Studies on Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase from *Escherichia coli*

VARIATIONS OF THE ENZYME ACTIVITY DURING GROWTH

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(Received 4 May 1972)

1. RNA polymerase activity of *Escherichia coli* extracts prepared from cells in exponential and stationary phases of growth, when measured in the presence and absence of external template, showed significant qualitative differences. 2. In both extracts, polymerase activity was higher when assayed with external template, suggesting the presence of a pool of enzyme not bound to cellular DNA. 3. In the crude extract, the fraction of enzyme bound to cellular DNA is higher during the exponential phase of growth. 4. A method is described for the purification of enzyme molecules not tightly bound to cellular DNA from exponential- and stationary-phase cultures. 5. Purified enzyme preparations showed differences in template requirement and subunit composition. 6. On phosphocellulose chromatography of stationary-phase enzyme, a major portion of polymerase activity eluted from the column with 0.25M-KCl. In the case of exponential-phase enzyme, polymerase activity eluted from a phosphocellulose column mainly with 0.35M-KCl. 7. Enzyme assays done with excess of bacteriophage T₄ DNA showed a strong inhibition of stationary-phase enzyme by this template. The exponential-phase enzyme was only slightly inhibited by excess of bacteriophage T₄ DNA.

It has been demonstrated that RNA polymerase purified from *Escherichia coli* by two different methods is not identical with respect to its ability to transcribe calf thymus DNA and bacteriophage T₄ DNA (Burgess *et al.*, 1969). A difference in template specificity was also shown between purified RNA polymerase from non-infected cultures of *E. coli* and from bacteriophage T₄-infected cultures (Walter *et al.*, 1968).

The experiments described in the present paper show differences in template specificity, subunit structure and other properties of RNA polymerase isolated essentially by the procedure of Chamberlin & Berg (1962), from cells harvested in the stationary and exponential phases of growth. By modification of the original procedure, an RNA polymerase of high specific activity was prepared free of nucleic acids. These results indicate that the physiological state of the enzyme is different in the exponential and stationary phase of growth.

Experimental

Methods

Organism and culture conditions. A mutant of *Escherichia coli* K₁₂ (M.R.E. 600) originally obtained from Dr. S. Brenner, F.R.S., Medical Research Council Laboratory, Cambridge, maintained by

monthly subculture in nutrient agar slopes, was used for these experiments.

Batches of cells (13 litres) were grown at 37°C in a New Brunswick bench fermentor with forced aeration, in a medium containing, per litre: KH₂PO₄, 4.5 g; K₂HPO₄·3H₂O, 15 g; (NH₄)₂SO₄, 2 g; MgSO₄·7H₂O, 0.3 g; glucose, 0.5 g. The inoculum was 25 ml of cells grown in the same medium containing 2.0 g of glucose/l. In the large-scale culture medium containing 0.5 g of glucose/l, growth was limited by exhaustion of glucose. After overnight growth to a limiting population, 4 g of glucose/l was added and cells were harvested in a Sharples continuous-flow centrifuge after four generations of continuous exponential growth ($E_{650} = 1.9$) or in the stationary-phase ($E_{650} = 3.2$). After reaching the maximum extinction value, the stationary-phase cultures were maintained at 37°C with aeration for 1 h before harvesting. E_{650} values did not change appreciably during this period.

Preparation of cell extracts. A buffer containing 10 mM-tris-HCl, pH 7.9 (5°C), 10 mM-MgSO₄, 0.1 mM-neutral EDTA and 1 mM-2-mercaptoethanol was used for preparation of the cell extract and purification of the enzyme.

Frozen cells were suspended in buffer (2 ml/g of frozen bacteria) and disrupted in a French press by using a pressure of 12.8–20.7 MN/m² (2000–3000 lbf/in²). The viscous extract was sonicated for 1 min in

a Branson B-12 model L sonifier at 50W. The insoluble debris was removed by sedimentation at 10000g for 30min. The clear supernatant fraction is referred to below as 'cell extract'.

Protein determinations. Protein concentrations in cell extracts and purified enzyme preparations were estimated by the method of Lowry *et al.* (1951) with salt-free bovine plasma albumin as standard.

Isolation of bacteriophage *T*₄ and *E. coli* DNA. DNA was isolated from purified *T*₄ bacteriophage (Yamamoto *et al.*, 1970) by the phenol-*p*-aminosalicylate method (Kelly, 1967), omitting, however, the ribonuclease treatment. *E. coli* DNA was prepared as described by Kelly (1967).

RNA polymerase assay. The routine assay of RNA polymerase was done as described by Abraham (1968). The reaction mixture contained 50mM-tris-HCl buffer, pH7.8, 10mM-MgCl₂, 160mM-KCl, 5mM-2-mercaptoethanol, 0.16mM each of GTP, UTP, CTP and ATP (with one of specific radioactivity 0.5 μ Ci/ μ mol), DNA and enzyme in a final volume of 0.5ml. The reaction was started by the addition of enzyme. After 10min of incubation at 37°C the reaction was stopped by 2ml of cold 5% (w/v) trichloroacetic acid. The precipitate was collected on a membrane filter (Sartorius MF 50) and washed twice with cold 5% trichloroacetic acid. Thymus DNA-primed assays contained 2mM-MnCl₂ in addition to the usual components. Radioactivity was measured with a Beckman LS-100 scintillation spectrophotometer.

Purification of RNA polymerase. Unless otherwise stated, all centrifugations were done for 30min at 10000g in a Servall RC II B centrifuge. RNA polymerase was isolated by the method of Chamberlin & Berg (1962), with the following modifications. To 400ml of cell extract containing 20mg of protein/ml, was added with stirring 20ml of freshly prepared aq. 10% (w/v) streptomycin sulphate. The pH of the extract was adjusted to 6.9 at this stage with 1M-tris base (usually the pH of cell extract is approx. 6.5). After being stirred for 30min, the solution was sedimented at 30000rev./min in a Spinco 30 rotor for 3h and the clear ribosome-free supernatant was collected.

To 360ml of ribosome-free supernatant containing 12mg of protein/ml was added slowly with stirring 15ml of aq. 1% protamine sulphate. The precipitate was removed by centrifugation and a second 15ml of protamine sulphate solution was added to the clear supernatant. The second protamine sulphate precipitate was suspended in 25ml of buffer containing 0.1M-ammonium sulphate with the help of a Potter-Elvehjem homogenizer. After 45min the suspension was centrifuged for 30min at 20000g. The supernatant (about 28ml) containing RNA polymerase was precipitated with 12ml of saturated ammonium sulphate (adjusted to pH6.7 with NH₃ at room temperature), and centrifuged after 30min. The super-

natant fraction was precipitated once again with 12ml of saturated ammonium sulphate. The second precipitate contained most of the RNA polymerase activity; it was collected and redissolved in 20ml buffer. After removing ammonium sulphate by dialysis against 2 litres of pH7.9 buffer for 2h, the residue was put on a DEAE-cellulose column (20cm $\times$ 1.5cm) which had been equilibrated with pH7.9 buffer the previous night. The column was eluted first with pH7.9 buffer and then successively with buffer containing 0.16M-KCl and 0.22M-KCl. In the early experiments Whatman DE23 was used and enzyme activity appeared both in 0.16M-KCl and 0.22M-KCl eluates. The 0.22M-KCl eluate contained small amounts of materials absorbing at 260nm. In the later experiments using Whatman DE 52, very little enzyme activity appeared in the 0.16M-KCl eluate; most of the RNA polymerase activity was eluted from the column free of nucleic acids by 0.22M-KCl buffer. This fraction usually incorporated 0.15–0.19 μ mol of ATP/mg of protein in a normal assay (Andersen & Abraham, 1970). The yield of pure enzyme from 100g (wet wt.) of bacteria harvested in the exponential and stationary phase was 4mg and 7mg respectively. The yield is rather low compared to the yield of 10–30mg of core enzyme from 100g of bacteria (Burgess, 1969). The low yield of pure enzyme by our method, and also in the original procedure of Chamberlin & Berg (1962), which also was of the order of 7mg of protein/100g wet wt. of bacteria, is most likely caused by removal of DNA during the streptomycin step.

Phosphocellulose chromatography. Phosphocellulose chromatography was done by the method of Burgess (1969). Enzyme was applied to the column in buffer C [0.05M-tris-HCl, pH7.9, 0.1mM-EDTA, 0.1mM-dithiothreitol, 5% (v/v) glycerol] containing 0.15M-KCl and eluted successively with the same buffer containing 0.15M-, 0.25M- and 0.35M-KCl. Only enzyme preparations having an E_{280}/E_{260} ratio over 1.7 were applied to the column. The flow-through fraction (peak A, Fig. 1) had no RNA polymerase activity.

Gel electrophoresis. Polyacrylamide-gel electrophoresis was done on 5% gels at pH7.1 in 0.1M-sodium phosphate buffer containing 0.1% sodium dodecyl sulphate (Shapiro *et al.*, 1967). The gels were run at room temperature for 7h at a current strength of 8mA/gel (0.6cm $\times$ 9.5cm). The proteins were denatured by incubation overnight in electrophoresis buffer containing 1% sodium dodecyl sulphate and 1% 2-mercaptoethanol. The gels were stained overnight in a 0.0125% aq. solution of Coomassie Brilliant Blue (R 250) containing 5.3% (v/v) acetic acid, 40% (v/v) methanol and 2.4% (v/v) glycerol, and destained with 7.5% (v/v) acetic acid. The subunits were identified by comparing their migration with polypeptides of known molecular weight.

Chemicals

Unlabelled ribonucleoside triphosphates, bovine plasma albumin, protamine sulphate (histone-free grade) and calf thymus DNA were purchased from Sigma Chemical Company, St. Louis, Mo., U.S.A. Streptomycin sulphate was obtained from Glaxo Laboratories, Greenford, Middlesex, U.K., and Whatman DEAE-cellulose DE 23, DE 52 and PC11 from H. Reeve Angel and Co. Ltd., London E.C.4, U.K. [$8\text{-}^{14}\text{C}$]ATP, [3H]ATP and [3H]UTP were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Results

RNA-synthesizing ability of cell extracts from exponential- and stationary-phase bacteria

RNA polymerase is found as a DNA-RNA-enzyme complex during RNA synthesis (Kodoya *et al.*, 1964; Bremer & Konrad, 1964). In the absence of active RNA synthesis, the enzyme tends to associate with DNA *in vitro*, although the DNA-enzyme complex is less stable than the complex active in RNA synthesis (Anthony *et al.*, 1966). Addition of external DNA to a crude extract of *E. coli* increased its ability to synthesize RNA. This suggested the presence of a pool of free RNA polymerase not actively engaged in RNA synthesis in these extracts.

The variations in the size of this pool during

growth was measured by comparing the incorporation of UTP into cold trichloroacetic acid-insoluble material in the presence and in the absence of external template. In exponentially growing cells approx. 58% of the total enzyme activity was bound to cellular DNA and was actively synthesizing RNA (Table 1). The remaining 42% became active when supplied with an external template like thymus DNA. This value was calculated on the assumption that the rates of RNA synthesis by the enzyme on cellular DNA and thymus DNA were comparable. The specific activity of the enzyme was higher in crude extracts of exponentially growing cells. In extracts prepared from stationary-phase cultures, approx. 35% of the total enzyme was active in RNA synthesis whereas the remaining 65% became active on addition of thymus DNA to these extracts. When *E. coli* or bacteriophage T₄ DNA served as external template the pool of RNA polymerase present in exponentially growing cells was not stimulated to synthesize RNA. The enzyme pool present in stationary-phase cells was stimulated, when *E. coli* or bacteriophage T₄ DNA served as external template (Table 1). The extent of stimulation however, was lower than that observed with thymus DNA. When equal parts of crude enzyme extracts from stationary and exponentially growing cells were mixed, the percentage of total enzyme activity dependent on external template was intermediate to that observed with either of the crude enzyme extracts alone.

Table 1. RNA-synthesizing ability of cell extracts from exponential- and stationary-phase bacteria

Enzyme assays were done as described in the text with [3H]UTP as labelled substrate.

Source of enzyme	Source of external template ...	UTP incorporated (nmol/min per mg of protein)				Percentage of total activity dependent on external template
		None	Thymus DNA	<i>E. coli</i> DNA	T ₄ DNA	
Crude cell extract from exponential phase of growth		0.59	1.02	0.59	0.57	42
Crude cell extract from stationary phase of growth		0.28	0.80	0.42	0.45	65
Equal parts of crude enzyme extracts from stationary and exponentially growing cells		0.45	0.92	0.50	0.51	51
Purified core enzyme (10 μg) from exponentially growing cells with optimum concentration of purified sigma factor (10 μg)		0.16	157	139	152	99.9

Table 2. *Purification of RNA polymerase from exponential- and stationary-phase cultures*

Enzyme assays were done as described in the text with [^3H]UTP or [^{14}C]ATP as the labelled substrate. RNA of crude cell extracts measured with thymus DNA was taken as 100% activity. In experiments where the streptomycin step was omitted, 25 ml of 1% protamine sulphate solution was used for the first protamine sulphate precipitation.

Source of enzyme	High-speed supernatant	Percentage of total activity recovered	
		Eluate from 2nd. protamine sulphate precipitate	Final stage (DEAE eluate)
Cell extract from exponentially growing culture	40	28	12
Cell extract from stationary-phase culture	67	32	20
Cell extract from exponentially growing culture (without the streptomycin step)	68	26	9
Cell extract from stationary-phase culture (without the streptomycin step)	71	38	20

Purification of RNA polymerase from exponential- and stationary-phase cultures

The basic procedure of Chamberlin & Berg (1962) has been the method of choice used in several laboratories to prepare RNA polymerase of high specific activity free of nucleic acids. The purification of this enzyme from *E. coli* (Chamberlin & Berg, 1962; Richardson, 1966; Abraham, 1968) involves high-speed centrifugation and streptomycin precipitation, steps that remove most of the cellular DNA. The enzyme is obtained in low yield by these procedures because of significant loss of enzyme during the elimination of DNA (Abraham, 1968). A comparison of the enzyme yield from exponential- and stationary-phase cultures at various stages of purification reveals several interesting facts. Only 12% of the total enzyme activity present in the crude extract of exponentially growing cells was recovered in the final enzyme fraction. Purification of enzyme from cells in the stationary phase of growth, having lower enzyme activity (Table 1), yielded 20% of the total enzyme activity in the purified fraction. There was a close correlation between the amount of enzyme lost at the streptomycin and high-speed-centrifugation steps, and the fraction of total enzyme activity bound to cellular DNA (Tables 1 and 2).

Omission of the streptomycin step did not increase the final yield of purified enzyme. More protamine sulphate was necessary to precipitate the enzyme in experiments where the streptomycin step was omitted. Since commercially available protamine sulphate contains significant amounts of salts, the exact quantity of 1% protamine sulphate solution (w/v) required to

precipitate the enzyme had to be determined for each batch of protamine sulphate. Protein determinations performed on the batch of protamine sulphate used in these experiments, by the method of Lowry *et al.* (1951), showed a protein content of 54%. Part of the enzyme lost together with cellular DNA in the streptomycin and first protamine sulphate precipitate could be recovered by extracting these precipitates with buffer containing 0.1 M-ammonium sulphate. The remaining activity could be solubilized with buffer containing 0.3 M-ammonium sulphate. Enzyme solubilized from these precipitates with 0.1 and 0.3 M-ammonium sulphate showed no dependence on external DNA. Our attempts to purify enzyme from these precipitates free of nucleic acids did not succeed. These results indicate that the purified enzyme obtained free of nucleic acids from cells either in the exponential or in the stationary phase represents the enzyme molecules not bound tightly to cellular DNA, whereas the enzyme lost during the elimination of DNA represents DNA-bound enzyme molecules.

Difference in the template specificity of enzyme purified from cells in exponential and stationary phase

Table 3 shows the specific activities of purified exponential- and stationary-phase enzymes. Though the activities of the two preparations with thymus DNA were comparable, with bacteriophage T₄ DNA as template the activity of the exponential-phase enzyme was significantly lower than that of the stationary-phase enzyme. Activity of exponential-phase enzyme with bacteriophage T₄ DNA, however,

Table 3. *Difference in the template specificity of RNA polymerase*

RNA polymerase assays were done as described in the text with 10μg of enzyme and the optimum DNA concentration. Sigma factor when present was 10μg. The sigma factor was isolated as described by Burgess (1969) from purified stationary-phase enzyme.

Source of purified enzyme	Sigma factor	ATP incorporated (nmol/min per mg of protein)	
		Thymus DNA	T ₄ DNA
Exponential-phase cells	+	160	51
	—	158	147
Stationary-phase cells	+	148	149
	—	156	160

could be stimulated twofold by supplementing this enzyme with sigma factor. The stationary-phase enzyme did not show a requirement for sigma factor. In this respect, the purified enzyme preparations reflected the properties of the free enzymes present in the corresponding crude extracts.

Subunit composition and phosphocellulose chromatography pattern of the two enzyme preparations

The exponential- and stationary-phase enzyme behaved differently during phosphocellulose chromatography. Phosphocellulose chromatography of RNA polymerase by the method of Burgess (1969) gives rise to three protein peaks; a flow-through peak (A) consisting mainly of sigma factor; peak B consisting of some core-enzyme subunits and sigma factor; and peak C, the core enzyme. The size of these three peaks varies from one enzyme preparation to another.

Chromatography patterns of exponential- and stationary-phase enzyme eluted from a phosphocellulose column are shown in Fig. 1. A major portion of stationary-phase enzyme was eluted in peak B, whereas the fraction of enzyme activity eluted in peak C was relatively small. The opposite was true for the exponential-phase enzyme. Polyacrylamide-gel electrophoresis of the two enzymes (Fig. 2) revealed one major difference: the sigma band which was predominant in the stationary-phase enzyme was very weak in the exponential-phase enzyme. Polyacrylamide-gel electrophoresis of the three protein peaks from the phosphocellulose column revealed a significant sigma band in the peak B fraction but not in peak C fraction (Fig. 3).

Difference in the response of the two enzyme preparations to increasing concentrations of DNA

During routine assay of RNA-polymerase activity, we observed an inhibition of the enzyme by excess of bacteriophage T₄ DNA. This inhibition was not due

Fig. 1. *Phosphocellulose chromatography of exponential- and stationary-phase enzyme*

Enzyme (9mg) was applied in each case on a phosphocellulose column (0.9cm×12cm), equilibrated with buffer C containing 0.15M-KCl (Burgess, 1969). Fractions (1ml) were eluted from the column, first with buffer C containing 0.15M-KCl (peak A), and subsequently with buffer C containing 0.25M- (peak B) and 0.35M-KCl (peak C). —, Exponential-phase enzyme; ---, stationary-phase enzyme.

to the presence of phenol, ribonuclease or some other impurity in the DNA preparation because the degree of inhibition was dependent on the enzyme/DNA ratio and not on the total amount of DNA. Figs. 4 and 5 show the activity of exponential- and

Fig. 2. Polyacrylamide-gel electrophoresis pattern of RNA polymerase isolated from exponential- and stationary-phase culture

The electrophoresis was done as described in the text. Stationary-phase enzyme (40 μ g) (a) and exponential-phase enzyme (40 μ g) (b) was applied to the gel.

stationary-phase enzymes at different DNA concentrations. The most pronounced inhibition was observed with stationary-phase enzyme (Fig. 5). Addition of sigma factor did not change the DNA optimum of the enzyme. Core enzyme obtained by phosphocellulose chromatography of stationary- or

Fig. 3. Polyacrylamide-gel electrophoresis pattern of the three protein fractions obtained on phosphocellulose chromatography of stationary-phase enzyme

(a), 10 μ g of peak A proteins; (b), 20 μ g of peak B proteins; (c), 20 μ g of peak C proteins.

exponential-phase enzyme was not inhibited by excess of bacteriophage T₄ DNA (Fig. 6).

Discussion

The low rate of RNA synthesis in cell extracts from stationary-phase cultures (Table 1) is in agreement with the finding that the number of growing RNA chains in *E. coli* is dependent upon the growth rate of the cells (Bremer & Yuan, 1968; Manor *et al.*,

Fig. 4. Template dependence of exponential-phase enzyme

Polymerase assays were done as described in the text by using either 10 μg of exponential-phase enzyme alone (○), or 10 μg of exponential-phase enzyme plus 5 μg of peak A fraction from a phosphocellulose column (●).

1969). The existence of a pool of polymerase not actively engaged in RNA synthesis is further supported by the finding of Maaløe & Kjeldgaard (1966) that bacteria synthesizing RNA at low or intermediate rates can be stimulated to increase their rate of RNA synthesis significantly in the absence of protein synthesis. The results given in the present paper show a compartmentalization of the enzyme within the cell into an active fraction tightly bound to cellular DNA, and a pool of enzyme able to synthesize RNA when supplied with a suitable template. Variation in the size of the active enzyme fraction is physiologically more significant than the variation in the total amount of enzyme within the cell (Table 1).

Our method for the purification of RNA polymerase, involving streptomycin precipitation, high-speed centrifugation and two protamine sulphate precipitations, separates the DNA-bound enzyme from the rest of the enzyme and gives a preparation with an E_{280}/E_{260} ratio of 1.8 (Andersen & Abraham, 1970).

The complete inability of the crude enzyme extracts from exponential-phase cells and the decreased ability of stationary-phase cells to synthesize RNA when provided with external templates like *E. coli*

Fig. 5. Template dependence of stationary-phase enzyme

Polymerase assays were done as described in the text by using either 10 μg of stationary-phase enzyme alone (○), or 10 μg of stationary-phase enzyme plus 5 μg of peak A fraction from a phosphocellulose column (Δ), or 10 μg of stationary-phase enzyme plus 10 μg of peak A fraction (□).

or bacteriophage T₄ DNA could be due to the presence of some interfering factors in the crude system. It is also possible that the sigma factor content of purified enzyme is not representative for the crude extract, since the purified enzyme represents less than 20% of the total enzyme activity.

From studies *in vitro* with purified enzyme and DNA, Su *et al.* (1970) concluded that RNA polymerase has greater affinity for bacteriophage T₄ DNA than for *E. coli* DNA. These authors also found that the rate of RNA synthesis is ten times greater with bacteriophage T₄ DNA as compared with *E. coli* DNA. On the basis of these results they suggested that the initial event in the viral control of host mechanisms occurs at the level of RNA transcription where competition occurs between bacteriophage T₄ and *E. coli* DNA for host polymerase. Our results (Table 1) with cell extracts, where the state of enzyme and cellular DNA mimics the situation *in vivo* more closely, do not support this hypothesis.

Fig. 6. Template dependence of core enzyme

Polymerase assays were done as described in the text by using 10 μg of core enzyme (o), or 10 μg of core enzyme plus 10 μg of peak A fraction from a phosphocellulose column (●).

Further, the ability of purified enzyme containing sigma factor to synthesize RNA with equal efficiency by using bacteriophage T₄ and calf thymus DNA speaks against a special affinity of the enzyme for viral DNA.

The dissociation pattern of RNA polymerase on phosphocellulose column chromatography, done by the method of Burgess (1969), varied depending on the enzyme preparations (Fig. 1). DNA-bound enzyme isolated by Babinet's (1967) procedure, when subjected to phosphocellulose chromatography, gave only two protein peaks corresponding to peaks A and C (Hirschbein *et al.*, 1969). Peak C which consists mainly of core enzyme has a subunit composition $\alpha_2\beta'\beta$ (Burgess, 1969). Peak B has been reported to have a subunit composition $\alpha\sigma\beta$ (Bautz *et al.*, 1969). Peak A consists of sigma factor and a few other minor proteins whose function is not known at present. Because most of the stationary-phase enzyme eluted in peak B on phosphocellulose chromatography (Fig. 1), it appeared initially that the stationary-phase enzyme had an abnormal subunit composition (Bautz *et al.*, 1969).

The first polyacrylamide-gel electrophoresis patterns of the stationary-phase enzyme, where we ob-

served a weak β' band compared with β , supported this belief. However, with improved techniques for the denaturation of enzyme samples, it was not possible to detect any difference in the amount of β' and β subunits in the two enzyme preparations (Fig. 2); the α band was very much diffused when the electrophoresis was done on 5% gels. By running enzyme samples on 7.5% gels we were able to obtain a sharp α band. The β and β' subunits did not separate properly under these conditions. The gel-electrophoresis pattern of the three fractions obtained on phosphocellulose chromatography on the enzyme (Fig. 3) indicates that peak B represents undissociated enzyme species. The only difference between peak B and peak C was the absence of sigma factor in the latter. From these data it seems reasonable to assume that the relative sizes of peaks B and C depend on the stoichiometry of the enzyme put on the column (Figs. 1 and 2), enzyme preparations deficient in the sigma subunit being eluted from the column predominantly in peak C. The absence of undissociated enzyme molecules (peak B) on phosphocellulose chromatography of DNA-bound enzyme observed by Hirschbein *et al.* (1969) can be attributed to the low sigma factor content of their preparation, as sigma factor is released from the enzyme-DNA complex after initiation of RNA chains.

Inhibition of RNA polymerase by excess of bacteriophage T₄ DNA has been observed in several laboratories (Abraham, 1968; Walter *et al.*, 1968). Any explanation to account for this template inhibition of the enzyme must take the following facts into consideration. (1) Inhibition is not observed with thymus or *E. coli* DNA. (2) A system consisting of core enzyme, sigma factor and bacteriophage T₄ DNA does not show template inhibition (Fig. 6). (3) Stationary-phase enzyme which elutes mainly in peak B from the phosphocellulose column shows a larger inhibition than exponential-phase enzyme (Figs. 4 and 5). (4) If the inhibition is caused by the presence of some minor protein factors in the enzyme, this factor elutes from the column in peak B. (5) If the inhibition is due to a functional heterogeneity of sigma factors (Travers, 1970), the several sigma factors that inhibit bacteriophage T₄ transcription have a greater affinity for core enzyme, as it appears in peak B on phosphocellulose chromatography.

The presence of a pool of RNA polymerase in the cell at all times, by the co-ordinated synthesis of β' , β and probably α subunits, indicates that the transcription process is not regulated at the level of enzyme synthesis. These findings are in agreement with the reported co-ordinated synthesis of β and β' subunits (Matzura *et al.*, 1971). Our results do not explain the variation in the amount of functionally active enzyme molecules during growth. But it seems highly improbable that the mere presence of sigma or sigma-

like proteins alone are involved in the regulation of gene activity in bacteria.

The authors thank Professor R. M. Dowben for helpful advice during preparation of the manuscript and Miss Sidsel Brakstad for excellent technical assistance during part of this work.

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