Protein Synthesis by Cell-Free Extracts from Castor Bean Seedlings. I. Preparation and Characteristics of the Amino Acid Incorporating System*

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ABSTRACT: A ribosomal system has been developed from castor bean embryos which appears to be capable of incorporating amino acids. The system meets the commonly accepted requirements for an *in vitro* protein synthesis since it shows an absolute requirement for ribosomes, 105,000*g* supernatant, adenosine triphosphate (ATP), an ATP-generating system, and magnesium ions. In addition, the activity of the system is enhanced by adding soluble ribonucleic acid (s-RNA), guanosine triphosphate (GTP), potassium ions, and a complete mixture of amino acids. Addition of polyuridylic acid brings about a 40-fold stimulation in the incorporation of phenylalanine; polycytidylic acid slightly stimulates the incorporation of proline. The cell-free incorporation of lysine is inhibited by ribonuclease and puromycin, whereas chloramphenicol, streptomycin, actinomycin, and other antibiotics known to interfere with protein and nucleic acids biosynthesis have no effect. Attempts to interchange ribosomes and 105,000*g* supernatant from castor bean embryos and *Escherichia coli* gave presumptive evidence for a marked degree of species specificity.

Protein synthesis has been conclusively demonstrated in many instances with ribosomal preparations from animal and microbial origin (for reviews, see, *e.g.*, Berg, 1961; Simpson, 1962; McQuillen, 1962). Evidence for such a synthesis has been obtained by using natural messenger RNA (homologous and heterologous) as well as synthetic polynucleotides (Nirenberg and Matthaei, 1961).

The development of such systems from higher plants has been less successful. Webster (1959, 1960) and Raacke (1959) have prepared cell-free systems from pea seedlings, capable of incorporating radioactive amino acids in what has been termed soluble protein. These results have been criticized by Lett and Takahashi (1962) who have shown that the production of proteins by the systems could be attributed to a leakage of protein from the particulate preparation rather than to a *de novo* synthesis. Webster *et al.* (1962) reexamined the problem and concluded that it was possible to prepare a system capable of net protein synthesis only from certain lots of peas. Some of the tested varieties of peas yielded systems in which amino acid incorporation into ribosomes was evident but there was no release of newly formed protein. Lett *et al.* (1963) concluded that, in any case, protein synthesis by pea ribosomes occurred only at a tracer level.

Rabson and Novelli (1960) and Mans and Novelli (1964) have prepared a system from maize endosperm which appears to incorporate amino acids into ribosomes. The system requires ATP,[1] Mg^{2+}, an ATP-generating system, and is stimulated by GTP and s-RNA (Mans *et al.*, 1964). The activity is inhibited by RNAase and chloramphenicol.

Morton and Raison (1964) have separated two amino acid incorporating systems from wheat endosperm; one of them, the so-called supernatant preparation, appears to be similar to other ribosomal systems.

Marcus and Feely (1965) prepared a system from peanut and wheat embryos that responded to both natural and synthetic messenger RNA's and demonstrated the characteristic requirements for a protein synthesizing system.

Other systems capable of incorporating amino acids have been prepared from plants. However, they were either from chloroplasts (Sissakian, 1963; Parthier and Wollgiehn, 1963; Spencer and Wildman, 1964; Spencer, 1965; Francki *et al.*, 1965) or from mitochondria (Das *et al.*, 1964).

In the present investigation, a ribosomal system has been developed from castor bean seedlings which appears to be capable of incorporating amino acids. The system appears to meet the commonly accepted requirements for being considered a protein synthesizing system.

Materials and Methods

Bacillus subtilis and *Escherichia coli* s-RNA were prepared according to the procedure of Holley *et al.* (1961). s-RNA from peanut cotyledons and castor bean

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[1] Abbreviations used: ATP, GTP, the triphosphates of adenosine and guanosine; PEP, phosphoenolpyruvic acid; RNAase, ribonuclease; DNAase, deoxyribonuclease.

endosperms was prepared essentially according to the procedure developed by Mans *et al.* (1964) for the s-RNA from maize seedlings except that the s-RNA was extracted from the defatted homogenates with aqueous phenol rather than with NaCl. Yeast s-RNA was purchased from Sigma Chemical Co.

ATP and GTP were obtained from Pabst Laboratories; PEP, pyruvate kinase, Tris, spermidine, spermine, and poly-L-proline from Sigma Chemical Co.; synthetic polynucleotides from Miles Chemical Co.; L-[^{14}C]lysine (180 to 222 μc/μmole), L-[^{14}C]phenylalanine (333 μc/μmole), L-[^{14}C]leucine (180 μc/μmole), L-[^{14}C]tyrosine (334 μc/μmole), L-[^{14}C]arginine (222 μc/μmole), L-[^{14}C]isoleucine (240 μc/μmole), L-[^{14}C]-valine (200 μc/μmole) from New England Nuclear Corporation; L-[^{14}C]proline (115 μc/μmole) from Volk Radiochemical Co.

Castor Bean Seedlings. The tegument of castor bean seeds (a commercial variety of *Ricinus communis* L.) was carefully removed with a small dissecting knife, and the seeds were washed three times with sterile water. The seeds were spread on Petri plates containing water-soaked sterile filter paper disks or vermiculite and allowed to germinate at 28° in the dark. Sterile water was added approximately every 12 hr. When the embryos reached a length of 1–1.5 cm (after 50–60 hr), they were removed from the endosperm and the cotyledons, and if not used immediately stored at −70°.

Preparation of Extract. The embryos were homogenized at 0° in a prechilled mortar for 5 min in the presence of approximately one-tenth of their weight of sand and one volume (equal to the embryos' weight) of a solution composed of Tris, pH 7.8 (0.3 M), sucrose (0.4 M), magnesium acetate (0.01 M), mercaptoethanol (0.02 M), KCl (0.06 M), and reduced glutathione (0.003 M).

The homogenate was centrifuged at 20,000*g* for 20 min and the residue was discarded. The supernatant was centrifuged first at 30,000*g* for 30 min, then at 105,000*g* for 120 min. The supernatant was dialyzed in the cold for 18 hr *vs.* a buffer containing Tris, pH 7.8 (0.02 M), magnesium acetate (0.01 M), mercaptoethanol (0.02 M), KCl (0.06 M), reduced glutathione (0.002 M), and Versene, pH 7.8 (0.0001 M) (standard buffer), and used as a source of 105,000*g* supernatant.

The ribosomal pellet from the ultracentrifugation was suspended in standard buffer and centrifuged, in the order, at 105,000*g* for 120 min, at 20,000*g* for 10 min, and, finally, again at 105,000*g* for 120 min to give the washed ribosomes. If the fractions were not used immediately they were stored frozen at −70° and aliquots thawed just before use.

All manipulations were carried in the cold. All glassware was washed with 0.001 M Versene, pH 7.8. The solutions used in the extraction procedure as well as for the assay mixtures were sterilized by passing through Millipore filters.

Assay for Amino Acid Incorporation. The standard assay contained, in a final volume of 1.0 ml, Tris, pH 7.8 (100 μmoles), magnesium acetate (10 μmoles), KCl (60 μmoles), ATP (3 μmoles), GTP (0.1 μmole), PEP (5 μmoles), pyruvate kinase (20 μg), mercaptoethanol (15 μmoles), washed ribosomes (0.2 to 1.0 mg of RNA), 105,000*g* supernatant (0.33 to 1.0 mg of protein), and s-RNA (0.012 to 0.25 mg). Uniformly [^{14}C]amino acid (1.5–4.3 mμmoles) (0.5 μc) was added to each assay together with 0.05 μmole each of the remaining 19 [^{12}C]amino acids. Other additions are reported in the text.

The reaction mixtures were incubated in glass centrifuge tubes at 37° for 30 min. The reaction was terminated by the addition of 40 μmoles of the [^{12}C]-amino acid which corresponded to the [^{14}C]amino acid used in the experiment and trichloroacetic acid to a final concentration of 5%. After heating for 10 min in boiling water, the precipitate was transferred onto filter paper disks (Schleicher and Schull No. 5893), washed with 5% trichloroacetic acid, ethanol–ethyl ether (1:1, v/v), and ethyl ether. In the experiments in which polycytidylic acid and L-[^{14}C]proline were employed, the reaction was terminated by adding 40 μmoles of [^{12}C]proline, 200 μg of poly-L-proline, and trichloroacetic acid to reach a final concentration of 20%.

Radioactivity was measured on a SELO gas flow counter (counting efficiency 10.2%). Counts were performed under conditions of negligible self-absorption and corrected for background activity. When feasible,

TABLE I: Characteristics of L-[^{14}C]Lysine Incorporation into Protein by the Cell-Free System from Castor Bean Seedlings.[a]

Additions or Omissions	[^{14}C]Lysine Incorp[b]	% Inhib
Complete system	16.01	0
− s-RNA	11.15	30
− 105,000*g* supernatant	0.66	95
− Ribosomes	0.07	99
− 105,000*g* supernatant and s-RNA	0.76	95
− Ribosomes and s-RNA	0.09	99
− ATP, PEP, pyruvate kinase, and GTP	0.41	97
− ATP, PEP, and pyruvate kinase	0.46	97
− ATP	2.45	84
− GTP	12.23	23
− 19 [^{12}C]amino acids	10.98	31
+ RNAase (30 μg)	0.17	99
+ DNAase (5 μg)	13.80	13
Complete, deproteinized at 0 time	0.04	..

[a] Composition of the assay mixture is reported in the Methods. When added, ribosomes were equivalent to 0.19 mg of RNA, *B. subtilis* s-RNA was equivalent to 0.15 mg of RNA, and 105,000*g* supernatant was equivalent to 0.61 mg of protein. [b] Values are micromicromoles of L-[^{14}C]lysine (specific activity 180 μc/μmole) per assay.

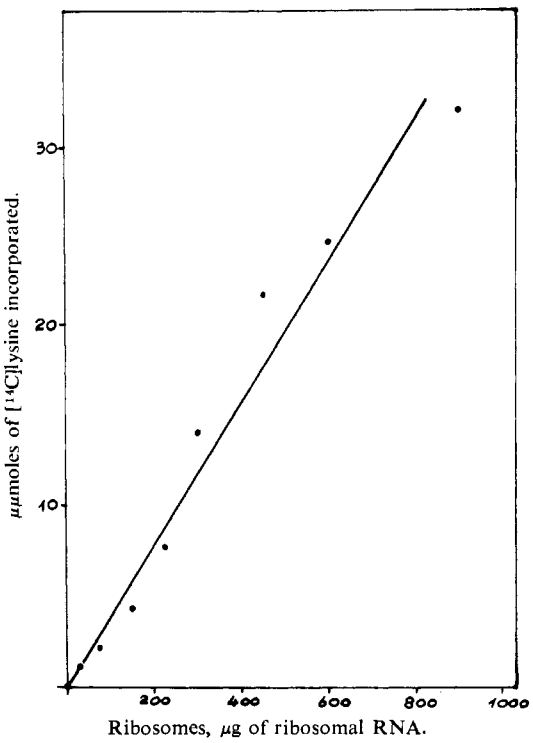

FIGURE 1: Effect of ribosomes concentration on the incorporation of L-[^{14}C]lysine (specific activity 180 μc/μmole) by the cell-free system. The composition of the assay mixture is reported in the Methods; 105,000g supernatant corresponding to 1 mg of protein and 0.1 mg of castor bean s-RNA were added to each assay mixture. The amount of ribosomes was varied as shown.

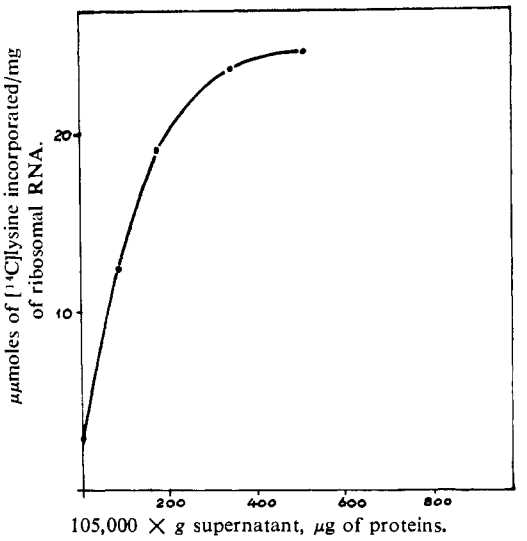

FIGURE 2: Effect of the concentration of the 105,000g supernatant on the incorporation of L-[^{14}C]lysine (specific activity 180 μc/μmole) by the cell-free system. The composition of the assay mixture is reported in the Methods; ribosomes corresponding to 0.48 mg of RNA and 0.1 mg of castor bean s-RNA were added to each assay mixture. The amount of 105,000g supernatant was varied as shown.

radioactivity was expressed as micromicromoles of [^{14}C]amino acid incorporated per mg of ribosomal RNA, otherwise as micromicromoles of [^{14}C]amino acid incorporated per assay.

Other Determinations. Protein was determined according to the method of Lowry *et al.* (1951) using crystalline egg albumin as a standard. RNA was determined by the orcinol method (Schneider, 1957) using yeast RNA (Pabst Laboratories) as a standard.

Results

Requirement for Amino Acid Incorporation. Table I reports the characteristics of the amino acid incorporating system. Like other *in vitro* protein-synthesizing systems, the preparation from castor bean embryos has an absolute requirement for the concomitant presence of ribosomes and 105,000g supernatant. s-RNA only stimulates the incorporation as would be expected because of the presence of s-RNA molecules in the 105,000g supernatant and on the ribosomes. In addition the activity of the system is strictly dependent on the presence of ATP and of an ATP-generating system. GTP appears to stimulate amino acid incorporation in a manner similar to that reported for one of the more purified protein-synthesizing systems from plants (Mans and Novelli, 1964). Omission of the mixture of unlabeled amino acids results in a depression of the incorporation of [^{14}C]lysine. The addition of RNAase to the reaction mixture completely inhibits amino acid incorporation whereas DNAase is only slightly inhibitory.

Increasing the concentration of ribosomes (Figure 1) stimulates a proportional increase in [^{14}C]lysine incorporation. The effect of various concentrations of 105,000g supernatant is reported in Figure 2; at high concentrations of supernatant, the incorporating activity levels off. This is probably due to the rate-limiting effect of some other factor, *e.g.*, ribosomes. In a few instances, the incorporation of amino acids by ribosomes in the absence of 105,000g supernatant was fairly high. This was probably due to an incomplete removal from the ribosomes of the soluble enzymes required for protein synthesis.

s-RNA from both castor bean endosperms and peanut cotyledons stimulates [^{14}C]lysine incorporation to approximately the same extent (Figure 3). As will subsequently be shown, the s-RNA's obtained from microorganisms are less active than plant s-RNA's in stimulating the incorporation of lysine by the system.

As mentioned previously, GTP appears to stimulate [^{14}C]lysine incorporation. Quantitatively, the requirement for GTP is very small since a concentration 6 ×

10^{-6} M is sufficient for maximal incorporation (Figure 4).

[^{14}C]Lysine incorporation is dependent on the concentration of Mg^{2+} in the assay mixture. The incorporating activity is restricted to a narrow range of concentrations (Figure 5). At Mg^{2+} concentrations below 10 μmoles/ml and above 30 μmoles/ml, the incorporation of [^{14}C]lysine is severely inhibited. The highest incorporation appears to take place at Mg^{2+} concentration of about 1.5×10^{-2} M. The effect of K^+ is less drastic since even in its absence the activity of the system is quite good (Figure 6). It should be remembered, however, that the system already contains *ca.* 7–10 μmoles/ml of K^+ since this ion is present in the standard buffer used in the procedure for the preparation of the active fraction. Nevertheless, K^+ stimulates [^{14}C]lysine incorporation up to a concentration of 70 μmoles/ml. Higher concentrations of potassium depress the activity of the system.

The incorporation of L-[^{14}C]lysine is presumably the result of the binding of the amino acid to a growing peptide chain rather than some other phenomena such as an exchange of amino acids in the terminal positions of proteins. The addition of an excess of unlabeled lysine at various time intervals does not decrease the amount of labeled amino acid rendered trichloroacetic-acid insoluble at the time of addition of the cold chaser (Table II). In addition, the incorporation of L-[^{14}C]-lysine at 0° for 30 min is identical with that of the controls in which trichloroacetic acid is added at 0 time.

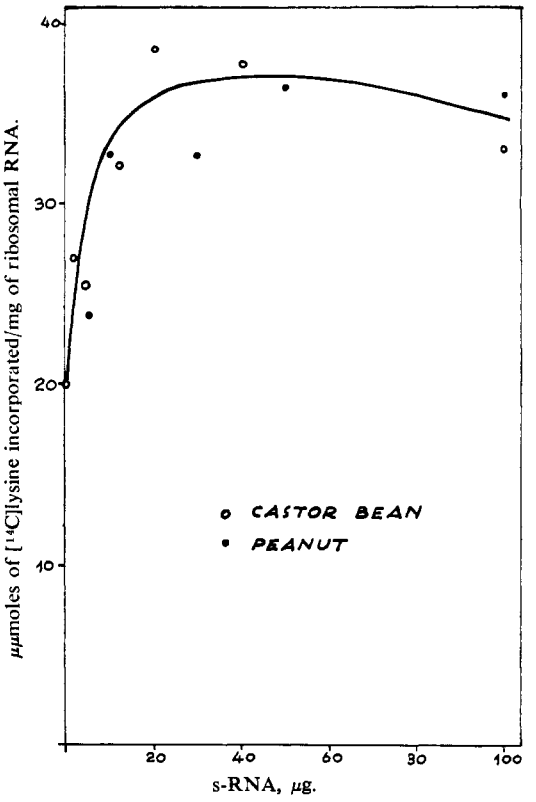

FIGURE 3: Effect of s-RNA concentration on the incorporation of L-[^{14}C]lysine (specific activity 180 μc/μmole) by the cell-free system. The composition of the assay mixture is reported in the Methods; ribosomes corresponding to 0.31 mg of RNA and 105,000*g* supernatant corresponding to 0.37 mg of protein were added to each assay mixture. The amount of s-RNA from castor bean endosperm and peanut cotyledons was varied as shown.

TABLE II: The Effect of an Excess of Unlabeled Amino Acid, Added at Various Time Intervals, on the Incorporation of L-[^{14}C]Lysine.[a]

Time of Addition	[^{14}C]Lysine Incorp[b]	
	Addition of Trichloroacetic Acid	Addition of an Excess of L-[^{12}C]Lysine
0	2.60	...
5	11.70	16.61
10	16.98	18.55
15	19.07	22.07
20	19.68	20.59
30	21.94	24.03
40	23.79	...

[a] Composition of the assay mixture is reported in the Methods; ribosomes equivalent to 0.60 mg of RNA, 105,000*g* supernatant equivalent to 0.29 mg of proteins, and 0.08 mg of castor bean s-RNA were added. A series of duplicate assay mixtures were arranged and at the indicated time intervals the reaction in one sample was stopped by adding trichloroacetic acid; to a second sample, approximately a 1000-fold excess (2 μmoles) of unlabeled L-lysine was added and the incubations continued up to 40 min. [b] Values are micromicromoles of L-[^{14}C]lysine (specific activity 180 μc/μmole) per mg of ribosomal RNA.

The system is also active with other amino acids; incorporations 8–50 times higher than the controls were obtained in the case of L-leucine, L-tyrosine, L-arginine, L-isoleucine, L-phenylalanine, and L-proline. On the other hand, the incorporation of L-valine was not higher than that of the controls.

Effect of s-RNA Obtained from Different Sources. Since s-RNA from different plants stimulates [^{14}C]-lysine incorporation to approximately the same extent (Figure 3), the effect of s-RNA's from other organisms was investigated. As reported in Table III, s-RNA isolated from *B. subtilis* and yeast slightly stimulates the system from castor bean. Thus, whereas [^{14}C]lysine incorporation in the presence of s-RNA from higher plants is 60–100% higher than that of the controls, s-RNA's from the two microorganisms stimulates approximately 30% (a stimulation of 43% by *B. subtilis* s-RNA was found in the experiments of Table I). Such an effect cannot be attributed to the absence (or the

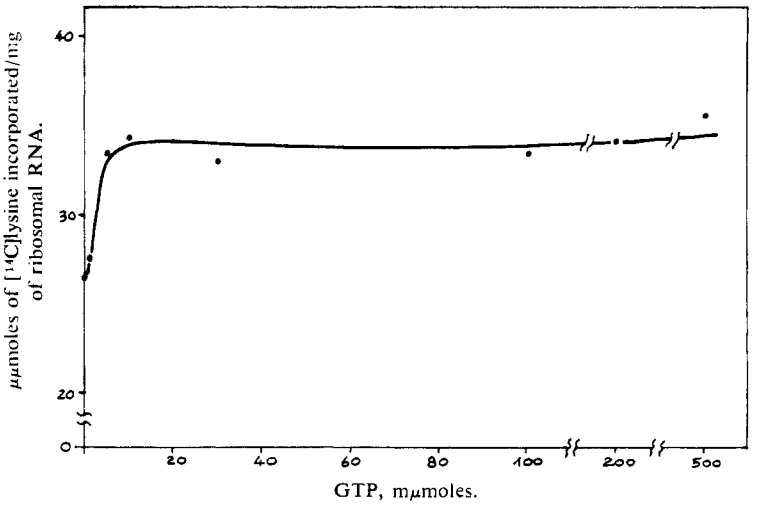

FIGURE 4: Effect of GTP concentration on the incorporation of L-[^{14}C]lysine (specific activity 180 μc/μmole) by the cell-free system. The composition of the assay mixture is reported in the Methods; ribosomes corresponding to 0.25 mg of RNA and 105,000*g* supernatant corresponding to 0.37 mg of protein were added to each assay mixture. The amount of s-RNA from castor bean endosperm was 0.02 mg per assay and that of GTP varied as shown.

TABLE III: Effect of s-RNA from Different Sources on the Incorporation of [^{14}C]Lysine.[a]

Source of s-RNA	[^{14}C]Lysine Incorp[b]	% Stimulation
Complete (control)	17.66	100
+ castor bean (100 μg)	28.32	161
+ castor bean (250 μg)	29.18	166
+ peanut (100 μg)	34.25	191
+ peanut (250 μg)	37.57	218
+ *B. subtilis* (100 μg)	22.56	128
+ *B. subtilis* (250 μg)	19.35	110
+ yeast (100 μg)	21.04	120
+ yeast (250 μg)	23.18	132
deproteinized at 0 time	1.93	...

[a] Composition of the assay mixture is reported in the Methods; ribosomes were equivalent to 0.29 mg of RNA and 105,000*g* supernatant was equivalent to 0.37 mg of protein. s-RNA was omitted from the control experiment. [b] Same as Table II.

lack of activity) of the s-RNA's specific for lysine in the preparations of microbial origin, since such preparations are charged with [^{14}C]lysine when activating enzymes of both bacterial and plant origin are employed. It could be postulated that the effect is due to a degradation of microbial s-RNA's specific for lysine which affects the capacity of transferring the amino acid to the growing peptide chain but not that of accepting the amino acid (Nishimura and Novelli, 1965). Alternatively, a marked species specificity of the s-RNA's from different origins could be a likely explanation. As previously reported (Jacobson *et al.*, 1964; Bennett *et al.*, 1965) there is often no uniform specificity when s-RNA from a given organism is used with aminoacyl-s-RNA synthetases of different origin.

Effect of Inhibitors. Puromycin (10 and 100 μg/ml) inhibited the incorporation of [^{14}C]lysine 71 and 91%, respectively. At a concentration of 30 and 300 μg/ml, daunomycin inhibited the reaction 32 and 46%, respectively. No effect was observed by employing concentrations of up to 100 μg/ml of the following antibiotics which are known to interfere with protein and/or nucleic acid synthesis in other systems: chloramphenicol, streptomycin, cycloheximide, riphamycin, chlortetracycline, neomycin, streptovitacin, actinomycin D, and mitomycin C. Therefore, it may be surmised that, as in the case of other plants, cell-free protein synthesis in castor bean embryos is less sensitive to certain antibiotics (*e.g.*, streptomycin, chloramphenicol) than microorganisms. The incorporation of amino acids directed by synthetic polynucleotides confirms this conclusion (see below).

Response to Synthetic Polynucleotides. In preliminary experiments, not reported here, as compared to control experiments lacking poly-U, magnesium at a concentration of 10 μmoles/ml induced only a twofold increase in L-[^{14}C]phenylalanine incorporation, whereas at 20 and 30 μmoles/ml the stimulation was seven–eightfold. Therefore, magnesium was used at a concentration 2×10^{-2} M in all experiments with synthetic polynucleotides. As reported in Table IV, the addition of poly-U

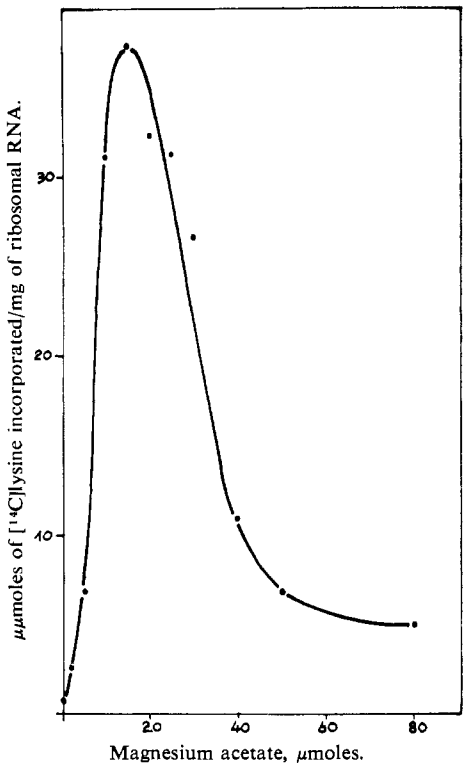

FIGURE 5: Effect of magnesium concentration on the incorporation of L-[^{14}C]lysine (specific activity 180 μc/μmole) by the cell-free system. The composition of the assay mixture is reported in the Methods; ribosomes, 105,000g supernatant, and s-RNA as in Figure 4. The amount of magnesium acetate was varied as shown.

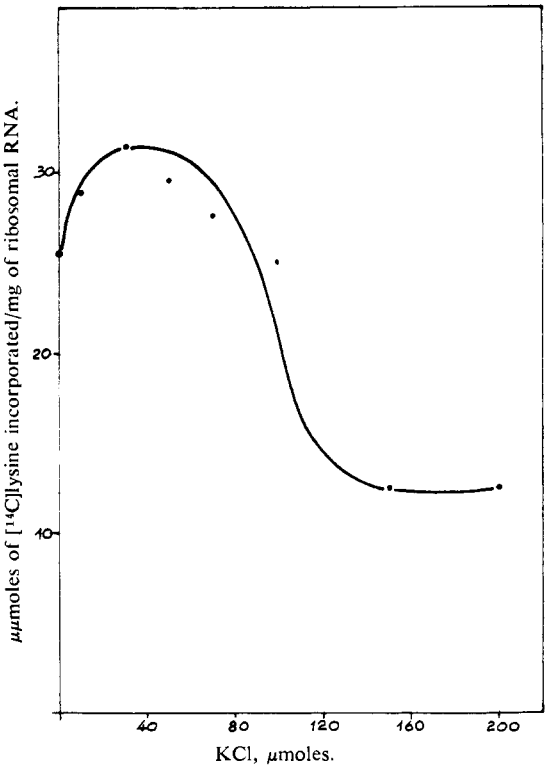

FIGURE 6: Effect of KCl on the incorporation of L-[^{14}C]lysine (specific activity 180 μc/μmole) by the cell-free system. The composition of the assay mixture is reported in the Methods; 105,000g supernatant corresponding to 0.33 mg of protein, ribosomes corresponding to 0.25 mg of RNA, and 0.012 mg of castor bean s-RNA were used. The amount of KCl was varied as shown.

stimulates the incorporation of L-[^{14}C]phenylalanine. The stimulation takes place with both nonpreincubated and preincubated ribosomes.

Of the tested inhibitors, only puromycin consistently inhibits the incorporation of phenylalanine directed by poly-U. The failure of streptomycin to miscode (Davies *et al.*, 1964) is of special interest and may indicate that the miscode effect induced by streptomycin is not universal.

As streptomycin, chloramphenicol is ineffective also in inhibiting protein synthesis directed by synthetic polynucleotides. Polycytidylate stimulates slightly the incorporation of L-proline but has no effect on L-phenylalanine incorporation.

Stability of the System. Storage of ribosomal preparations at +4, −12, and −70° resulted in a very rapid loss of the incorporating activity. However, it appears that if sodium dehydrocholate (0.5%) but not sodium deoxycholate was added to the ribosomal preparations, there was little loss of activity on storage at −70° for up to 6 weeks. At a concentration of 40 μg/ml spermine and spermidine did not improve the stability of the preparations of ribosomes. It may be added that the two polyamines known to stimulate cell-free protein synthesis (Bretthauer *et al.*, 1963) at a concentration of up to 50 μg/ml had no effect on the amino acid incorporation by both preincubated and nonpreincubated ribosomes in the presence of either natural m-RNA or synthetic polynucleotides. The preparations of 105,000g supernatant did not loose their activity when kept frozen at −70° for periods of up to a few months.

Crossed Experiments between the Systems from Castor Bean Seedlings and E. coli. Preliminary attempts were made to determine the degree of exchangeability of the components of the systems prepared from castor bean seedlings and those from *E. coli* (Nirenberg and Matthaei, 1961). Experiments performed on the incorporation of L-lysine, in the presence of natural messenger RNA's, as well as those performed on L-phenylalanine in the presence of poly-U, indicate that a marked degree of species specificity exists when the ribosomes from one source are employed together with the 105,000g supernatant from a different source. Indeed, in all experiments, the use of heterologous 105,000g supernatant causes a marked decrease in the incorporation of amino acids by the ribosomes. The presence of specific inhibitors and/or nucleases is ruled out when both

homologous and heterologous supernatants are employed in the same assay mixture. In addition, in all reaction mixtures, s-RNA from both organisms was added in order to prevent any effect due to the species specificity of amino acid activating enzymes and s-RNA's of different origin. However, it is also evident that in the controls containing ribosomes and supernatants from both sources, the amino acid incorporation was never the sum of the incorporations present in the homologous systems. At present, no convincing explanation can be offered for such results.

Discussion

The amino acid incorporating activity of the system prepared from castor bean embryos appears to have properties and requirements similar to those of other systems developed from plants, microorganisms, and animals. The procedure for the preparation of the castor bean system excludes the presence of mitochondria and chloroplasts from the active fraction so that the incorporating activity must be attributed to the ribosomes present in the cell cytoplasm.

The finding that once the incorporation of a labeled amino acid has taken place it is not affected by the addition, at a later time, of an excess of unlabeled amino acid, suggests that the amino acid is incorporated into a growing peptide chain. Hoagland (1960) reported that this is one of the requirements that must be met before equating amino acid incorporation to protein synthesis.

The incorporation of amino acids by the cell-free preparation is not due to bacterial contamination since counts performed on the intact embryos as well as on the assay mixtures showed that the bacterial population was negligible (*e.g.*, <100 colonies/ml of reaction mixture).

Synthetic polynucleotides may serve as m-RNA for both nonpreincubated and preincubated ribosomes, directing the synthesis of polyamino acids. Such a synthesis is quite evident with poly-U, but less so in the case of poly-C.

TABLE IV: Effect of Polynucleotides on the Incorporation of L-[^{14}C]Amino Acids.[a]

Amino Acid	Addition	[^{14}C]Amino Acid Incorp[b]	Stimulation,-Fold
L-[^{14}C]Phenylalanine	...	6.96	1
	Poly-U	292.52	42
	Poly-U + streptomycin	282.44	40.6
	Poly-U + chloramphenicol	324.04	46.5
	Poly-U + puromycin	63.80	9.2
	Poly-C	8.66	1.2
	Poly-A	9.13	1.3
L-[^{14}C]Isoleucine	...	8.83	1
	Poly-U	8.37	0.9
	Poly-U + streptomycin	9.54	1.1
L-[^{14}C]Proline	...	14.25	1
	Poly-C	61.45	4.3

[a] The composition of the assay mixture as described in the Methods except that Mg^{2+} concentration was raised to 20 μmoles/ml; ribosomes were equivalent to 0.30 mg of RNA, 105,000*g* supernatant to 0.40 mg of protein, and castor bean s-RNA to 0.1 mg. Before adding the L-[^{14}C]amino acid, each assay tube, containing 0.8 ml of the mixture, was preincubated for 20 min at 37°. After that period, 0.5 μc of L-[^{14}C]phenylalanine, 0.5 μc of L-[^{14}C]isoleucine, 0.5 μc of L-[^{14}C]proline, 100 μg of poly-U, 800 μg of poly-C, 200 μg of poly-A, 50 μg of streptomycin, 50 μg of chloramphenicol, and 50 μg of puromycin were added as indicated, and the volume was brought to 1 ml with distilled water. [b] Values are micromicromoles of L-[^{14}C]phenylalanine (specific activity 333 μc/μmole), of L-[^{14}C]isoleucine (specific activity 240 μc/μmole), or of L-[^{14}C]proline (specific activity 115 μc/μmole) per mg of ribosomal RNA.

Amino acid incorporation by the cell-free system is inhibited by puromycin, an antibiotic known to promote the release of the unfinished peptide chains from the ribosomes. RNAase completely inhibits amino acid incorporation whereas DNAase, streptomycin, and chloramphenicol have little or no effect.

Preliminary evidences indicate that amino acid incorporation, directed by either natural or synthetic polynucleotides, is depressed when ribosomes and 105,000*g* supernatants from different sources are employed. Such results may indicate that one or both components of the systems (ribosomes and 105,000*g* supernatant) are at least in part species specific.

TABLE V: [^{14}C]Lysine and [^{14}C]Phenylalanine Incorporation by Homologous and Heterologous Systems from Castor Bean Seedlings and *E. coli*.[a]

Ribosomes	105,000*g* Supernatant	[^{14}C]-Amino Acid Incorp[b]
	Expt I	
E. coli	...	4.93
E. coli	*E. coli*	26.41
E. coli	Castor bean	5.15
E. coli	*E. coli* + castor bean	18.53
Castor bean	...	2.46
Castor bean	Castor bean	20.01
Castor bean	*E. coli*	10.58
Castor bean	*E. coli* + castor bean	24.50
E. coli + castor bean	...	5.92
E. coli + castor bean	*E. coli* + castor bean	24.90
	Expt II	
E. coli	*E. coli*	220.15
E. coli	Castor bean	25.17
E. coli	*E. coli* + castor bean	225.46
Castor bean	Castor bean	159.15
Castor bean	*E. coli*	33.12
Castor bean	*E. coli* + castor bean	183.02
E. coli + castor bean	*E. coli* + castor bean	225.46

[a] Expt I: Composition of the assay mixture as described in the Methods. L-[^{14}C]Lysine (0.5 μc), 0.1 mg of castor bean s-RNA, and 0.1 mg of *E. coli* s-RNA were added to each sample; castor bean ribosomes equivalent to 0.45 mg of RNA, *E. coli* ribosomes equivalent to 0.67 mg of RNA, and castor bean and *E. coli* 105,000*g* supernatants equivalent to 0.4 mg of protein each were added when indicated. Expt II: Composition of the assay mixture as described in the Methods except that Mg^{2+} concentration was raised to 20 μmoles/ml. L-[^{14}C]Phenylalanine (0.5 μc), 0.1 mg of poly-U, 0.1 mg of castor bean s-RNA, and 0.1 mg of *E. coli* s-RNA were added to each sample. Castor bean ribosomes equivalent to 0.45 mg of RNA, *E. coli* ribosomes equivalent to 0.8 mg of RNA, and castor bean and *E. coli* 105,000*g* supernatants equivalent to 0.4 mg of protein each were added when indicated. [b] Values are micromicromoles of L-[^{14}C]lysine (specific activity 222 μc/μmole) or L-[^{14}C]phenylalanine (specific activity 333 μc/μmole) per assay.

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