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Ribonucleic Acid Polymerase from Eukaryotic Cells

Effects of Factors and Rifampicin on the Activity of RNA Polymerase from Chromatin of Coconut Nuclei

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The role of protein factor B and C on RNA synthesis by RNA polymerase CI isolated from chromosomal non-histone proteins of coconut nuclei has been studied further. Factor B has been implicated as the initiation factor on the experimental evidences that (a) in its absence, RNA polymerase CI shows only minimal activity; (b) it can bind with RNA polymerase and the enzyme · factor B complex then binds to DNA, but factor B alone can not bind to DNA; (c) it promotes the incorporation of [β,γ-$^{32}P_2$]ATP into RNA and this stimulation reaches a plateau rather quickly while the incorporation of [^{14}C]ATP in the interior of RNA chain continues; (d) it is active with native homologous DNA as template, but not with denatured or λ DNA; (e) RNA molecules synthesized in its presence are of higher sedimentation value (10–20 S) than that synthesized in its absence (4 S); (f) it can completely counteract the inhibitory effect of rifampicin, which is known to inhibit RNA synthesis at the initiation step.

Factor C seems to facilitate the release of synthesized RNA from the DNA template since (a) it stimulates RNA synthesis by polymerase CI when added on top of factor B, but in absence of factor B, C alone is inactive; (b) it can reinitiate RNA synthesis after the reaction has reached a plateau in a system where DNA is limiting, an effect similar to that obtained at higher ionic strength. Factor C, however, does not influence the molecular size of RNA synthesized. Furthermore, the RNA polymerase CI is sensitive to α-amanitin whereas the RNA polymerase CII is comparatively resistant. The former appears to synthesize the non-ribosomal RNA whereas the latter synthesizes ribosomal RNA.

In the earlier paper [1], we have reported the isolation and purification of two RNA polymerases and two protein factors from coconut endosperm chromatin. It was also reported earlier that, of the two RNA polymerases, one was sensitive to the antibiotic rifampicin [2]. In the present communication, we present further evidence to show that factor B acts as initiator and factor C probably as terminator for RNA synthesis. It is also shown that inhibition of RNA synthesis by rifampicin is reversible by factor B at high concentration. RNA polymerase CI seems to be sensitive to α-amanitin whereas RNA polymerase CII is comparatively resistant.

Abbreviations, QAE-Sephadex, quaternary aminoethyl Sephadex.

Definition. A_{260} unit, the quantity of material contained in 1 ml of a solution which has an absorbance of 1 at 260 nm, when measured in a 1-cm-pathlength cell.

Enzyme. RNA polymerase or nucleosidetriphosphate :RNA nucleotidyltransferase (EC 2.7.7.6).

MATERIALS AND METHODS

Sources of the labelled and non-labelled nucleotide triphosphates are given in the previous paper [1]. Acrylamide, N,N'-methylene bisacrylamide and N,N,N',N'-tetramethyl ethylenediamine were obtained from Eastman Kodak (Rochester, N.Y.). α-Amanitin was obtained as a gift from Dr T. Wieland (Max-Plank Institute, Heidelberg, Germany). All other chemicals were regent grade commercial products.

Enzymes

DNA-dependent RNA polymerases and factors were isolated from coconut endosperm nuclei by chromatography as described in the earlier paper [1]. DEAE-cellulose fractions of RNA polymerases and factors were used in most of these studies. For some experiments, RNA polymerase CI was further purified by QAE-Sephadex chromatography.

Polyacrylamide-Gel Electrophoresis

Disc electrophoresis of proteins (enzymes and factors) were carried out at pH 8.3 in 5% acrylamide gels by slight modification [3] of the original method of Davis [4]. The electrophoresis was carried out in a 4 °C cold room. After the run, the gels were sliced and assayed for polymerase and factors as described under respective figures.

Electrophoresis of RNA was carried out in 2.5% polyacrylamide gel according to Bishop *et al.* [5] with a modified technique for slicing the soft gels. After electrophoresis of the labelled RNA along with carrier *Escherichia coli* RNA, the gels were stained with toluidine blue and washed as usual. The stained gel was transferred without distortion on glass slides and a rectangular piece of filter paper was stuck on it. The gel was now easily transferred to the filter paper without any change in length and was kept in a 37 °C incubator. The gel dried on to the paper as a thin film in several hours. The gel was cut into 3-mm strips, digested with H_2O_2 and counted with dioxan-based scintillation fluid [6].

Preparation of [β,γ-$^{32}P_2$]ATP

[β,γ-$^{32}P_2$]ATP was prepared by the method [7] of [^{32}P]PP$_i$ exchange into β,γ-positions of ATP in the presence of amino acids and crude activating enzymes from *Azotobacter vinelandii*. *A. vinelandii* was grown in Burk's medium [8] and the enzymes were extracted from the bacteria by grinding with alumina in presence of Tris-Mg-K buffer (0.01 M Tris-HCl pH 7.0, 0.01 M $MgCl_2$, 0.05 M KCl, and 5 mM 2-mercaptoethanol). One ml of *A. vinelandii* extract (0.2 g protein) was incubated at 37 °C for 30 min in presence of 0.5 ml of ATP (10 µmol/ml), 0.02 ml 0.5 M NaF, 0.04 ml 1 M Tris-HCl pH 7.0, 0.1 ml of casamino acids (100 mg/ml) and 0.2 ml of [^{32}P]PP$_i$ (600 counts $\times$ min^{-1} $\times$ pmol^{-1}, 10 µmol/ml). The incubation was stopped by 1 M perchloric acid, chilled in an ice bath for 30 min and centrifuged. The supernatant was taken and neutralised with KOH, chilled for 20 min in ice. The clear supernatant was concentrated under vacuum and the [β,γ-$^{32}P_2$]ATP was separated by paper chromatography using the solvent isobutyric acid—ammonia—water (66:1:33, v/v/v). The spots were located under an ultraviolet lamp and ATP was eluted with distilled water in a saturated chamber. It was shown by partial hydrolysis followed by chromatographic separation of ATP, ADP and AMP that there was no incorporation of ^{32}P at the α-position. The calculated specific activity at each of β and γ position was 200 counts $\times$ min^{-1} $\times$ pmol^{-1}.

Competition-Hybridization Procedures

The ribosomal RNA was isolated by hot phenol-dodecylsulfate from ribosomes obtained from coconut endosperm tissue. ^{3}H-labelled RNA was synthesized *in vitro* by both the enzymes separately with the addition of factor B. The incubation mixture contained all the ingredients as described in earlier paper [1] except that both [^{3}H]GTP (spec. act. 9.9 Ci/mmol) and [^{3}H]ATP (spec. act. 3.2 Ci/mmol) were used to get higher specific activity in the RNA synthesized. The hybridization technique was that of Gillespie and Spigelman [9] with slight modification. In a hybridization mixture 20 µg heat-denatured coconut DNA with a fixed amount of [^{3}H]RNA synthesized by RNA polymerase CI (spec. act. 1.2 counts $\times$ min^{-1} $\times$ ng^{-1}) or by RNA polymerase CII (specific activity 1 count $\times$ min^{-1} $\times$ ng^{-1}) was simultaneously competed with increasing amounts of unlabelled rRNA in 3 ml of 2 $\times$ standard-saline-citrate buffer (0.15 M NaCl and 0.015 M sodium citrate = standard-saline citrate) at 70 °C for 12 h. After processing, Millipore HAWP filters were counted in a liquid scintillation counter with 10 ml dioxan-based liquifluor and 1 ml water in which the filters were completely dissolved giving a uniform phase.

RESULTS

Role of Factor B

Of the five protein peaks obtained from the DEAE cellulose column, the one eluted at 0.5 M KCl (fraction B) had a stimulatory activity on the RNA polymerases. The extent of stimulation depended on the amount of factor B added, when the amount of RNA polymerase CI was kept constant. Saturation point was reached when the ratio of RNA polymerase CI to factor B was 10:1. The enzyme RNA polymerase CI (and also RNA polymerase CII) was almost incapable of RNA synthesis *in vitro* without the addition of factor B (Fig. 1).

The mode of action of factor B on RNA polymerase was further elucidated. For this purpose, the incorporation of [β,γ-$^{32}P_2$]ATP (which is a measure of RNA chain initiation) and of [^{14}C]ATP (which is incorporated into the internucleotide linkages as well) into acid-insoluble products was studied. When assayed at 37 °C, it was found that the incorporation of [β,γ-$^{32}P_2$]ATP was very fast and reached a plateau within 2 min (results not given). To slow down this rate, the incubation was then carried out at 20 °C. In this case, the incorporation of [β,γ-$^{32}P_2$]ATP was linear from the beginning and reached a plateau around 6 min. The incorporation of [^{14}C]ATP, on the other hand, was much slower at the beginning and then increased linearly from 5—10 min under the assay conditions (Fig. 2). It appeared, therefore, that factor B might act as an initiation factor. Further evidence that B acts as the initiation factor came from the experiments to be described later.

As the fraction B from the DEAE-cellulose column was a mixture of proteins, as revealed by polyacryl-

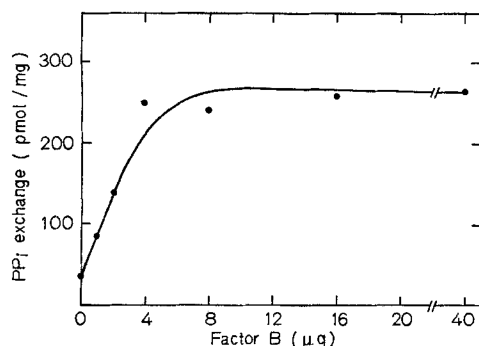

Fig. 1. *Optimal concentration of factor B for RNA polymerase CI.* The conditions of the experiment were the same as in Table 1. The enzyme activity was assayed by the pyrophosphate-exchange method as described in a previous paper [1]. Varying concentrations of factor B and 50 µg polymerase CI (DEAE-cellulose fraction) were used in each set of this experiment

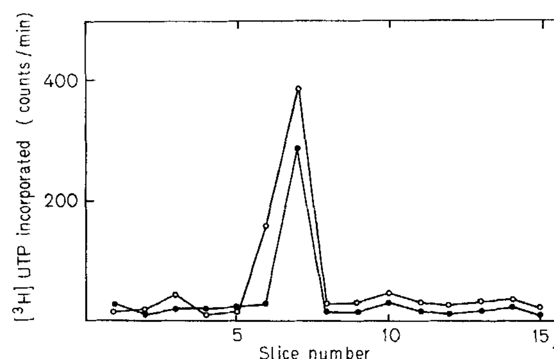

Fig. 3. *Activity of factor B with polymerase CI and CII.* 200 µg of factor B was separated by 5% polyacrylamide gel electrophoresis as described in the text, each 2-mm slice of the gel was extracted overnight with 0.2 ml Tris-glycerol buffer [1]. Then each extracted fraction was assayed separately with 50 µg polymerase CI and 50 µg polymerase CII. The assay mixture was the same as in Table 1

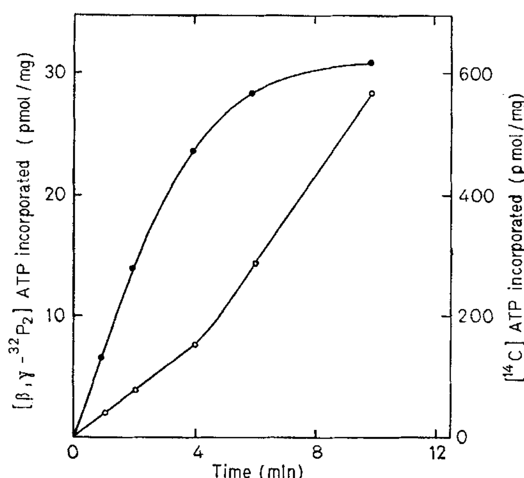

Fig. 2. *Kinetics of initiation and total synthesis of RNA.* The conditions of the experiment were the same as in Table 1. $[\beta,\gamma\text{-}^{32}P_2]$ATP (200 counts × min^{-1} × $pmol^{-1}$) was used for detection of the initiation (●) and $[^{14}C]$ATP (10 counts × min^{-1} × $pmol^{-1}$) was used for determining the total synthesis (○). Temperature was adjusted to 20 °C to follow the initial incorporation, 100 µg of polymerase CI (DEAE-cellulose fraction) and 10 µg of factor B were used

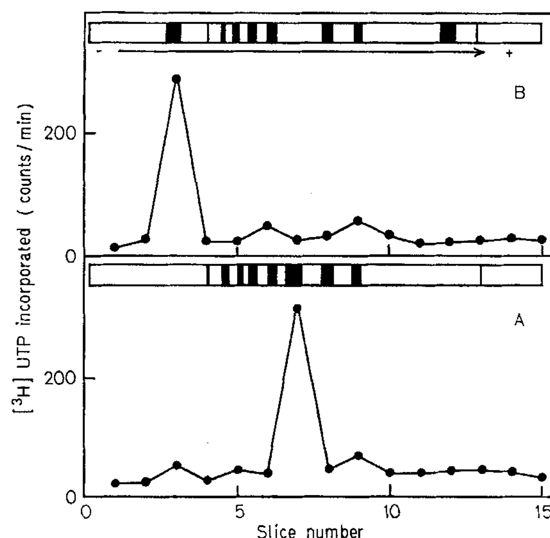

Fig. 4. *Binding of factor B with polymerase CI.* (A) 50 µg factor B alone, (B) 50 µg factor B with 100 µg polymerase CI were electrophoresed separately on 5% polyacrylamide gel as described in the text. The gels were sliced, extracted and assayed as described in Table 1. The activity of factor B in (A) was assayed with 50 µg polymerase CI added extraneously

amide gel electrophoresis, we wanted to see if the same protein was responsible for stimulating the activity of both the RNA polymerase CI and CII or if there were two different factors. We therefore subjected the fraction B to polyacrylamide gel electrophoresis, sliced the gel and the extract of each gel slice was assayed for the stimulatory activity for both RNA polymerase CI and CII. Results presented, in Fig. 3 indicate that either the same factor acts on both the polymerases or if the factors are different, then are they so very closely similar that they can not be separated by this method.

Complex Formation of Factor B with RNA Polymerase and DNA

It is known in the case of *E. coli* that the σ factor binds with the core RNA polymerase and the enzyme · factor complex again binds with the template DNA [10, 11]. A similar situation exists in the present case of coconut RNA polymerase and factor B. When the crude factor B preparation was separated by electrophoresis in polyacrylamide gel and the slices assayed for stimulatory activity with added RNA polymerase CI, the factor activity was located at slice No. 7 (Fig. 4A). But when a mixture of RNA

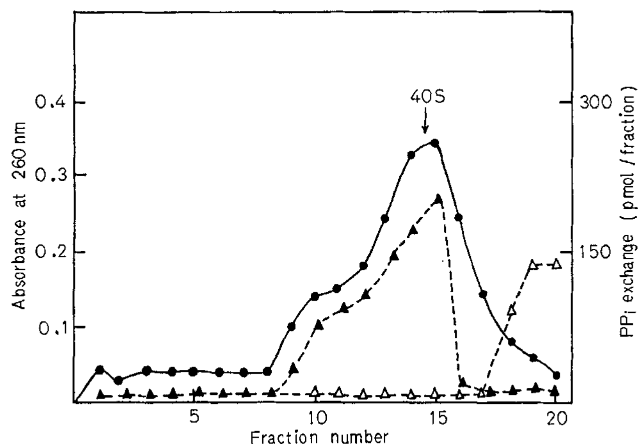

Fig. 5. *Binding of RNA polymerase CI and factor B with DNA.* A mixture of 100 μg RNA polymerase CI (DEAE-cellulose fraction) and 10 μg factor B was centrifuged in the presence or absence of 2.0 A_{260} units of coconut endosperm DNA on a 5–20% linear sucrose gradient in 0.01 M Tris, 0.1 M NaCl, pH 8.0 for 2 h at 37000 rev./min in the SW-39 rotor of Spinco model L-2 ultracentrifuge. After the run, fractions of 15 drops (about 0.25 ml) were collected from the bottom of the tubes. The fractions were assayed for RNA polymerase activity by $[^{32}P]PP_i$ exchange. Absorbance at 260 nm of DNA only (●——●); activity of RNA polymerase run with DNA and factor B (▲----▲); the activity of the same run with factor B only (△----△). The DNA was added extraneously in the latter set for detection of the activity

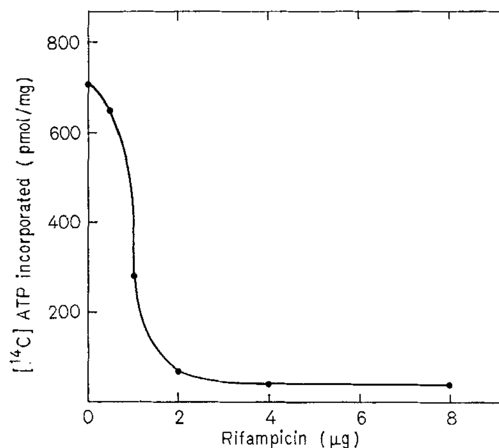

Fig. 6. *Minimal concentration of rifampicin to inhibit the RNA synthesis by RNA polymerase CI.* The conditions of the experiment were the same as in Table 1. Rifampicin in varying concentration was added to the incubation mixtures before the factor B was added (5 μg) to the RNA polymerase CI (50 μg) in each experiment

polymerase CI and factor B was run and similarly assayed without any external addition of enzyme or factor, the activity could be located in slice No. 3 (Fig. 4B). It was observed in a separate experiment that the activity of the enzyme when run alone in the electrophoresis was also located in slice No. 3. It is clear from these results that under the incubation conditions, factor B associates with RNA polymerase CI. The factor being presumably of much smaller molecular weight than the enzyme, its association with the enzyme does not appreciably change the size or charge of the latter.

When a mixture of RNA polymerase CI and factor B was centrifuged along with DNA in a sucrose gradient, both the enzyme and factor activities sedimented to the same region as that of DNA (Fig. 5). However, when RNA polymerase CI and factor B were separately centrifuged along with DNA, it was found that the enzyme was bound to DNA while the factor remained at the top of the gradient (results not presented). These experiments clearly show that the enzyme · factor complex can bind to DNA, though free factor can not.

Inhibition of RNA Polymerase CI by Rifampicin and Its Reversal by Factor B

Nuclear RNA polymerase systems from eukaryotic cells have generally been found to be insensitive [11,12] to the drug rifampicin which inhibits RNA polymerase from prokaryotes at the initiation step [13,14]. However, we found that of the two enzymes from coconut nuclei, RNA polymerase CI was inhibited by the drug whereas RNA polymerase CII was not [2]. Fig. 6 shows that maximum inhibition of RNA polymerase CI in the presence of a saturating amount of factor B (5 μg) is obtained at a rifampicin concentration of 2 μg/incubation volume of 0.5 ml. Furthermore, it was interesting to note that rifampicin was effective only when it was added to the polymerase before the addition of the factor B. The effects of the different orders of additions of the components

Table 1. *The effect of rifampicin on RNA polymerase CI*
The incubation system (either 0.5 ml or 0.25 ml) contained 0.04 M Tris-HCl pH 8.0, 0.2 M EDTA, 5 mM 2-mercaptoethanol, 2 mM $MnCl_2$, 0.4 mM K_2HPO_4, 0.16 mM KCl, the four triphosphates each 0.15 mM, of which one was labelled ($[^{14}C]$ATP, specific activity 10 counts × min^{-1} × $pmol^{-1}$, or $[^3H]$UTP, specific activity 4 counts × min^{-1} × $pmol^{-1}$), and 40 μg/ml coconut endosperm DNA. 50 μg polymerase CI, 5 μg factor B and 4 μg/ml rifampicin (rif.) were used in each set of this experiment. The components were added in a specific order as described below. The details of the assay procedure was described in the previous paper [1]

Order of addition	$[^{14}C]$ATP
	pmol/mg
1. NTP[a], DNA, CI, rif., factor B	32
2. NTP, CI, factor B, rif., DNA	790
3. NTP, CI, factor B, DNA, rif.	750
4. NTP, DNA, factor B, rif., CI	52
5. DNA, NTP, CI, factor B, rif.	800
6. DNA, CI, factor B, NTP, rif.	740
7. DNA, CI, rif., factor B, NTP	42

[a] NTP means the 4 nucleoside triphosphates including labelled ATP, other components not depicted in the tabular form were added as mentioned above.

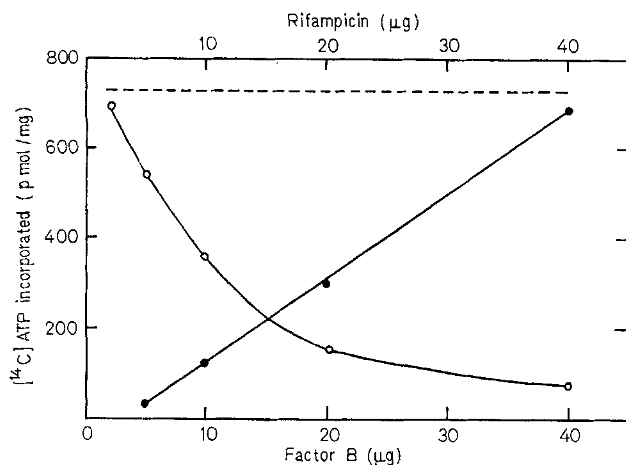

Fig. 7. *Competitive effects of rifampicin and factor B on the activity of RNA polymerase CI.* The conditions of the experiments were the same as in Table 1. In one set of experiments, 2 µg rifampicin was added to 50 µg RNA polymerase CI followed by factor B in varying concentration (●). In another set, 5 µg factor B was added to 50 µg polymerase CI followed by rifampicin in varying concentration (○). The dotted line (----) indicates the control activity obtained with 50 µg polymerase CI and 5 µg factor B in the absence of rifampicin

Table 2. *Effect of factor C on initiation and polymerization*
The incubation mixture (0.25 ml) contained the components as described in Table 1. Both 0.15 mM [β, γ-$^{32}P_2$]ATP 200 counts × min^{-1} × pmol^{-1}) and 0.15 mM [^{3}H]UTP (10 counts × min^{-1} × pmol^{-1}) were used in these experiments. In each incubation, 50 µg RNA polymerase CI (QAE Sephadex fraction) and 5 µg factor B were added at zero time. Then 5 µg of crude factor C (dialysed DEAE-cellulose fraction) or purified factor C (eluted from gel after electrophoresis and corresponding to 5 µg crude C) was added at the specified period. Aliquots from the incubation mixture were soaked in Whatman 3-MM filter paper discs and dried. The discs were treated with 10% trichloroacetic acid, washed with 5% acid containing 0.25 M sodium pyrophosphate, ethanol and finally with ether. The discs were then dried and counted

Incubation system	Time of addition of factor C	Total incubation period	[γ,β-$^{32}P_2$]-ATP incorporated	[^{3}H]UTP incorporated
	min	min	pmol/mg	nmol/mg
Polymerase CI + factor B	—	20	10	3.89
Polymerase CI + factor B	—	40	13	4.09
Polymerase CI + B + crude C	0	20	10	2.88
Polymerase CI + B + purified C	0	20	16	6.12
Polymerase CI + B + purified C	20	40	23	8.96

in the incubation mixture are presented in Table 1. This led us to believe that rifampicin binds with RNA polymerase CI probably at the same site where factor B can also bind. This was borne out by the competitive effects of rifampicin and factor B. Thus, the inhibitory effect of 2 µg rifampicin could be completely reversed by a high concentration (40 µg) of factor B. Similarly, 5 µg of factor B first added to the polymerase CI makes the activity resistant to a low concentration (2 µg) of rifampicin, but at high concentration of the drug (20—40 µg), the reaction was completely inhibited (Fig. 7). Also, rifampicin inhibited the formation of the polymerase-CI · factor-B complex (results not presented) as was evidenced from gel electrophoretic studies similar to that presented in Fig. 4.

Role of Factor C

The protein peak eluted from the DEAE-cellulose column at 0.58 M KCl after exhaustive dialysis (fraction C), had an inhibitory activity on RNA synthesis [2]. However, no RNAase or DNAase could be detected in this fraction [1]. Later, it was found that this fraction was heterogeneous and contained material ultraviolet-absorbing at 260 nm. When this material (presumably nucleic-acid) was isolated by deproteinization of fraction C and added to the incubation mixture, there was no inhibition of RNA synthesis (results not presented). The inhibition by the crude fraction C may be due to several reasons. One obvious possibility was that the C fraction had some component which might inhibit initiation of RNA synthesis. Results presented in Table 2 show that fraction C does not inhibit initiation of RNA synthesis as measured by incorporation of [β,γ-$^{32}P_2$]ATP. However, the total synthesis of RNA (as measured by the incorporation of [^{3}H]-UTP) is inhibited. This indicates that C has a component that inhibits RNA synthesis at some point beyond initiation.

Next we attempted to purify the factor C by polyacrylamide gel electrophoresis and the extract of gel slices was assayed for any inhibitory or stimulatory activity during RNA synthesis by polymerase CI and factor B (Fig. 8). Curiously enough, apart from the inhibitory band there is a stimulatory band of protein. Our attention was focussed on this band which stimulates the RNA synthesis by polymerase CI and factor B combination. However, in the absence of factor B, this protein had no stimulatory effect. It occurred to us that C might act for RNA chain termination and release from DNA template so that fresh initiation would be reflected in the stimulation. To test this, we allowed RNA synthesis by RNA polymerase CI and factor B in the presence of a limiting amount of DNA to proceed upto the plateau point when all the DNA is held in the enzyme · DNA · nascent-RNA complex. If at this point, either fraction C eluted from the gel after electrophoresis or 0.2 M KCl was added, a new spurt of RNA synthesis begins (Fig. 9). The reinitiation of RNA chain by the

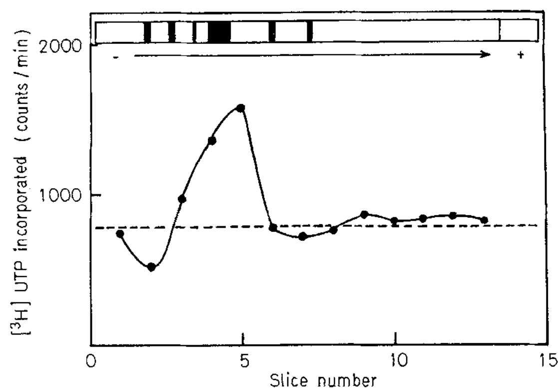

Fig. 8. *Scanning of factor C activity after gel electrophoresis.* 40 µg factor C was subjected to 5% polyacrylamide gel electrophoresis as described in the text. 2-mm gel slices were extracted with Tris-glycerol buffer [1] and each fraction was assayed for activity with 50 µg RNA polymerase CI (QAE-Sephadex fraction) along with 5 µg factor B added at zero minute. The assay mixture was the same as used in Fig. 3. The dotted line (----) indicated the control activity in the absence of factor C

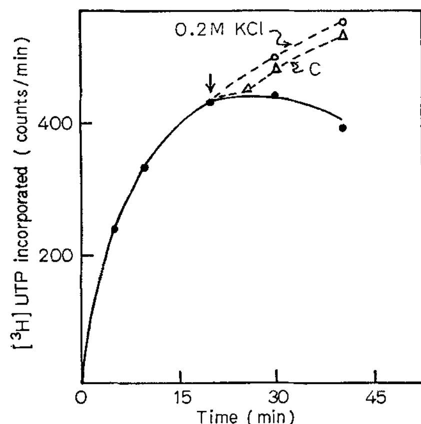

Fig. 9. *Role of factor C on RNA synthesis by RNA polymerase CI.* The ingredients of the experiments were the same as in Table 1. After 20-min incubation of 50 µg RNA polymerase CI (QAE-Sephadex fraction) with 5 µg factor B, 5 µg factor C (fraction C from DEAE-cellulose column exhaustively dialyzed and purified through gel electrophoresis) was added and the reactions were terminated at time intervals (△). One set (○) was exactly maintained as above with 0.2 M KCl minus factor C and (●), the control was without factor C and KCl

factor C was also indicated in Table 2. From the similarity of action of high ionic strength and factor C, the ability of factor C to release the synthesized RNA from the complex is indicated.

Effect of α-Amanitin on Enzymic Activity

Nucleoplasmic RNA polymerase from eukaryotes was reported to be sensitive to α-amanitin [15]. The drug inhibits the RNA synthesis totally even when used in very low concentration (0.4 µg/ml). To test

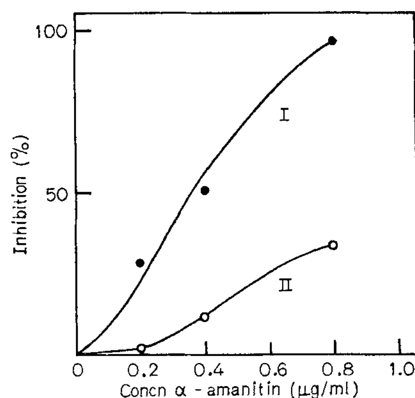

Fig. 10. *Effect of α-amanitin on RNA synthesis.* The conditions of the experiment were the same as in Table 1, except that α-amanitin was added in varying concentration. 50 µg RNA polymerase CI and 5 µg factor B were used in one set (●). 50 µg RNA polymerase CII and 5 µg factor B were used in other set (○)

whether the enzymes isolated by us are sensitive to the drug, we allowed the synthesis of RNA by the two enzymes separately in the presence of different amounts of α-amanitin. It is shown in Fig. 10, that RNA polymerase CI is highly sensitive to α-amanitin whereas RNA polymerase CII is comparatively resistant. α-Amanitin (0.8 µg/ml) completely inhibits the CI enzyme whereas CII is inhibited by 20% only.

Analysis of the RNA Product

The RNA synthesized by RNA polymerase CI alone and by RNA polymerase CI in the presence of factor B and C was then analyzed by electrophoresis on 2.5% polyacrylamide gel. From Fig. 11, it is clear that in the absence of any factor, RNA polymerase CI synthesizes most probably in a non-specific manner, small amounts of RNA of very low molecular weight (4 S and smaller). In the presence of the initiation factor B, the synthesis is considerably stimulated and in this case, RNA of higher molecular weight (10—20 S) is synthesized. However, there is no appreciable change in the size distribution of RNA synthesized by the simultaneous addition of factor B and C. Though, it can not be said from this experiment whether factor B initiates the synthesis of specific types of RNA, at least it helps to synthesize longer RNA molecules. When the same procedure was applied to the product of polymerase CII, 15 to 30 S-RNAs are indicated. That these RNAs are ribosomal and the former ones are non-ribosomal has further been confirmed by hybridization technique. It is seen from the Fig. 12 that 0.5 mg of unlabelled rRNA can not compete with the labelled RNA produced by RNA polymerase CI, whereas this can compete 90% with the labelled RNA produced by CII. This indicates that the CI enzyme can synthesize non-ribosomal and the CII ribosomal RNA.

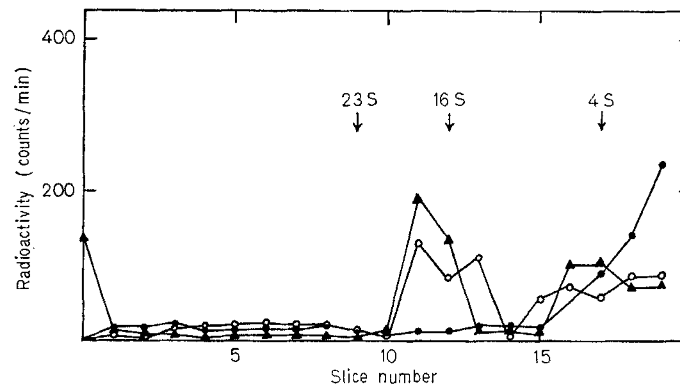

Fig. 11. *Polyacrylamide-gel electrophoresis of RNA synthesized* in vitro. The incubation mixture contained all the component as described in Table 4, except that both [^{14}C]GTP (spec. act. 17 mCi/mmol) and [^{3}H]UTP (spec. act. 2 Ci/mmol) were used to increase incorporated counts. 200 μg RNA polymerase CI, 30 μg factor B and 30 μg purified factor C were used in the appropriate cases: (○) with both factor B and C; (▲) with factor B only; (●) without any added factor. Labelled RNA was isolated from the incubation mixture by hot phenol-dodecylsulfate and precipitated by ethanol in the presence of 500 μg of *E. coli* RNA as carrier. Precipitated RNA was dissolved in 0.3 ml of electrophoresis buffer containing 10% glycerol. Samples containing 8000 acid-insoluble counts/min were run in 2.5% polyacrylamide gel for 1 h at a current of 4 milliamp/tube. Details of the electrophoresis, gel slicing and counting are given under Materials and Methods. Arrows mark the stained *E. coli* RNA bands

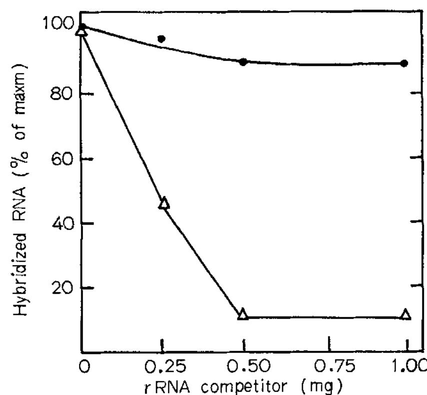

Fig. 12. *Competition for DNA sites, between [^{3}H]RNA synthesized* in vitro *and purified rRNA in the hybridization reaction.* In one set of experiments 20 μg denatured DNA was incubated with 50 μg ^{3}H-labelled RNA produced by polymerase CI and increasing amounts of rRNA. (●). The other set contained the ^{3}H-labelled RNA synthesized by polymerase CII along with other ingredients as before (△). The hybridization procedure was described in Materials and Methods

DISCUSSION

The role of factors on RNA synthesis by *E. coli* RNA polymerase has already been elucidated [16, 17]. The core enzyme binds with the initiation factor σ and then interacts with the promoter site of operon forming an initiation complex. Synthesis of RNA starts from that point, extends through the cistron and finally terminates with the help of ϱ factor [18].

It has also been shown that either σ or core polymerase is changed with the stages of development of phages (T_4) in *E. coli* [19, 20]. Similar phenomenon has been reported in the case of sporulation of *Bacillus subtilis* [21]. Interestingly, a single σ factor is present in the *E. coli* cell suggesting no specificity of σ for a particular gene or promoter site. Very recently, Travers *et al.* [22] has discovered in *E. coli* another factor (ψ_r) which is required in addition to the σ factor for the transcription of ribosomal RNA.

However, nothing was known about initiation or termination factors in eukaryotic cells. The report of a stimulatory factor for RNA polymerase isolated from calf thymus has appeared [23], but the mechanism of stimulation by this factor has not been clarified yet. Our intention was to find whether RNA polymerases and the factors are associated with the chromatin. Since chromatin contains DNA, RNA, histone and non-histone proteins, it is pertinent to ask the question whether the protein parts have any role in RNA synthesis. Histone has now been classified as general repressor for RNA polymerase [24]. This repression can however, be derepressed non-specifically by many substances [25]. Non-histone protein has been found to have some role in derepression [26]. Does non-histone protein contain any RNA polymerase activity? Is it possible that non-histone protein in the chromatin can modulate RNA synthesis in the eukaryotic cell by changing its composition and conformation during the division cycle? Present finding shows that RNA polymerases are present in the non-histone part of the chromatin [1]. Surprisingly it has also been found that both initiation and termination factors are present in the same fraction of chromatin. That the factor B in the present case acts as initiator has been supported from the following evidence. (a) It can form a complex with DNA and RNA polymerase; without RNA polymerase it can not bind with DNA alone. (b) There is no promotive

effect of factor B when λ DNA was used; natural homologous DNA is more effective than DNA from thymus. (c) Rifampicin which can inhibit initiation in the case of *E. coli* RNA polymerase also influences RNA polymerase in the present case if the factor B was added after the drug. When the addition of factor B was followed by rifampicin no inhibition was discernible; but the inhibition caused by rifampicin can be reversed by the addition of factor B in excess (Fig. 7). (d) Factor B promotes the [β,γ-$^{32}P_2$]-ATP incorporation which reaches a plateau quickly whereas the extension of RNA chain continues linearly with an initial lag (Fig. 2); the product of the reaction without factor B indicates the presence of low molecular weight (4S) RNA whereas the same with factor B shows 10 to 20-S RNA (Fig. 10).

Factor C, when purified to a single protein stimulates RNA synthesis by the polymerase CI and factor B combination. However, factor C can not influence the molecular size of RNA synthesized. Our preliminary studies indicated that the composition of the synthesized RNA in the presence of factor C did not differ significantly from that synthesized in its absence. The stimulatory effect of factor C may be explained by the fact that RNA synthesis would not proceed after a certain period if this is not terminated and dissociated from the template. At this point, should factor C be added there would be a spurt in the RNA synthesis. In fact, this is the case with factor C and this seems to be very similar to the effects caused by high ionic strength in the incubation medium. All these taken together suggest that factor C can act as terminator for RNA synthesis in the present case, though it may not be totally comparable to the ϱ factor from *E. coli* [18]. RNA polymerase CI in the present case has been found to be sensitive to α-amanitin. This characteristic seems to be shared with the RNA polymerase II or B from the mammalian sources [15,27—29]. But polymerase CII is less resistant to α-amanitin when compared to RNA polymerase I from mammalian sources. This perhaps suggests that the plant enzyme is at least partly different from that of the animal system as far as the action of α-amanitin is concerned. Alternatively, it might be speculated that the resistance to α-amanitin is conferred by a protein component (factor) and the degree of resistance is dependent on how tightly this factor is bound with the enzyme. Why the elution profile of the two RNA polymerases from the DEAE-cellulose column has been reversed in the present case is a matter for further studies. However, this might be due to dissociation of factor B from the polymerases of the chromatin isolated from the coconut nuclei. In this connection the interconvertibility of the two forms of RNA polymerase from mammalian sources recently described [30] may also be mentioned.

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