Amino Acid Incorporation and Aminoacyl Transfer in a Wheat Embryo System*

(Received for publication, June 2, 1966)

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SUMMARY

An amino acid-incorporating system has been obtained from ungerminated wheat embryos. This system has very low endogenous activity, but phenylalanine incorporation can be stimulated by the addition of polyuridylic acid. The poly U-stimulated system is similar to those from other sources in that it requires ribosomal particles, supernatant fluid, soluble ribonucleic acid, adenosine triphosphate, guanosine triphosphate, a nucleoside triphosphate-regenerating system, and magnesium and potassium ions for maximal activity. Puromycin and ribonuclease cause considerable inhibition of phenylalanine incorporation while chloramphenicol has little effect. The polymerization of phenylalanine from phenylalanyl-soluble ribonucleic acid in the presence of polyuridylic acid requires ribosomes, Mg++, K+, GTP, its generating system, and a factor present in the supernatant fraction. This aminoacyl transfer factor from wheat embryos has been partially purified by ammonium sulfate precipitation and DEAE-cellulose column chromatography. The specificity of the ribosomes for the supernatant factor in the transfer reaction was studied with ribosomes from Escherichia coli, guinea pig liver, wheat embryo, and tobacco leaf chloroplasts. The mammalian and wheat ribosomes could interchange their supernatant factors to give aminoacyl transfer but were not active with the E. coli factor. E. coli ribosomes could use only the homologous supernatant fraction. The tobacco leaf chloroplast ribosomes were tested with the wheat supernatant fluid and were found to be active.

The phenomenon of seed germination, triggered by water imbibition, offers a promising opportunity to investigate the early stages of cell differentiation. The synthesis of new proteins must play a very important role in the mechanism whereby the seed passes from a latent to a highly active state. It appears worthwhile, therefore, to study at different stages of germination the properties of the components that are known to be involved in the biosynthesis of proteins.

Cell-free systems obtained from seeds and that incorporated amino acids into proteins have been described previously by several investigators (1-3). Recently Marcus and Feeley (4) described systems from peanut cotyledons and wheat germ and investigated the effects of germination on their capacity to incorporate amino acids into protein. In this communication, we present some detailed characteristics of a cell-free system obtained from ungerminated wheat that incorporates amino acids into polypeptides utilizing either free amino acids or aminoacyl-soluble ribonucleic acids as the starting material. This system was used to obtain evidence regarding the genetic code of higher plants (5).

Materials

A genetically pure strain of wheat (Triticum durum C P x St 464 PM 60 C p 64N) was kindly supplied by the Instituto de Desarrollo Agropecuario of the Chilean Department of Agriculture. 14C-Phenylalanine was obtained from New England Nuclear. ATP, creatine phosphate, creatine phosphate kinase, and glutathione were purchased from Sigma. GTP was obtained from Schwarz BioResearch. Crystalline pancreatic ribonuclease was purchased from Worthington. Chloramphenicol was obtained from Parke, Davis and Company, and puromycin was generously provided by Dr. H. A. Jolley from Cyanamid International. Polyuridylic acid was purchased from Miles Laboratories and yeast sRNA1 was obtained from General Biochemicals. Phosphoenolpyruvate and pyruvate kinase were purchased from Calbiochem. Log phase Escherichia coli strain B paste was purchased from the Grain Processing Corporation.

Methods

Wheat germ was isolated according to the method of Johnston and Stern (6) and stored frozen at -20°C.

Preparation of Ribosomes and Supernatant Fraction—A typical ribosome preparation involved grinding, in the cold for 5 min, 8 g of germ with washed sea sand in the presence of 20 ml of medium containing 0.5 M sucrose, 0.05 M Tris-HCl at pH 7.5, 0.01 M MgCl2, 0.025 M KCl, and 0.005 M 2-mercaptoethanol. The extract was passed through cheesecloth, centrifuged for 20 min at 22,000 × g, and the supernatant fluid was finally

1 The abbreviations used are: sRNA, soluble ribonucleic acid; poly U, polyuridylic acid; and TCA, trichloroacetic acid.

* This work was supported by Project No. 164 of the Jane Coffin Childs Memorial Fund for Medical Research and by the University of Chile-University of California Program.

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5813
E. coli ribosomes were isolated and resedimented three times to make them dependant on supernatant transfer enzymes for polypeptide synthesis as described by Nathans and Lipmann (7). The E. coli supernatant fluid was reccentrifuged 5 hours at 105,000 x g to eliminate a small ribosome contamination.

Guinea pig liver ribosomes free of supernatant enzymes were prepared by the procedure described by Nathans and Lipmann (8) for rat liver ribosomes. Tobacco leaf chloroplast ribosomes which were predominantly of the 60 S variety were kindly supplied by Dr. C. R. Stocking.

Preparation of sRNA—Rat liver sRNA was prepared by the method of Brunngraber (9). For the preparation of wheat embryo sRNA, 80 g of wheat embryos were homogenized in a Waring Blender for 15 min in a medium containing 200 ml of 0.025 m Tris-HCl at pH 7.5, and 140 ml of water-saturated phenol. The isolation of the sRNA was performed essentially by the procedure of Brunngraber except that, previous to the last ethanol precipitation, the nucleic acid preparation was treated with DNase (5 mg per ml) in the presence of 0.001 M MgCl2. The yield was approximately 80 mg of the freeze-dried product. 14C-Phenylalanyl-sRNA was prepared with a rat liver sRNA and a rat liver supernatant fraction by the method of von Ehrenstein and Lipmann (10).

Assays—The amino acid-incorporating system contained in a total volume of 1 ml: 50 m M Tris-HCl at pH 7.5; 7.5 m M MgCl2; 25 m M KCl; 5 m M creatine phosphate; 10 m M creatine phosphate kinase; 1 m ATP; 0.5 m M GTP; ribosomes containing 150 pg of ribosomal RNA; 100 pg of wheat embryo sRNA; supernatant fraction with 0.2 mg of protein; 8 m M 14C-phenylalanine (specific activity, 25 m C per pmol); and 50 pg of poly U. This system was incubated for 45 min at 30 °C, and the reaction was stopped by addition of 5% TCA. After addition of 1 mg of casein as carrier, the precipitated protein was treated as described by Zamecnick et al. (11) and counted in a Nuclear Chicago gas flow counter with an efficiency of 39%.

The polymerization of phenylalanine starting from 14C-phenylalanyl-sRNA was measured in 1 ml of the following incubation mixture: 50 m M Tris-HCl, pH 7.5; 25 m M KCl; 7.5 m M MgCl2; 0.5 m M GTP; 150 pg of RNA as ribosomes; 0.5 mg of protein of supernatant fraction; 40 pg of poly U; 14C-phenylalanyl-sRNA, containing approximately 2500 cpm of 14C-phenylalanine (specific activity, 100 m C per pmol); 5 m M phosphoenolpyruvate; and 10 pg of pyruvate kinase. After a 15-min incubation at 37 °C, the reaction was stopped by the addition of 4 ml of 5% TCA. The precipitate was washed and counted as described above.

Results

General Properties of Amino Acid-incorporating System—The results shown in Fig. 1 confirm the report of Marcus and Feeley (4) with respect to the low endogenous activity of the system obtained from the ungerminated seed. However, polyuridylic acid is able to stimulate the incorporation of phenylalanine more than 100-fold. It seemed advantageous, therefore, to study the general properties of this system in the presence of the synthetic messenger.

Table I shows that this system has an absolute requirement for ribosomal particles, poly U, for ATP, together with its generating system, and for magnesium ions. Marked dependence
is observed for ATP, GTP, supernatant fraction, and sRNA, while glutathione and other 19 amino acids are not required. Wheat embryo sRNA can be replaced by similar preparations from yeast or rat liver.

Puromycin and ribonuclease inhibit most of the phenylalanine incorporation while chloramphenicol, at the concentration used, has a negligible effect.

The activity of the system with different concentrations of poly U is shown in Fig. 2. Maximal stimulation is obtained with 40 μg of the polynucleotide while no inhibition is observed with higher concentrations.

The influence of increasing amounts of ribosomal particles appears in Fig. 3. Optimal incorporation is attained with particles containing 115 μg of ribosomal RNA per ml of incubation mixture. The activity decreases gradually as the concentration is increased, possibly due to increase of nucleases or to an increased requirement of magnesium. Sucrose gradient sedimentation of the wheat embryo ribosomes (Fig. 4) shows that the particles obtained by the described procedure are mostly monomers with a sedimentation coefficient of approximately 80 S, and no appreciable amount of polysomes is observed. Aliquots from the gradient fractions corresponding to the single ribosomes were active in the incorporation of phenylalanine into polypeptide in the presence of poly U.

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**Fig. 2.** Effect of poly U in the incorporation of 14C-phenylalanine. The reaction tubes contained ribosomes with 150 μg of RNA, different amounts of poly U, and the other components as described in "Methods.”

**Fig. 3.** Effect of ribosome concentration expressed as micrograms of RNA on the incorporation of 14C-phenylalanine. The incubation mixture was the same as described in “Methods.”

**Table II**

Requirements of aminoacyl-sRNA transfer system

<table>
<thead>
<tr>
<th>System</th>
<th>CPM transferred</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>294</td>
<td>100</td>
</tr>
<tr>
<td>Without ribosomes</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Without supernatant fraction</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td>Without poly U</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Without magnesium</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Without KCl</td>
<td>56</td>
<td>19</td>
</tr>
<tr>
<td>Without GTP</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Without P-enolpyruvate</td>
<td>40</td>
<td>13</td>
</tr>
<tr>
<td>Without pyruvate kinase</td>
<td>87</td>
<td>29</td>
</tr>
<tr>
<td>Without P-enolpyruvate and pyruvate kinase</td>
<td>16</td>
<td>5</td>
</tr>
</tbody>
</table>

Magnesium ion shows a rather sharp optimal concentration at 7.5 μmoles per ml for phenylalanine incorporation. The system is likewise dependent on potassium ion, the maximal activity being obtained at 25 μmoles per ml.

Polyphenylalanine Synthesis from Phenylalanyl-sRNA—The wheat embryo system can bring about the synthesis of polyphenylalanine directly from phenylalanyl-sRNA by the amino acid “transfer reaction.”

Table II shows that when washed ribosomes are used, the
transfer reaction is dependent on the presence of supernatant fraction, GTP, poly U, Mg++, the GTP-generating system, and K+.

Partial purification of the transfer factors present in the supernatant fraction can be obtained by ammonium sulfate precipitation and chromatography on DEAE-cellulose exchanger as described in Fig. 5. The transfer activity coincides with the protein fraction that is eluted with 0.3 M KCl. This partially purified fraction is quite unstable, but can be stabilized by adding ammonium sulfate to 80% saturation and storing the precipitated protein at -20°C. Even under these conditions there is considerable loss of activity over a period of 4 or 5 days. Fig. 6A shows the effect of increasing concentrations of the purified factor on the transfer reaction.

The species specificity of the transfer factors for the ribosomes was tested with the use of systems from bacterial, mammalian,

![Figure 5. DEAE-cellulose chromatography of the wheat embryo transfer factor.](image)

and plant sources that showed absolute requirements for both ribosomal and supernatant fractions in order to carry out phenylalanine transfer in the presence of poly U. In all the experiments, the phenylalanine-sRNA used was from rat liver. Table III shows the results obtained. It is evident that the wheat embryo and the guinea pig liver systems can interchange supernatant fractions and ribosomes while the E. coli system is only active with the homologous components. The chloroplast ribosomes can carry out the transfer reaction with wheat embryo supernatant factor; they were not tested with the supernatant fluid of the other species because of the limited amount available. Fig. 6B shows the effect of increasing concentrations of crude wheat embryo supernatant fraction on the transfer reaction with wheat embryo ribosomes and guinea pig ribosomes. The wheat factor is somewhat more effective with the mammalian ribosomes at low concentrations of enzyme but maximal transfer is obtained at similar levels.

**DISCUSSION**

The system obtained from ungerminated wheat embryos is practically inactive in the incorporation of amino acids into protein. However, when supplemented with synthetic messenger in the form of polynucleotides, high polymerization of amino acids into polypeptides is observed. This result confirms the report of Marcus and Feeley (4) and supports their hypothesis that prior to imbibition all the components involved in protein
synthesis are present in the germ with the exception of active messenger.

The characteristics of the poly U-supplemented system are similar to those of systems obtained from other seeds (19) and many other sources. Thus, full activity is attained only in the presence of ribosomes, supernatant fraction, ATP, GTP, Mg++, K+, and sRNA.

With regard to inhibitors of protein synthesis, wheat germ is similar to mammalian systems in that puromycin and ribonuclease are potent inhibitors while chloramphenicol shows little effect.

In the poly U-stimulated system a high dependence on added sRNA was repeatedly observed. This finding is in contrast with the lack of requirement for additional sRNA in an imbibed peanut cotyledon system (13). Since the availability of sRNA has been postulated as a control mechanism in protein synthesis (14), the significance of this difference should be investigated further. It is interesting that wheat embryo sRNA can be replaced by similar preparations from rat or yeast. Mans and Novelli (15) have described a corn seedling system in which a pH 5 fraction obtained from the supernatant fluid was required for amino acid incorporation. This fraction, which presumably contained sRNA, aminomethyl-RNA synthetases, and aminomethyl transfer enzymes, could be replaced by a pH 5 fraction obtained from rat liver.

The sedimentation profile of the wheat embryo ribosomes in a sucrose density gradient showed a negligible amount of polyribosomes. This observation is in agreement with previous findings (13) with ungerminated seeds.

Polypeptide formation was studied more directly with phenylalanyl-sRNA as the initial substrate in the poly U-stimulated system. As previously shown for other systems (7, 16, 17), the transfer reaction requires ribosomes, GTP, Mg++, and K+ ions, messenger RNA, and factors present in the high speed supernatant fluid.

The transfer factor present in the wheat embryo supernatant fluid has properties expected of an enzyme, such as lability and its behavior to ammonium sulfate precipitation, gel filtration, and DEAE-cellulose chromatography.

In other systems, the transfer enzymes have been fractionated into two complementary components (17–19), and more recent evidence points to three such enzymes in bacterial systems (20). Further purification of the wheat embryo factor, which is presently being pursued, may well show the involvement of several enzymes in the transfer reaction.

The requirement for GTP in the transfer reaction is in agreement with the findings in other systems, but the absolute necessity for the GTP-generating system indicates the probable presence of contaminating GTPases. No attempt has yet been made to determine whether the transfer factor possesses a ribosome-dependent GTPase as described for E. coli (21, 22).

As reported previously (5) the binding of phenylalanyl-sRNA to the wheat embryo ribosomes in the presence of poly U does not require transfer factor or GTP.

The studies of Nathans and Lipmann (7) and Rendi and Ochoa (23) showed that rat liver ribosomes could carry out the transfer reaction in the presence of the supernatant fraction from the livers of various animal species, or from rabbit reticulocytes, but not in the presence of E. coli supernatant fraction. Bacterial ribosomes likewise cannot use the mammalian or yeast transfer factors (24).

A recent publication of Klink and Richter (25) showed that yeast supernatant was able to replace the animal factor in a rat liver system. The results of the present work confirm previous reports regarding the specificity of mammalian and bacterial systems and show that wheat ribosomes and supernatant factor are interchangeable with those from guinea pig liver but not with those of E. coli. Tobacco leaf chloroplast ribosomes which have a sedimentation coefficient of 66 S (26, 27), and therefore resemble bacterial ribosomes in this property, are able to use the wheat embryo supernatant enzymes for the transfer reaction.

The evidence, therefore, supports the idea that ribosomes and transfer factors from nucleated species are similar and interchangeable and are different from their bacterial counterparts. Comparative studies of transfer reaction components from these two groups may be useful in approaching such problems as susceptibility to antibiotics and the nature of the reaction steps involved in the transfer process. Techniques such as cesium chloride sedimentation of the particles which separates ribosomal proteins essential for their activity (28–30) may be valuable tools in these studies.

Acknowledgments—Part of this work was carried out at the laboratory of Dr. C. Ralph Stocking, Department of Botany, University of California, Davis, while one of us (J. E. Allende) was a Visiting Scholar of the University of Chile-University of California Program. We are extremely grateful to Dr. Stocking for his encouragement and advice. We acknowledge also the able technical assistance of Miss Lucinda Nuñez.

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