Labat, J., Baumann, F., & Courtois, J.-E. (1973) *Carbohydr. Res. 26*, 341-349.
Lowry, H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265-275.
Nakano, M. (1980) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 39*, 1919.
Nisizawa, K., & Hashimoto, Y. (1970) *The Carbohydrates* (Pigman, W., & Horton, D., Eds.) 2nd ed., Vol. 2, pp 241-300, Academic Press, New York.
Sacktor, B. (1972) *Biochemistry of the Glycosidic Linkage* (Piras, R., & Pontis, H. G., Eds.) pp 281-289, Academic Press, New York.
Saito, S. (1960) *J. Biochem. (Tokyo) 48*, 101-109.
Sawai, T. (1967) *Proceedings of the Amylase Symposium, 1967* (Society of Amylase Researchers, Eds.) pp 111-117, Osaka, Japan.
Williams, D. E., & Reisfeld, R. A. (1964) *Ann. N.Y. Acad. Sci. 121*, 373-381.

Formation of a Single Phosphodiester Bond by RNA Polymerase B from Calf Thymus Is Not Inhibited by α-Amanitin†

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ABSTRACT: The template-directed synthesis of a single phosphodiester bond by highly purified calf thymus RNA polymerase B is not inhibited by high concentrations of α-amanitin (10^{-6} M). However, a subsequent internucleotide bond is not synthesized in the presence of α-amanitin. These results suggest that translocation of the nascent RNA and RNA polymerase B along the DNA template is the enzymatic process inhibited by α-amanitin. It is also shown that the formation of a single phosphodiester bond by RNA polymerase B results in a stable ternary transcription complex, i.e., between the enzyme, the DNA, and the nascent RNA. Under reaction conditions which normally favor the elongation of RNA, the transcriptional process is arrested at initiation by α-amanitin. Such ternary initiation complexes have been isolated by agarose gel electrophoresis.

The cytotoxin α-amanitin is a bicyclic octapeptide occurring in high concentrations in the deadly, poisonous mushroom *Amanita phalloides*. The primary cytopathogenicity of the amatoxins is the inhibition of RNA polymerase B (ribonucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) which is the enzyme that transcribes the precursor of mRNA. RNA polymerases B from higher eukaryotes are typically half-maximally inhibited by α-amanitin at a concentration of 5×10^{-9} M [for a review, see Wieland & Faulstich (1978)].

In a very detailed study, Cochet-Meilhac & Chambon (1974) demonstrated that the amatoxins bind to RNA polymerase B with a 1:1 stoichiometry, that the affinity of the toxin for the enzyme is very strong with an equilibrium association constant of 3.5×10^8 M^{-1} at 30 °C, and that the dissociation is very slow and highly dependent upon temperature and ionic strength. Subsequently, it was shown by Brodner & Wieland (1976), using a carbodiimide condensation reaction of labeled amatoxin to calf thymus RNA polymerase B, that the 140-kilodalton subunit was a binding site for the amatoxins. Thus, it has been clearly shown that inhibition by α-amanitin is the result of a direct interaction of the toxin with RNA polymerase B.

The process of RNA synthesis catalyzed by DNA-dependent RNA polymerase is quite complex and may be described in several steps as follows: (a) formation of a stable binary complex with the DNA template; (b) RNA chain initiation; (c) translocation and elongation of the nascent RNA; (d) termination and dissociation of the RNA chain. Hence, there are a number of enzyme mechanisms which could possibly be specifically disrupted by α-amanitin. It has been shown that the binding of α-amanitin does not cause a dissociation of the DNA–RNA polymerase B binary complex or the dissociation of the DNA–enzyme–RNA ternary complex (Cochet-Meilhac & Chambon, 1974). In the same study, direct evidence was given that the binding of α-amanitin was responsible for the inhibition of RNA chain elongation as catalyzed by RNA polymerase B. The inhibition of chain initiation was suggested by indirect experimentation, i.e., by a failure to detect pyrophosphate exchange which is presumably the reverse reaction of phosphodiester bond formation (Krakow & Fronk, 1969). Therefore, it was concluded by Cochet-Meilhac & Chambon (1974) that the binding of amatoxin to RNA polymerase B prevents the formation of phosphodiester bonds.

In this paper, we report that calf thymus RNA polymerase B will catalyze a template-directed synthesis of a single phosphodiester bond in the presence of a high concentration of α-aminitin. However, we were unable to detect the subsequent synthesis of a second internucleotide bond. Our results suggest that translocation may be the enzymatic process blocked by α-amanitin. A preliminary report of these results was presented earlier (Vaisius & Wieland, 1981).

Materials and Methods

Biochemicals. Amanitin was a preparation from our laboratory. Ribonucleases and alkaline phosphatase (minimal nuclease) were commercial preparations from P-L Biochemicals. Nucleoside triphosphates, purified by high-performance liquid chromatography, were purchased from ICN Biochemicals. Radioactively labeled nucleoside triphosphates were from NEN and Amersham International. Dinucleoside (3′→5′) monophosphates and trinucleoside (3′→5′) diphosphates were from P-L Biochemicals. The synthetic templates poly(dA-dT) and poly(dI-dC) were purchased from Boehringer, Mannheim.

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Purification of RNA Polymerase B. The procedures for the purification of RNA polymerase B from calf thymus were modified from those previously published for the wheat germ enzyme (Jendrisak & Burgess, 1975). The purification procedures involve precipitation with poly(ethylenimine), selective elution of RNA polymerase B from the poly(ethylenimine), and also chromatography on DEAE-cellulose, P-cellulose, and DEAE-Sephadex and affinity chromatography on DNA–agarose. By these methods, RNA polymerase B was purified to near homogeneity. When proteins were stained with Coomassie brilliant blue R250, a single protein band was seen after nondenaturing polyacrylamide gel electrophoresis, and only the typical subunit bands (Kēdinger et al., 1974; Hodo & Blatti, 1977) were seen after sodium dodecyl sulfate–polyacrylamide gel electrophoresis. However, if the proteins were visualized after electrophoresis with a very sensitive silver stain (Merril et al., 1981), traces of contaminating proteins could be detected.

Initiation Assay. The standard reaction solution (50 μL) containing 0.75 M glycerol, 15 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid (Hepes) (pH 7.6), 50 mM KCl, 2 mM $MnCl_2$, 1 mM 2-mercaptoethanol, 2.5 μg of the designated DNA template, 0.5 μg of calf thymus RNA polymerase B, 50 μM initiator nucleotide, and 1 μCi/5 μmol [^{3}H]NTP or 2.5 μCi/5 μmol [^{32}P]NTP as indicated. In those reactions containing α-amanitin, the desired concentration was obtained by serial dilution of a standard sample whose concentration was determined spectrophotometrically by using ϵ_{310} = 13 500. All components, except the nucleotide substrates, were first incubated for 5 min at 25 °C, then the substrates were added, and the reaction solutions were incubated for an additional 5 min at 25 °C. Unless otherwise stated, the reactions were stopped by heating at 56 °C for 3 min. This effectively inactivates the enzyme and dissociates the ribonucleotide product from the ternary complex.

Alkaline Phosphatase Treatment of Reaction Products. After the reactions were heated to 56 °C, they were cooled to room temperature. The reactions were then incubated with 0.5 unit in 5 μL of alkaline phosphatase at 37 °C for 15 min. We have analyzed the alkaline phosphatase preparation for nuclease activity, and by any method, we could not detect contaminating nuclease activity.

Determination of the Transcriptional Product. Nucleotides and oligonucleotides were separated by continuous-flow thin-layer chromatography (Randerath & Randerath, 1967) on poly(ethylenimine)–cellulose sheets with a phosphate buffer system (Cashel et al., 1969). Prior to use, the sheets were washed as described by Ogawa & Okazaki (1979). A 5-μL sample was spotted from each reaction by using a [^{32}P]NTP label, and two 5-μL samples were spotted from each reaction by using a [^{3}H]NTP label. The chromatogram was first developed with 2 mM KH_2PO_4 (pH 3.5) for 15 min and then immediately transferred to 0.2 M KH_2PO_4 (pH 3.5) for a specified time. With continuous-flow thin-layer chromatography (TLC), we took 3 h to separate a trinucleotide diphosphate product and 2 h to separate a dinucleotide monophosphate product. After chromatography of reactions using a [^{3}H]NTP label, the chromatograms were dried and cut into 0.8-cm strips. Each strip was placed in a scintillation vial and then eluted with 1.5 mL of 1 N H_2SO_4. The samples were counted with a fine quality scintillant in a Searle Mark III scintillation counter equipped and programmed to determine disintegrations per minute. After chromatography of reactions using a [^{32}P]NTP label, the chromatograms were dried, and autoradiographs were then taken.

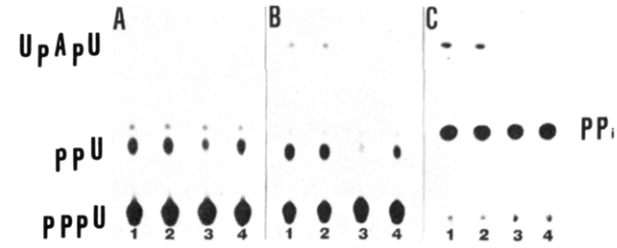

FIGURE 1: Autoradiograms of thin-layer chromatograms showing the products of single-step addition reactions with UpA and [α-^{32}P]UTP as substrates and poly(dA-dT) as the template. In all instances, the reactions were as follows: lane 1 is without α-amanitin; lane 2 is with 10^{-6} M α-amanitin; lane 3 is a blank without RNA polymerase B; lane 4 is without poly(dA-dT). (A) Initiation assays were incubated at 25 °C as described under Materials and Methods. (B) Samples from (A) were quickly heated to 56 °C for 3 min and then cooled to ambient temperature. (C) Samples from (B) were treated with alkaline phosphatase as described under Materials and Methods. Autoradiography was for 12 h in (A) and (C) and for 6 h in (B).

Electrophoresis of Ternary Complexes. After the assays were incubated at 25 °C as described above, to each reaction was added 10 μL of a 50% (w/w) sucrose solution, which contained 6 × 10^{-6} M α-amanitin for those reactions not previously having α-amanitin. The solutions were then cooled to 0 °C in an ice–water bath. Samples of 40 μL were loaded onto a 1% (w/w) agarose gel, which was precooled to 1 °C. The gel was formed by using a casting mold of the Cold Spring Harbor design (Sugden et al., 1975), and the electrophoresis buffer was similar to that described by the same authors. Electrophoresis was at 75 mA for 3.5 h, with the temperature of the entire system maintained at 1 °C. Immediately after electrophoresis, the gel was autoradiographed without removing it from the casting mold but by simply replacing the covering plate with a plastic wrap over which was laid the X-ray film and the intensifying screen. Exposure was for 6 h at 1 °C.

Results

In general, RNA polymerases may use a mono-, di-, or triphosphate nucleotide or even an oligoribonucleotide as the 5′ initiating substrate, whereas only nucleoside triphosphates may serve as subsequent substrates in the polymerization of RNA (Minkley & Pribnow, 1973; Oen & Wu, 1978; Shaw & Saunders, 1979). Therefore, by using a synthetic DNA template with a specific alternating sequence, along with an initiator which cannot serve as a substrate for elongation, a reaction can be designed to allow for the synthesis of a single phosphodiester bond by RNA polymerase. We have used such "single-step addition reactions" (Oen & Wu, 1978) to determine whether or not α-amanitin will inhibit RNA polymerase B under these conditions. For example, with poly(dA-dT) as a template, we have UpA with pppU, or ApU with pppA, as substrates for the synthesis of UpApU and ApUpA, respectively. Likewise, with poly(dI-dC) as a template, we have used CpG with pppC or GpC with pppG as substrates for the synthesis of CpGpC and GpCpG, respectively. The concentration of α-amanitin (10^{-6} M) in the single-step addition reactions was sufficient to be ~100% inhibitory in a typical RNA polymerase assay to determine the total mass of RNA synthesized.

The experiments described in Figure 1 were designed to permit the synthesis of UpApU. However, if the initiation assays were incubated at 25 °C and then, without further treatment, a sample was chromatographed, the only apparent reaction product is ppU, as shown in Figure 1A. We have observed the same phenomenon with initiation assays as de-

Table I: Synthesis of Oligoribonucleotides by RNA Polymerase B with Single-Step Addition Reactions[a]

substrates	template	product (phosphatase treated)	pmol incorporated −α-amanitin	pmol incorporated +α-amanitin (10^{-6} M)
pA, pppU*	poly(dA-dT)	ApU*	3.8	3.3
UpA, pppU*	poly(dA-dT)	UpApU*	4.1	3.9
pU, pppA*	poly(dA-dT)	none		
ApU, pppA*	poly(dA-dT)	ApUpA*	3.7	3.8
ApApU, pppA*	poly(dA-dT)	ApApUpA*	1.6	1.6
pG, pppC*	poly(dI-dC)	GpC*	2.9	2.5
CpG, pppC*	poly(dI-dC)	CpGpC*	1.1	1.0
pC, pppG*	poly(dI-dC)	none		
GpC, pppG*	poly(dI-dC)	GpCpG*	1.1	1.3

[a] All assays used a [^{3}H]NTP as the labeled substrate in a standard assay described under Materials and Methods. The reactions were then treated with alkaline phosphatase, and the product was separated by TLC as described.

scribed above which utilized ATP, GTP, or CTP as the penultimate substrate. Thus, the conversion of nucleotide triphosphates to their respective diphosphates is not nucleotide specific. The 5′-phosphohydrolase activity is not template dependent, but preliminary results indicate that there is a greater activity in the presence of a template under conditions which do not allow for elongation of the nascent RNA. Similarly, it has been shown that a nucleoside triphosphate phosphohydrolase activity is associated with purified but nonhomogeneous preparations of *Escherichia coli* RNA polymerase (Ninio et al., 1975; Volloch et al., 1979). This activity from *E. coli* was also not nucleotide specific and was greatest on nucleotide substrates which were noncomplementary to DNA of defined sequence (Ninio et al., 1975; Volloch et al., 1979). A full characterization of the 5′-phosphohydrolase activity which copurifies with RNA polymerase B will be described elsewhere. However, at this time, we consider it likely that the conversion reaction (pppN → ppN) may be coordinated with the action of RNA polymerase B by an analogous proofreading mechanism as suggested for the bacterial enzyme by Ninio et al. (1975) and Volloch et al. (1979).

If the reactions described in Figure 1A are stopped by heating to 56 °C or even 95 °C, a product which comigrates with UpApU is resolved. In our initial experiments, we observed similar results to those shown in Figure 1B by stopping the reactions with the addition of 5 μL of a solution containing 11% (w/w) sodium dodecyl sulfate and 0.11 M EDTA. Therefore, the product is not an artifact of heat catalysis, but rather it is most probable that both the heat and the sodium dodecyl sulfate treatment cause the dissociation of the product from a relatively stable transcriptional complex. For confirmation that the resolved product results from transcription, the reaction solutions used in Figure 1B were subsequently treated with alkaline phosphatase. As clearly shown in Figure 1C, the only product resistant to the phosphatase treatment is the template-dependent product which comigrates with UpApU, whereas the ^{32}P labels on the product identified as ppU, as well as on the residual pppU, are entirely hydrolyzed under the same conditions. Since alkaline phosphatase readily cleaves phosphomonoester bonds, this supports the conclusion that the α-^{32}P was incorporated into a diester internucleotide bond. With ApU and [α-^{32}P]ATP as substrates, we obtained results which were totally in agreement with those in Figure 1A–C. In that case, the only product resistant to the phosphatase comigrated with ApUpA. In parallel experiments with [^{3}H]NTP labeled on the bases, all radioactivity, except that of the expected product, migrated onto the paper wick during continuous-flow TLC. Because this latter procedure provided a very clean background, it was the method used for the

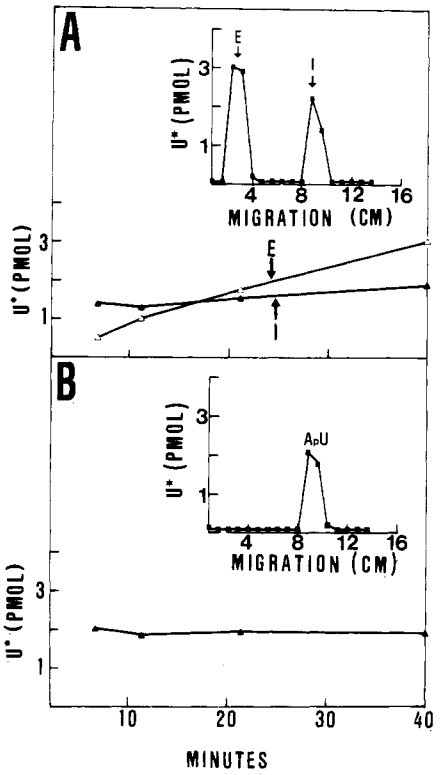

FIGURE 2: Time courses of single-step addition reactions by using ADP, which contained contaminating AMP and ATP, and [^{3}H]UTP as substrates and poly(dA-dT) as the template. Standard assays were incubated for various times, and the reaction products were digested with alkaline phosphatase and then chromatographed. All procedures are described under Materials and Methods. (A) Reactions without α-amanitin. Line I designates the synthesis of the initiation product ppApU, and line E designates the synthesis of elongated RNA. The insert is a profile of the chromatogram from 40-min reactions. (B) Reactions with 10^{-6} M α-amanitin. As shown in the insert, only the initiation product is synthesized after 40 min. The time course indicates that the amount of initiation product synthesized remains unchanged after the period of initial synthesis.

quantification of results given in Table I. For all the reactions described in Table I, the synthesis of an initiation product was very low and unaffected by the presence of 10^{-6} M α-amanitin. In fact, even at a concentration of 10^{-3} M α-amanitin, there was no inhibition of the synthesis of a single phosphodiester bond by calf thymus RNA polymerase B.

In many of the assays described in Table I, the initiation product synthesized was nearly stoichiometric with respect to RNA polymerase B in the reactions. Therefore, we sought to determine whether this resulted from a very slow turnover or from the formation of a stable ternary complex after the formation of a single phosphodiester bond. In the experiments

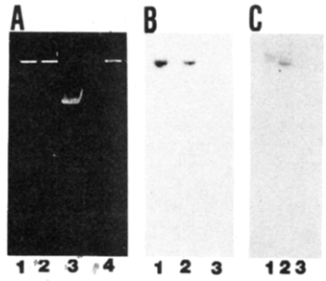

FIGURE 3: Agarose gel electrophoresis of ternary initiation complexes with RNA polymerase B, adenovirus-2 DNA, and labeled nascent RNA. Procedures for the agarose gel electrophoresis are described under Materials and Methods. In all instances, the reactions were as follows: lane 1 is without α-amanitin; lane 2 is with 10^{-6} M α-amanitin; lane 3 is without RNA polymerase B; lane 4 is without nucleotide substrates. (A) Ethidium bromide fluorescence of adenovirus-2 DNA from reactions with GpA and UTP as substrates. (B) Autoradiogram from reactions similar to those in (A). (C) Autoradiogram from reactions with 10 μM each of ATP, CTP, and GTP and 2.5 μCi/5 μmol [α-^{32}P]UTP as substrates.

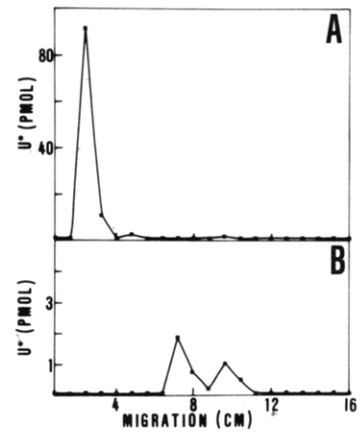

FIGURE 4: Thin-layer chromatograms from reactions permitting the elongation of RNA. The substrate concentrations were 50 μM each of ATP, CTP, and GTP and 1 μCi/5 μmol [^{3}H]UTP. The template was adenovirus-2 DNA. Assays were performed under standard conditions, and the reaction products were digested with alkaline phosphatase and then chromatographed. All procedures are described under Materials and Methods. (A) Reactions without α-amanitin. The major peak represents elongated RNA, and migrating just ahead of this peak is a minor peak presumably of oligonucleotides. (B) Reactions with 10^{-6} M α-amanitin. The only reaction products are dinucleotides which comigrate with GpU and ApU.

described in Figure 2, we measured the amount of initiation product synthesized with time. The initiator substrate was ppA which contained small amounts of contaminating pppA as well as pA. The contaminating pppA suited this experiment because it allowed for limited elongation of RNA. The reactions described in Figure 2A were incubated without α-amanitin, and the synthesis of the ppApU initiation product only slightly increased with time, whereas the synthesis of elongated RNA, although very slight, did increase by 6-fold. During the same time course, the reactions containing 10^{-6} M α-amanitin had a relatively uniform amount of initiation product synthesized, which indicates that the synthesis of a single phosphodiester bond may be sufficient to form a stable ternary initiation complex. For reactions without α-amanitin, one might expect that the amount of initiation product synthesized would decrease as slow elongation increased. However, a more likely explanation is that elongation is rapid even under very limiting conditions and that ternary initiation complexes are very stable. This suggestion would be in accord with the fact that we were unable to detect the synthesis of oligonucleotides with two, three, or four phosphodiester bonds, which would have been easily resolved with the TLC system used. In reactions containing α-amanitin, we were unable to detect the synthesis of any elongated RNA whatsoever.

In order to conclusively verify that RNA polymerase B and the initiation product are in a stable complex with the DNA template, we sought to isolate this complex by agarose gel electrophoresis. The procedures described for the electrophoresis of the ternary initiation complexes were determined by trial and error with an emphasis on maintaining a stable transcriptional complex. Because our initial experience indicated that the subsequent addition of α-amanitin to initiation assays without α-amanitin tended to stabilize the ternary complex, all reactions were brought to a final concentration of 10^{-6} M α-amanitin prior to electrophoresis. Similarly, it was necessary to keep the reaction products at 0–4 °C after the initiation assays were stopped. To maximize the detection of a ternary initiation complex carrying a single label, it was essential to use a DNA template of uniform size which permitted sufficient RNA polymerase B loading but which also allowed for the resolution of binary and ternary complexes. For these reasons and in anticipation of future work, we chose to use adenovirus-2 DNA. Ethidium bromide fluorescence shown in Figure 3A demonstrates that adenovirus-2 DNA complexed with RNA polymerase B (lane 4) migrates slower than the naked DNA and that DNA from the initiation assays (lanes 1 and 2) migrates identically with the binary complex. The autoradiograph in Figure 3B clearly shows that a radioactively labeled product from initiation assays comigrates with the DNA and is not inhibited by α-amanitin. If RNA polymerase B is omitted from the reactions (Figure 3B, lane 3), radioactive label does not become associated with the DNA. The reactions in Figure 3C were designed to allow for limited elongation. However, under conditions which are optimal for elongation using the same enzyme:DNA ratio described above, ternary complexes for reactions without α-amanitin will not enter the gel. The results show that the ternary complexes containing elongated RNA from reactions without α-amanitin migrate slower than those with α-amanitin which migrate identically with the DNA–RNA polymerase B binary complexes. Although we were unable to recover the initiation product after electrophoresis, parallel assays were treated with alkaline phosphatase and the products resolved by TLC, as previously described. The products for reactions carried out without α-amanitin consisted of RNA polymers (Figure 4A) and a low level (~5 pmol) of oligoribonucleotides. The only detectable products for reactions with α-amanitin are dinucleotide monophosphates (Figure 4B). We were also able to isolate the ternary initiation complex by microscale exclusion chromatography on Bio-Gel P-60 (unpublished results). The transcriptional initiation product, which chromatographed with the excluded volume, was verified by TLC. Thus, by agarose gel electrophoresis as well as by exclusion chromatography, we substantiate the conclusion that the transcriptional initiation complex can be fixed by α-amanitin even under conditions which favor elongation of the nascent RNA, presumably because the translocation process is blocked by the toxin.

Discussion

Using single-step addition reactions, we have shown that the catalysis of a single phosphodiester bond by RNA polymerase B from calf thymus is not inhibited by a high concentration of α-amanitin (10^{-6} M). Our results demonstrate that an internucleotide bond will be formed in the presence of α-amanitin regardless of whether the incoming substrate is a purine or a pyrimidine. This was interesting to us because prior to these findings, it remained possible that α-amanitin

could have inhibited RNA polymerase B in such a way as to prevent the enzyme from rotating the glycosidic bonds of pyrimidines into the proper angle for the base pairing with the template. In the presence of α-amanitin but under conditions which otherwise favored the elongation of RNA, the synthesis of an initiation product was stoichiometric with the amount of RNA polymerase B in the reactions, and the formation of a second phosphodiester bond was never detected. Therefore, we believe that α-amanitin inhibits the translocation mechanism of RNA polymerase B. This postulation might also explain the seeming contradiction between our results and those of Cochet-Meilhac & Chambon (1974), who showed that α-amanitin inhibited pyrophosphate exchange at the level of initiation. Pyrophosphate exchange by RNA polymerase only occurs with the penultimate nucleoside triphosphate and is thought to occur after polymerization of a phosphodiester bond but before translocation occurs (Krakow & Fronk, 1969). However, evidence that pyrophosphorolytic cleavage of the nascent phosphodiester bond occurs prior to translocation is limited and indirect. If the pyrophosphate exchange reaction requires translocation of the product prior to pyrophosphorolysis, i.e., an active site on the enzyme may need to be free to coordinate with the pyrophosphate, then α-amanitin could inhibit pyrophosphate exchange, whereas the synthesis of a phosphodiester bond would not be inhibited.

Recently, it has been reported that α-amanitin would inhibit the synthesis of a single internucleotide bond by RNA polymerase B from *Saccharomyces cerevisiae*. With a dinucleotide initiator and a single nucleoside triphosphate as substrates, the yeast enzyme incorporated an extraordinary 40% of the labeled substrate into an oligoribonucleotide (Lescure et al., 1981). The limited initiation which we observed is more in accord with the previously reported results obtained by terminally labeled RNA (i.e., γ-^{32}P-labeled ribonucleotide triphosphate) from reactions with calf thymus RNA polymerase B (Mandel & Chambon, 1974) or with yeast enzyme (Dezélée et al., 1974). Lescure et al. (1981) postulated that the high rate of oligonucleotide synthesis which they observed might reflect abortive transcription under standard conditions. As shown in our results, we were only able to detect a minimal synthesis of oligonucleotides under conditions which permitted elongation. Comparison of the results obtained with the yeast and the calf thymus RNA polymerase B is at best tenuous because there is obviously a fundamental difference in the activity of the two enzymes, which is difficult to explain at present. These differences may also be reflected in the fact that yeast RNA polymerase B is much less sensitive to α-amanitin (Schultz & Hall, 1976) than is the calf thymus enzyme (Kédinger et al., 1970). However, we believe that the results of Lescure et al. (1981) do not necessarily preclude the fact that the synthesis of a phosphodiester bond by the yeast enzyme can occur in the presence of a high concentration of α-amanitin. For example, if α-amanitin stabilized the ternary initiation complex, then there would be a pronounced inhibition effect by α-amanitin relative to the very high turnover rate without α-amanitin. Working with calf thymus RNA polymerase B, we believe that α-amanitin does indeed stabilize a ternary transcription complex, and at present, we are developing techniques to properly quantify this effect.

As shown by its resistance to exclusion chromatography and agarose gel electrophoresis, a ternary initiation complex is relatively stable. The initiation complex can be fixed with α-amanitin even under conditions which favor the elongation of RNA. The initiation of transcription is a critical control point in gene expression, but in the past, it has been difficult to study the initiation of transcription by RNA polymerase B because there was no apparent means to isolate this event under standard assay conditions. On the basis of the observations reported in this paper, the use of α-amanitin should provide a new dimension to the study of the mechanisms involved in the initiation of transcription by RNA polymerases B from higher eukaryotes.

References

Brodner, O., & Wieland, Th. (1976) *Biochemistry 15*, 3480–3484.

Cashel, M., Lazzarini, R., & Kalbacher, B. (1969) *J. Chromatogr. 40*, 103–109.

Cochet-Meilhac, M., & Chambon, P. (1974) *Biochim. Biophys. Acta 353*, 160–184.

Dezélée, S., Sentenac, A., & Framageot, P. (1974) *J. Biol. Chem. 249*, 5971–5977.

Hodo, H., & Blatti, S. (1977) *Biochemistry 16*, 2334–2343.

Jendrisak, J., & Burgess, R. (1975) *Biochemistry 14*, 4639–4645.

Kédinger, C., Gniazdowski, M., Mandel, J.-L., Gissinger, F., & Chambon, P. (1970) *Biochem. Biophys. Res. Commun. 38*, 165–171.

Kédinger, C., Gissinger, F., & Chambon, P. (1974) *Eur. J. Biochem. 44*, 421–436.

Krakow, J., & Fronk, E. (1969) *J. Biol. Chem. 244*, 5988–5993.

Lescure, B., Williamson, V., & Sentenac, A. (1981) *Nucleic Acids Res. 9*, 31–45.

Mandel, J.-L., & Chambon, P. (1974) *Eur. J. Biochem. 41*, 379–395.

Merril, C., Goldman, D., Sedman, S., & Ebert, M. (1981) *Science (Washington, D.C.) 211*, 1437–1438.

Minkley, E., & Pribnow, D. (1973) *J. Mol. Biol. 77*, 255–277.

Ninio, J., Bernardi, F., Brun, G., Assari, L., Lauber, M., & Chapeville, F. (1975) *FEBS Lett. 57*, 139–144.

Oen, H., & Wu, C.-W. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1778–1782.

Ogawa, T., & Okazaki, T. (1979) *Nucleic Acids Res. 7*, 1621–1633.

Randerath, K., & Randerath, E. (1967) *Methods Enzymol. 12*, 323–347.

Schultz, L., & Hall, B. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1029–1033.

Shaw, P., & Saunders, G. (1979) *FEBS Lett. 106*, 104–110.

Sugden, B., DeTroy, B., Roberts, R., & Sambrook, J. (1975) *Anal. Biochem. 68*, 36–46.

Vaisius, A. C., & Wieland, Th. (1981) *Biochem. Soc. Trans. 9*, 173P abstract.

Volloch, V., Rits, S., & Tumerman, Z. (1979) *Nucleic Acids Res. 6*, 1535–1546.

Wieland, Th., & Faulstich, H. (1978) *CRC Crit. Rev. Biochem. 5*, 185–260.