Factor Stimulating Transcription by RNA Polymerase

by
RICHARD R. BURGESS
ANDREW A. TRAVERS
Biological Laboratories,
Harvard University
JOHN J. DUNN
EKKEHARD K. F. BAUTZ
Institute of Microbiology,
Rutgers University

A protein component usually associated with RNA polymerase can be separated from the enzyme by chromatography on phosphocellulose. The polymerase is unable to transcribe T4 DNA unless this factor is added back.

IN *E. coli* the synthesis of all types of cellular RNA is thought to be mediated by a single enzyme, DNA-dependent RNA polymerase. The highly purified enzyme[1-6] can catalyse the synthesis of RNA *in vitro* in the presence of DNA and the ribonucleoside triphosphates. When an intact double helical DNA is used as template, transcription *in vitro* is asymmetric—only one of the complementary DNA strands is transcribed[7-10]. This is also characteristic of transcription *in vivo*[11-13]. Moreover, the selective transcription *in vitro* of certain regions of T4 and λ DNA[10,14-17], coupled with studies on the binding of RNA polymerase to DNA[18-22], suggests that the polymerase initiates RNA synthesis at specific sites on the DNA. The state of aggregation of the enzyme is strongly influenced by ionic strength[19,21,23,24], substrate[25], and possibly by enzyme concentration and temperature. Most investigators agree that the molecular weight of the active enzyme is in the range of 350,000–700,000 daltons. This large size is consistent with the observation that the enzyme is composed of several different polypeptide chains[26,27]. Furthermore, it has been assumed that "highly purified" RNA polymerase is a protein entity from which nothing can be further removed without destroying its enzyme activity. We report here, however, the separation of polymerase into two components. One contains enzyme activity, but its ability to transcribe certain DNA templates is greatly reduced; the other is a factor able to stimulate RNA synthesis on these restrictive templates to normal levels.

It was independently observed at Harvard and Rutgers that when RNA polymerase was purified by chromatography on a phosphocellulose column, the enzyme obtained, although able to transcribe calf thymus DNA almost normally, was much less active when assayed with T4 DNA as template. Enzyme purified by an alternative procedure, however, was almost equally active on both templates. This suggested that some component necessary for the transcription of T4 DNA was separated from RNA polymerase by the phosphocellulose column. Furthermore, the activity of the phosphocellulose-purified enzyme on T4 DNA could be greatly enhanced by the addition of another fraction from the phosphocellulose column. This fraction lacked significant RNA polymerase activity of its own. We describe here the identification and some properties of this stimulating component.

Isolation of RNA Polymerase

The RNA polymerase we used was purified as outlined in Fig. 1 from *E. coli* K12. Three methods were used to achieve the final purification of the polymerase. Enzyme purified with phosphocellulose (PC enzyme) and with glycerol gradient (GG enzyme) was prepared by the method of Burgess (manuscript in preparation). Using

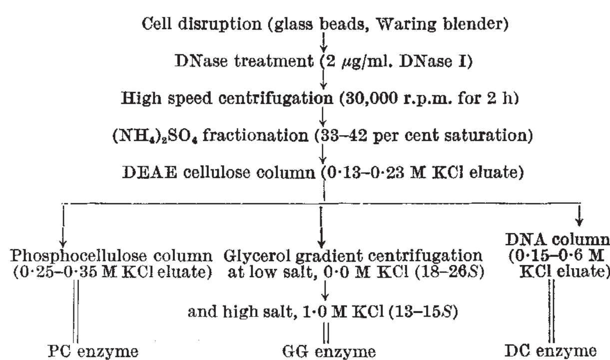

Fig. 1. Outline of enzyme purification.

the same method until the DEAE cellulose step, he purified the enzyme by binding it to a cellulose column containing immobilized T4 DNA and eluting the bound enzyme with high salt[28]. DNA column enzyme (DC enzyme) obtained in this way and GG enzyme are essentially identical and do not differ significantly from enzymes purified by the method of Chamberlin and Berg[1]. Unless otherwise stated, the work described here was carried out with GG enzyme, but similar results were obtained with DC enzyme.

Identification of the Component Stimulating Transcription of T4 DNA

To isolate the component necessary for the transcription of T4 DNA, GG enzyme was chromatographed on a

Table 1. ASSAY OF STIMULATING ACTIVITY OF PEAKS *A*, *B* AND *C*

Sample assayed for stimulation	μμmole AMP incorporated/min No PC enzyme added	4 μg PC enzyme added	Stimulation ratio
PC enzyme (4·0)	3	9	2
GG enzyme (1·1)	57	170	38
Peak *A* (1·0)	13	114	34
Peak *B* (1·3)	66	203	46
Peak *C* (1·9)	2	8	2

The assay mixture (0·25 ml.) contained 0·04 M *tris*-HCl buffer, pH 7·9, at 25° C, 0·01 M $MgCl_2$, 0·0001 M EDTA, 0·0058 M 2-mercaptoethanol, 0·15 M KCl, 0·5 mg/ml. bovine serum albumin (Calbiochem, crystalline), 0·15 mM CTP, GTP and UTP, 0·15 mM ^{14}C-ATP (1 mCi/mmole), 20 μg/ml. T4 phage DNA, and varying amounts of enzyme and stimulating factor. The mixture was incubated for 10 min at 37° C, chilled, precipitated with 5 per cent TCA, and filtered on 'Millipore' filters. Samples were counted on an end-window gas-flow counter. The amount of incorporation is expressed as μμmoles of AMP incorporated per min of incubation. To obtain a measure of the stimulating activity in a particular sample, that sample was assayed in the absence and presence of PC enzyme. The additional incorporation in the presence of PC enzyme was due to the stimulation of this added PC enzyme by the factor in the sample. When this additional incorporation is divided by the small amount of incorporation obtained with PC enzyme alone, the stimulation ratio is obtained. The values in parentheses indicate the amount of protein, in μg, added to the assay mixture.

©1969 Nature Publishing Group

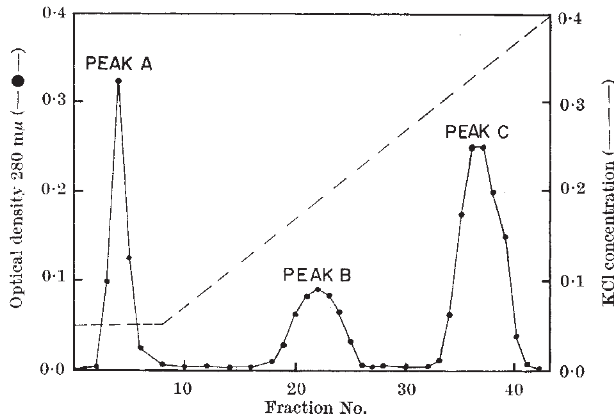

Fig. 2. Phosphocellulose column profile. Phosphocellulose (Whatman P11, 7·4 mequiv./g) was washed with base and acid and titrated to pH 7·9 at 25° C with KOH. This material was placed in a column (0·9 × 10 cm) and equilibrated extensively with a buffer containing 0·05 M *tris*-HCl, pH 7·9, at 25° C, 0·05 M KCl, 0·0001 M EDTA, 0·0001 M dithiothreitol and 5 per cent glycerol. The pH of the outflow was 8·1 ± 0·05 at 4° C. A sample containing 9 mg of GG enzyme in 4 ml. of this buffer was applied to the column. The column was then washed with 5 ml. of buffer and eluted with a 100 ml. gradient from 0·05 M to 0·4 M KCl. The flow rate was 0·5 ml./min and 2·2 ml. fractions were collected. Tubes 4, 22 and 36 were taken as representative of peaks *A*, *B* and *C* respectively. Assuming a value of $E^{1\%}_{280\text{m}} = 6{\cdot}6$ for all fractions, these tubes contain 0·5, 0·14 and 0·4 mg of protein per ml. respectively. A small amount of polymerase activity was present in the flow-through (peak *A*). If the sample is applied to the column too rapidly (at a rate greater than one column volume per 30 min), then much more enzyme will be found in the flow-through.

phosphocellulose column. This column separated the material present in GG enzyme into three peaks, *A*, *B* and *C* (Fig. 2). Each peak was assayed for ability to promote the transcription of T4 DNA in the presence and absence of PC enzyme. Peak *A* contains stimulating activity, peak *B* is a mixture of PC enzyme and stimulating activity, while peak *C* is identical to normal PC enzyme. The results (Table 1) show that, in the absence of PC enzyme, peak *B* can use T4 DNA as a template for RNA synthesis while peaks *A* and *C* have little activity. The addition of peak *A* material to PC enzyme, however, results in a stimulation of RNA synthesis which increases with increasing amounts of peak *A*, until a rate of RNA synthesis comparable with that obtained with GG enzyme is reached. Although peak *B* is able to stimulate PC enzyme, it can itself be stimulated by the addition of peak *A*, and thus is a mixture of PC enzyme and stimulating component.

Each of the fractions was analysed by polyacrylamide gel electrophoresis in various conditions to determine the species of protein present. These gels were run in the presence of either 8 M urea or 0·1 per cent sodium dodecyl sulphate (SDS), both of which can cause the dissociation of oligomeric proteins into single polypeptide chains. The protein bands observed in this analysis are shown in Fig. 3.

Previous studies[27] have shown that phosphocellulose enzyme is composed of two chief types of polypeptide chains, which we here designate α and β. These chains are present in equimolar amounts and have molecular weights of ~40,000 and ~160,000 respectively. α and β are present in all fractions but only in very small amounts in peak *A*. GG enzyme contains, in addition, two extra bands which we shall designate σ and τ. These bands are present in peak *A* in amounts greatly in excess of the amounts of α and β present. Peak *B* contains bands α, β and σ, but lacks τ. These patterns are observed on both the 8 M urea gels and the 0·1 per cent SDS gels. These data are consistent with band σ being the component responsible for the stimulating activity, but they do not exclude the possibility that band τ or even some other material might also stimulate.

The stimulating component was identified as σ by the zone sedimentation of peak *A* material on a glycerol density gradient (Fig. 4). The factor required for T4 transcription was found to sediment at ~5*S*. Analysis of the gradient fractions by electrophoresis on 8 M urea gels showed that band σ sedimented identically to the stimulating activity whereas band τ sedimented at about 8*S* in a region with no stimulating activity.

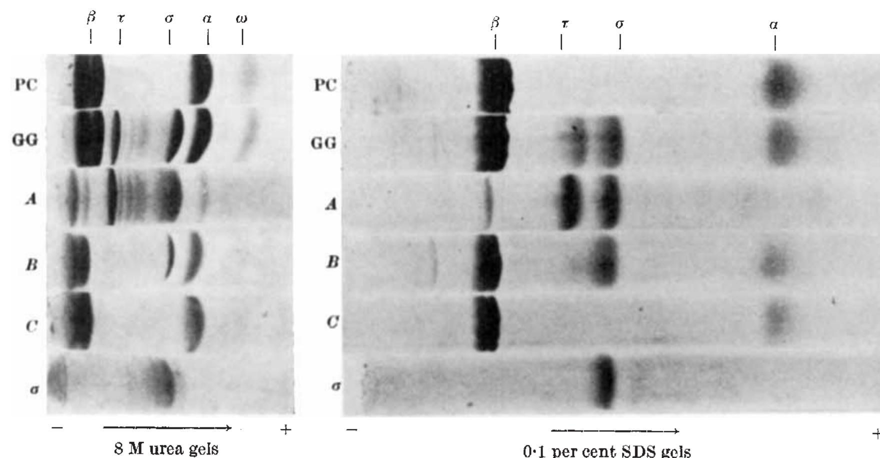

Fig. 3. Polyacrylamide gel electrophoresis patterns of different preparations of RNA polymerase and of purified factor. From top to bottom: PC enzyme (20 μg), GG enzyme (20 μg), peak *A* (10 μg), peak *B* (7 μg), peak *C* (8 μg) and purified factor (2 μg) from tube 17 of the glycerol gradient shown in Fig. 4. 8 M urea gels were prepared and run according to the general method described by Davis[34]. These gels contained 7·5 per cent acrylamide. 0·1 per cent SDS gels were run according to the procedure of Shapiro *et al.*[35] and contain 5 per cent acrylamide. In both cases the gels were stained by immersing them in a 0·25 per cent solution of Coomassie brilliant blue in methanol : acetic acid : water (5 : 1 : 5 v/v/v) for at least 2 h. The gels were then soaked in 7·5 per cent acetic acid, 5 per cent methanol for 0·5 h and finally destained electrophoretically in this same solvent. The bands observed are designated β, τ, σ, α and ω; α, β and ω are the bands normally seen in PC enzyme. The molecular weights of α and β are ~40,000 and ~160,000 respectively. The molecular weight of β was previously reported to be 110,000 (ref. 27), but recent measurements (Burgess, in preparation) indicate that 160,000 is a more accurate value. The band β as seen on 0·1 per cent SDS gels appears as two closely spaced bands of equal intensity which probably correspond to two different polypeptide chains, with molecular weights of about 155,000 and 165,000. For simplicity these are both called β in the text. In addition, a small polypeptide, ω, with a molecular weight of about 10,000 can be seen moving ahead of α on the 8 M urea gels. It is present in GG enzyme, PC enzyme and DC enzyme but it is not yet known whether it is a component of RNA polymerase or merely a tightly binding impurity. The amount of band τ observed in GG enzyme and also in peak *A* is variable. DC enzyme (not shown) contains only traces of τ and thus it is probably an impurity. From the SDS gels it is estimated that 45 per cent of peak *A* protein is σ. Purified factor is estimated to be about 80 per cent pure and is completely free of τ. The right hand two-thirds of the urea gels which contain no bands are not shown.

©1969 Nature Publishing Group

Table 2. HEAT INACTIVATION OF THE STIMULATING FACTOR

Treatment of factor before assay	μμmole AMP incorporated/min	Per cent inactivation of stimulating activity
5 min at 0° C	294	0
5 min at 37° C	295	0
5 min at 45° C	154	52
5 min at 50° C	31	98

Peak *A* protein (0·9 μg) was added to each assay tube which also contained assay solution lacking the four triphosphates and T4 DNA. Separate tubes were incubated at the indicated temperatures for 5 min and then chilled to 0° C. The triphosphates, T4 DNA, and PC enzyme (6 μg) were added and the assay performed as described in the legend to Table 1. With no added factor, PC enzyme incorporated 17 μμmoles of AMP. Factor alone incorporated 9 μμmoles of AMP. The percentage inactivation at a given temperature was calculated by subtracting 26 μμmoles from the total incorporation observed after treatment at that temperature and then setting the incorporation of the unheated sample equal to 100 per cent activity.

Stimulating Factor is a Protein

The association of the stimulating activity with a specific band on polyacrylamide gels suggests that the factor is a protein. To confirm this, we determined the heat stability of the stimulating activity (Table 2). Incubation of peak *A* material for 5 min at 45° C and 50° C resulted in an inactivation of the stimulating activity of 52 per cent and 98 per cent respectively. Furthermore, the activity was sensitive to trypsin. We conclude therefore that the factor is a heat labile protein, although it is still possible that the factor is associated with a nucleic acid component of low molecular weight.

The molecular weight of a protein can be estimated from its mobility on 0·1 per cent SDS polyacrylamide gels[29]. Using this procedure with β-galactosidase, bovine serum albumin and ovalbumin, with molecular weights of 130,000, 67,000 and 45,000 respectively, as molecular weight markers, the molecular weight of σ was calculated to be about 95,000 ± 5,000. This is consistent with the *S* value of about 5 obtained from the glycerol gradient.

Factor Requirements on Various DNA Templates

RNA synthesis in the presence and absence of factor was measured for several different DNA templates (Table 3). The greatest stimulation was observed with native T4 DNA, where the presence of factor increased the amount of synthesis seventy-five-fold. With all other templates tested the stimulation was considerably lower. This variation may be ascribed to two factors. First, the fully stimulated levels of RNA synthesis vary according to the template used: this has been observed by several investigators[1-3]. Second, in the absence of factor, different DNA templates direct the synthesis of differing amounts of RNA. From analytical gels we estimate that PC enzyme contains less than 2 per cent as much factor as GG enzyme. Thus it is possible that the very small amount of RNA synthesis off T4 DNA with PC enzyme is due to traces of remaining factor. With calf thymus DNA, however, this could not easily explain the results. A more likely possibility is that there are some sites at which initiation can occur in the absence of factor. It is clear that such sites are not merely fully denatured regions, for denaturation of T4 DNA does not result in increased synthesis with PC enzyme. Furthermore, φX-174 DNA is also a poor template. The behaviour of PC enzyme on

Table 3. STIMULATION ON VARIOUS DNA TEMPLATES

DNA template	PC enzyme	PC enzyme + factor	GG enzyme
	mμmoles AMP incorporated/min/mg enzyme		
T4—native	0·5	33·0	37·5
T4—denatured	0·5	6·1	3·0
Calf thymus—native	14·2	32·8	30·5
Calf thymus—denatured	3·3	14·5	10·7
φX-174	0·9	6·2	4·9

RNA synthesis in the presence and absence of factor was assayed as described in the legend to Table 1, except that the DNA concentration was 10 μg/ml. DNA was denatured by adding 1/10 volume of 2 N NaOH to a 20 μg/ml. DNA solution. After standing at 25° C for 10 min, the solution was neutralized. Almost identical results were obtained if the DNA was denatured by heating at 95° C for 10 min, and then rapidly chilling in ice. The concentrations of PC enzyme and GG enzyme in the reaction mixture were both 4 μg/ml. Peak *A* protein was present, where indicated, at a concentration of 4 μg/ml. This corresponds to 2 μg/ml. of factor. The ratio of factor to enzyme in the mixture of PC enzyme and factor was about twice that normally occurring in GG enzyme. Incorporation by factor alone was negligible for all types of DNA tested. In all cases saturating amounts of DNA were used. The φX-174 phage DNA was a gift from Dr D. T. Denhardt.

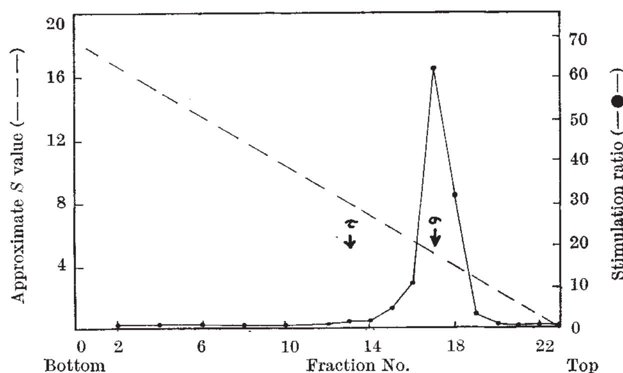

Fig. 4. Analysis of peak *A* by zone centrifugation. A 0·2 ml. sample containing 100 μg of peak *A* protein was layered on a 4·8 ml. linear 10–30 per cent glycerol density gradient containing 0·01 M *tris*-HCl buffer, *p*H 7·9, 0·01 M $MgCl_2$, 0·15 M KCl, 0·0001 M EDTA, and 0·0001 M dithiothreitol. The gradient was centrifuged for 11 h at 60,000 r.p.m. in a Spinco *SW*65 rotor at 4° C. 0·22 ml. fractions were collected. Each fraction was assayed for stimulating activity as described in the legend to Table 1. The proteins present in each fraction were analysed by electrophoresis on 8 M urea polyacrylamide gels. Band σ peaked in tube 17, band τ in tube 13. Molecular weight markers (*E. coli* β-galactosidase, human gamma-globulin, and egg lysozyme with sedimentation coefficients of 16*S*, 7*S* and 1·9*S*, respectively) were centrifuged on a parallel gradient. The sedimentation coefficients of σ and τ were estimated to be about 5*S* and 8*S*, respectively.

T4 DNA and calf thymus DNA is remarkably similar to that of the RNA polymerase isolated from T4 infected cells by Walter *et al.*[30].

Factor is not a Nucleus

It could be argued that σ is a type of nuclease which makes single-stranded breaks in the DNA, thus "activating" the DNA. One prediction of this hypothesis is that DNA which has been used as a template for transcription in the presence of both enzyme and factor should then be capable of supporting the initiation of RNA chains in the absence of factor. This is not the case. The experiment shown in Table 4 shows that DNA which has been used as a template for factor-stimulated transcription exhibits virtually the same unstimulated and stimulated levels of transcription on re-use as DNA which has not previously been incubated with factor. In addition, evidence obtained by several investigators[19-21,31] argues that DNA used for transcription by polymerase purified by the methods of Chamberlin[1] and Furth[2] remains intact. Because enzyme prepared in this way contains σ, it seems unlikely that the stimulation observed is due to nuclease action.

Formation of a Factor–Polymerase Complex

Several lines of evidence suggest that the factor can exist in a complex with RNA polymerase. First, the factor purifies with polymerase through steps involving protamine sulphate precipitation, ammonium sulphate fractionation and DEAE–cellulose chromatography. Furthermore, it remains with polymerase during low and high salt glycerol gradient centrifugation ($\Gamma/2 = 0{\cdot}04$ and 1·0, respectively) where the polymerase sediments at 24*S* and 14*S*. The free factor sediments at about 5*S*, so it must be tightly bound to polymerase in all these conditions. Second, from the molecular weights of α, β and σ, and from the intensity of their stained bands on polyacrylamide gels, it is possible to make an estimate of the relative amounts of each band present in GG enzyme and also in peak *B*. In both, such an estimate yields a very approximate molar ratio of α : β : σ of 2 : 2 : 1. Third, complex formation between PC enzyme and factor can be demonstrated by running standard *p*H 8·7 polyacrylamide gels in the absence of dissociating agents (Fig. 5). In these conditions PC enzyme is resolved into several bands, which probably represent aggregates. Peak *B*, which contains the complex of α, β and σ, migrates as a single band which moves ahead of the PC enzyme bands. Purified σ moves

©1969 Nature Publishing Group

Table 4. EFFECT OF PREINCUBATION WITH FACTOR AND PC ENZYME ON THE ABILITY OF T4 DNA TO DIRECT FACTOR-DEPENDENT TRANSCRIPTION

Material present during preincubation of DNA	μμmoles AMP incorporated/min PC enzyme alone	PC enzyme + factor
No additions	8	264
PC enzyme (10 μg)	10	282
Factor (1·5 μg)	17	238
PC enzyme (10 μg) + factor (1·5 μg)	15	259
No preincubation	12	259

Four reaction mixtures (0·25 ml. each) for RNA synthesis were set up as described in the legend to Table 1, with the modification that BSA was omitted and non-radioactive ATP replaced ^{14}C-ATP. PC enzyme and factor were added as indicated. Each reaction mixture was incubated for 10 min at 37° C. They were then diluted to 1 ml. with distilled water and extracted with 1 ml. of water-saturated phenol. The DNA was precipitated from the aqueous phase by the addition of 2 ml. ethanol. The precipitates were washed twice with 2 ml. of ethanol, collected by centrifugation and dried *in vacuo* to remove all traces of ethanol. Finally the DNA samples were re-dissolved in 0·2 ml. 0·01 M *tris* buffer *p*H 7·9. The ability of this DNA to direct RNA synthesis by PC enzyme in the presence and absence of factor was then assayed as described in the legend to Table 1 with the modification that the reaction volume was 0·10 ml. and BSA was omitted. Each tube contained 4 μg of PC enzyme. 0·6 μg factor (1·3 μg of peak *A* material) was also added where indicated.

faster than the complex. If σ and PC enzyme are mixed in approximately equivalent amounts and then subjected to gel electrophoresis, a single band corresponding to the complex is seen.

Even though PC enzyme and σ form a complex, for example as in peak *B*, the addition of either factor or PC enzyme to this complex results in stimulation. We are investigating whether the enzyme and factor are in rapid equilibrium with complex or whether factor is released for re-use in other complexes by the act of initiation.

Enzyme and complex elute at different ionic strengths from a phosphocellulose column, so it seems possible that they would elute from a DNA cellulose column at different ionic strengths. We found, however, that they behave identically on such a column. This provides some indication that the factor does not function merely by increasing the affinity of the polymerase for DNA. Furthermore, the free factor was not retained by the DNA cellulose column at 0·1 M KCl. This suggests that the free factor does not bind to DNA in conditions where the basic enzyme and the complex do so.

The Function of σ

The results clearly show that PC enzyme can by itself initiate the synthesis of RNA chains, and can catalyse chain elongation. Thus it is possible that this enzyme is the fundamental RNA polymerase. The presence of the stimulating factor, σ, greatly enhances the amount of RNA synthesis, the degree of enhancement being dependent on the DNA template used. Several possible modes of action of σ can be proposed. It could stimulate initiation, increase the rate of polymerization or prevent unusually early cessation of chain growth. Preliminary evidence (Travers and Burgess, manuscript in preparation) indicates that σ markedly increases the number of

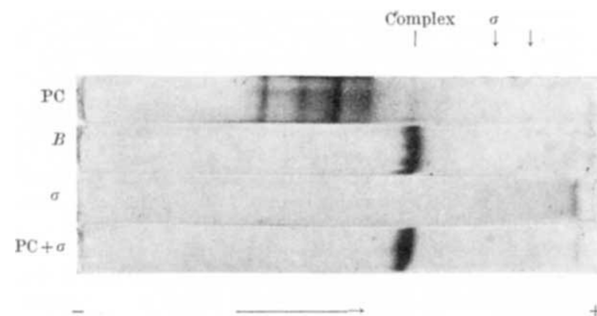

Fig. 5. Reconstitution of the enzyme–factor complex as demonstrated by electrophoresis on polyacrylamide gels. From top to bottom: PC enzyme (4 μg), peak *B* (4 μg), purified factor (1 μg) from tube 17 of the glycerol gradient shown in Fig. 4, and a mixture of PC enzyme and purified factor. Polyacrylamide gels, *p*H 8·7, 4 per cent acrylamide, were prepared and run as described by Davis[34], and stained as described in the legend to Fig. 3. σ appears as two very faint bands which move about 20 and 30 per cent faster than the complex and are indicated by arrows. The marker dye, seen very near the right end of the third gel from the top, just ran off of the other three gels.

RNA chains initiated. This suggests that σ acts at the level of initiation.

We can thus pose the question: is the additional initiation observed in the presence of σ merely due to an increase in the rate of initiation at sites poorly utilized in its absence, or does this initiation occur at sites which absolutely require σ for their expression? The first possibility implies that the PC enzyme determines the specificity of initiation and that σ may have some other function in the process of initiation. If σ itself determines the specificity of initiation, however, the interesting possibility arises that several similar factors could exist, each with a specificity for a different type of initiation site. This latter idea is attractive, for recent studies using the antibiotic rifamycin suggest that *in vivo* only one kind of RNA polymerase exists[32]. Yet there is also much evidence to indicate that *in vivo* the control of *m*RNA, *t*RNA and *r*RNA synthesis is not coordinate[33]. σ and similar factors could then act as positive control elements regulating the amount of synthesis of different classes of RNA, including the late RNA of certain bacteriophages.

We thank Professor J. D. Watson for his interest and support, Professor K. Weber, Mr J. Roberts and Dr J. Tkacz for helpful discussions and suggestions, and Miss Anne-Marie Piret and Mrs Christine Roberts for technical assistance. This work was supported by grants from the US National Science Foundation and the US Public Health Service. One of us (A. A. T.) is a postdoctoral fellow of the Damon Runyon Memorial Fund for Cancer Research, and R. R. B. is a National Science Foundation predoctoral fellow.

Received December 2, 1968.

1 Chamberlin, M., and Berg, P., *Proc. US Nat. Acad. Sci.*, **48**, 81 (1962).
2 Furth, J. J., Hurwitz, J., and Anders, M., *J. Biol. Chem.*, **237**, 2611 (1962).
3 Stevens, A., and Henry, J., *J. Biol. Chem.*, **239**, 196 (1964).
4 Fuchs, E., Zillig, W., Hofschneider, P. H., and Preuss, D., *J. Mol. Biol.*, **10**, 546 (1964).
5 Richardson, J. P., *Proc. US Nat. Acad. Sci.*, **55**, 1616 (1966).
6 Babinet, C., *Biochem. Biophys. Res. Commun.*, **26**, 639 (1967).
7 Hayashi, M., Hayashi, M. N., and Spiegelman, S., *Proc. US Nat. Acad. Sci.*, **51**, 351 (1964).
8 Geiduschek, E. P., Tocchini-Valentini, G. P., and Sarnat, M., *Proc. US Nat. Acad. Sci.*, **52**, 486 (1964).
9 Green, M., *Proc. US Nat. Acad. Sci.*, **52**, 1388 (1964).
10 Luria, S. E., *Biochem. Biophys. Res. Commun.*, **18**, 735 (1965).
11 Hayashi, M., Hayashi, M. N., and Spiegelman, S., *Proc. US Nat. Acad. Sci.*, **50**, 664 (1963).
12 Tocchini-Valentini, G. P., Stodolsky, M., Sarnat, M., Aurisicchio, A. Graziosi, F., Weiss, S. B., and Geiduschek, E. P., *Proc. US Nat. Acad. Sci.*, **50**, 935 (1963).
13 Marmur, J., and Greenspan, C. M., *Science*, **142**, 387 (1963).
14 Khesin, R. B., Shemyakin, M. F., Gorlenko, Zh. M., Bogdanova, S. L., and Afanaseva, T. P., *Biokhimiya*, **27**, 1092 (1962).
15 Geiduschek, E. P., Snyder, L., Colvill, A. J. E., and Sarnat, M., *J. Mol. Biol.*, **19**, 541 (1966).
16 Naono, S., and Gros, F., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 363 (1966).
17 Cohen, S. N., Maitra, U., and Hurwitz, J., *J. Mol. Biol.*, **26**, 19 (1967).
18 Crawford, L. V., Crawford, E. M., Richardson, J. P., and Slayter, H. S., *J. Mol. Biol.*, **14**, 593 (1965).
19 Richardson, J. P., *J. Mol. Biol.*, **21**, 83 (1966).
20 Jones, O. W., and Berg, P., *J. Mol. Biol.*, **22**, 199 (1966).
21 Pettijohn, D., and Kamiya, T., *J. Mol. Biol.*, **29**, 275 (1967).
22 Sentenac, A., Ruet, A., and Fromageot, P., *Europ. J. Biochem.*, **5**, 385 (1968).
23 Priess, H., and Zillig, W., *Biochim. Biophys. Acta*, **140**, 540 (1967).
24 Stevens, A., Emery, jun., A. J., and Sternberger, N., *Biochem. Biophys. Res. Commun.*, **24**, 929 (1966).
25 Smith, D. A., Martinez, A. M., Ratliff, R. L., Williams, D. L., and Hayes, F. N., *Biochemistry*, **6**, 3057 (1967).
26 Zillig, W., Fuchs, E., and Millette, R., in *Proc. Nucleic Acid Res.* (edit. by Cantoni, G. L., and Davies, D. R.), 326 (Academic Press, New York, 1966).
27 Burgess, R. R., *Fed. Proc.*, **27**, 295 (1968).
28 Alberts, B. M., Amodio, F. J., Jenkins, M., Gutman, E. D., and Ferris, F. L., *Cold Spring Harbor Symp. Quant. Biol.*, **33** (in the press).
29 Shapiro, A., Vinuela, E., and Maizel, J. V., *Biochem. Biophys. Res. Commun.*, **28**, 815 (1967).
30 Walter, G., Seifert, W., and Zillig, W., *Biochem. Biophys. Res. Commun.*, **30**, 240 (1968).
31 Maitra, U., and Hurwitz, J., *J. Biol. Chem.*, **242**, 4897 (1967).
32 Tocchini-Valentini, G. P., Marino, P., and Colvill, A. J., *Nature*, **220**, 275 (1968).
33 Maaløe, O., and Kjeldgaard, N. O., *Control of Macromolecular Synthesis* (Benjamin, New York, 1966).
34 Davis, B. J., *Ann. NY Acad. Sci.*, **121**, 406 (1964).

©1969 Nature Publishing Group