[38] Lehman, I. R., G. G. Roussos, and A. Pratt, *J. Biol. Chem.*, in press.
[39] Lowry, O., J. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[40] Monod, J., *Ann. Inst. Pasteur*, **79**, 390 (1950).
[41] Wiesmeyer, H., and M. Cohn, *Biochim. Biophys. Acta*, **39**, 417 (1960).
[42] Lineweaver, H., and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).
[43] Doty, P., these PROCEEDINGS, **42**, 791 (1956).
[44] Markham, R. and J. P. Smith, *Biochem. J.*, **52**, 552 (1952).
[45] Magasanik, B., E. Vischer, R. Doniger, D. Elson, and E. Chargaff, *J. Biol. Chem.*, **186**, 37 (1950).
[46] Weiss, S. B., and T. Nakamoto, these PROCEEDINGS, **47**, 1400 (1961).
[47] Geiduschek, E. P., T. Nakamoto, and S. B. Weiss, these PROCEEDINGS, **47**, 1405 (1961).
[48] Hall, B. D., and S. Spiegelman, these PROCEEDINGS, **47**, 137 (1961).
[49] Sinsheimer, R. L., *J. Mol. Biol.*, **1**, 43 (1959).
[50] Rich, A., these PROCEEDINGS, **46**, 1044 (1960).
[51] Schildkraut, C. L., J. Marmur, J. R. Fresco, and P. Doty, *J. Biol. Chem.*, **236**, PC 2 (1961).
[52] Wyatt, G. R., and S. S. Cohen, *Biochem. J.*, **55**, 774 (1953).

---

*THE EFFECT OF ENZYMATICALLY SYNTHESIZED RIBONUCLEIC ACID ON AMINO ACID INCORPORATION BY A SOLUBLE PROTEIN–RIBOSOME SYSTEM FROM ESCHERICHIA COLI**

WILLIAM B. WOOD† AND PAUL BERG

DEPARTMENT OF BIOCHEMISTRY, STANFORD UNIVERSITY SCHOOL OF MEDICINE

Communicated by Arthur Kornberg, November 17, 1961

The enzymatic synthesis of RNA‡ by a mechanism requiring all four of the commonly occurring ribonucleoside triphosphates and DNA is now well established.[1–6] The DNA used to prime the reaction appears to dictate the base composition,[6–9] the nearest-neighbor frequency pattern,[9] and possibly the base sequence[10] of the RNA synthesized. It seems reasonable to postulate that this enzymatic mechanism accounts for the occurrence in normally growing microorganisms[11–13] and phage-infected bacteria[14–17] of a rapidly turning-over RNA fraction with a base composition closely resembling that of the cellular DNA or the infecting phage DNA, respectively.

The characteristics of this unique RNA fraction have prompted the hypothesis that it could function as a genetic information carrier, which, in combination with ribosomal particles, directs the assembly of free amino acids into specific proteins.[13, 17, 18] From studies on the participation of RNA polymerase[6] in amino acid incorporation by a soluble protein–ribosome system from *E. coli*, we have obtained evidence which bears on this hypothesis. The addition of purified RNA polymerase and exogenous DNA to the system markedly increases both the rate and extent of amino acid incorporation. This increase appears to be caused by the enzymatically synthesized RNA.

Materials and Methods.—DL-leucine-1-C^{14} (1.5×10^7 cpm per μmole), DL-valine-1-C^{14} (1.4×10^7 cpm per μmole) and unlabeled DL-amino acids were obtained from the California Corporation for Biochemical Research. The latter were used to prepare a mixture containing all of the naturally occurring amino acids except leucine, valine, asparagine, and glutamine, in the molar proportions found in the total protein of *E. coli*.[19] Quantities of the amino acid mixture used in

the various experiments are expressed on the basis of alanine, the most abundantly occurring amino acid (12.7 moles per cent). CTP^{32} containing P^{32} in the ester phosphate and unlabeled ATP, GTP, UTP, and CTP were obtained as previously described.[6]

DNA was prepared from calf thymus, salmon sperm, and bacteriophages ØX174,λ,T2, T5, and T6 as previously described.[6] *E. coli* DNA was prepared from fresh *soluble fraction I* (see below) by phenol extraction followed by exhaustive dialysis against 0.02 *M* Tris buffer, pH 7.8, containing 0.008 *M* $MgCl_2$ and 0.06 *M* KCl (buffer I). Amino acid–acceptor RNA was isolated from *E. coli* by the procedure of Ofengand *et al.*[20] The active RNA, capable of reacting with amino acids to form amino acyl derivatives, is referred to as RNA . . . pCpCpA. RNA . . . pCpCp denotes preparations which have been treated with periodate and an amine and are incapable of forming amino acyl derivatives.[21] Concentrations of all nucleic acids are expressed on the basis of nucleotide content.

RNA polymerase was the most highly purified fraction, prepared as previously described.[6] Crystalline beef pancreatic ribonuclease (RNase) and deoxyribonuclease (DNase) were obtained from the Worthington Biochemical Corporation.

E. coli B, grown and harvested as previously described,[6] were washed once by suspension in buffer I and stored at −15° for up to 3 months prior to extraction. During this period, the loss of amino acid–incorporating activity in extracts of the stored cells was less than 30%.

Preparation and fractionation of extracts were carried out at 4°. Forty gm of cell paste were ground with 100 gm of washed alumina for 5 min and then mixed evenly with 100 ml of buffer I. After centrifuging the suspension for 5 min at 10,000 × *g* to remove the bulk of alumina and whole cells, the resulting supernatant fluid was centrifuged for 30 min at 10,000 × *g*, and this final supernatant fluid (*crude extract*) was saved for further fractionation.

To the *crude extract* were added, with stirring, 1.0 *M* $MgCl_2$ and 0.5 *M* glutathione (GSH) to final concentrations of 0.015 *M* and 0.002 *M*, respectively, followed by 1.4 gm of solid ammonium sulfate per 10 ml of the *crude extract*. After 5 min, the mixture was centrifuged for 5 min at 10,000 × *g*, and the supernatant fluid was decanted and dialyzed for 9 hr against three 3-liter portions of buffer I. The dialyzed extract was centrifuged in a Spinco Model L preparative ultracentrifuge (#40 rotor, 13-ml tubes) for 120 min at 105,000 × *g*, and the upper 7 ml of the supernatant fluid (*soluble fraction I*) was withdrawn.

The transparent pellets were drained free of supernatant fluid, combined, and suspended using a glass pestle in 50 ml of buffer I containing GSH (10^{-3} *M*) and EDTA (10^{-4} *M*) (buffer II). The suspension was centrifuged for 5 min at 10,000 × g to remove unsuspended material, and then recentrifuged at 105,000 × *g* as described above. The clear colorless pellets were drained, combined, resuspended in 10 ml of buffer II, and centrifuged briefly at low speed as above. The opalescent supernatant fluid (*ribosomal fraction*) was decanted and stored at 0 to 1°.

Soluble fraction I was further fractionated by the addition of 2 ml of freshly prepared protamine sulfate solution (1%) per 10 ml of soluble fraction followed by centrifugation for 5 min at 10,000 × *g*. The supernatant fluid (*soluble fraction II*) was decanted and stored at 0 to 1°. As a result of this procedure, the ratio of absorbencies at 280 and 260 mμ increased from 0.67 in *soluble fraction I* to 1.04 in *soluble fraction II*, while the absorbence at 260 mμ decreased from 44.5 to 11.8 in a typical fractionation. As estimated from these data using the absorption constants of Warburg and Christian,[22] the protamine sulfate treatment removed more than 85% of the nucleic acid present in *soluble fraction I*.

During one week of storage under the conditions indicated, *soluble fraction II* and *ribosomal fraction* lost approximately 5% and 20%, respectively, of their original activity as measured under the conditions given in Figure 2.

The reaction components for the various experiments are described in the legends to tables in the text. All incubations were carried out at 37°. The conversion of C^{14}-labeled leucine and valine to a form insoluble in hot perchloric acid (PCA) was used as a measure of the incorporation of amino acid into peptide linkage.[23, 26] Incubations were terminated by the addition of 7% PCA, and bovine serum albumin was added to bring the protein content of each reaction mixture to approximately 1 mg. After heating in a boiling water bath for 10 min, the precipitates were centrifuged and washed twice by solution in 0.1 *M* NaOH followed by reprecipitation with 7% PCA and centrifugation. The final precipitate from each reaction mixture was dissolved in 1.0 ml of 0.3 *M* NH_4OH; and 0.50 ml aliquots were plated on aluminum planchets, dried, and counted in a windowless gas-flow counter. Radioactivity values are given as counts per minute (cpm) over

a background of 20 to 25 cpm, with no corrections for self-absorption. The over-all precision of the assay was estimated at ±10%.

Protein concentrations were estimated by the method of Lowry *et al.*[27]

Results.—Characteristics of amino acid incorporation by the soluble protein-ribosome system: In agreement with the reports of other investigators,[23–26] incorporation of leucine and valine into peptide linkage requires both ribosomal and soluble protein fractions, Mg^{++}, and ATP (Table 1). The incorporation observed in the

TABLE 1

CONDITIONS FOR AMINO ACID INCORPORATION IN THE SOLUBLE PROTEIN–RIBOSOME SYSTEM

Reaction mixture	Amino acid incorporation (*per cent*)
Complete	100
plus DNase	93
plus RNase	4
plus chloramphenicol	10
plus UTP, CTP	103
minus *soluble fraction II*	<2
minus *ribosomal fraction*	<2
minus Mg^{++}	9
minus ATP	11
minus amino acid mixture	55
minus K^+	57
minus RNA . . . pCpCpA	65
minus GTP	70

Incubation time was 30 min. Incorporation is expressed as a percentage of 960 cpm, the value observed in the complete system. The complete reaction mixture contained, in a total volume of 0.25 ml, 4.0 μmoles of Tris buffer, pH 7.8, 2.0 μmoles of magnesium acetate, 12.5 μmoles of KCl, 3.1 μmoles of β-mercaptoethanol, 50 mμmoles of amino acid mixture, 500 mμmoles of ATP, 100 mμmoles of GTP, 25 mμmoles of DL-leucine-1-C^{14}, 14 mμmoles of DL-valine-1-C^{14}, 160 mμmoles of RNA . . . pCpCpA, 700 μg of *ribosomal fraction* protein, and 400 μg of *soluble fraction II* protein. Where indicated, 1.0 μg of DNase, 0.25 μg of RNase, 4.0 μg of chloramphenicol, and 33 mμmoles each of UTP and CTP were added to the complete reaction mixture.

complete system is reduced by 30 to 50 per cent when either the mixture of unlabeled amino acids, K^+, RNA . . . pCpCpA, or GTP is omitted from the reaction mixture. Added UTP and CTP have no effect on amino acid incorporation by the complete system, but small amounts of chloramphenicol or RNase block the reaction almost completely. In contrast to results obtained using crude extracts [25, 26, §] DNase does not significantly inhibit the incorporation by the soluble protein-ribosome system.

The initial rapid rate of amino acid incorporation in the complete system decreases sharply after 10 min, and the reaction is essentially complete within 30 min (Fig. 1). In estimating the rate and extent of incorporation, incubations of 8 and 30 min, respectively, were therefore employed. As demonstrated in Figure 2*a*, the *initial rate* of amino acid incorporation is, within the limits shown, proportional to the amount of *soluble fraction II* in the reaction mixture and independent of the amount of *ribosomal fraction.* Conversely, the *extent* of incorporation (Fig. 2*b*) is, within the limits shown, proportional to the amount of *ribosomal fraction* and independent of the amount of *soluble fraction II.*

The effects of added RNA polymerase and DNA on amino acid incorporation: The addition of purified RNA polymerase alone to the soluble protein–ribosome system has almost no effect on the amino acid incorporation reaction (Fig. 3, curve *A*). When, however, T2 phage DNA is added as well (curve *B*), the initial rate of incorporation is increased 4-fold, and the duration of the reaction is extended from 30 min to over an hour. The net effect is an increase in the extent of amino acid incorporation of up to 20-fold (Table 2).

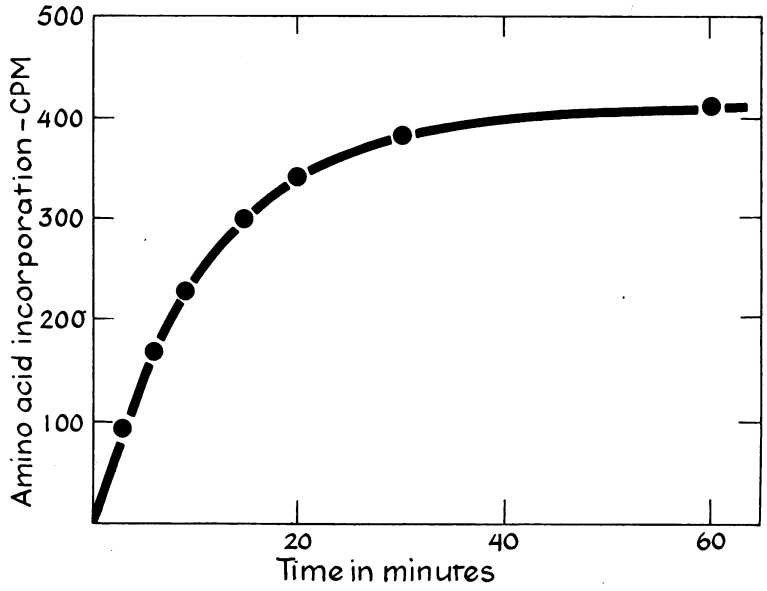

FIG. 1.—Kinetics of amino acid incorporation in the soluble protein–ribosome system. Reaction conditions were as described in Table 1.

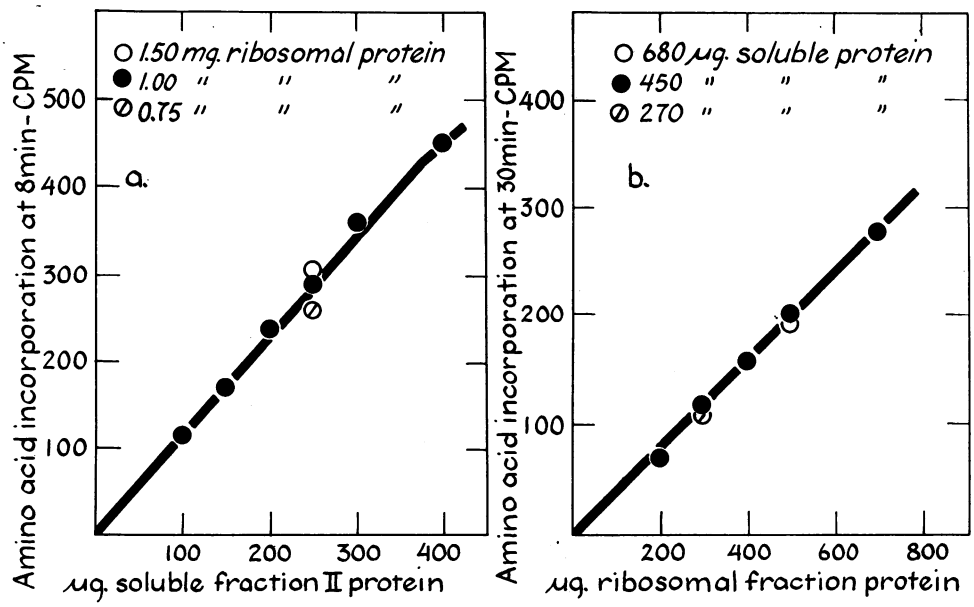

FIG. 2.—The effect of varying the amounts of protein components on the rate and extent of amino acid incorporation in the soluble protein–ribosome system. Reaction conditions were as described in Table 1 with varying amounts of *soluble fraction II* and *ribosomal fraction* protein in the reaction mixture as indicated.

Under these conditions, the requirements for maximal incorporation are similar to those observed in the unsupplemented soluble protein-ribosome system, with certain exceptions. The absolute dependence on both *ribosomal* and *soluble fractions* remains unchanged. Omission of either RNA polymerase or DNA from

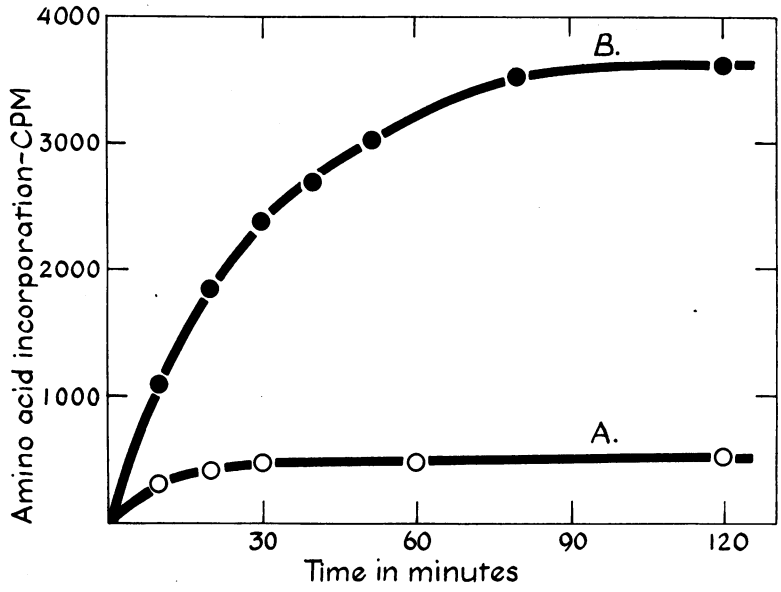

FIG. 3.—The effects of added RNA polymerase and T2 phage DNA on the kinetics of amino acid incorporation in the soluble protein–ribosome system. Reaction conditions were as described in Table 2, except that T2 phage DNA was omitted from the reaction mixtures of curve *A*.

TABLE 2

CONDITIONS FOR AMINO ACID INCORPORATION IN THE SOLUBLE PROTEIN–RIBOSOME SYSTEM SUPPLEMENTED WITH RNA POLYMERASE AND T2 PHAGE DNA

Reaction mixture	Amino acid incorporation (*per cent*)
Complete	100
plus DNase	17
plus RNase	1
plus chloramphenicol	5
minus T2 phage DNA	7
minus RNA polymerase	14
minus *soluble fraction II*	1
minus *ribosomal fraction*	3
minus Mg^{++}	4
minus RNA . . . pCpCpA	29
minus RNA . . . pCpCpA, plus RNA . . . pCpCp	31
minus K^{+}	45
minus amino acid mixture	64

Incubation time was 60 min. Incorporation is expressed as a percentage of that observed in the complete system, which was between 2,000 and 3,000 cpm in three experiments. The complete reaction mixture was as described in Table 1, except that the *ribosomal fraction* protein was reduced to 250 μg, and 33 mμmoles each of UTP and CTP, 60 mμmoles of T2 phage DNA, and 30 μg of RNA polymerase were included. Where indicated, 1.25 μg of DNase, 0.5 μg of RNase, and 4.0 μg of chloramphenicol were added to the complete reaction mixture.

the complete system reduces the incorporation to the level observed in the unsupplemented system. In contrast to the data in Table 1, DNase as well as RNase now markedly inhibits the reaction. In addition, the requirement for RNA . . . pCpCpA appears to be increased. That this effect is specifically related to the amino acid–acceptor function of the RNA is suggested by the fact that RNA . . . pCpCp will not restore the activity lost upon omission of RNA . . . pCpCpA.

As shown in Figure 3, amino acid incorporation in the soluble protein-ribosome system supplemented with RNA polymerase and T2 phage DNA ceases entirely between 60 and 90 min. The reaction cannot be prolonged by a second addition of RNA polymerase and T2 phage DNA at 60 min. As in the unsupplemented soluble soluble protein–ribosome system, this final extent of incorporation is linearly related to the amount of *ribosomal fraction* present (Fig. 4, curve *A*). Comparison with the

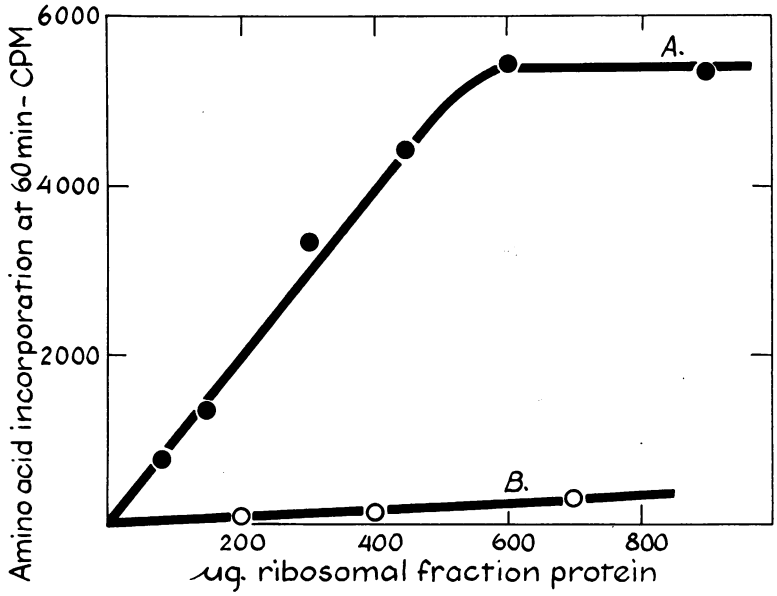

FIG. 4.—The effect of varying the amount of *ribosomal fraction* on the extent of amino acid incorporation in the soluble protein–ribosome system supplemented with RNA polymerase and DNA (curve *A*). Reaction conditions were as described in Table 2, with varying amounts of *ribosomal fraction* protein in the reaction mixture as indicated. The lower curve (*B*), showing the results of the same experiment in the unsupplemented soluble protein–ribosome system is replotted from Figure 2*b* for comparison.

corresponding proportionality curve obtained in the unsupplemented system (curve *B*) indicates that the action of RNA polymerase and DNA produces a 20-fold increase in the specific activity of the ribosomal fraction.

The marked stimulation of amino acid incorporation by exogenous DNA in the presence of RNA polymerase is observed not only with T2 phage DNA but also with the DNA of two other virulent coliphages, T5 and T6 (Table 3). Considerably less stimulation (3.5- to 1.5-fold) is seen with DNA preparations from *E. coli*, λ phage, salmon sperm, or calf thymus, and no effect is observed with ØX 174 phage DNA. The relative activities of the DNA preparations tested show no correlation with their efficiencies as primers for RNA polymerase.[6] It is interesting to note that when T2 phage DNA is heated under conditions leading to collapse of its double-stranded structure[28] its ability to stimulate amino acid incorporation is eliminated. This finding will be examined further in a subsequent experiment.

Evidence for stimulation of amino acid incorporation by enzymatically synthesized RNA: The above observations are consistent with the possibility that the in-

TABLE 3

STIMULATION OF AMINO ACID INCORPORATION BY DNA FROM VARIOUS SOURCES

DNA source	Amino acid incorporation (*cpm*)
Control (no DNA)	130
T6 phage	2,860
T2 phage	2,280
T5 phage	1,560
λ phage	450
E. coli	430
Salmon sperm	330
Calf thymus	210
T2 phage, heated	140
ØX 174 phage	130

Incubation time was 60 min. Conditions were as described in Table 2, except that the T2 phage DNA was replaced by 30 mμmoles of the indicated DNA preparation. Heated T2 phage DNA was prepared by heating at a concentration of 2 μmoles per ml in 0.05 M NaCl for 10 min at 100°, followed by rapid cooling.

creased amino acid incorporation in the supplemented system is due to the formation of an active RNA component in the RNA polymerase reaction. Evidence supporting this hypothesis was obtained from the following three experiments, in which RNA synthesis and amino acid incorporation were carried out separately.

In the first experiment (Table 4), RNA synthesis was allowed to proceed for a

TABLE 4

THE FORMATION OF AN ACTIVE RNA WHICH STIMULATES AMINO ACID INCORPORATION

Reaction mixture for stage I	RNA synthesis during stage I (mμmoles)	Amino acid incorporation during Stage II (cpm)	Increase in amino acid incorporation over control (cpm)
Complete	71.2	550	430
plus DNase	2.2	120*	—
minus RNA polymerase	<0.1	110	0
minus T2 phage DNA	<0.1	110	0
minus UTP	<0.1	130	10

* Taken as the control value of amino acid incorporation.

The experiment was performed in two stages. Stage I reaction mixtures contained the components necessary for RNA synthesis and were made up in duplicate, one group containing CTP^{32} and the other unlabeled CTP. After a 20-min. incubation, reaction mixtures containing CTP^{32} were assayed as described previously;[6] the total amount of RNA synthesized during Stage I was calculated from the CMP^{32} incorporation and the base composition of the T2 phage DNA.[6] To reaction mixtures containing unlabeled CTP were added 10 μg of DNase (except where DNase had been added at the beginning of the Stage I incubation), followed by the components necessary for amino acid incorporation. After a second incubation for 60 min, the reaction mixtures were assayed for amino acid incorporation as usual. Complete Stage I reaction mixtures contained, in a volume of 0.18 ml, 4.0 μmoles of Tris buffer, pH 7.8, 2.0 μmoles of magnesium acetate, 12.5 μmoles of KCl, 3.1 μmoles of β-mercaptoethanol, 100 mμmoles of $MnCl_2$, 50 mμmoles of amino acid mixture, 500 mμmoles of ATP, 200 mμmoles of GTP, 100 mμmoles each of UTP and CTP (or CTP^{32}), 60 mμmoles of T2 phage DNA, 30 μg of RNA polymerase, and, where indicated, 10 μg of DNase. In addition to the components of Stage I, all Stage II reaction mixtures contained, in a final volume of 0.25 ml, 10 μg of DNase, 25 mμmoles of DL-leucine-1-C^{14}, 14 mμmoles of DL-valine-1-C^{14}, 160 mμmoles of RNA . . . pCpCpA, 400 μg of *soluble fraction II* protein, and 200 μg of *ribosomal fraction* protein.

short time (Stage I) and was then terminated by the addition of DNase. The reaction mixture was then combined with the soluble protein–ribosome system, and amino acid incorporation was assayed after a second incubation period (Stage II). If, during Stage I, DNase is present or T2 phage DNA or UTP is absent, little or no RNA formation occurs, and a basal level of amino acid incorporation is observed during State II. However, under conditions allowing RNA synthesis during Stage I, the extent of amino acid incorporation is increased by approximately 5-fold.

The second experiment (Table 5) was carried out in a similar manner, except that RNA synthesis during Stage I was terminated by heating at 100°. If, during Stage I, heated RNA polymerase is substituted for the active enzyme or UTP is omitted from the reaction mixture, no RNA synthesis occurs, and a basal level of amino acid incorporation during Stage II is observed. When native DNA and active RNA

TABLE 5

THE FORMATION OF AN ACTIVE RNA WHICH STIMULATES AMINO ACID INCORPORATION

Reaction mixture for Stage I	RNA synthesis during Stage I (mμ moles)	Amino acid incorporation during Stage II (cpm)	Increase in amino acid incorporation over control (cpm)
Complete	54.5	1020	740
Complete; heated T2 phage DNA	41.2	370	90
Complete; heated RNA polymerase and heated T2 phage DNA	<0.1	280*	—
Complete; heated RNA polymerase	<0.1	320	40
Complete; minus UTP	<0.1	300	20

* Taken as the control value of amino acid incorporation.

The experiment was performed in two stages as described in Table 4. The complete reaction mixtures for Stage I and II incubations were as given in Table 4, except that no DNase was used. Where indicated, heated RNA polymerase and heated T2 phage DNA, prepared by heating 2 min at 100°, were included in Stage I reaction mixtures in place of the active enzyme and native DNA, respectively. After Stage I incubation (15 min), all reaction mixtures were heated 2 min at 100°. Those containing CTP^{32} were assayed for CMP^{32} incorporation, and the amount of RNA synthesis during Stage I was calculated as indicated in Table 4. Those containing unlabeled CTP were supplemented with the components necessary for amino acid incorporation, incubated, and assayed as described in Table 4.

polymerase are present in the complete Stage I reaction mixture, allowing RNA synthesis to occur, the amino acid incorporation during Stage II is stimulated 4-fold over the basal level. If heated T2 phage DNA is substituted for the native material during Stage I, RNA formation is decreased only slightly (25%), but the stimulation of amino acid incorporation during Stage II is almost completely eliminated.

In the third experiment, increasing amounts of enzymatically synthesized RNA were added to the soluble protein–ribosome system, and the effect on the extent of amino acid incorporation was determined. RNA formation was allowed to proceed for 15 min under the conditions previously described for net synthesis[6] and was terminated by heating at 100° for 2 min, followed by centrifugation to remove denatured protein. The supernatant fluid contained newly synthesized RNA at a concentration of 200 mμmoles per ml (calculated from CMP^{32} incorporation as indicated in Table 4). When aliquots of this solution corresponding to 8, 16, and 24 mμmoles of RNA were added to the soluble protein–ribosome system in the absence of RNA polymerase and DNA, the increase in amino acid incorporation over the basal level was 75, 150, and 200 per cent, respectively. This increase was unaffected (< 3%) by the presence of 1.0 μg of DNase in the reaction mixture.

Discussion.—Ribosomes are believed to contain the sites at which amino acids are arranged in sequence and linked together to form protein molecules (for recent review see ref. 29). However, attempts to study the mechanism of this process in cell-free extracts have been hampered by the limited extent and short duration of the amino acid incorporation reaction. As suggested by the linear relationship which we observe between the extent of incorporation and the amount of *ribosomal fraction* present, the early cessation of incorporation may be due to the consumption or inactivation of one or more essential ribosomal components during the incubation. Since the addition of RNA polymerase and DNA to the soluble protein–ribosome system can increase the extent of amino acid incorporation 20-fold and triple the duration of the reaction, it appears that one of the essential ribosomal components is produced as a consequence of the RNA polymerase reaction.

This suggestion is supported by the markedly increased specific activity of the *ribosomal fraction* in the presence of RNA polymerase and T2 phage DNA. Under conditions where RNA polymerase, DNA, and the soluble protein fraction are in excess, up to 4 mμmoles of leucine can be incorporated per mg of ribosomal protein.

Assuming that other amino acids are incorporated in proportions corresponding to the amino acid composition of T2 phage structural protein,[30] this figure represents a total incorporation of 9 μg of amino acids per mg of ribosomal protein. A similar, though smaller, effect results from the addition of enzymatically synthesized RNA to the soluble protein–ribosome system in the absence of RNA polymerase and DNA. It therefore seems likely that the increase in ribosomal specific activity is caused by newly synthesized RNA *per se* or a component containing this RNA, which either activates previously nonfunctional ribosomes or stimulates those which are already active. The nature of this activation process and the identity of the RNA-containing component are currently under study.

The eventual cessation of amino acid incorporation at 90 min, even in the presence of continuing RNA synthesis, indicates that under these conditions some other component of the system becomes limiting. In addition, it is not clear why less stimulation of amino acid incorporation occurs when RNA polymerase and DNA are replaced by enzymatically synthesized RNA in the soluble protein–ribosome system, but two possible reasons can be suggested: (1) endogenous RNase in the soluble protein–ribosome system[31] may compete more effectively for RNA added at the start of the incubation than for RNA generated throughout the reaction, or (2) the procedures used to terminate RNA synthesis (addition of DNase, heating) may affect the activity of the enzymatically synthesized product.

In contrast to our results with the soluble protein–ribosome system, two groups of investigators[25,26] have reported that DNase produces up to 70 per cent inhibition of amino acid incorporation in *E. coli* preparations resembling our *crude extract.*§ The DNase sensitivity observed in our RNA polymerase–soluble protein–ribosome system, which is consistent with the known requirement for DNA in the RNA polymerase reaction, offers an explanation for this apparent discrepancy. In *crude extract,* which contains the bulk of the cellular DNA as well as RNA polymerase activity, part of the observed amino acid incorporation is very likely a consequence of DNA-dependent RNA synthesis and can therefore be blocked by DNase. On the other hand, very little DNA remains in the soluble protein–ribosome system (less than 3 mμmoles of DNA per 100 μg of protein in *soluble fraction II* as estimated spectrophotometrically). Since under these conditions essentially no DNA-dependent RNA synthesis could occur, it is not surprising that almost all (>90%) of the amino acid incorporation is insensitive to DNase. Consistent with this interpretation is the observation that in the presence of DNase the kinetics of amino acid incorporation by a "crude extract" (S-30 fraction[26]) are very similar to those which we observe in the unsupplemented soluble protein–ribosome system.

It is difficult to rationalize at present the widely varying activities of DNA from various sources in stimulating amino acid incorporation, particularly since there appears to be no relationship between these activities and the relative efficiencies of the same preparations in priming enzymatic RNA synthesis.[6] Perhaps the most striking discrepancy is observed in the experiments with native and heat-denatured T2 phage DNA. The rate of RNA synthesis observed with the denatured DNA is almost as high (75%) as that observed with the native material, but the product formed in the presence of denatured DNA appears to be completely inactive in stimulating amino acid incorporation. It is unlikely that this is due to thermal degradation of the primer DNA, since the rate of polynucleotide strand

breakage at 100° is less than 0.6 scissions per 2 min per 20 million molecular weight.[32] This observation, in conjunction with the complete inactivity of ØX 174 phage DNA, raises the question of whether the double-stranded form of DNA is required for the production of active RNA. Meaningful interpretation of these results will require a more detailed understanding of the mechanism of RNA polymerase and the critical structural parameters of its primer and product.

Two other questions raised in the course of these experiments are (1) whether the increase in amino acid incorporation resulting from the RNA polymerase reaction represents the *de novo* synthesis of complete specific proteins, and (2) if so, whether the proteins formed will vary according to the origin of the DNA used to prime RNA synthesis. These questions are currently under investigation.

Summary.—1. Amino acid incorporation has been studied in a partially resolved *E. coli* extract from which the bulk of the DNA has been removed. Incorporation is dependent on both a ribosomal and a soluble protein fraction, ATP, and Mg^{++}. It is stimulated by a mixture of amino acids, KCl, amino acid acceptor RNA, and GTP; these components are together referred to as the soluble protein–ribosome system. Incorporation in this system is blocked by RNase or chloramphenicol but is unaffected by DNase.

2. Addition of purified *E. coli* RNA polymerase to this system has no effect on amino acid incorporation; however, addition of T2 phage DNA as well results in an increase of 4-fold in the rate and up to 20-fold in the extent of the reaction. Conditions for the increased incorporation are the same as above, except that DNase is now strongly inhibitory.

3. DNA from T5 or T6 phage stimulates incorporation as effectively as T2 phage DNA. DNA from λ phage, *E. coli*, salmon sperm, or calf thymus is much less active, and ØX 174 phage DNA is inactive. T2 phage DNA is completely inactivated by brief heating at 100° followed by rapid cooling.

4. The extent of amino acid incorporation in the soluble protein–ribosome system can be increased 3- to 5-fold by the addition of enzymatically synthesized RNA in the absence of RNA polymerase and DNA. This increase is not affected by DNase.

* This work was supported by Public Health Service Research Grant No. RG6814 and Public Health Service Training Grant No. 2G 196.

† Pre-doctoral Fellow.

‡ The abbreviations used in this paper are RNA and DNA for ribonucleic and deoxyribonucleic acid, respectively; EDTA for ethylenediaminetetraacetate; and ATP, GTP, CTP, and UTP for the ribonucleoside triphosphates of adenosine, guanosine, cytidine, and uridine, respectively.

§ Unpublished experiments, using *crude extract* in place of *ribosomal fraction* and *soluble fraction II*, show that under the conditions given in Table 1, the presence of DNase in the incubation mixture reduces the extent of amino acid incorporation up to 50 per cent.

[1] Weiss, S. B. and T. Nakamoto, these PROCEEDINGS, **47**, 694 (1961).

[2] Weiss, S. B. and T. Nakamoto, *J. Biol. Chem.*, **236**, PC 18 (1961).

[3] Furth, J. J., J. Hurwitz and M. Goldman, *Biochem. Biophys. Res. Comm.*, **4**, 362 (1961).

[4] Stevens, A., *Biochem. Biophys. Res. Comm.*, **3**, 92 (1960).

[5] Burma, D. P., H. Kröger, S. Ochoa, R. C. Warner, and J. D. Weill, these PROCEEDINGS, **47**, 749 (1961).

[6] Chamberlin, M., and P. Berg, these PROCEEDINGS, preceding paper.

[7] Stevens, A., *J. Biol. Chem.*, **236**, PC 43 (1961).

[8] Furth, J. J., J. Hurwitz and M. Goldman, *Biochem. Biophys. Res. Comm.*, **4**, 431 (1961).

[9] Weiss, S. B. and T. Nakamoto, these PROCEEDINGS, **47**, 1400 (1961).
[10] Geiduschek, E. P., T. Nakamoto, and S. B. Weiss, these PROCEEDINGS, **47**, 1405 (1961).
[11] Yčas, M., and W. S. Vincent, these PROCEEDINGS, **46**, 804 (1960).
[12] Gros, F., H. Hiatt, W. Gilbert, C. G. Kurland, R. W. Risebrough, and J. D. Watson, *Nature*, **190**, 581 (1961).
[13] Havashi, M., and S. Spiegelman, these PROCEEDINGS, **47**, 1564 (1961).
[14] Volkin, E., and L. Astrachan, *Virology*, **2**, 149 (1956).
[15] Nomura, M., B. D. Hall and S. Spiegelman, *J. Mol. Biol.*, **2**, 306 (1960).
[16] Volkin, E., these PROCEEDINGS, **46**, 1336 (1960).
[17] Brenner, S., F. Jacob and M. Meselson, *Nature*, **190**, 576 (1961).
[18] Jacob, F., and J. Monod, *J. Mol. Biol.*, **3**, 318 (1961).
[19] Roberts, R. B., P. H. Abelson, D. B. Cowie, H. T. Bolton, and R. J. Britten, *Studies of Biosynthesis in E. coli*, Carnegie Institution of Washington Publication 607 (1955).
[20] Ofengand, E. J., M. Dieckmann, and P. Berg, *J. Biol. Chem.*, **236**, 1741 (1961).
[21] Priess, J., P. Berg, E. J. Ofengand, F. H. Bergman, and M. Dieckmann, these PROCEEDINGS **45**, 319 (1959).
[22] Warburg, O., and W. Christian, *Biochem. Z.*, **310**, 384 (1942).
[23] Lamborg, M. R. and P. C. Zamecnik, *Biochim. Biophys. Acta*, **42**, 206 (1960).
[24] Nathans, D. and F. Lipmann, these PROCEEDINGS, **47**, 497 (1961).
[25] Tissières, A., D. Schlessinger, and F. Gros, these PROCEEDINGS, **46**, 1450 (1960).
[26] Matthaei, J. H. and M. W. Nirenberg, these PROCEEDINGS, **47**, 1580 (1961).
[27] Lowry, O., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[28] Doty, P., these PROCEEDINGS, **42**, 791 (1956).
[29] Hoagland, M. B., in *The Nucleic Acids*, ed. E. Chargaff and J. N. Davidson (New York: Academic Press, 1960), vol. 3, chapter 37.
[30] Luria, S. E., *General Virology* (New York: Wiley, 1953), p. 104.
[31] Spahr, P. F. and B. Hollingworth, *J. Biol. Chem.*, **236**, 823 (1961).
[32] Doty, P., J. Marmur, J. Eigner and C. Schildkraut, these PROCEEDINGS, **46**, 461 (1960).

AN INTERMEDIATE IN THE BIOSYNTHESIS OF POLYPHENYLALANINE DIRECTED BY SYNTHETIC TEMPLATE RNA

BY MARSHALL W. NIRENBERG, J. HEINRICH MATTHAE,* AND OLIVER W. JONES

NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Communicated by Richard B. Roberts, November 24, 1961

We have recently found that simple, synthetically prepared polyribonucleotides such as polyuridylic† acid and polycytidylic acid function as RNA templates in a cell-free protein-synthesizing system prepared from *E. coli*.[1, 2] In this system, poly U contains the information for the synthesis of polyphenylalanine; therefore, the code for phenylalanine is one or more uridylic acid residues. Poly U was much more effective in increasing the rate of cell-free protein synthesis than naturally occurring informational RNA,[2] possibly because the synthesis of a protein containing only one amino acid was faster than the synthesis of a protein containing 20 amino acids. We are using this model system currently to study the enzymatic sequence of protein synthesis.

Although sRNA and amino acid–activating enzymes have been studied extensively, there is controversy concerning their relationship to protein synthesis.[3–6]