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SPECIFIC TEMPLATE ACTIVITY OF RIBONUCLEOPROTEIN PARTICLES ISOLATED FROM GERMINATING *TRITICUM AESTIVUM* EMBRYO

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Key Word Index—*Triticum aestivum*; Gramineae; wheat embryo; germination; ribonucleoprotein particles; informosomes; template activity; methionine.

Abstract—A ribonucleoprotein (RNP) complex formed in wheat embryo at early germination stage was shown to be composed of 40-S RNP particle monomers and oligomers. RNA purified from this complex stimulated incorporation *in vitro* of various amino acids with efficiences dependent upon the frequencies of the corresponding codons calculated for this RNA from its base composition. Methionine was incorporated over the expected rate when the crude RNP complex, instead of the purified RNA, was used as the template. It is believed that the RNP complex represents a crude informosome fraction. The informosomes seem to contain a protein component that promotes the initiation of translation but does not involve the subsequent production of protein.

INTRODUCTION

It has been previously [1] shown that RNA synthesis is rapidly activated in wheat seeds when suitable changes in the environment allow them to germinate. The newly synthesized RNA complexes *in vivo* with a pre-existing protein component to form ribonucleoprotein (RNP) particles which were interpreted as representing either newly synthesized ribosomes [2] or informosomes [3–6]. To make a critical distinction between these two possibilities, the template activity of RNA contained in these particles was now tested *in vitro*. Some accounts of this work have already been presented in a preliminary form [7].

RESULTS

Isolation of 40-S RNP particle monomers and oligomers

The crude RNP fraction was isolated from 3-hr-germinated wheat embryos as previously described [3]. When the freshly-prepared RNP fraction was layered on a sucrose gradient and centrifuged, the material absorbing at 260 nm sedimented heterogeneously (Fig. 1). The main part of the material was located in the 40-S region and several additional, partially separated peaks at the higher S values. The sedimentation pattern of the RNP fraction changed considerably already after a short time of storage. After 6 hr at 0° prior to centrifugation the preparation sedimented as a single peak at approximately 40 S. The 40-S particles as well as the crude RNP fraction were composed of protein and RNA in a mass ratio of 4:1. Probably, the native RNP fraction represents oligomers of the 40-S particles which are rapidly degraded to the monomeric form during their isolation and storage.

Stimulation of amino acid incorporation

The ability of the isolated RNP fraction to stimulate incorporation of amino acids into protein was tested in a cell-free system which responded to exogenous templates but was not influenced by non-informational RNA (Table 1). In such a system, both RNP fraction and RNA puri-

Table 1. Stimulation of the *in vitro* incorporation of phenylalanine into protein by RNP and RNA preparations

Additions	[^{14}C]-Phenylalanine incorporation (cpm/assay)	Stimulation (%)
None	838	—
Poly(U)	4860	580
Ribosomal RNA	820	−5
Crude RNP fraction	1552	85
Isolated RNA	1548	85
Stored RNP fraction	1410	68

Standard assay conditions were followed except for the indicated additions. These were used in quantities equal to 20 μg of RNA in each case.

fied from this fraction stimulated incorporation of [^{14}C]-phenylalanine significantly. The capacity was reduced slightly after the storage period long enough to result in a complete disappearance of 40-S particle oligomers (compare Fig. 1).

Further attempts were made to establish whether the observed stimulation of phenylalanine incorporation was due to template activity of the added RNP fraction. Advantage was taken of the fact that the RNA contained in this fraction has a specific and unusual base composition [3]; the molar proportions of the four bases being: A, 16·4; G, 36·4; C, 14·5; and U, 32·7. If RNA of such a specific composition is translated in the cell-free system used, then the resulting protein should have a specific amino acid composition. The most probable composition of the expected protein was calculated by assuming that nucleotides are randomly distributed along the polynucleotide chain, that all information contained in the putative *m*RNA is translated and that the translation proceeds according to the known meaning of the code [8] (Table 2). As these assumptions certainly over-simplify the situation, only a rough correspondence between the amino acid composition of the predicted and actually formed product may be expected. For this reason only some amino acids, most typical of the calculated composition, were chosen to test the template activity of the RNA. It was found that added RNA stimulated incorporation of various amino acids with different efficiencies (Table 3), but in general, there was a good correlation between the expected and observed effect. In particular, tryptophan and methionine, whose codons were calculated to occur most frequently in the putative *m*RNA, were incorporated most efficiently. These observations seem to indicate that the isolated RNP fraction does contain *m*RNA (or its precursor) as the RNA component and therefore can be referred to as an informosome fraction.

Data presented in Table 3 make it also possible to compare the translation of *m*RNA added either as an informosome fraction or as deproteinized RNA. The only significant difference concerns the

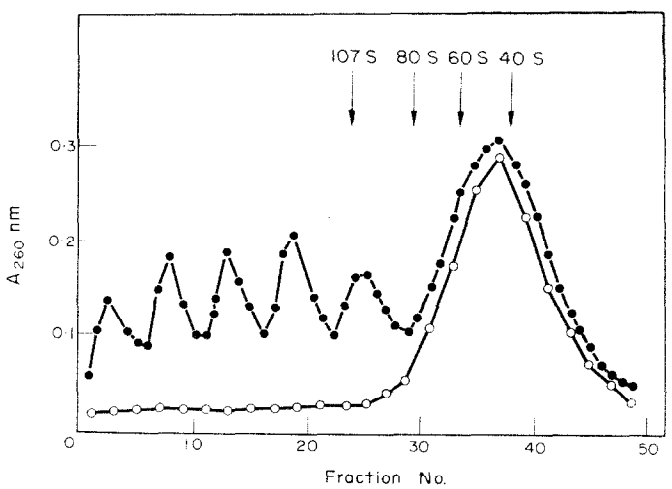

Fig. 1. Sedimentation profiles of freshly isolated and stored RNP fraction preparations. The preparations were isolated and centrifuged as described under Experimental. ●—● Freshly prepared RNP fraction; ○—○, the preparation stored for 6 hr at 0°.

Table 2. Calculated amino acid composition of the protein product coded for by the RNA component of the isolated RNP fraction

Amino acid	Mol %	Amino acid	Mol %
Tryptophan	7·7	Tyrosine	4·7
Cysteine	7·3	Serine	4·6
Valine	7·3	Isoleucine	4·5
Methionine	7·0	Arginine	4·5
Glycine	6·6	Asparagine	3·7
Aspartic acid	6·1	Glutamine	3·4
Glutamic acid	5·9	Histidine	3·3
Alanine	5·5	Threonine	3·0
Leucine	4·8	Lysine	3·0
Phenylalanine	4·7	Proline	2·4

The composition was calculated as explained in the text.

incorporation of [1-^{14}C]-methionine. Incorporation of this amino acid was stimulated fivefold more efficiently by the RNP than by the purified RNA. Since methionine is known as the initiating amino acid [9], it may be assumed that the informosome fraction contains a protein component that promotes the initiation of translation and does not involve the subsequent production of protein.

DISCUSSION

While the previous experiments [3] have shown that the RNA component of the subcellular RNP fraction of wheat embryo is labelled extensively at the onset of germination, the present data indicate that the same component is able to support protein synthesis *in vitro*. The stimulation of various amino acids with different efficiencies, dependent on frequencies of the corresponding codons in the RNA component, strongly suggests that this fraction does carry a specific template activity. It seems reasonable to assume, therefore, that the isolated RNP fraction represents a crude informosome preparation. Sedimentation properties of the freshly prepared and stored informosome fraction preparations indicate that the nascent *m*RNA-protein complex may occur within the cell nuclei as 40-S RNP particle oligomers of structural organization corresponding to the informosome model proposed by Samarina *et al.* [10].

The observation that RNP stimulates methionine incorporation over the expected rate as compared to the purified mRNA obtained from it may indicate a specific role of protein in the RNP in the translation process. Recently, Ilan and Ilan [11] reported the occurrence of a specific initiation factor bound to *m*RNA in the insect *Tenebrio*. In this connection, our observation may be understood as resulting from the direct involvement of a protein component of the added RNP preparation in polypeptide chain initiation. This assumption is supported by the fact that in germinating wheat embryo, protein synthesis is initiated only after certain steps in informosome formation are completed [1, 3, 6].

The data have reported here do not confirm our preliminary suggestion [6] that the early synthesized RNA of the informosome fraction may code for the synthesis of histones. Both calculated values and actually observed differences in the stimulation of incorporation of various amino acids indicate that the amino acid composition of the protein product is more like that of non-histone chromatin protein. The synthesis of such regulatory proteins during the early germination stage might be essential for further activation of genome transcription.

Table 3. Stimulation of incorporation of various amino acids by purifed *m*RNA and crude informosome fraction (*m*RNP)

Amino acid	Expected stimulation (%)	Actual stimulation by *m*RNA (%)	Actual stimulation by *m*RNP (%)
Tryptophan	139	138	146
Methionine	127	106	510
Leucine	87	79	84
Phenylalanine	85	80	85
Arginine	81	<5	25
Lysine	54	20	<5

Experimental conditions were similar to those given in Table 1 except that the indicated [^{14}C]-amino acids were used as the radioactive substrates. The expected stimulation was calculated from the data of Tables 1 and 2.

EXPERIMENTAL

Plant material. A sample of 350 wheat grains (*Triticum aestivum*, cv. Kutnowska) was surface sterilized with 2% NaOCl, preimbibed in H_2O at 2° for 8 hr, and germinated at 22° for 3 hr. (The preimbibition period was not essential for the purposes of the present investigation; it was retained, however, to keep the experimental conditions comparable with those used previously [3]). At the end of the germination period embryos were separated manually from the reamining parts of the grain.

Preparation of crude RNP fraction. The wheat embryos were homogenized and the homogenate fractionated according to the Weeks and Marcus' procedure [12]. The subcellular α-mes-

senger fraction (23000–39000 *g* pellet) was used as the crude RNP preparation. Advantages of this method have been emphasized previously [3].

Sucrose density gradient centrifugation. The crude RNP fraction was resuspended in 1 ml of 20 mM $KHCO_3$, 20 mM KCl, 1 mM Mg acetate and 10 mM dithiothreitol and layered on a 15–30% sucrose gradient made up in the same solution. After centrifugation at 85000 *g* (MSE-65, rotor 3 × 25) for 2 hr at 0°, fractions of 0·5 ml were collected from the gradient and measured for the absorbancy at 260 nm. Sedimentation coefficients were calculated by assuming that the gradient was linear using 40-S and 60-S ribosomal subunits, 80-S ribosomes, and 107-S ribosome dimers as markers.

Cell-free amino acid incorporating system. Ribosomes and S-100 supernatant were prepared from ungerminated wheat embryos according to the method of Svetailo *et al.* [13]. The standard reaction mixture (0·35 ml) contained: 0·14 mg of ribosomes, 0·05 ml of S-100 supernatant (0·10 mg of protein), 0·05 mM of each 19 unlabelled amino acids, 2·5 μCi of [1-^{14}C]-phenylalanine (7·8 μCi/μmol), 35 mM Tris–HCl buffer (pH 7·4), 1 mM ATP, 0·2 mM GTP, 25 mM KCl, 3 mM Mg acetate, 2 mM dithiothreitol, 10 mM phosphoenolpyruvate and 15 μg of phosphoenolpyruvate kinase. Other labelled amino acids as well as exogeneous templates were used as specified in the tables. The mixture was incubated at 37° for 30 min. The $HClO_4$-precipitable material was collected on a filter paper disk [14] and used for the measurement of radioactivity in a scintillation counter.

General. The pHOH–cresol extraction procedure [12] was used to isolate RNA from the subcellular fractions. RNA was measured by absorbancy at 260 nm, assuming an E of 25 for 1 mg of RNA per ml. Protein was determined by the method of Lowry *et al.* [15].

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