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Effect of salts on abortive and productive elongation catalysed by wheat germ RNA polymerase II

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ABSTRACT

Modification of the ionic conditions in reaction assays containing wheat germ RNA polymerase II and poly(dAT) as template markedly alters the catalytic properties of the transcription complexes. These effects have been studied by measuring the rate of abortive initiation and the extent of productive RNA synthesis. Using combinations of metal ions or various salts, a marked inhibition of abortive initiation was always associated with an increased length of RNA chains. These results are discussed in terms of modulation of the stability of transcription complexes induced by salts or divalent cations. The behavior exhibited by wheat germ RNA polymerase II is also discussed in comparison with previously reported results for procaryotic and eucaryotic RNA polymerases.

INTRODUCTION

The discovery and quantitation of the abortive initiation reaction described by McClure et al. (1-7) have allowed a precise description of the initiation step in reactions of RNA synthesis catalysed by E.coli RNA polymerase. During this process, the enzyme catalyses the reaction of single step addition of ribonucleoside triphosphates to substrates that cannot elongate, such as ribonucleoside monophophates or dinucleoside monophosphates (8-10). These reactions provided a direct assay for the formation of the first phosphodiester bond in RNA chain initiation. Both holoenzyme and core enzyme from E.coli are active in such reactions and in both cases, open transcription complexes have been characterized (5). However, the presence of sigma subunit significantly alters the nature and extent of open complex formation through modification of the kinetic constants for incorporation of nucleotide substrates as well as the binding constant of enzyme to DNA (5). Using poly(dAT) as template, Sylvester and Cashel (9) demonstrated that stable transcription complexes can accompany formation of a single phosphodiester bond. This was only observed with holoenzyme since in the absence of sigma subunit, the reactions of single step addition of AMP to ApU resulted in catalytic production of the trinucleotide ApUpA, instead of stable ternary complexes. The study of the mechanisms governing the stability of transcription ternary complexes is interesting in the view that even in the presence of all nucleotide substrates

necessary for RNA chain elongation, the RNA polymerase can form and release short oligonucleotides, rather than to continue to elongate RNA chains (1). Thus, abortive initiation has been suggested to be possibly a normal accompaniment of RNA chain initiation (3), and a recycling model for initiation has been proposed to account for the high yield of oligonucleotides compared to long RNA transcripts (11). Production of a long RNA chain is essentially an escape from this cycling reaction (11). Thus, the initiation of RNA synthesis is now defined as the sequence of events up to and including sigma subunit release (6). Furthermore, it appears that the strength of a promoter site on DNA depends on the amount of abortive initiation that occurs at this site (12,13). Recent progress has been made in the study of the enzymatic properties of eucaryotic RNA polymerases II in the catalysis of the abortive initiation reaction, allowing some comparison with the well documented bacterial enzyme. For instance, it was shown that wheat germ RNA polymerase II is able to catalyse efficiently the primer-independent abortive initiation (14). In contrast to previous attempts (15), we have also recently shown that wheat germ RNA polymerase II can catalyse the reaction of condensation of ribonucleotides to dinucleotides, leading to trinucleotide products formation (16). Using poly(dAT) as template, we found that experimental conditions can be selected under which catalytic production of trinucleotides occurs, a behavior which is reminiscent of that of E.coli core RNA polymerase (9). In this report, we show that the abortive reaction with the plant enzyme is extremely sensitive to the ionic conditions of the reaction medium. In particular, it is possible to select experimental conditions under which the rate of the abortive reaction is markedly reduced. This reduction in rate is not due to a change in the number of enzyme molecules involved in transcription complexes or to changes in the catalytic constants for the dinucleotide primer or the nucleotide substrate, but rather to an increased stability of the ternary transcription complexes. Indeed, parallel experiments in which the reaction mixtures leading to trinucleotide products are supplemented with the missing nucleoside triphosphates necessary for RNA chain elongation indicate that the smaller is the level of abortive initiation, the longer are the RNA chains. Although the role of divalent cations in formation of the naturally occuring 3'-5' linkages during DNA transcription with simple model systems seems well established (17), we would like to point out in this report that metal ions can act at several levels in reactions of transcription catalysed by eucaryotic RNA polymerases.

MATERIALS AND METHODS

Reagents

Nucleoside triphosphates and the dinucleoside monophosphates were purchased from Sigma. Nucleotide concentrations were calculated using a molar extinction coefficient

of 15.4 $mM^{-1}cm^{-1}$ at 259 nm for ATP and of 10.0 $mM^{-1}cm^{-1}$ at 262 nm for UTP. 3H-ATP and 3H-UTP (25 and 16.2 Ci/mmol, respectively) were from ICN. (α^{32}P)UTP (410 Ci/mmol) was from Amersham. Poly(dAT) was from PL Biochemicals. All buffer components were reagent grade.

RNA polymerase

Wheat germ RNA polymerase II was purified by the method of Jendrisak and Burgess (18), as previously described (19), except that the DEAE-cellulose column was replaced by a Fractogel TSK-DEAE-650 (S) column from Merck. E.coli RNA polymerase was a gift fom M. Schnaar and M. Daune (IBMC-CNRS, Strasbourg).

Reaction assays

Unless otherwise noted in the legends of figures, the reaction mixtures contained 40 nM enzyme, 5 µg/ml DNA, 0.9 mM UpA, the nucleoside triphosphates and metal salts ($MnCl_2$ and/or $MgCl_2$) as appropriate. 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 % (v/v) Triton X100, 1.1 mM DTT and 1.5 mM NaF. Final volumes were 10 µl and assays were usually incubated for 60 minutes at 35°C. Reactions were stopped by mixing the 10 µl reaction mixtures with 20 µl stop buffer containing 1 mM EDTA, 80 % formamide and 0.1 % xylene cyanol.

Activity measurements

For activity measurements, the above reaction mixtures were processed using three different methods, as previously reported (16,20). First, 10 µl of reaction mixtures were adsorbed on Whatman GF/C filters and washed eight times with an ice-cold solution containing 5 % trichloroacetic acid and 0.04 M sodium pyrophosphate. After drying, the filters were counted for radioactivity in 5 ml of toluene -PPO-POPOP liquid scintillation cocktail. Second , 2 µl of same reaction mixtures were spotted on polyethyleneimine-cellulose thin layer sheets (Macherey-Nagel, 20 x 10 cm). After drying, the sheets were washed in a bath of ultra pure water in order to remove glycerol, for 30 minutes, with gentle agitation. Then, ascending chromatography in 1.0 M formic acid + 0.25 M LiCl was used, according to Randerath and Randerath (21). After drying, the sheets were autoradiographed, usually for 18 hours at -70°C, using Fuji RX films and Cronex Li-Plus intensifying screens from Dupont. For each lane of the PEI-cellulose sheets, all spots containing radioactive material were cut and counted for radioactivity in vials containing 5 ml toluene-PPO-POPOP. Third, 2 to 6 µl of the reaction mixtures were analysed by gel electrophoresis on 15 % polyacrylamide, 7 M urea, 50 mM Tris-borate (pH 8.0) gels (0.03 x 30 x 40 cm), using the Poker face equipment from Hoefer Scientific Instruments. Electrophoresis was conducted at a constant 100 W until the xylene cyanol had migrated for 15 cm.

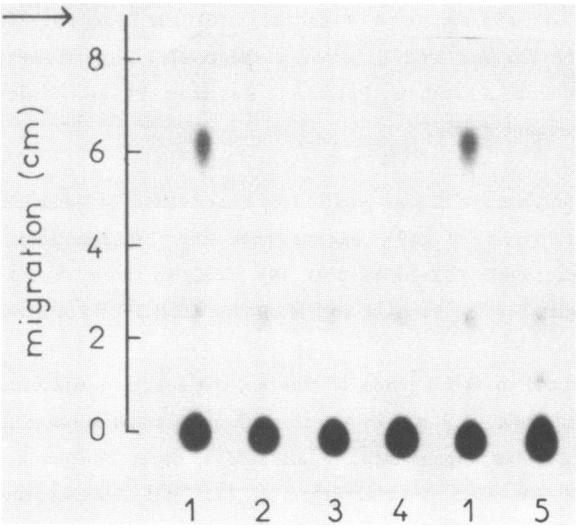


Figure 1: Effect of divalent cations and ammonium sulfate on poly(dAT) dependent formation of UpApU.
Reactions were conducted as described under Materials and Methods, in the presence of 40 nM wheat germ RNA polymerase II, 5 µg/ml poly(dAT), 0.9 mM UpA and 10 nM (α^{32}P)UTP (410 Ci/mmol). Incubation was conducted for 60 minutes at 35°C. Aliquots of 2 µl were removed from 10 µl reactions + 20 µl stop buffer and chromatographed as described under Materials and Methods. Autoradiography was performed at -70°C for 18 hours. The upper arrow represents the front of solvent. Reaction mixtures contained 2 mM MnCl2, 2 mM MnCl2 + 0.5 µg/ml α-amanitin, 2 mM MnCl2 + 10 mM EDTA, 2 mM MnCl2 + 20 mM MgCl2 or 2 mM MnCl2 + 40 mM ammonium sulfate for lanes 1 to 5, respectively.

Under these conditions, the marker dye comigrates with oligonucleotide of length 40 (22). The gels were autoradiographed at -70°C as above.

RESULTS

Effect of ionic conditions on UpApU synthesis

As previously reported (16), in a typical reaction assay as described under Materials and Methods, consequent production of UpApU occured after incubation for 60 minutes at 35°C. However, Figure 1 shows that such a reaction only occured in the presence of $MnCl_2$ as a cofactor. The addition of either 20 mM $MgCl_2$ or EDTA in the standard reaction mixture completely abolished the reaction. Marked inhibition was also observed in the presence of 40 mM ammonium sulfate, in agreement with preliminary experiments (16).

Effect of MgCl2 on UpApU and on poly(rAU) synthesis

The mechanism of inhibition of UpApU formation by $MgCl_2$ was investigated in more

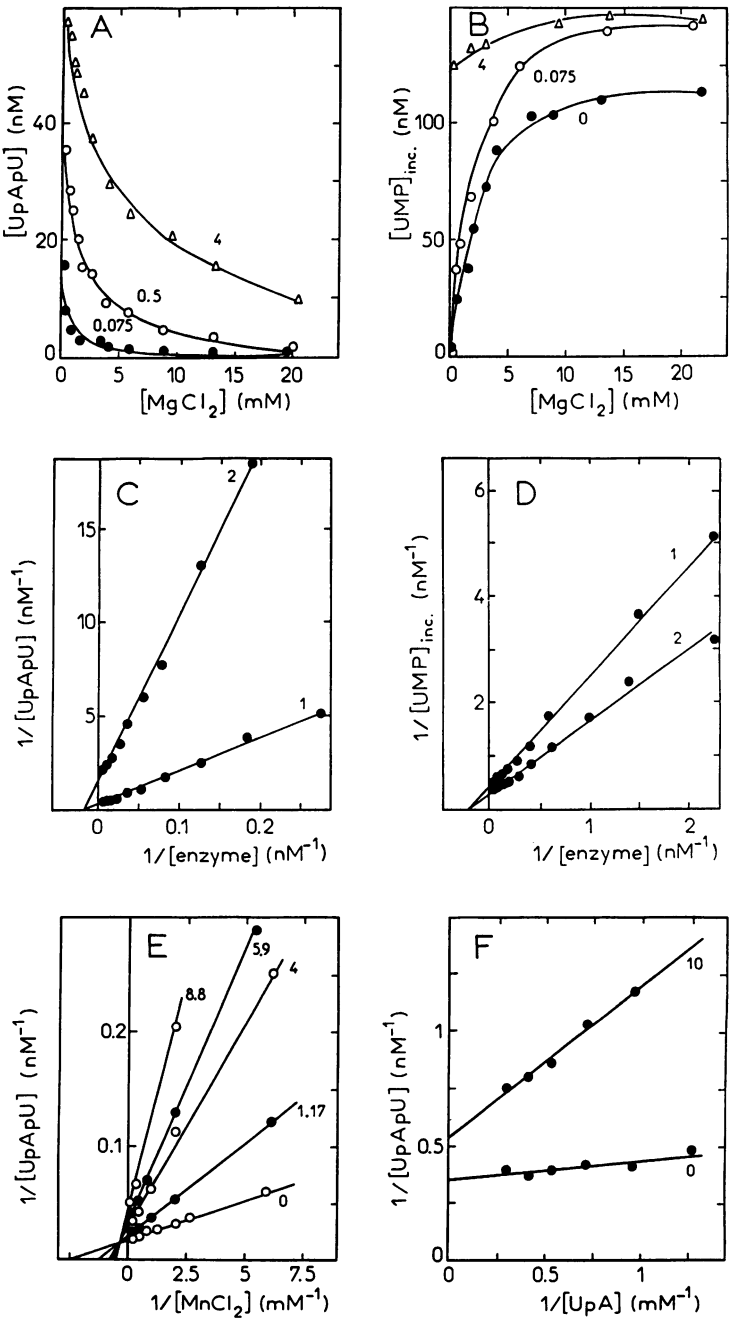
details. In parallel experiments, the effect of $MgCl_2$ on poly(rAU) synthesis was also examined. Figure 2, A and B shows that the inhibition of trinucleotide synthesis is not associated with a loss of RNA synthesis activity. On the contrary, under the experimental conditions of Figure 2, A and B, $MgCl_2$ activated poly(rAU) synthesis, at any $MnCl_2$ concentration in the transcription assay.

Figure 2, C and D shows titration curves of poly(dAT) template by wheat germ RNA polymerase II for both reactions of UpApU synthesis and poly(rAU) synthesis, in the absence or in the presence of excess $MgCl_2$. Again, $MgCl_2$ causes an inhibition of UpApU synthesis and an activation of poly(rAU) synthesis, at any enzyme concentration in the reaction assay. Furthermore, Figure 2, C and D shows that the apparent Km for enzyme is not affected by the presence of $MgCl_2$ in reaction assays leading to UpApU synthesis or poly(rAU) synthesis. This means that the addition of $MgCl_2$ in the reaction mixtures does not result in a change in the number of enzyme molecules involved in UpApU synthesis or in poly(rAU) synthesis. Interestingly, the Km value associated with UpApU synthesis is much higher (65 nM) than that corresponding to the reaction of poly(rAU) synthesis (5 nM). From the amount of poly(dAT) in the reaction assays, one can calculate that these Km values correspond to occupancy of 100 or 1250 (A-T) base pairs per enzyme molecule, for the reactions of abortive synthesis or poly(rAU) synthesis, respectively.

Figure 2, E shows the rate plots corresponding to the reaction of UpApU synthesis obtained by varying the concentration of $MnCl_2$ in the presence of fixed amounts of $MgCl_2$. In the concentration range studied (0-5 mM $MnCl_2$, 0-15 mM $MgCl_2$), the double reciprocal plots 1/v versus $1/(MnCl_2)$ are linear but have no common intercept on the 1/v axis. This indicates that $MgCl_2$ behaves as an uncompetitive inhibitor of $MnCl_2$ in the reaction of UpApU synthesis. Nevertheless, when same data are plotted in the form 1/v versus $MgCl_2$ concentration at fixed amounts of $MnCl_2$, the resulting plots are linear, indicating that the ternary complexes $MgCl_2$-transcription complex-$MnCl_2$ are inactive in the abortive reaction (not shown).

Figure 2, F shows the rate plots corresponding to UpApU synthesis at 2 mM $MnCl_2$, obtained by varying the concentration of UpA primer, in the absence or in the presence of 10 mM $MgCl_2$. These results show that $MgCl_2$ behaves as an uncompetitive inhibitor with respect to the primer, which therefore indicates that the ternary complexes $MgCl_2$-transcription complex-$MnCl_2$ can bind the primer.

Since we have previously shown that, in the presence of MnCl2, poly(rAU) synthesis proceeds as if wheat germ RNA polymerase II is a non-processive enzyme (16,23), we investigated the effect of $MgCl_2$ on the RNA products distribution. The results are shown in Figure 3. As previously noted (16,23), when poly(rAU) products synthesized in the presence of $MnCl_2$, are analysed by high resolution gel electrophoresis, a


A
[UpApU] (nM)
40
20
0
4
0.5
0.075
0 5 10 15 20
[MgCl2] (mM)
B
[UMP]inc. (nM)
100
50
0
4
0.075
0
0 5 10 15 20
[MgCl2] (mM)
C
1/[UpApU] (nM-1)
15
10
5
2
1
0 0.1 0.2
1/[enzyme] (nM-1)
D
1/[UMP]inc. (nM-1)
6
4
2
1
2
0 1 2
1/[enzyme] (nM-1)
E
1/[UpApU] (nM-1)
0.2
0.1
5.9
4
8.8
1.17
0
0 2.5 5 7.5
1/[MnCl2] (mM-1)
F
1/[UpApU] (nM-1)
1
0.5
0
10
0
0 0.5 1
1/[UpA] (mM-1)

ladder of RNA chains of increasing lengths is obtained. It has been proposed that RNA product dissociation occurs after UMP incorporation (16). Figure 3 also shows that the distribution of RNA products can be shifted readily by varying the concentration of $MgCl_2$ in the reactions: increasing the $MgCl_2$ concentration shifted the distribution to longer RNA products.

Effect of salts on UpApU synthesis and on poly(rAU) synthesis

Preliminary experiments (16) indicated that the reaction of UpApU formation catalysed by wheat germ RNA polymerase II was extremely sensitive to the presence of salts in the reaction assays. Experiments were thus carried out to investigate whether the behavior encountered with $MgCl_2$ was specific of the metal ion or related to more general ionic effects. Figure 4 shows the effect of NaCl, $(NH_4)_2SO_4$ and buffered sodium phosphate (pH 7.8) on the rates of UpApU synthesis and poly(rAU) synthesis at 1 mM $MnCl_2$. In all cases, moderate concentrations of salts produce a marked inhibition of the reaction of UpApU formation, whereas the effect on the rate of poly(rAU) synthesis is rather small.

The effect of salts on the distribution of poly(rAU) products was analysed by high resolution gel electrophoresis. The results are shown in Figure 5: it can be seen that increasing the salt concentration in the reactions shifts the distribution to longer RNA products.

Comparison with UpApU synthesis catalysed by E.coli RNA polymerase

It is well known that the bacterial enzyme can catalyse the abortive initiation reaction, however, the reactions with this enzyme are usually conducted in the presence of $MgCl_2$. To decipher whether the effects of salts observed in this study were specific of the plant enzyme, comparative experiments such as those in

Figure 2: Effect of MgCl2 on UpApU synthesis and on poly(rAU) synthesis.
Reaction mixtures leading to labeled UpApU formation were prepared as described in Figure 1 and under Materials and Methods (Figure 2, A, C, E, F). In parallel experiments, the reaction mixtures also received 100 µM ATP, necessary for poly(rAU) synthesis, which was quantitated using the GF/C filters, as described under Materials and Methods (Figure 2, B, D). The final (α32P)UTP concentration was 1 µM (7.5 Ci/mmol) in A, B, E or 9 nM (410 Ci/mmol) in C, D and F.
(A):rate of formation of UpApU and (B):rate of incorporation of UMP into poly(rAU) as a function of concentration of MgCl2. For each plot, the reaction mixtures contained a fixed concentration (mM) of MnCl2 indicated.
(C): saturation of poly(dAT) template for the reaction of UpApU synthesis and (D): for poly(rAU) synthesis at 2 mM MnCl2, in the absence (1) or in the presence of 15 mM MgCl2 (2).
(E): reciprocal velocities corresponding to the reaction of UpApU synthesis are plotted as a function of 1/MnCl2 concentration, at the fixed concentrations (mM) of MgCl2 indicated.
(F): reciprocal velocities corresponding to the reaction of UpApU synthesis at 2 mM MnCl2 are plotted as a function of 1/UpA primer concentration, at the fixed concentrations (mM) of MgCl2 indicated.

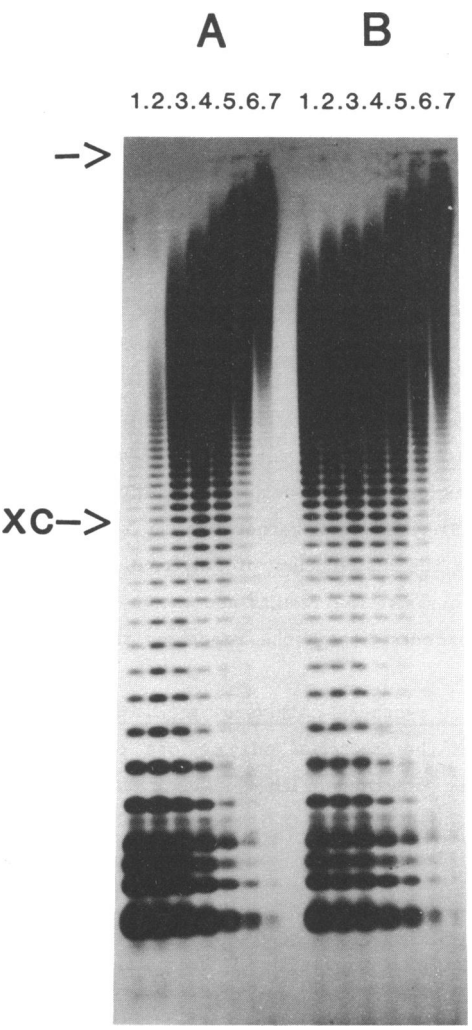


Figure 3: Effect of MgCl2 on product distribution from transcription of poly(dAT) in the presence of MnCl2.
Reaction mixtures leading to poly(rAU) synthesis were prepared as described under Materials and Methods, in the presence of 0.9 mM UpA, 1 μM (α32P)UTP at a specific activity of 7.5 Ci/mmol, 100 μM ATP, 40 nM wheat germ RNA polymerase II and 5 μg/ml poly(dAT). MnCl2 was present at a concentration of 0.5 mM (A) or 2 mM (B). MgCl2 was present at a concentration of 0, 0.52, 1.17, 2.63, 5.92, 13.33 and 20 mM for lanes 1 to 7, respectively.
Final volumes were 10 μl. Incubation time was 60 minutes at 35°C. Transcription was stopped by adding 20 μl stop buffer. 6 μl of the above reaction mixtures were loaded on a 15% polyacrylamide-7 M urea gel. Electrophoresis and autoradiography were conducted as described under Materials and Methods. The upper arrow represents the origin, and XC stands for xylene cyanol.

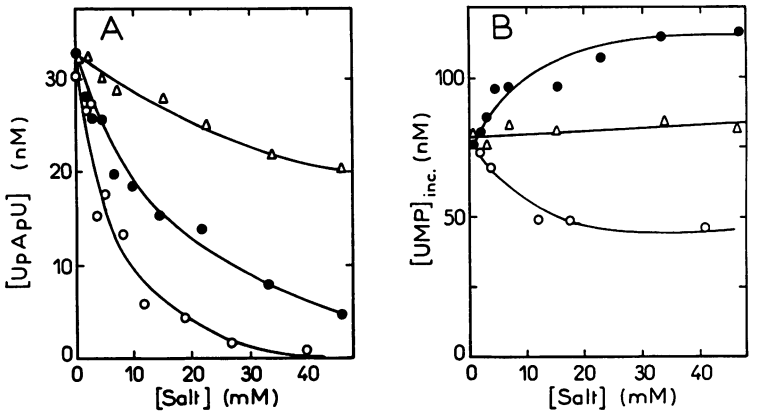


Figure 4: Effect of salts on UpApU synthesis and poly(rAU) synthesis.
Reaction mixtures leading to UpApU synthesis (A) or poly(rAU) synthesis (B) were prepared as described under Materials and Methods. Final (α32P)UTP concentration was 1 µM (6.8 Ci/mmol), MnCl2 was at 1 mM. In (B) the mixtures also received 100 µM ATP, resulting in poly(rAU) synthesis which was quantitated using the GF/C filters. Incubation time was 60 minutes at 35°C. For both reactions, the mixtures received various amounts of sodium chloride (Δ), ammonium sulfate (●) or buffered (pH 7.8) sodium phosphate (O) .

Figures 1, 2 and 4 were performed with E.coli RNA polymerase. At 2 mM $MnCl_2$, condensation of UMP to UpA could be detected. The radioactive product had exactly same Rf value as the product obtained with wheat germ RNA polymerase II, after chromatography on PEI-cellulose sheets. The reaction was absolutely dependent on the presence of enzyme, primer, divalent cation, UTP and poly(dAT) template (not shown). However, the reaction with the bacterial enzyme differs from that with the eucaryotic enzyme on the following points: 1) the specific activity of the bacterial enzyme is much higher: for instance under the conditions of Figure 2 (absence of salts, 2 mM $MnCl_2$, 1 µM UTP), about 5% of the input UTP is converted into UpApU with 40 nM plant enzyme, whereas about 40% of the input UTP is converted into UpApU in the presence of 6 nM of the bacterial enzyme; 2) with the procaryotic enzyme, the rate of UpApU synthesis at 2 mM $MnCl_2$ is not affected by addition of $MgCl_2$ (up to 20 mM) or ammonium sulfate (up to 30 mM) in the reaction assay, in contrast to the results in Figures 1, 2 and 4. This is shown in Figure 6.

DISCUSSION

From the results presented, it appears that the abortive initiation reaction catalysed by wheat germ RNA polymerase II is extremely sensitive to the nature of the metal ion and to the presence of salts in the reaction assays. Thus, in contrast to the

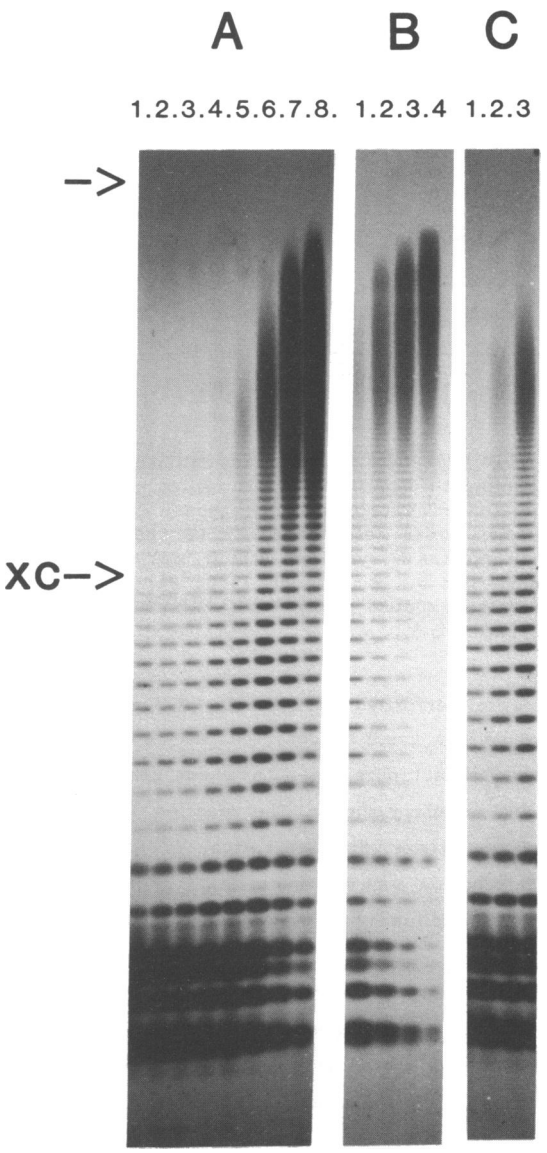


Figure 5: Effect of salt on product distribution from transcription of poly(dAT) in the presence of MnCl2.
Reactions were conducted at 1 mM MnCl2 as described in Figure 3 and Figure 4,B. Reaction mixtures also contained 0, 2.92, 6.58, 9.87, 14.8, 22.2, 33.3 and 50 mM ammonium sulfate (A: lanes 1 to 8, respectively) or 11.8, 17.7, 26.6 and 40 mM buffered (pH 7.8) sodium phosphate (B: lanes 1 to 4, respectively) or 14.8, 22.2 and 33.3 mM sodium chloride (C: lanes 1 to 3, respectively). Electrophoresis and autoradiography were carried out as described under Materials and Methods and in Figure 3. The upper arrow represents the origin, XC stands for xylene cyanol.

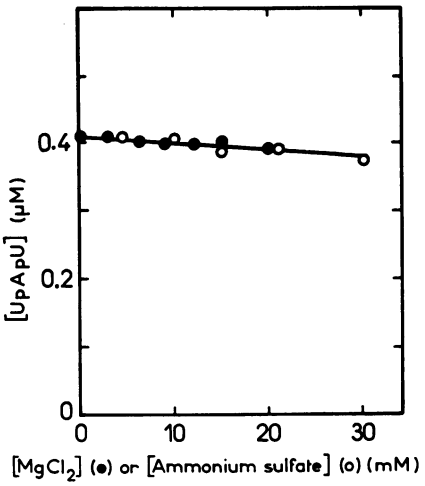


Figure 6: Effect of MgCl2 and ammonium sulfate on UpApU synthesis with E.coli RNA polymerase.
Reactions leading to UpApU synthesis were conducted as described under Materials and Methods, in the presence of 6 nM E.coli RNA polymerase, 5 μg/ml poly(dAT), 0.9 mM UpA, 1 μM (α32P)UTP (7 Ci/mmol), 2 mM MnCl2 and various amounts of MgCl2 (●) or ammonium sulfate (O). Incubation time was 60 minutes at 35°C. Reactions were quantitated as described under Materials and Methods.

results obtained with E.coli RNA polymerase (8,9, this study) and same template, $MgCl_2$ is a potent inhibitor of UpApU synthesis, and moderate concentrations of salts almost completely abolish the reaction. There are several reports mentioning the effect of divalent cations or salts on the reaction of transcription by eucaryotic RNA polymerases (24-31). From these studies, it appears that the observed effects could be explained by DNA conformational changes and/or enzyme conformational changes. In this context, the interaction between DNA and ions is well documented (32-35), and in some cases the effect of alteration of DNA conformation has been investigated at the level of transcription (25,36-37). Alternately, the possible effect of divalent cations on enzyme itself might be supported by the observation that E.coli RNA polymerase contains several Mn^{++} binding sites (38). Therefore, two different explanations are a priori available to account for the results in Figure 2. First, the effect of $MgCl_2$ and $MnCl_2$ can be exerted through poly(dAT) modifications which in turn may affect the RNA polymerase reaction. It is interesting to note that such conformational changes of DNA will affect the plant enzyme but not the procaryotic enzyme. Second, the effect of divalent cations would occur through modifications of enzyme itself. The experiments reported in this study do not allow to make a clear distinction between these two possibilities. However, whatever

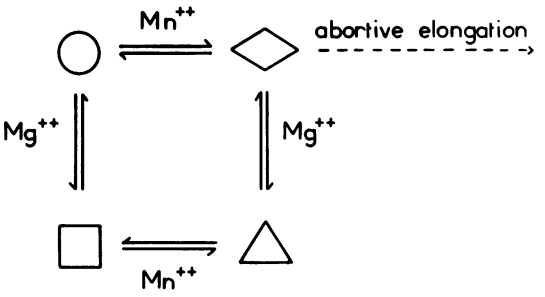


Figure 7: Simple reaction scheme to account for the effect of MgCl2 on the UpA primed synthesis of UpApU catalysed by wheat germ RNA polymerase II with poly(dAT) as template.
In this reaction scheme divalent cations affect the activity of transcription complexes (denoted by geometric symbols) in the abortive process leading to UpApU synthesis. As discussed in the text, the effect of divalent cations can occur through modification of poly(dAT) and/or enzyme conformation. The kinetic results in Figure 2 indicate the existence of a ternary complex MgCl2-transcription complex-MnCl2 (denoted by the triangle) which is inactive in the synthesis of UpApU. However, this complex can bind the UpA primer, as shown in Figure 2, F.

the step altered in the presence of divalent cations, the direct consequence is that divalent cations can modify the catalytic properties of the transcription complexes, poly(dAT)-enzyme-primer.

This is represented by the reaction scheme in Figure 7. The results in Figure 2 indicate that $MgCl_2$ does not change the number of enzyme molecules bound to poly(dAT) template. Furthermore, $MgCl_2$ is not a competitive inhibitor of $MnCl_2$ or UpA primer. This latter observation is in agreement with the results of Samuels et al. (39) showing that calf thymus RNA polymerase II can utilize dinucleoside monophosphates for priming of promoters specific RNAs in the presence of $MgCl_2$. Therefore, the enzyme-poly(dAT) complex can bind both $MnCl_2$, $MgCl_2$ and the primer. However, the resultant complex is not active for UpApU production, in contrast to the complex generated in the presence of $MnCl_2$ alone. On the other hand, under experimental conditions allowing poly(rAU) synthesis, i.e. in the additional presence of ATP, much longer RNA chains are obtained by increasing the $MgCl_2$ concentration in the reaction assay, at any $MnCl_2$ concentration. In a previous report (16), we have shown that the extent of abortive initiation as well as the non-processive behavior exhibited by wheat germ RNA polymerase II is at least in part, dependent on the stability of transcription complexes. In particular, using the poly(dAT) template, we found that the stability of transcription complexes is smaller after incorporation of UMP than after incorporation of AMP, which results in the preferential release of the U terminated products. This was observed either for

trinucleotide synthesis or for poly(rAU) synthesis (16). In this context, the present results would therefore suggest that transcription complexes are significantly more stable in the presence of $MgCl_2$ than with $MnCl_2$.

It is interesting to note that in a comparative study of the binding of procaryotic and eucaryotic RNA polymerases II on simian virus 40 DNA, Saragosti et al. (40) showed that the formation of a phosphodiester bond between a specific dinucleotide and the incoming ribonucleoside triphosphate can stabilize the initiation complex with E.coli RNA polymerase (in the presence of 10 mM $MgCl_2$), calf thymus RNA polymerase II (in the presence of 2 mM $MnCl_2$ and 50 mM $(NH_4)_2SO_4$) or wheat germ RNA polymerase II (in the presence of 1 mM $MnCl_2$, 10 mM $MgCl_2$ and 40 mM $(NH_4)_2SO_4$). This indicates that stabilization of initiation complexes with eucaryotic RNA polymerases II can be obtained under very similar ionic conditions, independently of the nature and conformation of the DNA template, which would may be favor the hypothesis that the observed transition is occuring by modification of the enzyme molecule. In this context, there are indications that mutations in the largest subunit of Drosophila or yeast RNA polymerase II alter RNA chain elongation in vitro (41,42). Termination of transcription during in vitro synthesis with calf thymus RNA polymerase II has also been shown to be relatively unimportant in the presence of $MgCl_2$ (43).

In the case of E.coli RNA polymerase, it has been proposed that there exists an inverse correlation between the amount of abortive oligonucleotide synthesis and the extent of productive synthesis leading to long RNA transcripts (12,13). Our results would suggest that such a behavior may also be exhibited by the plant enzyme, since the less abortive synthesis proceeds, the longer are the poly(rAU) chains. Abortive initiation seems to be a major process with RNA polymerase II and complete reconstituted transcription systems, as compared to the low level of synthesis of full size runoff RNAs (44).

Essentially similar results are obtained by addition of $MgCl_2$ or various salts in the reaction assays leading to UpApU synthesis or to poly(rAU) synthesis. Therefore, it seems possible that at least part of the effects of $MgCl_2$ can be explained by modifications of the ionic conditions rather than by specific effects. The importance of the electrostatic potential in reactions involving polyelectrolyte-enzyme complexes (45-47) has been examined in the view that the electrostatic potential may provide a regulatory mechanism via ionic control of enzyme activity: thus, in many cases, including nucleic acid-enzyme complexes, relatively mild changes in the ionic strength markedly affect enzyme activity. The nature of electrostatic interactions between E.coli RNA polymerase and DNA has been reviewed (48,49), and it has been shown that the binding of enzyme to DNA involves the release of counterions (50).

Another interesting observation is that divalent cations (in the same concentration range as investigated in this study) also affect transcription by RNA polymerase II in complete reconstituted transcription systems (51). It is therefore possible that the transition induced by divalent cations on the activity of a highly purified RNA polymerase II may also occur in more complex transcription systems. As suggested by Lescure et al. (52), in the absence of additional transcription factors, Mn^{++} ions may be important to facilitate enzyme-DNA interactions. These observations may raise the possibility that divalent cations could, at least in part, mimic the effects of some of the factors involved in the formation of "activated " initiation complexes (53), in RNA chain elongation (44) and/or in termination of transcription from these complexes.

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