AMINO ACID TRANSFER FROM AMINOACYL-RIBONUCLEIC ACIDS TO PROTEIN ON RIBOSOMES OF ESCHERICHIA COLI*

BY DANIEL NATHANS† AND FRITZ LIPMANN

THE ROCKEFELLER INSTITUTE, NEW YORK CITY

Communicated February 28, 1961

We are continuing attempts to understand the mechanism by which peptide bonds are formed in protein synthesis. Progress depends largely on the characterization of the enzymic processes involved in polypeptide synthesis from the amino acid esters of soluble ribonucleic acid (sRNA), the biosynthetically active amino acids. In a previous study,[1, 2] we used the aminoacyl-sRNA microsome system of rat liver. The recently developed *E. coli* ribosome system[3, 4, 17] seemed to hold more promise. The present report deals with an analysis of the components of the *E. coli* aminoacyl-sRNA ribosome reaction.

Methods.—Preparation of ribosomes: *E. coli* B was grown in a Biogen (American Sterilizer Co., Erie, Pa.), using a medium composed of 1 per cent dextrose, 1 per cent yeast extract, 0.25 *M* potassium phosphate pH 6.5; it was continuously harvested in a refrigerated Sharples centrifuge at the end of logarithmic growth. The cells were washed once and stored at −20° in 100 gm batches of paste. Ribosomes were prepared as required from 100 gm of bacteria by grinding with 250 gm of alumina (Alcoa A-303) and extracting with 300 ml of 0.01 *M* Tris HCl, pH 7.4, and 0.01 *M* magnesium acetate. After centrifuging first at 10,000 × *g* for 20 minutes and then the super

natant at 20,000 × *g* for 40 minutes, the ribosomal fraction was spun down from the resulting supernatant fluid at 78,000 × *g* for 3 hours. The pellets were rinsed and lightly homogenized in 70 ml of 0.01 *M* potassium phosphate, pH 7.0, and 0.0005 *M* magnesium acetate. To deplete the ribosomes of transfer factor, this suspension was recentrifuged at 15,000 × *g* for 10 minutes and the particles were spun down from the supernatant fluid at 105,000 × *g* for 3 hours. This washing was repeated twice and the resulting ribosomes were then lightly homogenized in 0.01 *M* Tris HCl, pH 7.4, and 0.01 *M* Mg acetate, and stored at −20° in small batches. Such preparations retained activity for several weeks. Re-sedimentation of the ribosomes after suspension in 0.0005 *M* Mg acetate, 0.01 *M* phosphate results in a preparation with one-and-one-half times the activity of ribosomes maintained in 0.01 *M* Mg acetate, 0.01 *M* Tris HCl. We suspect that this is due to partial purification of the low Mg^{++}-resistant and highly active 70 S particles described by Tissières *et al.*;[4] preliminary studies by means of sucrose gradient centrifugation support this view.

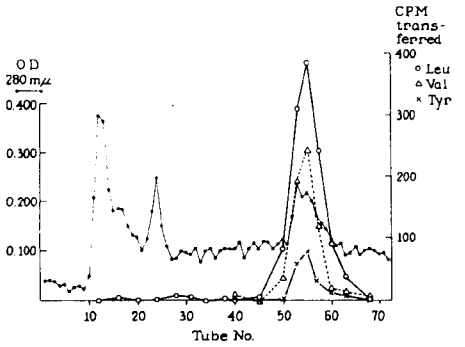


FIG. 1.—DEAE-cellulose column chromatogram of transfer factor. Application of 55 mg of the ammonium sulfate fraction was made to a 1.3 × 40 cm column equilibrated at 4° with 0.010 *M* potassium phosphate pH 7.4; 100 ml of 0.10 *M* potassium phosphate, pH 7.4, was passed through and a linear gradient of the same pH from 0.10 *M* to 0.25 *M* potassium phosphate started after tube 17. All buffers contained 0.004 *M* mercaptoethanol. Fraction volumes of 7 ml were collected and assayed for transfer factor with the labelled aminoacyl-sRNA's noted. Assay conditions were the same as given in Fig. 2, except as follows: with leucine and valine, 1.4 mg of ribosomal protein, 0.24 mg of sRNA (5000 cpm C^{14}-leucine, or 4040 cpm C^{14}-valine containing 1.23 × 10^7 cpm/μmole); with tyrosine, 3.5 mg of ribosomal protein, 0.57 mg of sRNA (1700 cpm C^{14}-tyrosine containing 5.2 × 10^6 cpm/μmole).

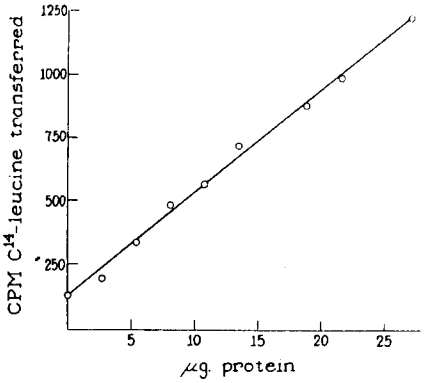


FIG. 2.—Concentration curve of transfer factor. The incubation mixture consisted of washed ribosomes (1.3 mg protein), 0.31 mg of sRNA charged with amino acids including 6660 cpm C^{14}-leucine (1.45 × 10^7 cpm/μmole), 0.0006 *M* GTP, 0.01 *M* PEP, 30 μg/ml PEP-kinase, 0.01 *M* GSH, 0.013 *M* $MgCl_2$, 0.03 *M* KCl, 0.05 *M* Tris HCl pH 7.4, and purified transfer factor as noted, in a volume of 0.50 ml. After 5 minutes at 30°, 5 per cent TCA was added, the precipitate extracted with 5 per cent TCA at 90° for 15 minutes, washed twice with TCA, once with 1:1 ethanol-ether, and counted in a windowless gas flow counter.

Preparation of sRNA charged with amino acids: *E. coli* sRNA was prepared by direct phenol treatment of the bacterial paste. After stripping of the sRNA by incubation in 0.5 *M* Tris HCl, pH 9, for 45 minutes at 36°, the sRNA was recharged with amino acids using the supernatant of alumina-ground *E. coli* as the enzyme source. For this purpose, the 105,000 × *g* supernatant fraction was treated with deoxyribonuclease and dialyzed against 0.02 *M* Tris HCl, pH 7.4, for 18 hours. A typical incubation mixture contained 3 mg of supernatant protein, 113 mg of sRNA, 0.0002 *M* of each of 21 amino acids including C^{14}-leucine, 0.003 *M* ATP, 0.01 *M* PEP, 30 μg/ml pyruvate kinase, 0.008 *M* GSH, 0.008 *M* $MgCl_2$, and 0.10 *M* Tris HCl, pH 7.2, in a volume of 4 ml. After incubation at 36° for 15 minutes, charged sRNA was re-isolated by phenol treatment and alcohol precipitation, and dialyzed against water. The concentration of sRNA was estimated by its optical density at 260 mμ, assuming 1.0 mg/ml equivalent to an optical density of 24.

Materials.—ATP, GTP, and CTP were products of Pabst Laboratories, Milwaukee. Phosphoenolpyruvate (PEP) silver barium salt and pyruvate kinase were obtained from C. F. Boehringer & Soehne, Mannheim, Germany. C^{14}-amino acids were from Volk Radiochemical Company, Chicago; and DEAE-cellulose was a product of Serva Entwicklungslabor, Heidelberg, Germany, and had a capacity of 0.74 mEq/gm.

Results.—Assay and Purification of the Amino Acid Transfer Factor.—Assay system: In preliminary experiments, it was found that in order to show the effect of supernatant fractions on amino acid transfer from sRNA to protein, the ribosomes had to be washed as described under Methods. Either 0.01 *M* phosphate, pH 7.0, and 0.0005 *M* Mg acetate, or 0.01 *M* Tris HCl, pH 7.4, and 0.01 *M* Mg acetate could be used for washing with equal effectiveness. With once-washed ribosomes, the supernatant fraction stimulated the transfer; with ribosomes

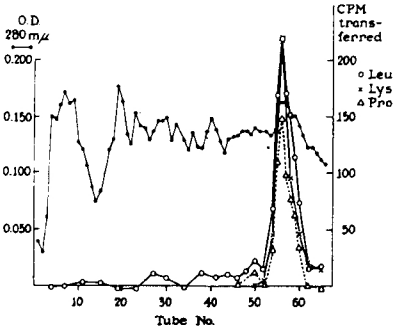


FIG. 3.—Coincidence of DEAE-cellulose eluate peak for different amino acids. From the *first ribosome wash*, 32 mg of the ammonium sulfate fraction was applied to a 1.3 × 40 cm column and protein eluted in 6 ml fractions as noted in Fig. 1, except that a linear gradient from 0.010 *M* to 0.25 *M* potassium phosphate pH 7.4 was used from the start. Transfer factor activity was assayed under conditions given in Fig. 2 except that sRNA was labelled with either C^{14}-leucine (0.28 mg sRNA, 3040 cpm), C^{14}-lysine (0.21 mg sRNA, 2350 cpm, 1.4 × 10^7 cpm/μmole), or C^{14}-proline (0.29 mg sRNA, 2050 cpm, 1.4 × 10^7 cpm/μmole).

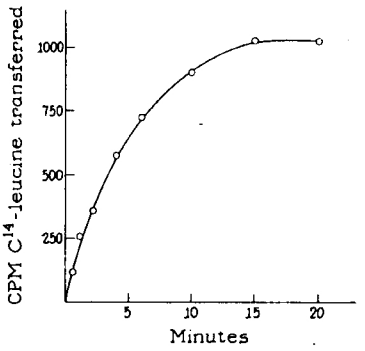


FIG. 4.—Time curve of leucine transfer from sRNA to protein. Incubation conditions as given in Fig. 2, except that the volume was 6.0 ml and contained ribosomes (20 mg protein), 7.2 mg of transfer factor, 5.0 mg of sRNA charged with amino acids including 51,000 cpm C^{14}-leucine. At each time point, 0.50 ml was pipetted into 5 per cent TCA and the precipitate treated as described in Fig. 2.

washed three times, an almost absolute requirement for supernatant appeared. This preparation then served for the assay of the transfer factor. The factor was found to lose activity completely after 1 minute at 70° and to be non-dialyzable. Its presence could be demonstrated not only in the 105,000 × *g* supernatant fraction, but also in the first ribosome wash. The wash fluid had twice the specific activity of whole supernatant, but only a small fraction of the total activity of the supernatant. The factor was partially purified from both sources by essentially similar procedures.

Purification.—After removal of nucleic acids with streptomycin, the solution was brought to 50 per cent saturation with solid ammonium sulfate, the pH being maintained at 7.4, and the precipitate was discarded. The precipitate obtained at 63 per cent saturation was dissolved in 0.01 *M* potassium phosphate pH 7.4,

with 0.004 M β-mercaptoethanol and was dialyzed against the same. This fraction was chromatographed on DEAE-cellulose as shown in Figure 1. Transfer factor activity was eluted at about 0.20 M phosphate concentration. On re-chromatography, the activity peak appeared in the same region of the chromatogram. Marked losses in activity occurred during chromatography and while the column fractions were kept at 4°, resulting in only 3 per cent over-all recovery of activity and 10–15-fold purification. When stored at −20°, however, the concentrated solution retained activity for several weeks. Leucine transfer with increasing amounts of the purified fraction is shown in Figure 2; maximum transfer occurred with 50 μg of protein.

Properties of the Purified Fraction.—Although the purified preparation showed some pyrophosphate exchange with ATP when the complete mixture of amino acids was added, the absence of leucine activating enzyme was shown by the failure of this fraction to transfer radioactive leucine to sRNA (Table 1). This excludes implication of the activating enzymes in amino acid transfer from sRNA. The

TABLE 1

TEST OF PURIFIED TRANSFER FACTOR FOR LEUCINE ACTIVATING ENZYME

Enzyme preparation	C^{14}-leu in sRNA (cpm)
105,000 × g supernatant, 0.30 mg	4,220
Transfer factor, 0.16 mg	58

Each incubation mixture contained: 2.0 mg of sRNA, 4×10^{-5} M C^{14}-leucine (3.7×10^6 cpm/μmole), 0.003 M ATP, 0.01 M PEP, 30 μg/ml pyruvate kinase, 0.008 M GSH, 0.008 M $MgCl_2$, and 0.10 M Tris HCl pH 7.2 in a volume of 0.25 ml. After 20 minutes at 36°, C^{12}-leucine and carrier RNA were added, and RNA precipitated by an equal volume of cold 1 N perchloric acid. The precipitate was washed four times with cold 0.5 N perchloric acid, once with 1:1 ethanol-ether, and counted.

question whether there is a general transfer factor was approached in the experiments shown in Figures 1 and 3. When the DEAE-cellulose column fractions were assayed with sRNA charged with different C^{14}-amino acids, a single identical peak of activity resulted whether C^{14}-leucine, lysine, proline, valine, or tyrosine was used. This is strong evidence in favor of a general transfer factor in contrast to the report of von der Decken and Hultin[5] whose data on differences between valine and tyrosine transfer in the rat liver system appear unconvincing.

Ribosome Specificity of Transfer Factor.—In a previous report[2] we noted that rat liver DOC-particles required a soluble factor partially purified from liver supernatant for transfer of amino acid from sRNA; there the supernatant fraction from rabbit, pigeon, chicken, or calf liver, or from rabbit reticulocytes could replace rat liver supernatant. *E. coli* supernatant, however, was found to be ineffective with rat liver DOC-particles and rat liver supernatant was without effect with *E. coli* ribosomes even though aminoacyl-sRNA of *E. coli* was used as amino acid donor in all cases (Table 2). Hence, the transfer factor has relative specificity for the particle preparation. Similar results have been obtained by Rendi and Ochoa.

TABLE 2

RIBOSOME SPECIFICITY OF TRANSFER FACTOR

Ribosomes	Supernatant fraction	C^{14}-leu trans. (cpm)
E. coli	None	90
E. coli	Liver	80
E. coli	*E. coli*	665
Liver	None	21
Liver	*E.* coli	19
Liver	Liver	244

Incubation conditions for *E. coli* ribosomes were the same as those in Figure 2, except as follows: 1.7 mg ribosomal protein, 0.26 mg of sRNA with 4,190 cpm C^{14}-leucine, 0.8 mg of *E. coli* supernatant, or 1.6 mg of rat liver supernatant. Conditions for rat liver DOC-particles as described previously.[2] *E. coli* sRNA was used with both ribosome preparations.

Properties of the Purified System for Transferring Amino Acids to Protein.—Figure 4 presents a time curve for amino acid transfer in the purified system. From a very early time, there is a fall in rate of transfer which is due largely to loss of amino acid from sRNA; addition of fresh amino acyl-sRNA after 10 minutes resulted in further amino acid transfer.

The cofactor requirement of the system is generally similar to that reported for mammalian preparations except for the high Mg^{++} concentration (0.012 *M*–0.016 *M* is optimal) (Table 3). Magnesium ion, however, can be replaced, at least partially, by spermidine which Cohen and Lichtenstein[6] have shown will replace Mg^{++} in stabilization of heavier ribosomes. In contrast to the liver microsome or DOC-particle system,[1] the effect of SH-compounds is not as striking, although GSH generally stimulates. Both puromycin[7] and chloramphenicol markedly inhibit transfer.

When sRNA charged only with C^{14}-leucine is substituted for fully charged sRNA, transfer is diminished by more than half, indicating that other aminoacyl-sRNA's are required for maximal transfer. Presumably this effect would be more striking

TABLE 3

COFACTOR REQUIREMENTS AND EFFECT OF INHIBITORS

Conditions	C^{14}-leu trans. (cpm)
Complete system	1,690, 1,760
− GSH[1]	1,420
− PEP, kinase, GTP	194
− GTP	319
− PEP, kinase	427
− added Mg^{++}[2]	87
− added Mg^{++}[2] + spermidine phosphate, 0.01 *M*	1,470
sRNA charged with C^{14}-leu, but no other amino acids	653
+ C^{12}-leu, 0.0008 *M*	1,630
+ Puromycin, 0.0004 *M*	46
+ Chloramphenicol, 0.00019 *M*	577
− Ribosomes	17

[1] Transfer factor dialyzed to remove mercaptoethanol.
[2] 0.0008 *M* Mg^{++} present from ribosome solution.
Conditions were the same as in Figure 2; 54 μg transfer factor was present in each tube.

TABLE 4

RETENTION OF ACTIVITY OF sRNA AFTER AMINO ACID TRANSFER

	Pretreatment	Charging of Re-isolated RNA	
Conditions	Leu trans. to protein (%)	C^{14}-leu, cpm / 0.23 mg RNA	mμmoles leu / mg RNA
1. Complete system	27	4,650	1.05
2. Complete system + puromycin	1.9	4,900	1.09

Pretreatment: 4.4 mg of sRNA charged with amino acids, including 12,700 cpm C^{14}-leucine (3.74×10^6 cpm/μmole), ribosomes (40 mg protein), 4 mg of transfer factor, plus cofactors and salts as in Figure 2 were incubated in a volume of 4.0 ml for 10 minutes at 30°; in a second tube (subsequent No. 2), 0.0005 *M* puromycin was included. At the end of incubation, sRNA was recovered and stripped of amino acids as noted in the text. Recovered sRNA was tested for leucine acceptance as described in Table 1, except that 0.23 mg of sRNA was used and 2.7×10^{-5} *M* C^{14}-leucine was present with 1.93×10^7 cpm/μmole.

if purified leucine activating enzyme were used to charge sRNA instead of the crude dialyzed supernatant. Similar results with the liver microsome system were reported by Acs.[8] As shown in Table 3, addition of unlabeled free leucine does not affect the transfer of the sRNA bound leucine. In a separate experiment it was shown, furthermore, that free C^{14}-leucine, C^{14}-threonine, and C^{14}-proline were

not incorporated into protein with the purified system even when ATP was included.

Recovery of Active sRNA after Transfer.—Following incubation of aminoacyl-sRNA with ribosomes, the sRNA was recovered by phenol treatment of the incubation mixture and extraction of the alcohol-precipitated RNA with cold 1 *M* NaCl. This RNA was stripped of residual amino acids by incubation at pH 9 with 0.5 *M* Tris HCl at 36° for 45 minutes. It was then re-precipitated and dialyzed, and tested for acceptance of leucine and of AMP. A similar experiment was carried out with incubation in the presence of 0.0005 *M* puromycin which inhibits incorporation (Table 3). As shown in Table 4, sRNA which has functioned in amino acid transfer has the same activity for accepting leucine as the control. Moreover, recovered sRNA does not accept AMP end groups (Table 5), a result in agreement with findings in whole cells.[11, 12] These data indicate that sRNA remains intact and active after amino acid transfer, and functions as a cofactor in the over-all incorporation of free amino acids being successively charged with amino acid and discharged at the template.

Deacylation and Transfer.—In the experiment recorded in Table 4, the total loss of amino acid from the aminoacyl-sRNA after incubation with and without puromycin was determined, including a rather constant chemical hydrolysis. From these values together with the transfer of leucine into protein, the over-all balance of the amino acid that is liberated is computed. As indicated in Table 6, in addition to transfer there occurs a hydrolysis of amino acids from the RNA. What is particularly significant is that in the presence of puromycin this hydrolysis is larger by an amount similar to the transfer that was prevented by the puromycin. These observations prompted a further exploration of this apparently enzymatic hydrolysis. It appears from Table 7 to be dependent on the same factors that are operating in amino acid transfer into protein. The experiments were carried out in the presence of a puromycin concentration where transfer is inhibited and only hydrolysis is observed. This hydrolysis obviously has a relation to the transfer reaction, in particular since it increases, by blocking the transfer, comparably to inhibition. So far analysis of the hydrolyzed leucine has indicated it to be electrophoretically comparable to free leucine.

Comments.—Nature and generality of transfer factor: Since the transfer factor could be separated from the activating enzyme for the amino acid which it transfers, it appears that the activating enzyme is not part of the peptide linking system. Further proof for this may be seen in the non-specificity of the transfer factor. The activity for all amino acids tested, including leucine, valine, tyrosine, lysine, and proline, was found in the same rather sharp peak on elution from a DEAE-cellulose column. By implication we assume that the peptide linking enzyme does not carry specificity for amino acids.

The reasons for the apparent fragility of transfer protein fractions are not explained. It is not impossible, although no indications have been found so far, that we are dealing not with a single but rather with a multiple fraction. A recombination of various column fractions so far has not shown encouraging results. The function of GTP in the process and its possible relationship to the transfer factor are in urgent need of explanation, and we will return to this in a subsequent communication.

TABLE 5

TEST FOR INTACTNESS OF ADENOSINE TERMINAL OF RECOVERED sRNA

Source of sRNA	cpm / 0.23 mg RNA	mμmoles AMP / mg RNA
1. From complete system	83	0.90
2. From complete system + puromycin	83	0.90
3. Venom-degraded sRNA	2,500	27

The following were incubated in a volume of 0.30 ml at 36° for 30 minutes: 0.23 mg of sRNA pretreated as described in Table 4, 0.00016 *M* C^{14}-ATP (4.02 × 10^8 cpm/μmole), 0.01 *M* PEP, 30 μg/ml pyruvate kinase, 0.00016 *M* CTP, 0.008 *M* GSH, 0.10 *M* Tris HCl pH 7.5, 0.006 *M* $MgCl_2$, and 0.57 mg of a 0–40 per cent saturated ammonium sulfate fraction of *E. coli* 105,000 × *g* supernatant.[9] RNA was precipitated and washed as noted in Table 1 with C^{12}-ATP present. Venom-degraded sRNA was prepared by incubating sRNA with venom phosphodiesterase[10] at pH 8.8 and 35° until 4 per cent degradation occurred.

TABLE 6

BALANCE OF DEACYLATION OF AMINOACYL-sRNA WITH C^{14}-LEUCINE AS MARKER

	No inhibitor	0.0005 *M* puromycin
	Per cent original C^{14}-leucine	
1. Total loss	76	71
2. Transfer	27	2
3. Chemical deacylation	17	17
4. Enzymatic deacylation*	32	52

* No. 1—(No. 2 + 3).

TABLE 7

REQUIREMENTS FOR ENZYMATIC DEACYLATION OF AMINOACYL-sRNA

Conditions	Experiment 1: cpm liberated	Experiment 1: % enzymatic deacylation	Experiment 2: cpm liberated	Experiment 2: % enzymatic deacylation
Chemical hydrolysis	310	—	1,070	—
Complete system	780	26	2,680	39
− ribosomes	300	0	1,170	2.4
− transfer factor	400	5.0	1,400	7.9
− GTP, PEP, & kinase	300	0	1,620	13
Ribosome concentration doubled	1,050	41	—	—
Incubation time	10 min.	10 min.	15 min.	15 min.

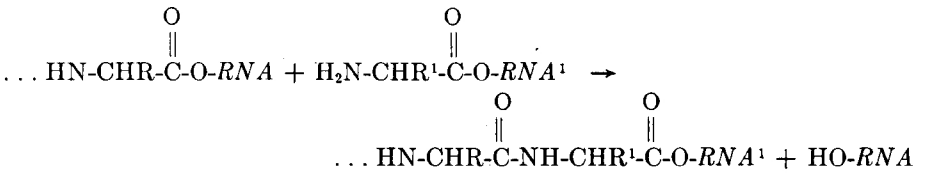
Incubation conditions as in Figure 2, except as follows: Experiment 1, 0.66 mg of ribosomal protein, 0.09 mg of sRNA, 0.0005 *M* puromycin in a total volume of 0.25 ml; Experiment 2, 4.2 mg of ribosomal protein, 0.18 mg of sRNA, 0.15 mg of transfer factor, 0.0005 *M* puromycin in a total volume of 0.50 ml. After incubation at 30°, RNA and protein were precipitated and washed as noted in Table 1, and the precipitate counted. In Experiment 1, 1,790 cpm were present in the precipitate at zero time; in Experiment 2, 4,180 cpm. Liberated cpm is the difference between the zero time and incubated values. Enzymatic hydrolysis is taken as total cpm liberated minus chemical hydrolysis.

Species specificity of transfer factor: In view of the interchangeability of aminoacyl-sRNA's derived from microbial or mammalian cells, it was somewhat surprising that the transfer factor, i.e. the peptide linking enzyme, displays a specificity for the ribosome on which the reaction takes place. The same aminoacyl-sRNA of *E. coli* may be used with different particles but to be joined into the peptide chain, *E. coli* ribosomes will respond only to *E. coli* factor, and mammalian ribosomes will respond only to mammalian transfer factor. The peptide linking factor, therefore, might relate to the protein part of the ribosome, which would most easily explain such a specificity.

Catalytic function of sRNA: The functional integrity of sRNA is maintained after discharging of amino acids on the ribosome; used RNA can be recharged and the accepting adenylic acid terminal remains untouched. In a protein synthesis cycle, therefore, RNA's act in a cyclic fashion as coenzymes which accept the matching amino acid on the activating enzyme, carry it to the microsome, and transfer it into a peptide link.

The meaning of the transfer of some sRNA to the ribosome[13, 14] remains to be

further explored. It would be expected that on every growing peptide chain the terminal amino acid carries its corresponding sRNA:[15]



This could explain the presence of a fraction of the sRNA's on the template.

Enzymatic deacylation of aminoacyl-sRNA and the effect of puromycin: The deacylation of charged sRNA in the puromycin-inhibited system seems to be one of the most promising observations made in the course of these studies. The dependence of this deacylation on the completeness of the system seems to indicate that one is dealing here with a degenerate reaction where, through the action of puromycin, hydrolysis partly takes the place of condensation. These results also provide some clues to the mechanism of inhibition by puromycin. We have found that puromycin acts directly on the ribosome, irreversibly and independently of transfer factor and GTP.[16] The deacylation experiments indicate, however, that the poisoned ribosomes which no longer transfer amino acids to protein are still active in enzymatic deacylation. This suggests that puromycin still leaves intact a partial reaction in aminoacyl transfer to protein, but rather specifically prevents the final condensation of the activated amino acids to peptides.

* This work was supported by research grants from the National Science Foundation and the National Cancer Institute, National Institutes of Health, United States Public Health Service.

† U.S. Public Health Service Postdoctoral Fellow.

[1] Hülsmann, W. C., and F. Lipmann, *Biochim. Biophys. Acta*, **43**, 123 (1960).

[2] Nathans, D., and F. Lipmann, *Biochim. Biophys. Acta*, **43**, 126 (1960).

[3] Lamborg, M. R., and P. C. Zamecnik, *Biochim. Biophys. Acta*, **42**, 206 (1960).

[4] Tissières, A., D. Schlessinger, and F. Gros, these PROCEEDINGS, **46**, 1450 (1960).

[5] von der Decken, A., and T. Hultin, *Biochim. Biophys. Acta*, **45**, 139 (1960).

[6] Cohen, S. S., and J. Lichtenstein, *J. Biol. Chem.*, **235**, 2112 (1960).

[7] Yarmolinsky, M. B., and G. L. de la Haba, these PROCEEDINGS, **45**, 1721 (1959).

[8] Lipmann, F., W. C. Hülsmann, G. Hartmann, H. G. Boman, and G. Acs, *J. Cell. Comp. Physiol.*, **54**, Sup. 1, 75 (1959).

[9] Berg, P., personal communication.

[10] Koerner, J. F., and R. L. Sinsheimer, *J. Biol. Chem.*, **228**, 1049 (1957).

[11] Scott, J. F., Cited in Hoagland, M. B., and L. T. Comly, these PROCEEDINGS, **46**, 1554 (1960)

[12] von Ehrenstein, G., and F. Lipmann, these PROCEEDINGS, in preparation.

[13] Hoagland, M. B., and L. T. Comly, these PROCEEDINGS, **46**, 1554 (1960).

[14] Bosch, L., H. Bloemendal, and M. Sluyser, *Biochim. Biophys. Acta*, **41**, 444 (1960).

[15] Bishop, J., J. Leahy, and R. Schweet, these PROCEEDINGS, **46**, 1030 (1960).

[16] Nathans, D., and F. Lipmann, unpublished observations.

[17] Zillig, W., D. Schachtschabel, and W. Krone, *Z. Physiol. Chem.*, **318**, 100 (1960).

[18] Rendi, R. Robert, and S. Ochoa, personal communication.