Transfer of Amino Acids from Aminoacyl Soluble Ribonucleic Acid to Protein by Cell-Free Extracts from Yeast*

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ABSTRACT: The characteristics of the transfer of phenylalanine from phenylalanyl soluble ribonucleic acid to polypeptides have been studied using cell-free extracts from yeast. Optimal conditions for the transfer reaction have been found to be pH 6.5 and 20°. Tris significantly accelerates the spontaneous hydrolysis of phenylalanyl-s-RNA at pH values above 6.5. The transfer of phenylalanine shows an absolute requirement for the 105,000 × *g* supernatant fraction. The over-all process requires magnesium and ammonium, and guanosine triphosphate (GTP) as the only nucleotide. Guanosine diphosphate (GDP) acts as an effective inhibitor by competing with GTP. The binding of phenylalanyl-s-RNA to the ribosome has been isolated as a step prior to amino acid polymerization. No requirement for GTP nor inhibition by GDP could be demonstrated for the binding reaction. Transfer enzymes from yeast and *Bacillus cereus* are not interchangeable.

It has been well established that aminoacy soluble ribonucleic acid, aminoacyl-(s-RNA), is an intermediate in protein biosynthesis. Not only are amino acids transferred from aminoacyl-s-RNA to proteins, but also enzymes catalyzing this reaction have been detected and partially purified from several cellular extracts (Nathans and Lipmann, 1961; Fessenden and Moldave, 1961; Bishop and Schweet, 1961; Takanami, 1961). Two separate stages have been distinguished in the sequence of reactions leading from aminoacyl-s-RNA to polypeptides. However, the mechanisms involved are as yet unclear. Whereas in reticulocytes each of these two distinguishable steps is catalyzed by a different protein fraction (Arlinghaus *et al.*, 1964), this has not as yet been demonstrated with others systems (Nakamoto *et al.*, 1963; Allende *et al.*, 1964).

In yeast there is not much information on the properties of the transfer reaction, although cell-free protein-synthesizing systems have been described for the hybrid *Saccharomyces fragilis* × *Saccharomyces dobzanskii* (Bretthauer *et al.*, 1963), *Saccharomyces fragilis* (So and Davie, 1963), and *Saccharomyces cerevisiae* (Maeda and Imahori, 1963; Lucas *et al.*, 1964). Only very recently, a work has appeared dealing with the transfer reaction in *Saccharomyces fragilis* (Downey *et al.*, 1965). The present paper is concerned with the study of the transfer of amino acids from aminoacyl-s-RNA to polypeptides in cell-free fractions from the yeast hybrid. The poly-U stimulated polyphenylalanine synthesis has been used as the test model.

Material and Methods

Preparation of Yeast Extracts. The yeast hybrid *S. fragilis* × *S. dobzanskii* was used in these experiments. Conditions of growth, composition of the growth medium, and preparation of the crude extracts have been previously described (Bretthauer *et al.*, 1963). Extracts were prepared in the following standard buffer: 10 mM Tris–HCl, pH 7.5, 5 mM magnesium acetate, 10 mM mercaptoethanol, and 10 mM KCl. Ribosomes were prepared by centrifugation of the 20,000 × *g* supernatant at 105,000 × *g* for 1.5 hr in a Spinco Model L centrifuge. The supernatant fluid was passed through a Sephadex G-25 column equilibrated with the standard buffer to remove low molecular weight components. The ribosomal fraction was resuspended in approximately 100 times its volume of the standard buffer and resedimented at 105,000 × *g* for 1 hr. This process was repeated three more times, following which ribosomes were suspended in the standard buffer to give a final concentration of approximately 100 $OD_{260m\mu}$/ml. The ribosome suspension and the 105,000 × *g* supernatant (usually containing 5–7 mg of protein/ml) were stored in small aliquots under liquid nitrogen. *B. cereus* ribosomes were prepared as described elsewhere (Kobayashi and Halvorson, 1966).

Composition of the Reaction Mixture. The following reaction mixture (0.4 ml) was used to assay the transfer activity: 50 mM maleate (ammonium) buffer, pH 6.5 (about 90 mM in ammonium), 1 mM spermidine, 13 mM magnesium acetate, 2.5 mM mercaptoethanol, 1.25 mM

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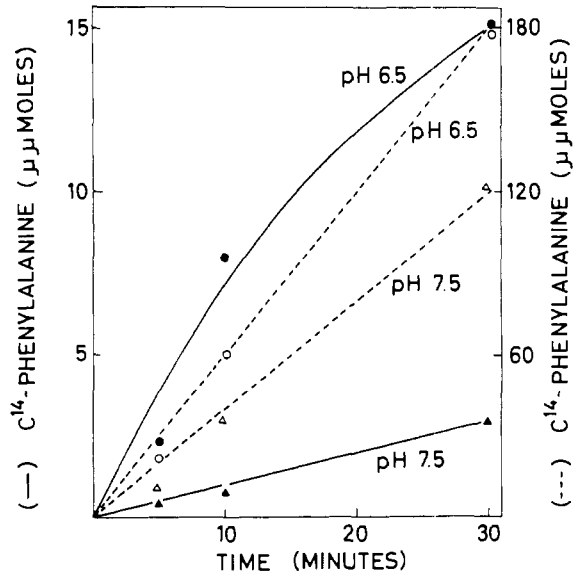

FIGURE 1: Effect of pH on the transfer of phenylalanine. Reaction was carried out using 0.1 mg of [^{14}C]phenylalanyl-s-RNA containing 45 $\mu\mu$moles of phenylalanine (solid lines) or 0.1 mg of stripped s-RNA, 2.5 mμmoles of [^{14}C]phenylalanine, 0.4 μmole of ATP, 1 μmole of phosphoenolpyruvate, and 10 μg of pyruvate kinase (dashed lines). Other conditions as described in Methods except that at pH 7.5 the ammonium maleate buffer was replaced by 25 mM Tris–HCl, pH 7.5, and 0.1 M ammonium chloride. The amount of ribosomal protein was 35 μg.

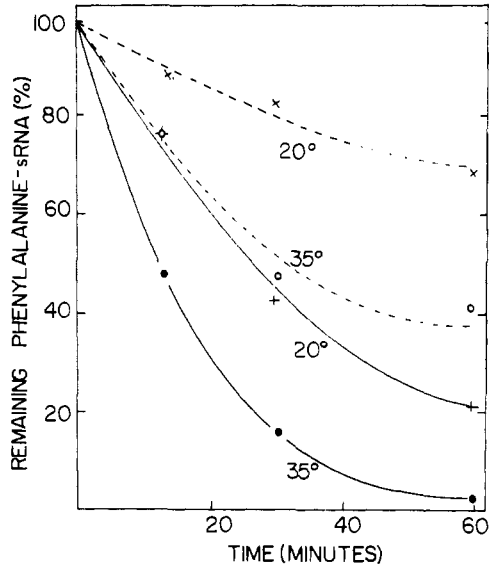

FIGURE 2: Spontaneous hydrolysis of the phenylalanyl-s-RNA at pH 7.5. Mixtures containing 50 mM Tris–HCl (solid lines) or 50 mM phosphate buffer (dashed lines), pH 7.5, and 0.5 mg of s-RNA charged with 35 $\mu\mu$moles of [^{14}C]phenylalanine were incubated in 0.6-ml volume at the temperatures indicated. Aliquots of 0.1 ml were removed at intervals, precipitated with 2 ml of 5% cold TCA, filtered, washed on millipore filters with 15 ml of 5% cold TCA, dried, and counted.

GTP[1]; 50 μg of poly-U, 200–300 μg of supernatant protein, and ribosomes and phenylalanyl-s-RNA as specified. Incubation was at 20°. The reaction was stopped, after addition of 0.8 mg of bovine albumin as carrier, with 3 ml of cold 5% trichloroacetic acid. The mixture was heated at 90° for 15 min and cooled, and the precipitate was washed twice with 3 ml each time of 5% trichloroacetic acid. The protein precipitated was dissolved in 1 ml of hydroxide of Hyamine 10-X (Packard Instruments Co.) and counted in a Tri-Carb liquid scintillation counter with 10 ml of toluene–PPO–POPOP solution (4 g of PPO and 50 mg of POPOP per liter of toluene).

The binding of the phenylalanyl-s-RNA to the ribosomes was measured as described by Nirenberg and Leder (1964).

Preparation of RNA. Soluble RNA was prepared from exponentially growing cells of *S. fragilis* × *S. dobzanskii* (Cherayil and Bock, 1965). Stripped soluble RNA was prepared by incubation with 0.5 M Tris–HCl buffer, pH 9, at 35° for 30 min. Tris was removed by exhaustive dialysis against 200 volumes of 10 mM potassium maleate–10 mM potassium chloride, pH 5.5. The soluble RNA was charged with [^{14}C]phenylalanine using the G-25-treated supernatant as the source of activating enzymes (von Ehrenstein and Lipmann, 1961). After phenol extraction and ethanol precipitation, the aminoacyl-s-RNA precipitate was washed three times with 70% cold ethanol, dissolved in 10 mM potassium maleate–10 mM potassium chloride buffer, pH 5.5, and dialyzed for 20 hr with three changes against 200 volumes of the same buffer.

Protein concentrations were determined by the method of Lowry *et al.* (1951). RNA concentrations were calculated by assuming 1 mg/ml has an optical density at 260 mμ of 20.

Materials. Unless otherwise specified, uniformly labeled L-[U-^{14}C]phenylalanine, with a specific activity of 50 μc/μmole (Schwarz BioResearch, Inc.), was used to charge the s-RNA. All the nucleotides were purchased from Pabst Laboratories. Pyruvate kinase and phosphoenolpyruvate were obtained from California Corp. for Biochemical Research. Poly-U, of an average sedimentation coefficient of 8, was from Miles Chemical Co. Puromycin was purchased from Nutritional Biochemical Corp. PPO and POPOP were products from Packard Instruments Co.

Results

Effect of the pH on the Transfer of Phenylalanine. The

[1] Abbreviations used in this paper: ATP, CTP, GTP, UTP, the 5′-triphosphates of adenosine, cytidine, guanosine, and uridine; GDP, guanosine 5′-diphosphate; TCA, trichloroacetic acid; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-2-(5-phenyloxazolyl)benzene.

transfer of phenylalanine from phenylalanyl-s-RNA to polypeptide is greatly influenced by the pH. At pH 7.5 the rate of incorporation is only 20% of that observed at pH 6.5 (Figure 1). In contrast, phenylalanine incorporation in a mixture including activating enzymes, ATP, and s-RNA is less affected by the pH and occurs at higher rates (Figure 1). During the transfer of phenylalanine at pH 7.5, a rapid deacylation of the phenylalanyl-s-RNA occurred without a concomitant incorporation of the amino acid into proteins. This process appears to be nonenzymatic and is greatly accelerated by the presence of Tris in the incubation mixtures (Figure 2). No breakdown of the s-RNA to small oligonucleotides was observed under the conditions of these experiments. No deacylation of the phenylalanyl-s-RNA was detected in 1 hr at pH 6.5 and 20°, conditions in which maximal rates and extent of the transfer of phenylalanine were observed.

Requirements for the Transfer Reaction. Phenylalanine incorporation is proportional to the amount of ribosomes in the range 0–0.1 mg of ribosomal protein under our assay conditions. At higher concentrations the reaction is eventually inhibited, presumably due to the higher levels of interfering enzymes, such as nucleases, contaminating the ribosomes preparations (Bretthauer *et al.*, 1964). Unwashed ribosomes do not require supernatant for the transfer of amino acids from aminoacyl-s-RNA to proteins. After washing three times with a Tris–Mg buffer (see Methods) a requirement for the supernatant was observed (Table I). Saturating levels of supernatant were usually reached using 0.2–0.3 mg of protein. Bruening (1965) described a more effective method for the purification of yeast ribosomes.

TABLE I: Effect of Various Components of the Reaction Mixture on the Transfer of Phenylalanine.[a]

Conditions	[^{14}C]Phenylalanine Incorporated ($\mu\mu$moles)
Complete	14.2
−Supernatant	0.2
−Ribosomes	1.6
−Poly-U	0.8
−Magnesium	2.1
−Ammonium[b]	0.4
Ammonium replaced by potassium	8.6

[a] The complete reaction mixture is described under Methods. [^{14}C]Phenylalanyl-s-RNA (0.1 mg containing 45 $\mu\mu$moles of [^{14}C]phenylalanine) and 0.04 mg of ribosomal protein were used. Incubation time was 45 min. [b] Tris–HCl (50 mM), pH 6.5, was used as buffer.

The dependence of the transfer of amino acids upon various components of the incubation mixture is presented in Table I. In this experiment, optimal magnesium concentration was attained at 13 mM. The system is *ca.* 50% stimulated by addition of 1 mM spermidine. No basal incorporation was usually observed in the absence of poly-U. A monovalent cation is necessary for activity, ammonium being the most effective (Lubin, 1964; Conway, 1964; Spyrides, 1964). The optimal ammonium concentration is in the neighborhood of 0.1 M.

GTP is the only nucleotide necessary for the transfer reaction (Table II). In some instances, ATP together with GTP has been shown to be necessary for maximal transfer of amino acids (Maeda and Imahori, 1963). As shown in Figure 3 at saturating levels of GTP (see below) the rate and extent of the transfer of phenylalanine from phenylalanyl-s-RNA is independent of ATP, whereas, as should be expected, ATP is absolutely required when the reaction is started with the free amino acid (lower curves in the graph). The transfer reaction is 50% inhibited by 0.05 mM puromycin. Saturating levels of mercaptoethanol were reached at 2.5 mM.

TABLE II: Nucleotide Specificity of the Transfer of Phenylalanine.[a]

Assay Conditions	[^{14}C]Phenylalanine Incorporated ($\mu\mu$moles)
No added triphosphates	0.6
0.12 mM GTP	1.9
1.2 mM GTP	5.2
1.2 mM ATP	0.9
1.2 mM CTP	0.9
1.2 mM UTP	0.8

[a] Reaction mixtures (see Methods), containing nucleotides as indicated, were incubated for 15 min. [^{14}C]-Phenylalanyl-s-RNA (0.35 mg) containing 45 $\mu\mu$moles of [^{14}C]phenylalanine and 0.1 mg of ribosomal protein were used.

Stability of the System. Instability of the transfer enzyme(s) has handicapped its purification. A 5–10-fold purification was achieved by fractionation with ammonium sulfate (40–60% fraction). Fractionation with organic solvents or column chromatography, before or after ammonium sulfate treatment, results in a high degree of inactivation without any improvement in the purification. The supernatant as well as the ribosomes were stored routinely under liquid nitrogen. Under these conditions, the activity is constant over a month; at −20° the transfer activity is lost in a few days; at 4° 50% of the activity is lost in 20 hr. No inactivation could be observed under the conditions of assay for at least 1 hr. A new cycle of freezing and thawing after

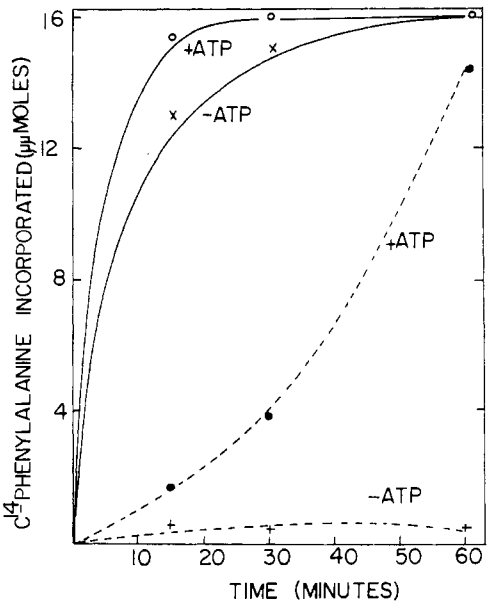

FIGURE 3: Effect of the ATP on the transfer of phenylalanine. Conditions as described in Methods, using 0.35 mg of [^{14}C]phenylalanyl-s-RNA (containing 25 $\mu\mu$moles of [^{14}C]phenylalanine) (solid lines), or 2.5 mμmoles of [^{14}C]phenylalanine (dashed lines). A mixture of 0.5 μmole of ATP and ATP-generating system (1 μmole of phosphoenolpyruvate and 10 μg of pyruvate kinase) was added as indicated. The amount of ribosomal protein was 0.05 mg.

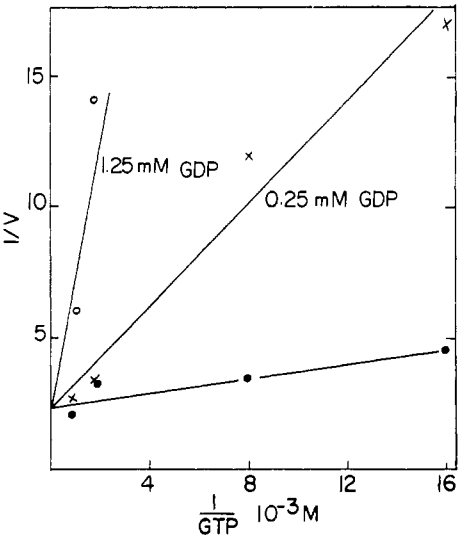

FIGURE 4: Inhibition by GDP of the transfer of phenylalanine. Reaction mixtures (see Methods) containing nucleotides as indicated were incubated for 20 min. [^{14}C]Phenylalanyl-s-RNA (0.14 mg containing 30 $\mu\mu$moles of [^{14}C]phenylalanine) and 0.02 mg of ribosomal protein were used.

storage in liquid nitrogen results in a complete loss of the activity of the ribosomes.

Nucleotide Specificity. The results presented in Table II show that omission of GTP from the incubation mixture results in an almost complete loss of the amino acid incorporation from aminoacyl-s-RNA. The requirement for GTP is quite specific. Neither ATP, CTP, nor UTP can be substituted for GTP (Table II). Incubation over 20 min at high concentrations of nucleotides (0.6–1 mM) eventually results in appearance of activity without added GTP, presumably due to the generation of this nucleotide. A similar phenomenon has been described in *E. coli* (Conway and Lipmann, 1964). GDP is an effective inhibitor by competing with GTP. From a Lineweaver and Burk plot (Figure 4) a K_i very similar (3×10^{-5} M) to the K_m for GTP (10^{-5} M) was calculated.

Binding of the Phenylalanyl-s-RNA to the Ribosomes. The dependence on poly-U of the binding reaction is shown in Figure 5. Under these conditions, saturating levels were reached at 30 mμmoles of base residues. The binding requires a critical concentration of magnesium (optimum, 20 mM). No requirements for nucleotides could be demonstrated. In the absence of GTP, the binding of phenylalanyl-s-RNA to the ribosomes takes

TABLE III: Effect of GTP on the Binding of [^{14}C]Phenylalanyl-s-RNA to the Ribosomes.[a]

Ribosomal Protein (mg)	[^{14}C]Phenylalanyl-s-RNA Bound ($\mu\mu$moles)	
	−GTP	+GTP (2 mM)
35	1.3	1.1
70	3.5	3.1
140	6.1	6

[a] Purified ribosomes (Bruening, 1965) were incubated for 30 min with 10 μg of poly-U, 36 μg of [^{14}C]phenylalanyl-s-RNA containing 20 $\mu\mu$moles of [^{14}C]phenylalanine, and nucleotides as indicated. For other conditions see Methods.

TABLE IV: Differential Effect of GDP on the Binding of Phenylalanyl-s-RNA to the Ribosomes and on the Polypeptide Formation.[a]

	[^{14}C]Phenylalanine ($\mu\mu$moles)		
	−GDP	5 mM GDP	Inhibition (%)
Binding	4.9	4.7	4
Hot TCA ppt[b]	3.4	0.9	73

[a] Conditions as in Figure 5 using 10 μg of poly-U. [b] GTP (1 mM) was present.

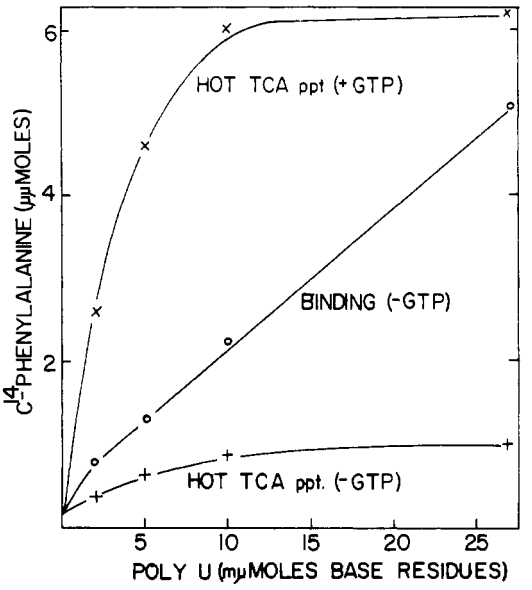

FIGURE 5: Differential effect of GTP on the binding of phenylalanyl-s-RNA to the ribosomes and on the polypeptide formation. Once washed ribosomes (0.14 mg of protein) were incubated for 20 min with [^{14}C]-phenylalanyl-s-RNA (35 μg containing 18 μμmoles of [^{14}C]phenylalanine of a specific activity of 165 μc/μmole) and poly-U under the conditions described for the binding reaction (see Methods). GTP (1 mM) was added when indicated.

place without concomitant protein synthesis (Figure 5) and it is not influenced by addition of 2 mM GTP (Table III). Moreover, concentrations of GDP which effectively block polypeptide synthesis have practically no effect on the binding reaction (Table IV). This lack of requirement for GTP and of inhibition by GDP in the binding of phenylalanyl-s-RNA was also observed using *Bacillus cereus* ribosomes (Y. Kobayashi, 1965, personal communication).

Species Specificity. Soluble RNA is not strictly species specific. Synthesis of hemoglobin was demonstrated using *E. coli* (von Ehrenstein and Lipmann, 1961) or guinea pig (von Ehrenstein and Lipmann, 1961; Bishop *et al.*, 1961) aminoacyl-s-RNA and rabbit reticulocytes ribosomes. We have observed a similar lack of specificity between yeast and *Bacillus cereus*. Yeast aminoacyl-s-RNA is as effective an amino acid donor for protein synthesis with the yeast system as it is with the *B. cereus* system (Table V). However, in heterologous systems, when yeast ribosomes were used with *B. cereus* supernatant fraction or *vice versa*, very little polypeptide synthesis was observed (Table V). The binding of yeast phenylalanyl-s-RNA to the ribosomes was the same with both yeast and *B. cereus* ribosomes (Table V).

Discussion

It has been well established that aminoacyl-s-RNA is an obligatory intermediate in *in vitro* protein synthesis in yeast (Bretthauer *et al.*, 1963; Maeda and Imahori, 1963) as in other organisms. Our previous attempts to characterize the transfer reaction in yeast were handicapped by the low amount of amino acid transferred from aminoacyl-s-RNA to proteins under conditions found to be optimal for the amino acid incorporation with yeast preparations (pH 7.5, Tris buffer, 35°) (Bretthauer *et al.*, 1963). One reason for this difference is the extreme instability of the phenylalanyl-s-RNA at alkaline pH. At pH 7.5 and 35°, a rapid spontaneous deacylation of the phenylalanyl-s-RNA occurs which is accelerated by Tris (Figure 2) (Sarin and Zamecnick, 1964). Under conditions in which the aminoacyl-s-RNA is stable (pH 6.5, maleate buffer, 20°), the rate and extent of the phenylalanine transferred is greatly increased (Figure 1). That this improvement is largely due to the protection of the aminoacyl-s-RNA is suggested by

TABLE V: Species Specificity of the Transfer Reaction.

Yeast		*B. cereus*		[^{14}C]Phenylalanine Transferred	[^{14}C]Phenylalanyl-s-RNA Bound to the Ribosomes (μμmoles)[b]	
Ribosomes	Supernatant	Ribosomes	Supernatant	(μμmoles)[a]	Yeast	*B. cereus*
+	+			12.5		
		+	+	10		
+			+	1.5	5.5	5
	+	+		0.8		

[a] Conditions as described in Methods, using 70 μg of [^{14}C]phenylalanyl-s-RNA, containing 40 μμmoles of [^{14}C]-phenylalanine, 0.04 mg of ribosomal protein, and 0.1 mg of supernatant. Incubation was for 45 min. [b] The binding reaction was measured as indicated in Methods using 0.04 mg of ribosomal protein, 10 μg of poly-U, and 36 μg of [^{14}C]phenylalanyl-s-RNA containing 20 μμmoles of [^{14}C]phenylalanine. Incubation time 30 min.

the fact that the relative activities under the two foregoing pH conditions are almost the same and the total incorporation is greater when an aminoacyl-s-RNA generating system is present (Figure 1). Additional factors superimposed on and presumably derived from the breakdown of the aminoacyl-s-RNA may also contribute for the low activity at pH 7.5, since at 20° the rate of spontaneous deacylation of the phenylalanyl-s-RNA cannot by itself fully account for the low incorporation observed.

The requirements of the transfer reaction in yeast are similar to those reported for other systems. An absolute dependence on magnesium and a monovalent cation (ammonium or potassium) has been observed. In contrast to what happens with preparations from *S. fragilis* (Downey *et al.*, 1965), washed ribosomes showed a requirement for the supernatant fraction (Table I). Compounds able to complex magnesium produce inhibitions that can be reversed by excess of this cation. Inhibitions produced by excess of RNA which have been difficult to explain in some cases (Maeda and Imahori, 1963) may be due to this effect. The transfer reaction requires GTP; other nucleotides are inactive (Table II) (Conway and Lipmann, 1964; Arlinghaus *et al.*, 1964; Fessenden and Moldave, 1963). The stimulatory effect of ATP (Maeda and Imahori, 1963) may be due to the generation of GTP by the action of transphosphorylating enzymes. Guanosine diphosphate acts as inhibitor by competing with GTP (Figure 4) (Conway and Lipmann, 1964). The affinity of the system for both nucleotides is of the same order of magnitude.

Further evidence for the binding of aminoacyl-s-RNA to the ribosomes as an obligatory step in peptide synthesis is presented (Figure 5). In reticulocytes, this reaction is stimulated by GTP and catalyzed by one of the two protein fractions isolated from the supernatant (Arlinghaus *et al.*, 1964). The characteristics of phenylalanyl-s-RNA binding to yeast ribosomes described here are more similar to those found in *E. coli* (Spyrides, 1964; Nakamoto *et al.*, 1963) in which neither requirement for the supernatant fraction nor stimulation by GTP could be demonstrated. The nonessentiality of GTP at this step is supported by the fact that under conditions in which the polypeptide synthesis is completely abolished by GDP, the binding reaction is unaffected (Table IV). The strict dependence on poly-U of the binding of phenylalanyl-s-RNA to the yeast ribosomes (Figure 5) demonstrates the specificity of this reaction.

The species specificity between ribosomes and transfer enzymes from mammalian and bacterial cells (Nathans *et al.*, 1962; Rendi and Ochoa, 1961) have been extended to yeast and *B. cereus* (Table V). Heterologous systems formed by combination of yeast ribosomes with *B. cereus* supernatant fraction or *vice versa* are ineffective for polypeptide synthesis. Since yeast phenylalanyl-s-RNA was used in all cases and both kinds of ribosomes showed the same ability to bind yeast phenylalanyl-s-RNA (Table V), the specificity appears related to the transfer enzymes and their ability to interact with homologous but not heterologous ribosomes.

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The Physical and Chemical Properties of an Immunologically Cross-Reacting Protein from Avian Egg Whites*

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ABSTRACT: The physical and chemical properties of an immunologically cross-reacting macroglobulin in avian egg whites were studied. This protein has comparatively high antigenicity in rabbits and cows and shows extensive immunological cross-reactivity when the proteins prepared from the egg whites of different avian species are tested against antibodies to the chicken protein. The chicken protein was a large protein and had a molecular weight of approximately 0.8×10^6 g mole^{-1} by sedimentation-velocity and light scattering measurements and had an isoelectric pH of approximately 4.5.

In an effort to understand the relatively extensive cross-reactivity, studies were made of the properties of the protein from four distantly related avian species: chicken, duck, penguin, and tinamou. All four proteins had very similar physical and chemical properties.

The avian egg white proteins have provided an extensive and unique system for studying the comparative biochemistry of proteins (Feeney *et al.*, 1960a; Clark *et al.*, 1963; Feeney, 1964). Comparison of structures and functions have shown widely varying properties. These proteins have also been used in fundamental studies of immunochemical phenomena by many investigators. Landsteiner *et al.* (1938) in earlier classical studies found cross-reactions among several ovalbumins. Deutsch (1953) and Wetter *et al.* (1952, 1953) found that components seen in moving boundary electrophoresis cross-reacted with varying degrees in the turkey, pheasant, duck, and chicken. Extensive studies of cross-reacting proteins from several species were recently reported from this laboratory (Miller and Feeney, 1964). In general, it was shown that the more closely related two species are, the greater was the similarity of the corresponding proteins; however, it was found that the various proteins differed in their degree of similarity. The conalbumins gave the most extensive cross-reaction of the major proteins but it was shown that antiserum to a minor component from chicken egg white cross-reacted strongly with the homologous protein from all other egg whites tested, except where this protein did not exist. This minor constituent described by Lush (1961) and later confirmed in our laboratory (Feeney *et al.*, 1963) has been previously called component 18 or C-18.[1] This protein was the only component from egg whites found to have this wide spectrum of cross-reactivity.

The present investigation has been made in order to describe more fully the physical and chemical properties of this purified constituent from chicken egg white and to attempt to understand the reasons for the extensive cross-reactivity between species. The approach to understanding the cross-reactivity has been through a comparative study of the purified component from four distantly related avian species: the chicken, tinamou, duck, and penguin.

Materials and Methods

Procurement of Eggs. The eggs of the chicken (*Gallus gallus*), Japanese quail (*Coturnix coturnix Japonica*), and turkey (*Meleagres gallopavo*) were obtained from the Poultry Department of the University of California at Davis. The eggs of the duck (*Anas platyrhynchos*) were obtained locally. Tinamou eggs (*Eudromia elegans*) were obtained from the San Diego Zoo. The eggs of the penguin (*Pygoscelis adeliae*) were obtained directly from a natural rookery in Antarctica, and those of the kiwi (*Apteryx mantelli*) from a game sanctuary in New Zealand. All eggs were refrigerated within 24 hr after

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[1] The authors propose that component 18 (C-18) be named ovomacroglobulin.