T)]·poly[d(T-T-A)] was synthesized as follows: first, the oligonucleotides d(A-A-T)₁₀ and d(T-T-A)₄ were constructed, then mixed in a 1:3 molar ratio and submitted to T4 DNA ligase at

Abbreviation used: ATP[S], adenosine 5'-[γ -thio]triphosphate.

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22 °C for 4 h. After denaturation at 100 °C for 2 min, the DNA fragments were separated on a 5%-polyacrylamide/50%-urea gel. Fragments of size greater than 120 nucleotides were electroeluted from the gel, and further purified on a DE-52 column (Maniatis *et al.*, 1982). Fractions containing the synthetic DNA were precipitated with 67% (v/v) ethanol, washed and freeze-dried. All buffer components were reagent grade.

Wheat-germ RNA polymerase II was purified by the method of Jendrisak & Burgess (1975), as previously described (Job *et al.*, 1984; Dietrich *et al.*, 1985, 1986).

Reaction assays

Unless otherwise noted in the Figure legends, the reaction mixtures contained 40 nM enzyme, 5 µg of DNA/ml, 0.9 mM dinucleotide primer, 5 µM-[α -³²P]UTP, 2 mM-MnCl₂ and appropriate amounts of unlabelled nucleotide substrates or derivatives. In all assays, other components were 64 mM-Tris/HCl buffer, pH 7.8, 12.5% (v/v) glycerol, 12.5 mM-2-mercaptoethanol, 5 mM- α -thioglycerol, 0.05 mM-EDTA, 0.05% Triton X-100, 1.1 mM-dithiothreitol and 1.5 mM-NaF. Final volumes were 10 µl, and assays were usually incubated for 60 min at 35 °C. Reactions were stopped by mixing the 10 µl reaction mixtures with 20 µl of stop buffer, containing 1 mM-EDTA, 80% formamide and 0.1% xylene cyanol. Control experiments lacking the DNA template were performed routinely, and all the rate measurements shown in the Figures were done relative to these blanks.

Activity measurements

For activity measurements, the above reaction mixtures were processed by three different methods. Since the methodology employed has been described in detail (Job *et al.*, 1984; Dietrich *et al.*, 1985, 1986), it will only be briefly mentioned here: (i) total RNA synthesis was measured by trichloroacetic acid precipitation of reaction mixtures spotted on Whatman GF/C filters; (ii) abortive synthesis was quantified after t.l.c. on poly(ethyleneimine) sheets (Randerath & Randerath, 1967); (iii) distribution of RNA chain length was analysed by gel electrophoresis on 20% polyacrylamide/7M-urea/50 mM-Tris/borate (pH 8.0) gels (0.03 cm × 30 cm × 40 cm). Electrophoresis was conducted at a constant 80 W until the xylene cyanol had migrated 15 cm. Rate measurements corresponding to synthesis of poly[r(A-U)] and trinucleotide were normalized to their $V_{\max}$ values and assembled to form the rate plots shown in the Figures. Calculations and plots were effected with a Plessey 6623 computer system and a HP 7475 A plotter.

RESULTS

Effect of ATP concentration on U-A-U synthesis

Reaction mixtures were prepared as described in the Materials and methods section, in the presence of U-A as primer and [α -³²P]UTP as substrate, thereby leading to labelled U-A-U formation. In addition, before incubation at 35 °C, they received various amounts of unlabelled ATP. In agreement with previous results (Dietrich *et al.*, 1985), Fig. 1(a) shows that increasing the ATP concentration leads to two effects: (i) there is a decrease in the rate of U-A-U synthesis and an increase in the rate of poly[r(A-U)] formation; (ii) at saturating ATP concentrations (> 10 µM), about 20% of U-A-U

still remains synthesized. The disappearance of U-A-U occurs for such low amounts of ATP that experiments such as those shown in Fig. 1(a) were performed in a smaller concentration range, i.e. lower than 1 µM (Fig. 1b). We investigated the possibility that these results could be explained by a competition between primer-dependent elongation and primer-independent initiation: the rate measurements in Fig. 1(b) were analysed by a Dixon plot, in the form 1/velocity of U-A-U synthesis versus inhibitor (ATP) concentration (Fig. 1c). This plot is not linear, which is an indication that ATP behaved as a non-competitive inhibitor, i.e. the enzyme-poly[d(A-T)] complex can bind both the U-A primer and ATP. However, the resulting transcription complex catalyses the abortive synthesis of U-A-U at a lower rate as compared with the transcription complex formed in the absence of ATP. It should be noted that the two rate plots in Fig. 1 do not vary concomitantly: thus, the apparent K_m for ATP utilization is of the order of 0.1 µM for the U-A-U reaction, in contrast with a value close to 3 µM from the poly[r(A-U)] rate plot. Although the rate plots shown in Fig. 1 could be fitted independently with simple hyperbolic relationships, the simplest empirical rate equations (Fromm, 1980; Hurst, 1967) allowing a simultaneous fitting of these data are of the form:

for U-A-U

$$v/V = \frac{(1 + c_1[\text{ATP}] + c_2[\text{ATP}]^2)}{(1 + a_1[\text{ATP}] + a_2[\text{ATP}]^2)} \quad (1)$$

for poly[r(A-U)]

$$v/V = \frac{(c_3[\text{ATP}] + a_2[\text{ATP}]^2)}{(1 + a_1[\text{ATP}] + a_2[\text{ATP}]^2)} \quad (2)$$

Eqns. (1) and (2) have been used to fit the experimental data in Fig. 1, for the numerical values of the parameters listed in the legend to Fig. 1. Similar results were obtained by studying the effect of low UTP concentrations on the reactions involving A-U as primer and [α -³²P]ATP as substrates: thus the rate plots assembled from 96 individual determinations of the rate of disappearance of labelled A-U-A and appearance of poly[r(A-U)] yielded an apparent K_m for UTP utilization of 0.1 µM for the A-U-A reaction, in contrast with a value of 0.6 µM for the poly[r(A-U)] rate plot (not shown).

The poly[r(A-U)] chains synthesized under the above conditions were analysed by high-resolution gel electrophoresis. The results in Fig. 1(d) show that: (i) in the low ATP concentration range, there is a decrease in the amount of U-A-U synthesized; (ii) as soon as poly[r(A-U)] can be detected on the autoradiogram of the gel (i.e. for ATP concentrations > 0.2 µM), the chain-length distribution is not drastically affected by ATP concentration; (iii) at high ATP concentrations there is still detectable U-A-U synthesis. Therefore, these results reinforce the validity of the data in Figs. 1(a) and 1(b).

Effects of ATP derivatives and non-complementary nucleotides on U-A-U synthesis

We found that ATP[S] can replace ATP with the same efficiency in the transcription of poly[d(A-T)] catalysed by wheat-germ RNA polymerase II (results not shown).

Fig. 1. Effect of ATP concentration on poly[d(A-T)]-dependent U-A-U and poly[r(A-U)] synthesis

(a) Transcription assays were prepared as described in the Materials and methods section, in the presence of wheat-germ RNA polymerase II, poly[d(A-T)] as template, U-A as primer and [α - ^{32} P]UTP as substrate, thereby leading to labelled U-A-U formation. In addition, before incubation for 60 min at 35 °C, they received various amounts of unlabelled ATP, necessary for poly[r(A-U)] synthesis. The total UTP concentration was held constant at 5 μ M. U-A-U was quantified after t.l.c. of reaction mixtures on poly(ethyleneimine)-cellulose sheets (Dietrich *et al.*, 1985; Randerath & Randerath, 1967). The concentration of labelled nucleotide was such that total radioactivity spotted on to each lane was close to 20000 c.p.m. The maximum amount of U-A-U synthesized (V), measured in the absence of ATP, corresponded to about 2000 c.p.m. The background in these experiments was 40 c.p.m. These data are represented by black circles (curve 2). Poly[r(A-U)] synthesized under these experimental conditions was quantified under trichloroacetic acid precipitation of reaction mixtures spotted on GF/C filters (Dietrich *et al.*, 1985; Job *et al.*, 1984). Consumption of substrates was less than 10%, ensuring steady-state conditions. The background in these experiments is less than 1/2000 of the input radioactivity (Job *et al.*, 1984). The data are represented by open circles (curve 1). In both cases, rate measurements were effected relative to blanks lacking the DNA template and were normalized to their corresponding $V_{\max}$ values. The continuous lines are computer simulations of eqns. (1) and (2), corresponding to disappearance of U-A-U and synthesis of poly[r(A-U)] respectively, for the following values of the parameters: $c_1 = 4$, $c_2 = 0.85$, $c_3 = 0$, $a_1 = 10$ and $a_2 = 4.7$. (b) Conditions were the same as in (a), except for ATP, which was varied in a smaller concentration range, i.e. < 1 μ M. Symbols and computer simulations have the same significance as in (a). (c) The results corresponding to the disappearance of U-A-U shown in (b) were analysed by a Dixon plot. Symbols and computer simulations have the same significance as in (b). (d) Product distribution from transcription of poly[d(A-T)] in the presence of various amounts of ATP: length distribution of poly[r(A-U)] chains in reaction mixtures as shown in (a) was examined by high-resolution gel electrophoresis, as indicated in the Materials and method section. ATP concentration was 25, 16.66, 11.11, 7.41, 4.94, 3.29, 2.2, 1.46, 0.97, 0.65, 0.43, 0.29, 0.19, 0.13, 0.085, 0.057, 0.038, 0.025 and 0 μ M, for lanes 1–19 (from left to right) respectively. The arrow indicates the position of xylene cyanol.

The data in Fig. 2(a) indicates that it behaves very similarly to ATP in the reaction of disappearance of labelled U-A-U. The results in Fig. 2(b) show that GTP, CTP, AMP and dATP do not affect the rate of abortive U-A-U synthesis. Under the same experimental condi-

tions, none of these nucleotides affected the rate of poly[r(A-U)] synthesis (results not shown).

Cordycepin triphosphate (3'-dATP) is a competitive inhibitor of ATP in the reaction of poly[r(A-U)] synthesis catalysed by wheat-germ RNA polymerase II,

Fig. 2. Effect of nucleotides and ATP derivatives on poly[d(A-T)]-dependent U-A-U synthesis

(a) Effect of ATP[S]: reaction mixtures were prepared as described in Fig. 1 and in the Materials and method section, thereby leading to labelled U-A-U formation. In addition, before incubation at 35 °C for 60 min, they received various amounts of unlabelled ATP[S]. The U-A-U synthesis was quantified as in Fig. 1. The black circles represent the experimental data; the continuous line is the computer simulation of eqn. (1), corresponding to the effect of ATP on the rate of U-A-U formation shown in Fig. 1, for the values of the parameters listed in the legend to Fig. 1. (b) Effect of GTP, CTP, AMP and dATP: reaction mixtures were prepared as described in (a), thereby leading to labelled U-A-U formation. In addition, before incubation for 60 min at 35 °C, they received various amounts of unlabelled GTP (○), CTP (●), AMP (■) or dATP (△), and the U-A-U synthesis was quantified as described in Fig. 1 and in the Materials and methods section. For comparison is shown the computer simulation of eqn. (1), fitting the data corresponding to the effect of ATP on the rate of U-A-U formation, for the values of the parameters listed in the legend to Fig. 1 (as the lower curve without symbols).

on a poly[d(A-T)] template (Dietrich *et al.*, 1985). The derivative behaves as a very potent inhibitor of translocation, and, at least in the low concentration range studied here and by Dietrich *et al.* (1985), it is apparently not incorporated into poly[r(A-U)] chains (Dietrich *et al.*, 1985). A series of experiments was thus performed in order to investigate whether this nucleotide could affect any of the steps involving the participation of ATP in the reactions shown in Fig. 1.

First, we investigated the effect of 3'-dATP on the reaction of formation of labelled U-A-U, in the absence of ATP. The results in Figs. 3(a) and 3(b), obtained after t.l.c. and high-resolution gel electrophoresis of reaction mixtures, indicate that U-A-U is not elongated to a tetranucleotide U-A-U-A in the presence of 3'-dATP. These results show that, if some tetranucleotide is formed, it is not accumulated by the abortive reaction and thereby escapes detection. However, it is clear that, in the experimental conditions investigated, 3'-dATP is much less efficient than ATP in stabilization of transcription complexes. These results also show that 3'-dATP does not compete with U-A primer in the reaction of abortive elongation.

Second, we investigated the effect of 3'-dATP on the reaction of formation of labelled U-A-U, but in the presence of a fixed amount of ATP (Fig. 3c). Since these experimental conditions allow the synthesis of poly[r(A-U)], we also measured the effect of 3'-dATP on the rate of RNA formation. The results are presented in Fig. 3(d). As previously noted (Dietrich *et al.*, 1985), 3'-dATP is a very potent inhibitor of poly[d(A-T)] transcription: a concentration of 0.3 μM is sufficient to produce a 50% inhibition of poly[r(A-U)] synthesis, although in these experiments the ATP concentration was held constant at 5 μM (Fig. 3d). In contrast with the results obtained in the absence of ATP (Fig. 3a), when these reaction mixtures are analysed for the reaction of

abortive elongation, the presence of 3'-dATP is associated with a decrease in the rate of labelled U-A-U synthesis: thus a 50% decrease in the rate of U-A-U formation is obtained at less than 1 μM 3'-dATP (Fig. 3c). It is important to note that, although poly[r(A-U)] synthesis was totally inhibited by 3'-dATP concentrations of the order of 3 μM , the inhibitory effect of 3'-dATP on productive elongation was not associated with an increased rate of abortive elongation, to that obtained in the absence of ATP.

Poly[d(A-A-T)]·poly[d(T-T-A)]-dependent abortive elongation

Experiments with *E. coli* RNA polymerase and T7 DNA as template indicated that productive initiation at the A1 promoter is activated by the third and the fourth nucleotides (Shimamoto *et al.*, 1981). Owing to the repetitive nature of poly[d(A-T)], it is difficult from the experimental results in Fig. 1 to decipher whether the two steps involving the elongating substrate require the same nucleotide base, or if these nucleotides might be selected at different sites on the DNA template. To answer this question, we have investigated the template properties of the synthetic polymer poly[d(A-A-T)]·poly[d(T-T-A)]. Abortive synthesis involving ATP as substrate were too low to allow quantification (results not shown). Fig. 4(a) shows that the various combinations of dinucleotide primers and substrate UTP give rise to the abortive products expected from the sequence of the template. Thus both reactions A-A+UTP and A-U+UTP lead to trinucleotide formation, whereas the combination U-A+UTP leads to formation of U-A-U and to a longer product, presumably U-A-U-U. As expected, UTP cannot be condensed to U-U. It should be noted that the extent of abortive elongation differs considerably for the A-A-, A-U- and U-A-primed reactions, although the concentration of the potential corres-

Fig. 3. Effect of cordycepin triphosphate on poly[d(A-T)]-dependent U-A-U synthesis

Reaction mixtures were prepared as described in Fig. 1 and in the Materials and methods section, thereby leading to labelled U-A-U formation. They were incubated for 60 min at 35 °C in the presence of various amounts of 3'-dATP, in the absence (a, b) or in the presence (c, d) of ATP. The synthesis of U-A-U and poly[r(A-U)] was quantified as described in Fig. 1 and in the Materials and methods section. (a) Effect of 3'-dATP on U-A-U synthesis in the absence of ATP. (b) Reaction mixtures shown in (a) were analysed by high-resolution gel electrophoresis. The concentration of 3'-dATP was 2.25, 1.6, 1.26, 0.95, 0.71, 0.53, 0.4, 0.3, 0.225, 0.17 and 0 μM for lanes 1–11 (from left to right) respectively. The arrow shows the position of xylene cyanol. (c) Effect of 3'-dATP on U-A-U synthesis in the presence of 5 μM -ATP. (d) Effect of 3'-dATP on poly[r(A-U)] synthesis. Reaction mixtures, as shown in (c) were assayed for poly[r(A-U)] synthesis. A replot of these data, in the form V/v versus 3'-dATP concentration, is linear. The line, obtained by linear least-squares analysis, has a slope of $3.27 \pm 0.15 \mu\text{M}^{-1}$ and a V/v -axis intercept of 0.98 ± 0.14 . Correlation coefficient is 0.953 (results not shown).

ponding sites on the template where abortive synthesis could occur is the same. Thus, in the experimental conditions of Fig. 4(a), the relative amounts of A-A-U, (U-A-U + U-A-U-U) and A-U-U synthesized were 1, 0.4 and 0.07 respectively. The effect of UTP concentration on the relative amounts of U-A-U-U and U-A-U for the U-A-U-primed reaction is shown in Fig. 4(b). In the experimental concentration range studied, this relative variation is a decreasing function with respect to UTP, suggesting that the stability of ternary transcription complexes is higher when formed with U-A-U-U than with the shorter product U-A-U.

DISCUSSION

The present results show that the rate of trinucleotide formation is diminished in the presence of the elongating substrate. However, the rate plots corresponding to disappearance of abortive products and formation of

poly[r(A-U)] chains do not vary concomitantly, i.e. they are not characterized by same apparent K_m value for the elongating substrate. This complex behaviour apparently does not arise from a direct competition between primer-dependent elongation and primer-independent initiation (Fig. 1c), in agreement with the finding that wheat-germ RNA polymerase II has been shown to incorporate dinucleotide primers into RNA in the presence of a poly[d(A-T)] template (Yarbrough, 1982). Fig. 3 shows that cordycepin triphosphate affects the rate of U-A-U synthesis in the additional presence of ATP, and this effect is associated with inhibition of productive elongation. These combined results may suggest the participation of more than one molecule of the elongating substrate in the process of condensation of a nucleotide. With *E. coli* RNA polymerase, kinetic results obtained with a poly[d(A-T)] template also suggest the binding of more than one UTP molecule to the enzyme-DNA complex during the initiation process

Fig. 4. Poly[d(A-A-T)]·poly[d(T-T-A)]-dependent abortive synthesis catalysed by wheat-germ RNA polymerase II

Reactions were conducted as described in the Materials and methods section, in the presence of 40 nM enzyme, 0.5 μg of poly[d(A-A-T)]·poly[d(T-T-A)] template/ml, 0.5 μM-[α-³²P]UTP (410 Ci/mmol), 2 mM-MnCl₂ and 0.9 mM dinucleoside monophosphates. After incubation for 60 min at 35 °C, reactions were analysed by gel electrophoresis as described in the Materials and methods section. Fig. 4(a) shows part of the autoradiogram of the gel, where radioactive spots could be detected. The arrow shows the position of xylene cyanol. Reactions were conducted in the presence of A-A, A-U, U-A and U-U as primer for lanes 1, 3, 5 and 7 respectively. In lanes 2, 4, 6 and 8 are shown the corresponding reactions performed in the absence of template. In lane 9 is shown for comparison the trinucleotide U-A-U synthesized on the poly[d(A-T)] template. Fig. 4(b) shows the effect of UTP concentration on the poly[d(A-A-T)]·poly[d(T-T-A)] dependent U-A-primed abortive synthesis catalysed by wheat-germ RNA polymerase II. Reactions were conducted as above, in the presence of 40 nM enzymes, 1.5 μg of poly[d(A-A-T)]·poly[d(T-T-A)]/ml, 0.9 mM-U-A, 0.5 μM-[α-³²P]UTP (410 Ci/mmol), 2 mM-MnCl₂ and various amounts of unlabelled UTP. After incubation for 60 min at 35 °C, reactions were analysed by gel electrophoresis as described in the Materials and methods section. R is the ratio of the amounts of the longer and the shorter products synthesized under these conditions (Fig. 4a). Measurements were effected by densitometric recordings of the autoradiography.

(Shimamoto & Wu, 1980a,b). It has been proposed that the additional UTP molecule acts as an activator or a discriminator in the catalytic pathway of RNA synthesis, and may be crucial in driving the initiation reaction or in discriminating between abortive or productive initiation (Shimamoto & Wu, 1980a,b).

The experiments conducted with the non-complementary nucleotides and with the ATP derivatives were performed in order to investigate the substrate requirements for the disappearance of abortive products. A specific role for ATP can be ruled out, since we found that UTP behaves similarly in the A-U-primed reaction. ATP[S] and adenosine 5'-[βγ-imido]triphosphate show altered sensitivity to hydrolysis of the γ-β bond (Yount *et al.*, 1971; Bunick *et al.*, 1982). The results in Fig. 2 indicate that ATP hydrolysis is not necessary for the stabilization of the enzyme-U-A-U-poly[d(A-T)] complex. GTP, CTP, AMP, dATP and 3'-dATP could not replace ATP in the stabilization of transcription complexes yielding U-A-U (Figs. 2 and 3). Therefore the stabilization step observed in the 1 μM concentration range is base-specific and necessitates both a triphosphate chain in the 5' position and hydroxy groups in the 2' and 3' positions of the incoming nucleotide, which are the requirements expected for the natural substrate to be incorporated. On the other hand, the results obtained with 3'-dATP in the presence of ATP (Fig. 3) suggest that the 2'-hydroxy group of the nucleotide is essential for occurrence of the second step of disappearance of U-A-U. Interestingly, with *E. coli* RNA polymerase, the abortive elongation reactions involving 3'-dATP are almost totally inhibited by a concentration of rifampicin that partially inhibits the parallel reaction involving ATP (Oen & Wu, 1978). With other RNA polymerases, such as the enzyme from *Pseudomonas putida* (Towle *et al.*, 1975) or the enzymes

I and II from *Dictyostelium discoideum* cells (Saneyoshi *et al.*, 1981), the K_i values associated with the inhibitory effect of 3'-deoxynucleoside 5'-triphosphates are significantly smaller than the corresponding K_m values for the natural substrates, a behaviour which is also encountered with wheat-germ RNA polymerase II (Dietrich *et al.*, 1985; the present work). The importance of the 2'-hydroxy group for the binding of nucleotides (substrates or inhibitors) is also strengthened by the observation that ara-ATP and 2'-amino-ATP are not inhibitors of transcription by *E. coli* RNA polymerase, in contrast with xylo-ATP and 3'-amino-ATP (Dennis *et al.*, 1980; Armstrong & Eckstein, 1976). Bis-(3'→5')-cyclic dinucleotides also behave as very potent inhibitors of the initiation phase of the polymerization with *E. coli* RNA polymerase (Hsu & Dennis, 1982). Therefore it seems possible that 3'-dATP might, to some extent, mimic the role of the activator or discriminator nucleotide described for the prokaryotic RNA polymerase reactions (Shimamoto & Wu, 1980a,b).

The results obtained with poly[d(A-A-T)]·poly[d(T-T-A)] as template (Fig. 4) show that the extent of abortive elongation differs considerably for the A-A-, A-U- and U-A-primed reactions. In addition, the effect of UTP concentration on the relative amounts of U-A-U-U and U-A-U for the U-A-primed reaction suggests that the stability of ternary transcription complexes is higher when formed with U-A-U-U than with the shorter product U-A-U. Since these experiments were conducted in the sole presence of UTP as substrate, the results in Fig. 4 would not favour the hypothesis that substrate and 'activator' correspond to the same nucleotide. On the other hand, if one numbers as '1' the base in DNA where UTP can be incorporated for the various primer-UTP combinations, it would appear that, when position 3 on the template is

complementary to UTP, transcription complexes are stable. This is the case for the reactions leading to A-U-U and U-A-U-U. Conversely, when position 3 on the template is not complementary to the UTP substrate, the transcription complexes are not stable, resulting in the synthesis of large amounts of abortive products. This is the case for the reactions leading to A-A-U and U-A-U (Fig. 4). These observations would therefore support the contention that 'activator' and substrate are selected at different sites on the DNA template, as proposed for *E. coli* RNA polymerase (Shimamoto *et al.*, 1981). It follows that the mechanisms involved in abortive and productive elongation appear remarkably similar for both the prokaryotic and wheat-germ RNA polymerases. The functional significance of such a behaviour has been questioned for *E. coli* RNA polymerase, and, as stressed by Shimamoto & Wu (1980*a,b*) and Shimamoto *et al.* (1981), the possibility of discriminating between the abortive and productive pathways by the involvement of regulatory nucleotides may offer some means of modulating the quantity of a specific RNA transcript and to ensure the fidelity of the initiation of transcription.

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REFERENCES

- Armstrong, V. W. & Eckstein, E. (1976) *Eur. J. Biochem.* **70**, 33–38
- Bunick, D., Zandomeni, R., Ackerman, S. & Weinmann, R. (1982) *Cell* **29**, 877–886
- Chamberlin, M. J. (1982) *Enzymes* 3rd Ed. **15**, 61–86
- Dennis, D., Jurgensen, S. & Sylvester, J. (1980) *Biochem. Biophys. Res. Commun.* **94**, 205–210
- Dietrich, J., Teissère, M., Job, C. & Job, D. (1985) *Nucleic Acids Res.* **13**, 6155–6170
- Dietrich, J., Teissère, M., Job, C. & Job, D. (1986) *Nucleic Acids Res.* **14**, 1583–1597
- Durand, R., Job, C., Teissère, M. & Job, D. (1982) *FEBS Lett.* **150**, 477–481
- Fromm, H. J. (1980) *Methods Enzymol.* **63**, part B, 84–103
- Hsu, C. Y. J. & Dennis, D. (1982) *Nucleic Acids Res.* **10**, 5637–5647
- Hurst, R. O. (1967) *Can. J. Biochem.* **45**, 2015–2039
- Jendrisak, J. J. & Burgess, R. R. (1975) *Biochemistry* **14**, 4639–4645
- Job, D., Durand, R., Job, C. & Teissère, M. (1984) *Nucleic Acids Res.* **12**, 3303–3319
- Kumar, S. A. (1982) *Prog. Biophys. Mol. Biol.* **38**, 165–210
- Lescure, B., Williamson, W. & Sentenac, A. (1981) *Nucleic Acids Res.* **9**, 31–45
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Cold Spring Harbor
- Manley, J. L., Fire, A., Cano, A., Sharp, P. A. & Geftter, M. L. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3855–3859
- Mosig, H., Schaffner, A. R., Sieber, H. & Hartmann, G. R. (1985) *Eur. J. Biochem.* **149**, 337–343
- Nierman, W. C. & Chamberlin, M. J. (1980) *J. Biol. Chem.* **255**, 4495–4500
- Oen, H. & Wu, C. W. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1778–1782
- Randerath, K. & Randerath, E. (1967) *Methods Enzymol.* **12**, part A, 323–347
- Saneyoshi, M., Tohyama, J., Nakayama, C., Takiya, S. & Iwabuchi, M. (1981) *Nucleic Acids Res.* **9**, 3129–3138
- Shimamoto, N. & Wu, C. W. (1980*a*) *Biochemistry* **19**, 842–848
- Shimamoto, N. & Wu, C. W. (1980*b*) *Biochemistry* **19**, 849–856
- Shimamoto, N., Wu, F. Y. H. & Wu, C. W. (1981) *Biochemistry* **20**, 4745–4755
- Sylvester, J. E. & Cashel, M. (1981) *Biochemistry* **19**, 1069–1074
- Towle, H. C., Jolly, J. S. & Boezi, J. A. (1975) *J. Biol. Chem.* **250**, 1723–1733
- Vaisius, A. C. & Wieland, T. (1982) *Biochemistry* **21**, 3097–3101
- von Hippel, P. H., Bear, D. G., Morgan, W. D. & McSwiggen, J. A. (1984) *Annu. Rev. Biochem.* **53**, 389–446
- Weil, P. A., Luse, D. S., Segall, J. & Roeder, R. G. (1979) *Cell* **18**, 469–484
- Yarbrough, L. R. (1982) *J. Biol. Chem.* **257**, 6171–6177
- Yount, R. G., Babcock, D., Ballantyne, W. & Ojala, D. (1971) *Biochemistry* **10**, 2484–2489

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