may be metastable so that it should at equilibrium decompose into the denatured DNA-mercury complex. We propose that on adding mercury ion to the end-to-end aggregate mercury cross-links are formed between the two complementary single strands that are the cohesive ends. However, when this DNA is diluted into the high chloride ion medium used in the transformation assay the mercury is removed in two steps. First the end-to-end aggregate dissociates, leaving a mercury-denatured DNA complex for each single strand end; then the mercury is complexed away from each end leaving the dissociated half molecules.

Acknowledgments

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Characterization of the Polynucleotide-dependent Transfer Reaction in Protein Biosynthesis Employing a Cell-free System from the Yeast *Saccharomyces fragilis**

Kathleen M. Downey,† Antero G. So,† and Earl W. Davie

ABSTRACT: The transfer of radioactive phenylalanine and lysine from soluble ribonucleic acid to peptide linkage in washed yeast ribosomes, in the presence of polyuridylic acid and polyadenylic acid, respectively, has been studied. For optimal activity, both reactions require guanosine-5′-triphosphate (2–4 mM), Mg^{2+} (6 mM), NH_4Cl (100 mM), spermine (1 mM), polynucleotide (100 μg/ml), aminoacyl-soluble ribonucleic acid, and ribosomes. A supernatant fraction was not required even with ribosomes that were washed with buffer for as many as five times. Ribosomes that were washed with 0.5 M NH_4Cl or 0.5% deoxycholate solutions, however, occasionally showed a significant stimulation by supernatant, but the total activity recovered in these preparations was 10% or less of that present prior to treatment. Observations presented in this report were obtained with buffer-washed ribosomes in the absence of supernatant. The transfer of both amino acids has a *p*H optimum of 7.0. Both reactions are inhibited by preincubation of the ribosomes with *p*-mercuribenzoate. Chloramphenicol, griseofulvin, amphotericin B, and mycostatin have little or no effect on either reaction; puromycin inhibits the incorporation of lysine and phenylalanine about 50%.

Studies by density-gradient sedimentation indicate that the radioactive polyphenylalanine is associated with a monosomal component. Thus far, no evidence has been obtained which would suggest that separate enzymes are involved in the transfer of phenylalanine and lysine.

The final stages in protein biosynthesis involve the transfer of amino acids from aminoacyl-s-RNA into polypeptides bound to ribosomes. In addition to ribosomes, a requirement for at least two soluble enzymes, guanosine triphosphate, messenger RNA, Mg^{2+}, K^+ or NH_4^+, and a sulfhydryl compound have been established in this reaction (Hülsmann and Lipmann, 1960; Nathans and Lipmann, 1960, 1961; Von Ehrenstein and Lipmann, 1961; Nathans *et al.*, 1962; Bishop and Schweet, 1961b; Fessenden and Moldave, 1961; Takanami, 1961; Allende *et al.*, 1962; Lamfrom and Squires, 1962; Nakamoto *et al.*, 1963; Arlinghaus *et al.*, 1963). The present paper describes the characteristics of the transfer reaction with phenylalanyl- and lysyl-s-RNA in the presence of poly-U and poly-A,

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respectively, in a cell-free system from the yeast *Saccharomyces fragilis*.

Materials

Guanosine triphosphate (GTP)[1] and crystalline ATP were purchased from Pabst Laboratories, Milwaukee, Wis. L-[U-^{14}C]Phenylalanine and L-[U-^{14}C]-lysine were purchased from New England Nuclear Corp., Boston, Mass. Poly-U and poly-A were purchased from Miles Chemical Co., Elkhart, Ind. According to the manufacturer, both preparations have sedimentation coefficients ranging from 4 to 7 S. A base analysis of these preparations has not been done. Puromycin and spermine were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, and chloroamphenicol was purchased from Parke Davis and Co., Detroit, Mich. Erythromycin was a gift of Dr. John Corcoran. Griseofulvin was a gift of Ayerst Laboratories Inc., New York City. Both amphotericin B and mycostatin process C were gifts of E. R. Squibb and Sons, New Brunswick, N.J. *p*-Mercuribenzoate, sodium salt, crystalline, was purchased from Sigma Chemical Co., St. Louis, Mo. All other chemicals were commercially available reagent grade.

Methods

Saccharomyces fragilis (American Type Culture 10022) was grown and stored as described by So and Davie (1963). Cells were broken by grinding at 0–4° with two to three times their weight of alumina (Alcoa 305) and extracted with buffer consisting of 0.01 M imidazole, *p*H 7.0, 0.004 M $Mg(OAc)_2$, 0.02 M NH_4Cl, and 0.0005 M β-mercaptoethanol. The suspension was centrifuged at 30,000 × *g* for 30 minutes at 4° and the centrifugation of the crude extract was repeated. In order to sediment the ribosomes, the extract was further centrifuged at 140,000 × *g* for 1.5 hours. The supernatant fraction was decanted and the pellet was suspended by homogenization in ice-cold buffer consisting of 0.01 M imidazole, *p*H 7.0, 0.004 M $Mg(OAc)_2$, and 0.0005 M β-mercaptoethanol, and the ribosomes were resedimented by centrifugation. This washing procedure was repeated once and the final pellet was suspended in 1 ml of buffer containing dimethyl sulfoxide (15% by volume). The ribosomes were rapidly frozen in an ethanol-CO_2 bath and stored at −18° for up to 1 month with little loss of activity. Before use, the ribosomes were thawed and washed for the third time with 0.01 M imidazole, *p*H 7.0, 0.004 M $Mg(OAc)_2$, and 0.0005 M β-mercaptoethanol. The ribosomal pellet was then resuspended in the same buffer with a final concentration of approximately 10 mg/ml ribosomal protein.

The s-RNA was prepared from *S. fragilis* by the method of Holley *et al.* (1961). It was freed of residual amino acids by the procedure of Nathans and Lipmann (1961).

For the preparation of [^{14}C]phenylalanyl-s-RNA, the incubation mixture consisted of 0.10 M Tris, *p*H 7.0, 0.006 M $Mg(OAc)_2$, 0.056 M NH_4Cl, 0.002 M ATP, 0.007 M β-mercaptoethanol, 0.003 mM [^{14}C]phenylalanine (1.0 μc/ml), yeast s-RNA (approximately 1 mg/ml), and 105,000 × *g* yeast supernatant (3 mg protein/ml) prepared according to So and Davie (1963) except that NH_4Cl was substituted for KCl. Fifty-ml reaction mixtures were incubated at 37° for 20 minutes, and the reaction was stopped by the addition of 0.10 volume of 20% potassium acetate, *p*H 5.0, and an equal volume of cold, saturated phenol. The mixture was stirred for 15 minutes at 4° and then centrifuged at 30,000 × *g* for 30 minutes. The water layer was carefully removed with a pipet and the phenol layer was reextracted with 25 ml of 0.2% potassium acetate, *p*H 5.0. The water layers were combined and 2 volumes of 95% ethanol, precooled to −70°, was added with stirring. The suspension was allowed to stand in ice for 30 minutes and was then centrifuged at 30,000 × *g* for 30 minutes. The pellet was washed once with 67% ethanol and dissolved in 1 M NaCl. The residue was removed by centrifugation at 12,000 × *g* for 30 minutes and the aminoacyl-s-RNA was reprecipitated with 2 volumes of cold 95% ethanol. The [^{14}C]phenylalanyl-s-RNA was dissolved in 0.2% potassium acetate, *p*H 5.0, dialyzed against the same buffer overnight, and stored at −18°. Preparations varied in final activity from 50,000 to 100,000 cpm/mg s-RNA. Assuming no dilution by endogenous phenylalanine in the enzyme or s-RNA preparations, 330 cpm is equivalent to 1 μμmole.

For the preparation of [^{14}C]lysyl-s-RNA the reaction mixture consisted of 0.10 M Tris, *p*H 7.0, 0.009 M $Mg(OAc)_2$, 0.05 M KCl, 0.001 M ATP, 0.005 M PEP, 0.1 mg/ml of pyruvate kinase, 0.006 M β-mercaptoethanol, 0.005 mM [^{14}C]lysine (1.0 μc/ml), *Escherichia coli* s-RNA (1 mg/ml), and *E. coli* supernatant (3 mg protein/ml). The rest of the procedure was the same as that described for the preparation of [^{14}C]phenylalanyl-s-RNA. The final activity of the lysyl-s-RNA preparations varied from 100,000 to 300,000 cpm/mg s-RNA. With the previously mentioned qualifications, 1 μμmole of lysine is equivalent to 200 cpm.

For [^{14}C]phenylalanine incorporation, the radioactive product was precipitated and washed in the presence of nonradioactive phenylalanine and counted in a Packard liquid scintillation counter as previously described (So and Davie, 1963). For poly-A-directed amino acid incorporation, the reactions were terminated by the addition of 8 volumes of a trichloroacetic acid–tungstate reagent and washed as previously described for polycytidylic acid–directed amino acid incorporation (So and Davie, 1964). In the present experiments, however, the trichloroacetic acid–tungstate reagent contained 15% trichloroacetic acid.

Density-Gradient Sedimentation. Linear gradients of 4.5 ml containing 5–20% (w/w) sucrose in 0.1 M imidazole, *p*H 7.2, 0.0058 M $Mg(OAc)_2$, and 0.1 M NH_4Cl were prepared employing a mixing chamber

[1] Abbreviations used in this work: GTP, ATP, CTP, UTP, the 5′-triphosphates of guanosine, adenosine, cytidine, and uridine, respectively; GDP, guanosine-5′-diphosphate; PEP, phosphoenolpyruvate.

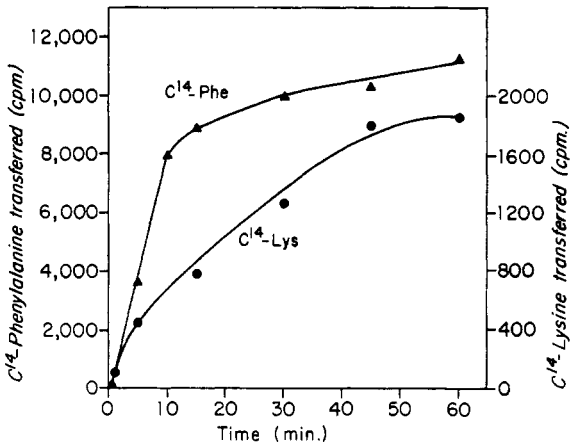

FIGURE 1: The transfer of [^{14}C]phenylalanine and [^{14}C]lysine with time. The incubation conditions are the same as those described in Table I. ▲—▲, transfer of [^{14}C]phenylalanine; ●—●, transfer of [^{14}C]lysine.

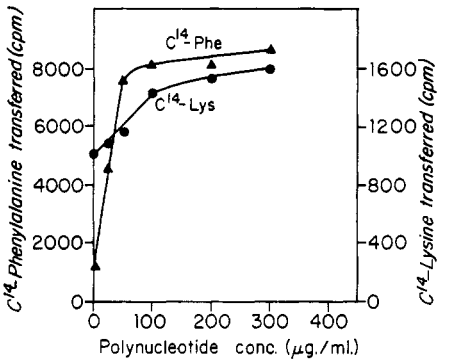

FIGURE 2: The effect of polynucleotide concentration on the transfer reaction. See Table I for incubation conditions. ▲—▲, transfer of [^{14}C]phenylalanine; ●—●, transfer of [^{14}C]lysine.

described by Britten and Roberts (1960), and allowed to stand at 4° overnight. For analysis, 0.3 ml of reaction mixture was layered over each gradient tube and the tubes were centrifuged at 4° in the SW-39 rotor of the Spinco Model L ultracentrifuge for 2 hours at 28,000 rpm. The 2-hour centrifugation included a gradual acceleration period of 3–4 minutes. It did not include the deceleration period of approximately 15 minutes. The contents of the tubes were analyzed by puncturing a small hole in the bottom of each tube and collecting fractions of 8 drops by gravity flow. Each tube yielded approximately thirty-six fractions. Duplicate tubes were run for measuring radioactivity and absorbance. In order to determine the distribution of radioactivity, fractions were collected in tubes containing 1.0 ml of 98% formic acid, and the radioactivity was determined directly in a liquid scintillation counter according to the method of So and Davie (1963). For measuring absorbance, nonradioactive phenylalanyl-s-RNA was used in the reaction mixture, and 1.0 ml of water, instead of formic acid, was added to each fraction. Absorbance readings were then made at 260 mμ in a Beckman DU spectrophotometer.

Sedimentation coefficients for the yeast ribosomes were determined in a Spinco Model E ultracentrifuge at a rotor speed of 29,500 rpm. A complete reaction mixture containing poly-U and phenylalanyl-s-RNA was incubated for approximately 5 minutes and immediately centrifuged at 20° employing schlieren optics. The ribosome concentration was varied from 1 to 4 mg protein/ml and s_{20} values were extrapolated to zero concentration. No corrections were made for buffer salts.

Results

General Requirements for Amino Acid Transfer. The requirement for phenylalanine and lysine transfer from aminoacyl-s-RNA to peptide linkage in yeast ribosomes is shown in Table I. The addition of radioactive phenylalanyl-s-RNA, containing 35,000 cpm, led to the transfer of [^{14}C]phenylalanine (10,850 cpm) into polypeptide in the complete system after a 10-minute incubation at 25°. [^{14}C]Lysine (2900 cpm) was transferred when radioactive lysyl-s-RNA, containing 81,000 cpm, was incubated in the complete system at 25° for 30 minutes.

When various components such as ribosomes, polynucleotide, GTP, spermine, NH_4Cl, or $Mg(OAc)_2$

TABLE I: Requirements for the Transfer Reaction in Yeast.[a]

Conditions	[^{14}C]Phenylalanine Transferred (cpm)	[^{14}C]Lysine Transferred (cpm)
Complete	10,850	2,900
Minus ribosomes	0	0
Minus poly-U or poly-A	1,190	1,890
Minus GTP	660	310
Minus spermine	2,810	1,460
Minus NH_4Cl	800	80
Low Mg^{2+} (0.8 mM)	2,900	220

[a] The complete reaction mixture contained 0.1 M imidazole, *p*H 7.0; 5.8 mM $Mg(OAc)_2$; 100 mM NH_4Cl; 0.8 mM spermine; 2.0 mM GTP; 100 μg/ml poly-U or poly-A; 1–2 mg/ml ribosomal protein; and approximately 0.5 mg/ml [^{14}C]phenylalanyl-s-RNA or [^{14}C]-lysyl-s-RNA. The reaction mixtures were preincubated for 3 minutes at 25° and the reaction was started by the addition of either [^{14}C]phenylalanyl-s-RNA or [^{14}C]-lysyl-s-RNA. The tubes containing [^{14}C]phenylalanyl-s-RNA were incubated for 10 minutes at 25°, while those containing [^{14}C]lysyl-s-RNA were incubated for 30 minutes at 25°.

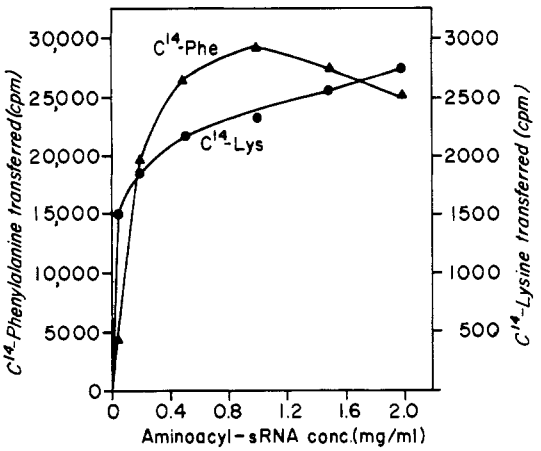

FIGURE 3: The effect of aminoacyl-s-RNA concentration on the transfer reaction. The incubation conditions are the same as those described in Table I. ▲—▲, transfer of [^{14}C]phenylalanine; ●—●, transfer of [^{14}C]-lysine.

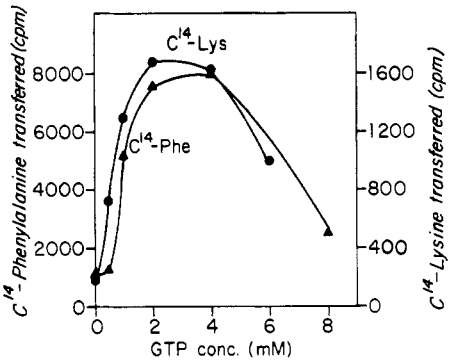

FIGURE 4: The effect of GTP concentration on the transfer reaction. ▲—▲, transfer of [^{14}C]phenylalanine; ●—●, transfer of [^{14}C]lysine. See Table I for incubation conditions.

were omitted or lowered, the amino acid incorporation was considerably reduced.

Neither transfer reaction required a supernatant fraction even with ribosomal preparations that were washed five times. Indeed, the addition of crude supernatant (2–3 mg protein/ml) to the reaction mixture inhibited the transfer of both phenylalanine and lysine about 50%.

Ribosomes treated with 0.5 M NH_4Cl or 0.5% deoxycholate solutions showed a phenylalanine incorporation ranging from approximately 400 to 1500 cpm/mg ribosomal RNA, in contrast to untreated ribosomes (washed three times with buffer) which routinely display an activity ranging from about 10,000 to 25,000 cpm/mg ribosomal RNA. When supernatant was added to the 0.5 M NH_4Cl or 0.5% deoxycholate-treated ribosomes, activity ranging from approximately 1000 to 2800 cpm/mg ribosomal RNA was obtained which in some cases amounted to a stimulation of up to 5-fold. This stimulation, however, was not always observed and, furthermore, the maximal activity obtained with treated ribosomes was less than 10% of that with untreated ribosomes. Thus, all succeeding experiments were performed with buffer-washed ribosomes in the absence of supernatant.

Characteristics of the Two Transfer Reactions. A time curve for the transfer of phenylalanine and lysine is shown in Figure 1. It can be seen that the transfer reaction of both amino acids continues for about 45 minutes, although the rate falls off in each reaction. In these experiments, 74% of the [^{14}C]phenylalanine and 5% of the [^{14}C]lysine were transferred after the 60-minute incubation at 25°.

The effect of polynucleotide concentration is shown in Figure 2. With the large polymers employed in the present experiments, transfer of both [^{14}C]phenylalanine and [^{14}C]lysine leveled off at approximately 100 μg/ml of poly-U and poly-A, respectively. Transfer of phenylalanine, however, was considerably more dependent on the presence of poly-U than was the transfer of lysine on poly-A. The effect of increasing concentrations of aminoacyl-s-RNA is shown in Figure 3. The optimal concentration of phenylalanyl-s-RNA was about 0.5–1.0 mg/ml. Higher concentrations (2.0 mg/ml) inhibited the reaction approximately 20%. Little inhibition of phenylalanine incorporation was observed in the presence of crude uncharged s-RNA. Thus, the addition of 1.0 mg of crude uncharged s-RNA reduced the incorporation of phenylalanine about 10% in the presence of 0.5 mg of substrate phenylalanyl-s-RNA. With lysyl-s-RNA, a gradual increase in activity was observed in the concentration range of 0.1–2.0 mg/ml.

The optimal concentration of GTP for the transfer of either phenylalanine or lysine was found to be about 2–4 mM (Figure 4). The optimal concentration of GTP was reduced about 10-fold (0.2 mM) in the presence of 2.5 mM PEP and pyruvic kinase (0.1 mg/ml). The addition of 2 mM ATP, CTP, or UTP in the presence or absence of 2 mM GTP had no effect on the reaction. GDP was about 30% as active as GTP at its optimal concentration, which was about 1.0 mM. Arlinghaus *et al.* (1964) have observed similar results with reticulocyte ribosomes, and suggested that GDP was converted to GTP by kinase reactions. It is probable that the same reaction occurs in the present system.

As in other microbial systems, the polyamine, spermine, has a stimulatory effect on the transfer of both phenylalanine and lysine. The optimal spermine concentration for transfer of both amino acids was found to be 0.8 mM in the presence of 6.0 mM magnesium acetate. These results are similar to those of Martin and Ames (1962) who first demonstrated a polyamine effect on amino acid incorporation in *Salmonella typhimurium* and those of Bretthauer *et al.* (1963) who observed a similar effect in the yeast *Saccharomyces dobzhanski* × *Saccharomyces fragilis.*

Figure 5 shows the effect of magnesium acetate concentration on the transfer reaction. Approximately 6

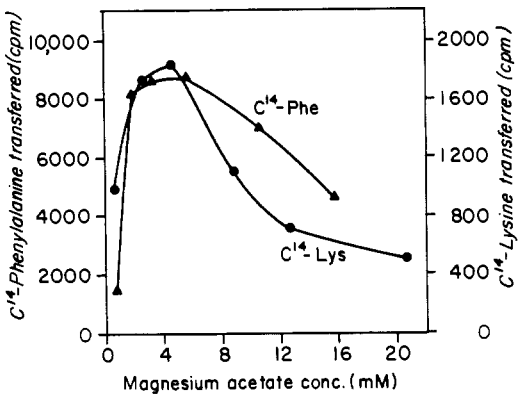

FIGURE 5: The effect of $Mg(OAc)_2$ concentration on the transfer reaction. ▲—▲, transfer of [^{14}C]phenylalanine; ●—●, transfer of [^{14}C]lysine. See Table I for incubation conditions.

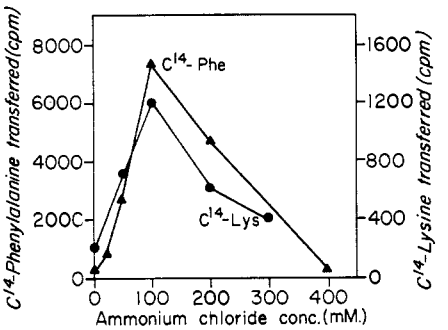

FIGURE 6: The effect of NH_4Cl on the transfer reaction. ▲—▲, transfer of [^{14}C]phenylalanine; ●—● refers to the transfer of [^{14}C]lysine. See Table I for incubation conditions.

mM magnesium ion was optimal for both reactions in the presence of 0.8 mM spermine. In the absence of spermine, the optimal magnesium ion concentration was increased to about 10 mM.

Both transfer reactions were found to be stimulated dramatically by ammonium ions (Figure 6). The optimal concentration was approximately 100 mM for both lysine and phenylalanine. The *p*H optimum for both transfer reactions was found to be 7.0 (Figure 7). This is similar to that found for the overall reaction for the incorporation of [^{14}C]leucine in the crude yeast system (So and Davie, 1963).

Effect of Inhibitors. The effect of a series of antibiotics on the two transfer reactions in yeast is shown in Table II. Chloramphenicol and erythromycin A have little effect on the transfer of phenylalanine and inhibit the transfer of lysine only 15–20%. Puromycin was only a slightly better inhibitor of both reactions, causing an inhibition ranging from 40 to 60% even at a concentration of 0.6 μmole/ml. The fungicides griseofulvin, amphotericin B, and mycostatin, at concentrations up to 0.5 mg/ml, had no effect on either reaction. *p*-Mercuribenzoate was found to be a good inhibitor of the two reactions (Figure 8). Inhibition of 50% was found for both amino acids by a 3-minute preincubation of the ribosomes with 0.8–1.0 mM *p*-mercuribenzoate.

TABLE II: Effect of Antibiotics on the Transfer Reaction in Yeast.[a]

Additions	[^{14}C]-Phenylalanine Transferred (cpm)	[^{14}C]-Lysine Transferred (cpm)
None	4470	3900
0.1 μmole chloramphenicol	4210	3400
1.0 μmole chloramphenicol	4230	3170
0.1 μmole erythromycin A	3990	3270
1.0 μmole erythromycin A	4300	3040
0.1 μmole puromycin	3520	2230
0.6 μmole puromycin	2620	1380

[a] The reaction conditions were the same as those described in Table I except that the tubes were preincubated for 3 minutes in the presence of the various antibiotics.

Nature of the Ribosome Complex. Various investigators have shown that amino acid incorporation is primarily associated with polysomes which apparently are held together by messenger ribonucleic acid. The polysomes are readily formed in the presence of messenger polyribonucleic acids such as poly-U (Barondes and Nirenberg, 1962; Spyrides and Lipmann, 1962; Gilbert, 1963). Thus, it was of interest to examine the present amino acid–incorporating system for similar effects. A density-gradient centrifugation pattern for the reaction mixture in the presence and absence of poly-U is shown in Figure 9. It can be seen that the radioactive phenylalanine in the presence of poly-U was associated with the main ribosomal peak. In the absence of poly-U, no radioactive phenylalanine was bound to the ribosomes. Control experiments have shown that approximately 70% of the ribosome-bound radioactivity was precipitable by hot trichloroacetic acid, which indicates that it was present in polypeptide linkage. Experiments employing the analytical ultracentrifuge showed that the main ribosomal peak has an s^0_{20} value of 74 S. This value was not corrected for viscosity and density, and it is assumed that this particle corresponds to the yeast ribosome, described by Chao and Schachman (1956), which had a sedimentation coefficient of 80 S.

Discussion

In the present experiments employing ribosomes from the yeast *S. fragilis*, a supernatant requirement was not

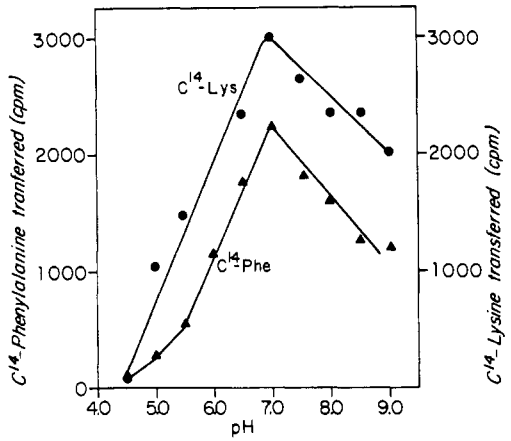

FIGURE 7: The effect of *p*H on the transfer reaction. ▲—▲, transfer of [^{14}C]phenylalanine; ●—●, transfer of [^{14}C]lysine. The incubation conditions were the same as in Table I except that the buffer consisted of 0.05 M Tris, 0.05 M imidazole, and 0.05 M acetic acid, titrated to the appropriate *p*H.

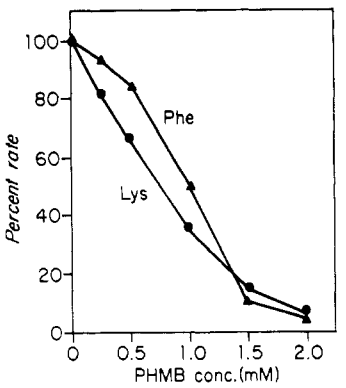

FIGURE 8: The effect of *p*-mercuribenzoate (PHMB) on the transfer reaction. ▲—▲, transfer of[^{14}C] phenylalanine; ●—●, transfer of [^{14}C]lysine. The incubation conditions are the same as those described in Table I except that the tubes were preincubated 3 minutes with increasing concentrations of *p*-mercuribenzoate.

readily demonstrated in the transfer of [^{14}C]phenylalanine from phenylalanyl-s-RNA or [^{14}C]lysine from lysyl-s-RNA to peptide linkage in the ribosomes. Repeated washing of the ribosomes with buffer failed to release the transfer enzyme(s). On occasion, ribosomes that were treated with 0.5 M NH_4Cl or 0.5% solutions of deoxycholate were stimulated by supernatant, but these preparations had very low activity. In contrast, ribosomes prepared from *Bacillus subtilis* or *E. coli* by procedures similar to those presently employed for yeast (washed with buffer) showed an absolute dependence on supernatant in the transfer reaction (K. M. Downey, unpublished observations). Thus, with the yeast preparations, the transfer and polymerizing enzymes are tightly bound to the ribosomes perhaps in a manner similar to reticulocyte ribosomes (Arlinghaus *et al.*, 1963). Siegel and Sisler (1964) reported that a supernatant fraction was not required in the transfer reaction with *Saccharomyces pastorianus*; however, Lucas *et al.* (1964) and Heredia and Halvorson (1965) observed a supernatant requirement in the transfer reaction for *Saccharomyces cerevisiae* and the hybrid yeast *S. dobzhanski* × *S. fragilis*, respectively.

In our previous studies of the incorporation of free amino acids, supernatants from *E. coli* and rat liver were active with yeast ribosomes (So and Davie, 1963). It is apparent from the present experiments that the *E. coli* and rat liver supernatants merely provided activating enzymes and s-RNA. It was also observed that amino acid incorporation with yeast ribosomes and yeast supernatant or yeast ribosomes and *E. coli* supernatant was not affected by chloramphenicol (So and Davie, 1963). Thus it was suggested that the site of chloramphenicol sensitivity in the *E. coli* system was localized in the ribosomal fraction. Since the yeast transfer enzymes are tightly bound to the yeast ribosomes, transfer enzymes from *E. coli* were not required in the mixing experiments with *E. coli* supernatant and yeast ribosomes. Thus the *E. coli* ribosomal fraction, the transfer enzymes, or both may be sensitive to chloramphenicol, since the aminoacyl-s-RNA synthetases from *E. coli* are resistant to this antibiotic. This is consistent with the findings of Nathans and Lipmann (1961) which showed that chloramphenicol blocks the transfer of amino acids from aminoacyl-s-RNA to the ribosome-messenger complex.

The inhibition of the transfer reaction by crude supernatant was due only in part to the presence of s-RNA in this fraction. Additional experiments will be required to clearly define this inhibition that might be due in part to nuclease activity. Previously we noted an inhibition of the yeast system by crude s-RNA for the incorporation of free amino acids (So and Davie, 1963). This inhibition may be due to a sensitivity of one or more of the aminoacyl-s-RNA synthetases to crude s-RNA. Inhibition of the transfer of other amino acids from aminoacyl-s-RNA to ribosomes, however, has not been ruled out.

Essentially identical optimal conditions were obtained in this system for both the transfer of lysine and phenylalanine from their respective aminoacyl-s-RNA's to ribosomes. Furthermore, no differential inhibition by *p*-mercuribenzoate or puromycin was noted. Thus, the possibility that separate enzymes are involved in the transfer of phenylalanine or lysine has not been established. Von der Decken and Hultin (1960) presented evidence that different enzymes might be involved in the transfer of [^{14}C]valine and [^{14}C]tyrosine from aminoacyl-s-RNA in a rat liver system. With the *E. coli* transfer factor, Nathans and Lipmann (1961) found transfer activity for all amino acids tested including leucine, valine, tyrosine, lysine, and proline.

In the present experiments, the polyphenylalanine formed in the transfer reaction was found to be as-

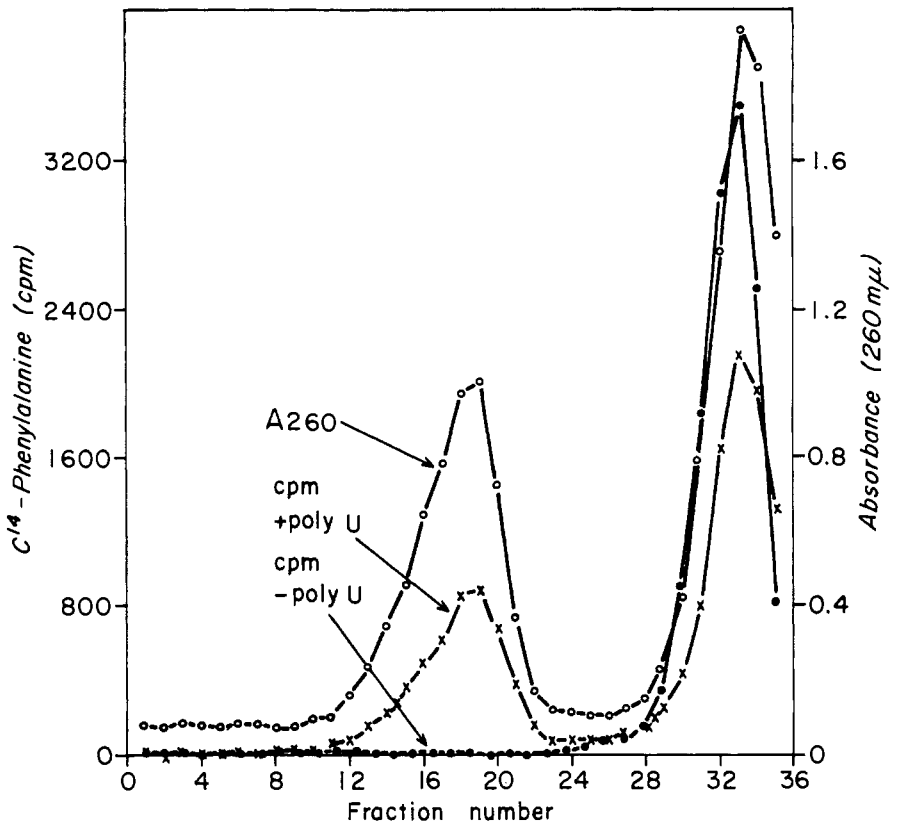

FIGURE 9: Labeling pattern of yeast ribosomes on a sucrose density gradient. The ribosomes were incubated in the complete system with [^{14}C]phenylalanyl-s-RNA as described in Table I. After the incubation period, the incubation mixtures were chilled in ice for 2 minutes and 0.3 ml was then layered over each gradient as described under Methods. O—O, absorbance at 260 mμ in the presence and absence of poly-U; ●—●, [^{14}C]phenylalanine in the absence of poly-U; X—X, [^{14}C]phenylalanine in the presence of poly-U.

sociated with single ribosomes. This is probably caused by the high ratio of polynucleotide to ribosomes employed in the incubation mixture, which would not be particularly favorable for polysome formation (Takanami and Okamoto, 1963). With reticulocyte ribosomes, both single ribosomes and aggregates have been observed in the presence of poly-U (Rich *et al.*, 1963; Hardesty *et al.*, 1963; Allen and Zamecnik, 1963; Gierer, 1963). Marcus *et al.* (1963) have noted that, in the presence of short-chain poly-U, single ribosomes from the yeast *S. dobzhanski* × *S. fragilis* function in polypeptide synthesis. With long-chain poly-U, polysomes were formed. In the *E. coli* system, however, the incorporation of phenylalanine occurs in the polysome fractions in the presence of poly-U (Barondes and Nirenberg, 1962; Spyrides and Lipmann, 1962; Gilbert, 1963). The possibility of converting polysomes into single ribosomes during the *in vitro* incubation, however, has not been ruled out. Degradation of radioactive poly-U was noted under conditions similar to those used in the regular reaction mixture. Approximately 35% of the [^{14}C]poly-U became soluble in an alcohol–magnesium acetate mixture after a 10-minute incubation at 25° in the presence or absence of aminoacyl-s-RNA. This is in contrast to the *E. coli* system where 85% of the [^{3}H]poly-U was found to be degraded in 3 minutes at 37° (Barondes and Nirenberg, 1962). However, the degradation of polysomes by nuclease activity is still a distinct possibility.

Previous studies with *E. coli*, reticulocytes, and rat liver transfer enzymes demonstrated the importance of sulfhydryl groups in the transfer step (Von der Decken and Hultin, 1960; Nathans and Lipmann, 1960, 1961; Hülsmann and Lipmann, 1960; Bishop and Schweet, 1961a). The inhibition of the yeast-transfer reactions by *p*-mercuribenzoate suggests a similar sulfhydryl requirement in this system.

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