volume and a given surface area. It follows, then. that the ratio p represents at least some part of the structural coding of a protein molecule, and possibly a very considerable part.

I thank Dr. Harold C. Choitz for much helpful discussion.

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*MECHANISM OF PEPTIDE BOND FORMATION IN POLYPEPTIDE SYNTHESIS**

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One of the major problems in molecular biology is the mechanism involved in "reading" the genetic message. This is believed to occur in the reaction sequence which leads to the transfer of amino acids from amino acyl-RNA to their proper sequential position in the polypeptide chain. Here we report that the reaction

sequence consists of two stages, catalyzed by different enzymes, that GTP is utilized in the first reaction prior to peptide bond formation, and that when the second reaction is separated from the first, a dipeptidyl-RNA is the product.

Amino acyl-RNA, GTP, ribosomes, and soluble enzymes are generally agreed to be involved in the transfer reaction.[1] Several groups have presented evidence that more than a single enzyme might be involved in these reactions.[2-6] However, in these earlier studies it was not possible to define the role of individual enzyme fractions or a reaction sequence. We have previously reported that the same 2 enzyme fractions were required for the transfer of C^{14}-phenylalanyl-RNA to polyphenylalanine using a poly U-stimulated reticulocyte system.[7, 8] Nakamoto *et al.*[9] have reported that 2 enzyme fractions were required for a similar system from *E. coli*.

Earlier studies with the reticulocyte system[7, 8] indicated that the first enzyme (TF-1) catalyzed a reaction measured by the binding of C^{14}-phenylalanyl-RNA to the ribosome, which was stimulated by GTP, while the second enzyme (TF-2) was required for peptide bond formation. We propose to name these enzymes the "binding" and "peptide synthetase" enzymes, respectively.

Methods.—Ribosome preparation: Unwashed ribosomes (1X) were prepared as described.[10] These ribosomes (9.5 mg) in 0.25 *M* sucrose were incubated with 50 μmoles of Tris HCl buffer, pH 7.5, 100 μmoles of KCl, and 20 μmoles of GSH in a final volume of 1 ml for 25 min at 37°. The effect of this "shock" treatment has been described.[11] The remaining constituents of the complete amino acid incorporating system[10] were then added, using C^{12}-amino acids, and incubation continued for an additional 40 min. The mixture was chilled and diluted 8-fold with a solution containing 0.25 *M* sucrose, 0.002 *M* $MgCl_2$, 0.0175 *M* $KHCO_3$, and 0.1 *M* KCl. The diluted mixture was centrifuged at 78,000 × *g* for 90 min and the supernatant removed. The pellets were rinsed with 0.25 *M* sucrose and gently homogenized in 0.25 *M* sucrose. The final solution contained 14 mg per ml of ribosomes (ribonucleoprotein). This was centrifuged at 10,000 × *g* for 10 min to remove a small amount of aggregated material. Deoxycholate (0.25 vol of 5% solution) was added and the mixture incubated 3 min at 37°. Ten volumes of cold medium B[10] was added, and the ribosomes were sedimented as before. The homogenization and centrifugation at 10,000 × *g* were repeated to give a final solution containing 15 mg ribosomes per ml.

Enzyme preparation: The starting material was the fraction of the high-speed supernatant which precipitated between 40 and 70% saturation with ammonium sulfate at pH 6.5 after protamine treatment.[10] This fraction was adsorbed onto calcium phosphate gel, and the peptide synthetase (TF-2) eluted with 0.1 *M* phosphate buffer, while the binding enzyme (TF-1) was eluted with 0.3 *M* phosphate buffer. The fractions were further purified on DEAE-cellulose columns, where the TF-2 fraction was eluted with 0.1 *M* NaCl and the TF-1 at 0.25 *M* NaCl. Details of this fractionation will be published elsewhere.[12]

RNA analyses: After incubation as described in the particular figures, ribosomes were isolated by centrifugation, and bound phenylalanyl-RNA or peptidyl-RNA was isolated and studied on a sucrose density gradient. Ribosomes were suspended in 0.5% Na dodecyl sulfate in 0.01 *M* sodium phosphate, pH 6.3. The mixture was shaken for 5 min at 25°, an equal volume of phenol (90% w/w) was added, and the RNA extracted at 0°. Linear sucrose density gradients were run in 0.01 *M* sodium acetate, pH 5.1, and the fractions counted directly.[12]

Assay procedures: Labeled phenylalanyl-RNA was prepared by a modification of earlier procedures[13] from guinea pig liver pH 5 enzyme. Either UL-C^{14}-phenylalanine (specific activity, 60 mC per mmole) or H^3-phenylalanine (specific activity, 500 mC per mmole) at a concentration of 8.3 μmoles per ml was used. The amino acyl-RNA was extracted and dissolved in 0.05 *M* KCl and 0.01 *M* potassium acetate, pH 5.1. The solution was dialyzed against 100 vol of the same buffer for 6–8 hr and then passed through a 2 × 40-cm column of Sephadex G-25 equilibrated with the same buffer. The peak tubes were pooled, adjusted to 3 mg RNA per ml, and stored at −90°. These preparations usually contained about 0.3 mμmoles of phenylalanine per mg RNA.

The complete reaction mixture for the binding (TF-1) reaction contained 0.1 μmole GTP, 100 μmoles KCl, 10 μmoles $MgCl_2$, 50 μmoles Tris-HCl, pH 7.5; 0.1 μmole L-C^{12}-phenylalanine,

usually 300 μg of the TF-1 enzyme fraction, 1.5 mg ribosomes, 100 μg poly U, and 300 μg labeled phenylalanyl-RNA in a final volume of 1.5 ml. The mixture was incubated for 10 min at 37° and diluted to 11 ml with a cold solution containing 0.01 *M* Tris buffer, pH 7.5 plus 0.002 *M* $MgCl_2$. The diluted sample was centrifuged for 1 hr at 40,000 rpm, and the pellet homogenized in 0.1 *M* Tris, pH 6.5. The solution was precipitated twice with cold 5% TCA, dissolved in 0.5 ml of 1 *N* NaOH, and after 2 min the protein was precipitated with acid-acetone. The supernatant solution, after centrifugation, was transferred to a counting vial, evaporated to dryness, and counted in a liquid scintillation counter.[12]

For the peptide synthetase (TF-2) reaction, after incubation as described above, the ribosomes were isolated by centrifugation from the reaction mixture after dilution with 2 vol of 0.01 *M* Tris, pH 7.5 containing 0.002 *M* $MgCl_2$. The ribosomes were homogenized in the same medium and incubated with 300 μg of TF-2 enzyme, 100 μmoles KCl, 15 μmoles of GSH, 10 μmoles $MgCl_2$, and 50 μmoles Tris buffer, pH 7.5 in a final volume of 1.5 ml for 10 min at 37°. In some experiments, the binding reaction was run for 20 min with one fifth the usual level of GTP (1.3×10^{-5} *M*). Under these conditions, the GTP was largely destroyed during the incubation, and the peptide synthetase reaction was initiated without centrifugation by the addition of the TF-2 enzyme and GSH. After incubation, in this case, the ribosomes were isolated by centrifugation before washing. The mixture in either case was chilled, and then cold 5% TCA was added and the same TCA and NaOH washes used as for the binding reaction. To the acid-acetone supernatant was added one μmole each of carrier phenylalanine, diphenylalanine, and in some cases triphenylalanine. The solution was evaporated to dryness, and water added to make a solution 0.2 *N* in sodium ion. The pH was adjusted to 2.5, and the solution applied to a small (1×3 cm) column of Dowex 50-X2 which had been equilibrated with sodium citrate buffer (0.35 *N* in sodium ion, pH 5.2).[14] The same buffer was used for elution, and the separated phenylalanine and diphenylalanine were counted separately.

GTP hydrolysis studies: Components of the binding assay were incubated for 10 min at 37° using 8-C^{14}-GTP (specific activity, 7.0 mC per mmole) at a concentration of 1.3×10^{-5} *M*. 12 mg of casein and 1 μmole each of GMP, GDP, and GTP were added followed by precipitation in the cold with 0.25 *M* perchloric acid. The pellet was discarded, and the nucleotides were absorbed to 40 mg of charcoal and eluted with 10% pyridine in 50% ethanol. Aliquots were chromatographed on Whatman #3 in isobutyric acid:ammonia:water (57.7:3.8:38.5). Spots were detected with UV light, cut out, and placed in vials for counting in the liquid scintillation spectrometer.

Results.—Properties of the binding (TF-1) reaction: The binding of phenylalanyl-RNA to the ribosome required poly U, $MgCl_2$, and the TF-1 enzyme fraction (Table 1). Boiling the enzyme, or replacing TF-1 with TF-2, gave no reaction. Pretreatment of the ribosomes as described (see *Methods*) was essential to destroy endogenous hemoglobin synthesis. This results in an absolute requirement for poly U for phenylalanyl-RNA (phe-RNA) binding. In other studies, it was found that the binding was time- and temperature-dependent, and proportional to the enzyme concentration.[12] Maximal binding was reached in 10 min at 37°. Leu-RNA, val-RNA, and lys-RNA were not bound under the conditions of these experiments.

It was reported previously that GSH was required for the *complete* transfer system.[15] This was not the case for the binding reaction; the enzyme is routinely prepared in the absence of GSH, and GSH may be omitted from the assay (Table 1). However, GSH is required for the peptide synthetase reaction (see below).

Product of the reaction: The product of the TF-1 reaction appears to be the original phe-RNA bound to the 80S ribosomes. To study the products, after incubation ribosomes were removed by centrifugation, treated with sodium dodecyl sulfate and phenol, and centrifuged in a sucrose gradient. The extracted material labeled with H^3-phe-RNA was mixed with carrier C^{14}-phe-RNA before centrifugation in the gradient. The movement of both types of RNA in the sucrose gradient

TABLE 1

REQUIREMENTS FOR TRANSFER OF PHENYLALANYL-RNA

Assay conditions	H^3-phenylalanyl-RNA bound ($\mu\mu$moles)
Complete	38.0
Minus GTP	6.6
Minus TF-1 enzyme	2.4
Minus GTP and TF-1	2.5
Minus $MgCl_2$, or ribosomes, or poly U	0.5
TF-2 in place of TF-1	2.4
Boiled TF-1	4.0
Plus GSH	38.0

Assay conditions are given in the text. The incubation mixture contained 300 μg of H^3-phenylalanyl-RNA (83 $\mu\mu$moles of H^3-phenylalanine) and 1.5 mg ribosomes.

TABLE 2

GTP REQUIREMENT FOR TRANSFER OF PHENYLALANYL-RNA

Assay conditions	H^3-phenylalanyl-RNA bound ($\mu\mu$moles)
No added triphosphates	5.6
0.02 μM GTP	30.0
0.10 μM GTP	35.0
0.1 μM ATP	4.9
0.1 μM CTP	6.4
0.1 μM UTP	6.4
0.1 μM GDP	13.0

Assay conditions are similar to those in Table 1. A nonenzymatic blank of 4.0 was subtracted from the values given above. The TF-1 enzyme fraction (120 μg) used in these studies was purified by precipitation at pH 6.0, in addition to the described procedure (see *Methods*).

was identical (Fig. 1). The rate of cleavage with hydroxylamine of the material isolated by phenol treatment also indicated that this was phe-RNA. After NaOH hydrolysis, the products were fractionated on a Dowex-50 column. These consisted of 97 per cent phenylalanine and 3 per cent diphenylalanine (Fig. 2). The latter compound comes from a trace of peptide synthetase activity, as will be shown below. The absence of peptide bond formation in this first reaction was confirmed by paper chromatography of the alkali hydrolyzed material in several solvents and by quantitative decarboxylation of the product (when 1-C^{14}-phe-RNA was used) with chloramine-T.[12] When the reaction mixture was incubated, chilled, and centrifuged to pellet ribosomes and these were layered on a sucrose gradient containing 10^{-4} *M* $MgCl_2$, the major portion of the bound phe-RNA moved with the 80S ribosomes, and the radioactivity and optical density peak for 80S ribosomes were coincident. Ribosomes prepared as described (see *Methods*) were almost all monomeric, and no polyribosomes were found when poly U and the components of the binding reaction were incubated together.[12]

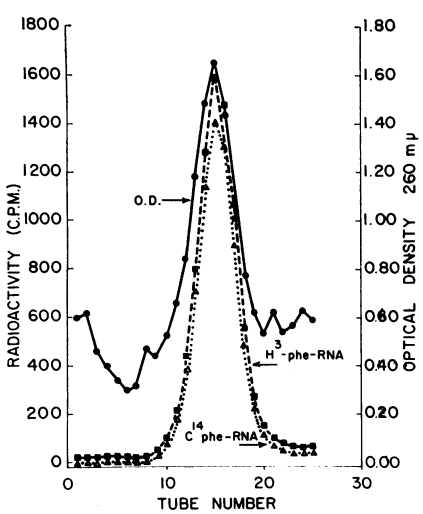

FIG. 1.—Sucrose density gradient of the phe-RNA intermediate from ribosomes. The binding reaction was run using H^3-phe-RNA. After incubation, ribosomes were isolated, and the bound intermediate was extracted with detergent and phenol (see *Methods*). The solid circles (●) show the optical density, and the open triangles (Δ) the radioactivity of carrier C^{14}-phe-RNA added to the intermediate, solid squares (■).

GTP requirement: The binding reaction required the addition of GTP. Almost maximal activity was obtained with 1.3 $\times$ 10^{-5} *M* GTP (Table 2). It is paradoxical that, as the TF-1 enzyme was purified, it became more difficult to show a GTP requirement. This was due to the removal of GTP-degrading enzymes, both hydrolytic and transphosphorylating. Small amounts of nucleoside triphosphates present in the amino acyl-RNA, poly U, and possibly ribosomes, were then sufficient to satisfy the requirement for GTP. It

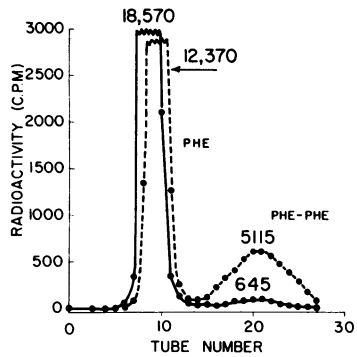

FIG. 2.—Column chromatography of reaction products after hydrolysis from ribosomes. The solid line (●) shows the products after the binding reaction. The dashed line (○) shows the same reaction mixture after the peptide synthetase reaction. In this experiment, the binding reaction was run for 20 min with one fifth the usual level of GTP. Ribosomes isolated from an aliquot were used for the data shown by the solid line (see *Methods*). The peptide synthetase enzyme and GSH were added to another aliquot, and incubation continued another 10 min. Ribosomes from this were used for the data shown by the dashed line. A similar experiment in which ribosomes were isolated by centrifugation after the binding reaction gave very similar results.

was only when the above-mentioned components were rigorously purified, as by passing the amino acyl-RNA through Sephadex, that a GTP requirement for the TF-1 reaction could be demonstrated. This accounts for our earlier, erratic results in this regard.[7] We have reported this new role for GTP previously.[8]

With the purified components, the reaction is almost completely specific for GTP (Table 2). The small stimulation with GDP is due to the formation of GTP from trace amounts of ATP or other nucleoside triphosphates still present, since enzymes which catalyze a reversible ATP-GDP transphosphorylation have not been completely removed. Not only is GDP itself not active, but nucleoside diphosphates in the presence of GTP are, in fact, quite inhibitory.[12]

TABLE 3

FORMATION OF GDP DURING BINDING OF PHENYLALANYL-RNA TO RIBOSOMES

Assay conditions	GDP formed (%)
GTP, only	20
Complete	49
Minus phe-RNA	39
Minus poly U	39
Minus phe-RNA and poly U	25

The binding of H^3-phe-RNA to ribosomes was as usual with the same enzyme fraction as used in Table 2, except that 0.02 μmoles of 8-C^{14}-GTP (specific activity, 7.0 mC per mmole) was used. GDP was determined as described in *Methods*.

During the binding reaction, GDP was formed from GTP (Table 3). Maximal GDP formation required all the reaction constituents. Thus, omission of any single reaction constituent reduced the GDP formation. There is no relation, however, between the amount of GDP formed and the amount of phe-RNA transferred, since this ratio is about 50. Despite this, it seems very likely that GDP formation is a result of the TF-1 binding reaction. Our present hypothesis is that an unstable intermediate is formed which continually breaks down and re-forms, resulting in the excess GDP formation. The GDP formation in the absence of poly U may result from a small amount of transfer of 18 different amino acyl-RNA's, which is related to the small residual hemoglobin synthesis.

Peptide bond formation (TF-2 reaction): Ribosomes with bound phe-RNA were centrifuged from the first reaction mixture and incubated with the peptide synthetase (TF-2) enzyme fraction. The products were isolated on a Dowex-50 column after NaOH hydrolysis. As shown in Figure 2, approximately 25 per cent of the original phe-RNA bound to the ribosome was converted to diphenylalanine (phephe). No other products were formed. The recovery of radioactivity in phe and phephe after the peptide synthetase reaction was 92–99 per cent of the control carried through the same procedure but without the peptide synthetase enzyme (Fig. 2). The addition of excess C^{12}-phenylalanyl-RNA or poly U, either singly or together, had no effect on the dipeptide formation. To determine whether GTP

could possibly be involved in this second reaction, these experiments were repeated with 8-C^{14}-GTP. The amount of C^{14}-nucleotide bound to the ribosome after the TF-1 reaction was too small to give any appreciable peptide bond formation if added to a complete transfer reaction mixture containing both transfer enzymes. We conclude that the peptide bond formation step does not require GTP. The material formed in the peptide synthetase reaction was identified as diphenylalanine (after NaOH hydrolysis) since the ninhydrin peak of carrier diphenylalanine (phephe) was identical with the radioactive peak (Fig. 2). Similar results were obtained on paper chromatograms, and this was confirmed by carrier recrystallization. The TF-2 enzyme fraction therefore can be called a peptide or polypeptide synthetase.

When the ribosomes were extracted with detergent and phenol after the peptide synthetase reaction and the product run in a sucrose density gradient, the material moved with the transfer RNA peak.[12] In this experiment the products were all small peptides bound to the RNA. Very little phe-RNA was present. The true product of the peptide synthetase reaction is therefore a peptidyl-RNA. This result provides strong evidence that the growing peptide chain is attached to transfer RNA. Similar results were reported by Gilbert, using an *E. coli* system.[16] Further evidence that the product of the reaction is phephe-RNA was shown by its rate of cleavage with hydroxylamine. The isolated phe-RNA and phephe-RNA mixture was incubated with hydroxylamine.[12] The rate of cleavage was a composite of the rate of breakdown of phe-RNA with a half-life of 2.1 min and another component with a half-life of 11.1 min. Analysis of the products (Fig. 2) identified this component as phephe-RNA. The conversion of phe-RNA to phephe-RNA thus gave a product which was 6 times as stable in hydroxylamine. This stabilization results from the formation of the first peptide bond, and the larger peptidyl-RNA species were only slightly more stable.

Formation of longer peptides: These results have the startling implication that only 2 sites on the ribosome can bind phe-RNA at one time. Thus, to make a larger polypeptide the two enzymes must act alternately over and over again. Another possibility was that the observations reported here pertained only to the formation of the first, N-terminal dipeptide of a chain. To test this, ribosomes containing bound C^{14}-phe-RNA were isolated from the first reaction mixture. An aliquot of these ribosomes containing approximately 16,000 cpm of C^{14}-phe-RNA and 1200 cpm of C^{14}-phephe-RNA was incubated with the peptide synthetase enzyme

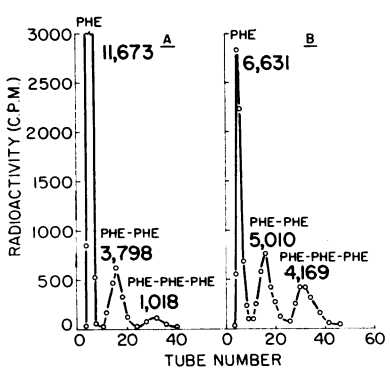

FIG. 3.—Column chromatography of reaction products after the peptide synthetase reaction. C^{14}-phe-RNA bound to ribosomes was prepared using the binding enzyme (see *Methods*). An aliquot of these ribosomes (after centrifugation) was incubated in the peptide synthetase reaction mixture with (*A*) 600 μg of C^{12}-amino acyl-RNA, and (*B*) with 600 μg of C^{12}-amino acyl-RNA and 6.7×10^{-5} *M* GTP. The products were prepared for chromatography as described in *Methods* except that 0.35 *N* (Na) citrate, pH 5.2 in 50% ethanol was used as eluting buffer after emergence of phenylalanine. A control set of ribosomes carried through the procedure shown in (*A*), but without GSH or peptide synthetase, contained approximately 15,500, 1100, and 160 cpm of phe, phephe, and phephephe, respectively.

only. After NaOH hydrolysis, the products were chromatographed as before, except that the eluting buffer was changed so that triphenylalanine (phephephe) was resolved. These results (Fig. 3*A*) were similar to the previous ones, except that a small peak of phephephe was observed. The amount of phephephe was equal to the original amount of dipeptide present as a result of the trace of synthetase reaction which occurred during the binding reaction. If *either* GTP, or C^{12}-phe-RNA was included in the peptide synthetase reaction mixture, the results were not changed greatly (Fig. 3*A*). However, when both GTP and C^{12}-phe-RNA were present, bound phe-RNA was decreased, and phephe-RNA and phephephe-RNA were increased (Fig. 3*B*). These results show that one more phe-RNA (C^{12}-phe-RNA in this case) has been added to the products shown in Figure 3*A*. In these cases, the recovery from the columns was about 98 per cent so that no other products were formed. Therefore, the same reaction sequence used to form a dipeptidyl-RNA also operates to form a tripeptidyl-RNA, and presumably is general for larger polyphenylalanyl-RNA species.

In all these experiments, no TCA-precipitable peptides (after NaOH hydrolysis) were formed. The length of the polypeptide chain depends on the conditions of the incubation. The presence of both enzymes at the start of the incubation with larger amounts of GTP, or a GTP-generating system, favor the formation of longer (TCA-precipitable) peptides. These are the expected results, if the binding step is needed after each peptide bond is made. These results explain our previously reported requirement for GTP in the peptide synthetase reaction,[7] since here only TCA-precipitable peptides were studied. As noted above, these are formed by an alternating sequence of binding reaction (requiring GTP) and peptide synthetase reaction.

In other studies, the peptide synthetase enzyme was treated with Sephadex to remove GSH. When this enzyme was used in the complete transfer system (containing both TF-1 and TF-2), at least a fivefold stimulation by added GSH was observed, as reported previously for hemoglobin synthesis.[15]

Discussion.—These results provide further evidence[7, 8] that phe-RNA bound to the ribosome is the first intermediate in the transfer of amino acid from amino acyl-RNA to polypeptide linkage. The specificity for phe-RNA when poly U is the messenger RNA, and the recovery from the ribosome of apparently unchanged phe-RNA after this reaction, indicate that this enzymatic step involves the binding of the specific species of transfer RNA to the poly U-ribosome template, and is therefore the key reaction in the translation of the genetic message. In addition, the failure of uncharged transfer RNA to inhibit the binding of phe-RNA[8] suggests that this reaction can distinguish between the amino acyl-RNA and the free transfer RNA species.

The requirement for GTP for the binding of phe-RNA to the ribosome suggests that this compound is utilized in the binding reaction. GTP is also hydrolyzed to GDP and Pi under these conditions. However, the breakdown is not completely dependent on added amino acyl-RNA. The peptide synthetase enzyme also catalyzes nonspecific GTP breakdown. It may not be possible to define the exact mechanism of GTP action until a chemical equation can be written for the binding reaction. It is possible that a high-energy intermediate is formed during the binding reaction, which breaks down rapidly, yielding apparent GTP hydrolysis. This

postulated intermediate could be necessary for the binding itself, or be related to the movement of ribosomes relative to the poly U which must occur as each peptide bond is formed.

The formation of dipeptidyl-RNA as the first product of the peptide synthetase reaction was unexpected. This result indicates that generally only two phe-RNA molecules can bind to a ribosome at any one time. The synthesis of a polypeptide must therefore involve an alternating sequence of binding reaction and peptide synthetase reaction. That this does occur was shown by the data of Figure 3, where a second cycle of reactions yielded the tripeptidyl-RNA. The data also indicate that when both enzymes and GTP are present, longer polypeptide chains on *fewer* ribosomes are formed. It appears that the binding reaction, and particularly the binding of the first amino acid in a chain, is the rate-limiting reaction. After the first peptide bond is made on a ribosome, chain elongation proceeds more rapidly. The binding and peptide synthetase enzymes probably remain on the ribosome, facilitating chain elongation. This was shown directly by demonstrating that after the reaction, these enzymatic activities were present on ribosomes centrifuged from the reaction mixture.

The data of Figure 3 show that a large number of ribosomes contain bound phe-RNA, but only about 25 per cent of these form dipeptide in the peptide synthetase step. More of these participate if C^{12}-phe-RNA and GTP are added in a second round of reactions. This suggests that a number of ribosomes contain only a single phe-RNA after the binding reaction takes place. It is possible therefore that one of the 2 binding sites on the ribosome has a higher affinity for phe-RNA than the other. In addition, preliminary studies indicate that dipeptidyl-RNA is bound more tightly to the ribosome-poly U complex than the phe-RNA. The failure of C^{12}-phe-RNA to influence peptide bond formation in the absence of GTP is strong evidence that the bound phe-RNA does not come off the ribosome during peptide bond formation.

The coincidence of the dipeptide and transfer RNA in a sucrose density gradient, and the hydrolysis with hydroxylamine are good evidence for dipeptidyl-RNA as the product of the peptide synthetase reaction. Evidence that a growing peptide chain terminates in transfer RNA has been reported by Gilbert,[16] by Takanami,[17] and by Bretscher.[18]

We conclude that the formation of the active amino acyl-RNA-messenger RNA-ribosome complex is an enzymatic reaction. The "binding" enzyme and GTP are required. The movement of the messenger RNA in relation to the ribosome requires peptide bond synthesis. Since uncharged transfer RNA does not participate in the binding reaction, after a peptide bond is formed, presumably the uncharged RNA leaves the ribosome by diffusion rather than by an exchange mechanism. This mechanism differs considerably from that proposed for *E. coli*.[16] The major experimental differences are the nonenzymatic nature of the binding and the participation of uncharged transfer RNA. In the *E. coli* system, Nakamoto *et al.*[9] have also reported the nonenzymatic binding of phe-RNA to ribosomes in the presence of poly U.

Note added in proof: The report of two transfer RNA molecules attached to each ribosome in polyribosomes [Warner, J., and A. Rich, these PROCEEDINGS, **51**, 1134 (1964)] provides independent evidence for two ribosomal binding sites.

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ELECTRON SPIN RESONANCE AND LUMINESCENCE STUDIES OF EXCITED STATES OF NUCLEIC ACIDS

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We have studied the optically excited states of purine nucleotides and their synthetic polynucleotides by Electron Spin Resonance (ESR) and optical emission. Properties and interactions of the excited states were determined from the preliminary results which are presented in this report.

All measurements reported were made at 77°K on samples dissolved in a 1:1 ethylene glycol-water (EG) glass, although exploratory measurements were made with other glasses. Concentrations used were in the range 10^{-4} to 10^{-3} M. Phosphate buffers were used to control the pH. The desired ionic strengths were obtained by varying the concentrations of NaCl. Luminescence measurements were made on an Aminco-Bowman spectrophotofluorometer, and ESR measurements with a Varian Associates X-band spectrometer with 100 kc/s modulation. For excitation in the microwave cavity, an Osram 200-watt Hg–Xe lamp was used. A water filter and Corning 7-54 filter eliminated all the radiation outside of the