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RNA SYNTHESIS DURING THE GERMINATION OF WHEAT SEED

EWA REJMAN and J. BUCHOWICZ

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

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Abstract—Incorporation of [^{14}C]uridine into various RNA fractions of germinating wheat embryo was studied. During the first 3 hr of germination the precursor was incorporated predominantly into a specific component of the RNA (messenger RNA). Neither ribosomal nor transfer RNA were labeled at this time. It is concluded that biosynthetic processes are resumed after the breaking of dormancy in a sequential manner. This sequence begins with the initiation of messenger RNA synthesis.

INTRODUCTION

ALTHOUGH early investigations[1-3] failed to reveal RNA synthesis in the initial phase of seed germination, more recent observations have indicated that RNA synthesis may be triggered in plant seeds immediately[4] or a few hours[5-12] after germination. These observations raised the question—what is the nature of the newly synthesized RNA? Main classes of RNA, ribosomal, transfer and messenger, were found to be present in mature unimbibed seeds.[13-19] The activation of the protein-synthesizing system on hydration, as studied *in vitro*,[13,14,18-21] was found to be associated with a spatial re-arrangement of its components rather than with an appearance of any newly made RNA molecules. Thus, an equal need for the synthesis of all RNA species might be expected once synthesis is triggered. Indeed, Melera[22] has shown that in germinating onion seeds the synthesis of all detectable RNA fractions began at approximately the same time. Similarly, Frankland *et al.*[11] have

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found that ^{32}Pi is incorporated into all RNA fractions in lettuce seeds germinated for 6 hr. However, Van de Walle and Bernier[5] and Deltour[7] have demonstrated that RNA synthesized in maize embryos during the period extending from 4 to 8 hr after soaking is localized exclusively in the chromatin, thus indicating that no *r*RNA is formed. MAK column chromatography[8] and polyacrylamide gel electrophoresis[9] have further shown that this RNA is highly heterodisperse and contains neither ribosomal nor soluble RNA species. On the other hand, Chen *et al.*[10] have found that only *r*RNA is synthesized in wheat embryos during the first 12 hr of germination. These contradictory reports make us believe that further investigations on the initiation of synthesis of the individual RNA species during the early stages of seed germination would be desirable.

Our approach was to label and isolate these RNA fractions synthesized in wheat grains exposed to the optimal germination conditions. The labeled precursors were introduced into the seed prior to the initiation of germination.[4] RNA species were then isolated by various methods, including a procedure worked out recently by Weeks and Marcus[19] for the preparation of messenger fraction RNA from wheat embryos.

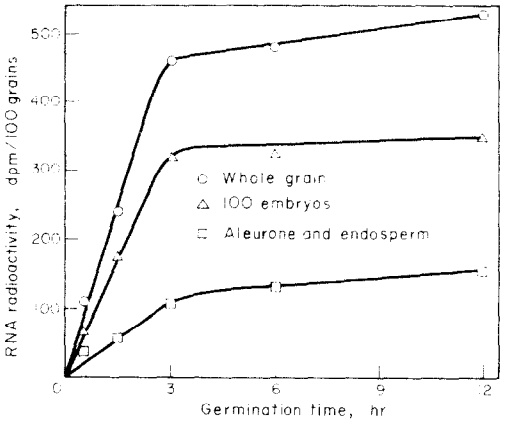

FIG. 1. COMPARISON OF [^{14}C]URIDINE INCORPORATION INTO THE EMBRYO AND ALEURONE-PLUS-ENDOSPERM RNA OF GERMINATING WHEAT GRAINS. RNA radioactivity is expressed as dpm per total RNA isolated from 100 embryos, aleurone and endosperm, or whole grains. At zero germination time 2·2, 4·5 and 6·9 mg of total RNA were isolated from 100 embryos, aleurone-plus-endosperm and whole grains, respectively, and these values did not change noticeably within the investigated period. Germination was preceded by 8-hr inbibition at 2° (see Experimental).

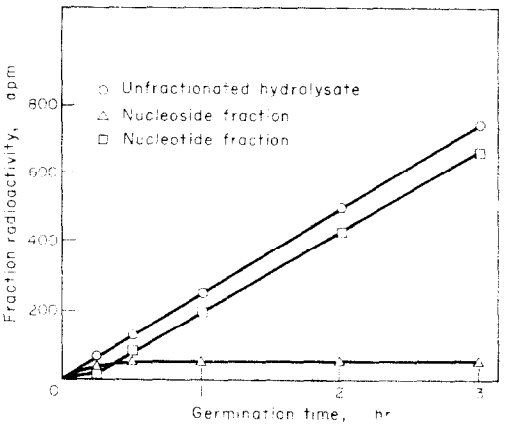

FIG. 2. DISTRIBUTION OF RADIOACTIVITY IN 3′-OH TERMINAL AND INTERNAL SEQUENCES OF RNA ISOLATED FROM THE GERMINATING WHEAT EMBRYOS. Conditions of labeling were similar to those given in Fig. 1, except that [^{14}C]orotate was used as the RNA precursor. Total RNA derived from 100 embryos was hydrolysed with KOH and the hydrolysate fractionated on Dowex 1 × 1 column (see Experimental).

RESULTS

As expected from previous work,[4] considerable radioactivity was detected in purified preparations of total RNA isolated from whole wheat grains administered [^{14}C]uridine and germinated for short periods (Fig. 1). Both embryo and aleurone RNA were labeled but the total count, and specific radioactivity in particular, were invariably much higher in the case of embryo RNA. For this reason in all further experiments the non-embryo moiety of the grain was discarded prior to the extraction of RNA.

When the radioactive embryo RNA was hydrolysed with 0·3 N KOH, acid-soluble products of the same total radioactivity were formed. Part of this radioactivity passed into the nucleotides and the remaining part into the nucleoside fraction when the hydrolysate was applied to a Dowex 1 × 1 column (Fig. 2). The ratio of nucleotide to nucleoside radioactivity was low for the hydrolysates of RNA isolated from embryos germinated for very short periods but thereafter rose rapidly. These results indicate that the precursor incorporated into polyribonucleotides during the first few minutes of germination occupies mostly the 3′-OH terminal positions. As germination proceeds, further chain elongation occurs and the radioactivity becomes located predominantly in the internal sequences of the synthesized RNA molecule.

In attempts to establish which classes of RNA are synthesized in response to the induction of germination, RNA isolated soon after germination was fractionated (see Experimental). The total amounts and specific radioactivities of various RNA fractions isolated from wheat embryos germinated for 3 and 12 hr respectively, are given in Table 1. When the method of Delihas and Staechelin was followed, a fraction corresponding to *t*RNA and a fraction containing a mixture of RNA species of higher MW were separated from each other. The *t*RNA fraction remained inactive throughout the investigated period and its quantity decreased with the germination time. The high MW RNA fraction was already highly radioactive after 3 hr germination and did not change noticeably within the investigated period. In this respect the high MW RNA fraction was similar to the total RNA (compare also the data of Fig. 1).

TABLE 1. DISTRIBUTION OF RADIOACTIVE RNA IN VARIOUS FRACTIONS ISOLATED FROM GERMINATING WHEAT EMBRYOS

Method of fractionation	Fractions obtained	3-hr germination RNA found (mg/100 embryos)	3-hr germination RNA activity (dpm/mg)	12-hr germination RNA found (mg/100 embryos)	12-hr germination RNA activity (dpm/mg)
Loening and Ingle	Total RNA	2·37	216	2·18	234
Delihas and Staehelin	*t*RNA	0·63	< 10	0·45	< 10
	High MW RNA	1·41	290	2·07	292
Caldwell and Henderson	*r*RNA	1·14	< 10	1·50	214
	Interfacial RNA	0·03	14 600	0·05	4150
	Soluble RNA	0·36	312	0·24	350
Weeks and Marcus	Non-nuclear	1·10	185	1·23	374
	23 000 *g* pellet	0·28	322	0·35	682
	39 000 *g* pellet (mF)	0·04	6670	0·06	2300
	39 000 *g* supernatant	0·23	70	0·29	126

The labeling conditions and methods of fractionation are described or quoted under Experimental.

Rapid incorporation of [^{14}C]uridine into the high MW RNA fraction might indicate that *r*RNA was synthesized during the first 3 hr of germination. However, isolation of *r*RNA by the method of Caldwell and Henderson, showed that *r*RNA remained completely inactive after 3 hr germination and became only moderately radioactive after the 12 hr germination period. Moreover, a fraction of non-ribosomal RNA (designated as interfacial RNA) was isolated and shown to contain most of the radioactivity incorporated during the first 3 hr of germination. Caldwell and Henderson's method of fractionation led also to the isolation of a soluble RNA fraction with considerable radioactivity. This fraction probably does not correspond exactly to the *t*RNA fraction which was obtained under the conditions of the method of Delihas and Staehelin.

The use of Weeks and Marcus' method of fractionation showed that the label incorporated into RNA during the first 3 hr of germination is localized predominantly in a

nuclear subfraction sedimenting between 23 000 and 39 000 *g*. The 39 000 *g* pellet, designated according to Weeks and Marcus as the messenger fraction (mF), contained only 1·7% of the total cellular RNA. Specific radioactivity of the *mF*RNA was however very high, 20- and 95-fold higher than that of the two other nuclear subfractions (23 000 *g* pellet and 39 000 *g* supernatant, respectively), and 35-fold higher than that of the crude cytoplasmic RNA. After the longer, 12-hr, germination period the radioactivity of *mF*RNA decreased, whereas the radioactivity of RNA species present in all other subcellular fractions increased significantly. The increase was similar in magnitude for all these fractions (Table 1).

Rapidly labeled RNA found in the mF was present in this subcellular fraction in the form of a ribonucleoprotein complex. This complex contained protein and RNA in a ratio of 4:1. Radioactive RNA could be released from the complex by a phenol treatment. The *mF*RNA thus obtained had a MW of *ca.* $1{\cdot}5 \times 10^6$ daltons and a rather unusual base composition. The molar proportions of the four bases were as follows: A, 16·4; G, 36·4; C, 14·5; and U, 32·7.

DISCUSSION

While the previously reported data[4] have shown that RNA synthesis is triggered in wheat grain immediately after the breaking of dormancy, results of the present investigation indicate that the bulk of the cellular RNA remains inactive during the first 3 hr of germination in the presence of [^{14}C]uridine. In particular, neither *r*RNA nor *t*RNA are labeled. Instead, most of the newly synthesized RNA molecules can be recovered in an RNA fraction which occurs in the wheat embryo in a quantity not exceeding 2% of the total RNA. Depending on the method of isolation this fraction may be obtained either as purified RNA or in the form of a ribonucleoprotein complex.

The ribonucleoprotein complex containing rapidly labeled RNA was obtained as a subcellular fraction, which was shown by Weeks and Marcus[19] to carry a template activity. For this reason, the radioactive RNA found in this complex is referred to as the *mF*RNA. It is not surprising that the newly made *mF*RNA appears in the germinating wheat embryo as a ribonucleoprotein complex. In many other materials, but most clearly in rat liver nuclei,[23–29] nascent *m*RNA was also found to be associated with a protein component to form ribonucleoprotein particles resembling informosomes first reported by Spirin[30] to occur in fish embryos. However, unlike the rat liver *m*RNA, the *mF*RNA isolated from 3-hr germinated wheat embryos was shown to represent a rather homogeneous population of RNA molecules, at least in respect to their size. Moreover, the synthesis of wheat embryo *mF*RNA could be observed under conditions which apparently do not allow the simultaneous synthesis of any other macromolecules. This advantage of our system is probably due to the natural synchronization of biosynthetic processes, which seem to be resumed after breaking of dormancy in a sequential manner. It was previously shown[4] that the synthesis of informational macromolecules is triggered *in vivo* in such an order that RNA synthesis precedes protein synthesis which in turn precedes the initiation of DNA replica-

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tion. It may be assumed now that the earliest activated transcription of the genome results mainly in the synthesis of only one class of RNA molecules which combine instantaneously with a pre-existing protein component to form an informosome-like material. Since the protein synthesis is not yet resumed, the possibility that the protein component may represent nascent polypeptides, formed on the RNA template, seems to be excluded. Similarly, the absence of *r*RNA synthesis eliminates the possibility of contamination of *mF*RNA with *r*RNA precursors.

These tentative conclusions are in full agreement with autoradiographic data of Van de Walle and Bernier[5] and Deltour[7] who demonstrated that RNA synthesized in maize embryos during the early hours of germination was localized exclusively in the chromatin. They are also consistent with the further data of Van de Walle,[8,9] showing that neither *r*RNA nor *t*RNA is synthesized under conditions leading to the selective labeling of the chromatin. It is also easy to reconcile our observations on the *mF*RNA synthesis during germination with evidence for the occurrence of *mF*RNA in unimbibed wheat embryos, presented by Weeks and Marcus.[19] Probably both stored and newly made messengers are necessary to code for different proteins, and further, to trigger DNA replication.

On the other hand, our conclusions differ from those of Chen *et al.*[10] who claim that only *r*RNA species are synthesized at the early stage of wheat embryo germination. To accept this point of view an assumption that the maturation of *r*RNA precursor molecules takes many hours should be made. It is known however, that in plant cells this process is rapid.[31–34] Usually both precursor *r*RNA and mature *r*RNA are radioactive within the first 20 min of labelling *in vivo*. In addition, the base composition of *mF*RNA differs markedly from that of *r*RNA. Therefore, instead of a precursor-product relationship existing between *mF*RNA and *r*RNA, it seemed more likely that the synthesis of *r*RNA precursor is initiated much later than, and independetly from, the synthesis of *mF*RNA. The validity of this assumption is also strengthened by observations[4] suggesting that the earliest-made RNA is degraded in germinating wheat grains rather than converted to any stable RNA species.

The initiation of *mF*RNA synthesis in the germinating wheat embryos resembles the induction of virus-specific RNA synthesis in phage-infected *E. coli* cells, first described by Volkin and Astrahan.[35] In both systems the RNA synthesis is followed by the initiation of synthesis of new protein molecules and then by DNA replication. This analogy, although remote, may indicate that some interrelationships between DNA, RNA and protein synthesis are common to these two completely different experimental systems. Probably, the direction and sequence of the flow of genetic information between DNA, RNA and protein molecules are the same in the simplest and in the most complex living forms.

EXPERIMENTAL

Materials. Wheat grain (*Triticum aestivum*) was similar to that described previously.[4] Commercial [2-^{14}C]uridine and [6-^{14}C]orotate were used as the radioactive precursors of RNA. The precursors were dissolved in H_2O to make 5 μM solutions, containing 50 000 dpm/ml.

Ingestion and germination. A sample of 350 wheat grains, weighing on average 16·5 g, was incubated with 10 ml of one of the precursor solutions at a subminimal germination temp. for 8 hr. To trigger germina-

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tion, the sample was exposed to the optimal temperature. Thus, in our experimental system, zero germination time corresponds to the moment at which the wet grain is transferred from 2 to 22°. Advantages and disadvantages of this approach were discussed 'previously,[4] where a more detailed description of the procedure was also given. It may be only once more emphasized that under these experimental conditions, at least during the first 3 hr of germination, microbial contamination does not contribute significantly to the observed incorporation of uridine. Such a possibility was reasonably well excluded by the observation that [1-^{14}C]leucine was not incorporated under the same conditions. In the case of a significant microbial development, both RNA and protein precursors should be incorporated.

Extraction and fractionation of RNA. At the end of a germination period, embryos were separated manually from aleurone and endosperm'. The aleurone and endosperm was usually discarded and the RNA in the embryos extracted according to one of the following procedures. Total RNA was isolated according to the method of Loening and Ingle.[36] For experiments referred to in Fig. 1, aleurone and endosperm and whole grains, in addition to the embryos, were used. The method of Delihas and Staehelin[37] was used for the separation of *t*RNA from high MW RNA species. The procedure of Caldwell and Henderson[38] was followed to separate *r*RNA from low MW and 'interfacial' RNAs. The method of Weeks and Marcus[19] was used in isolating the subcellular messenger fraction. The α-variant of this method was chosen as leading finally to RNA of the highest template activity. Each subcellular fraction that could be obtained under conditions of this procedure was checked for radioactivity and RNA. These determinations were performed after removing acid-soluble compounds with 0·3 M $HClO_4$. The messenger fraction was also treated with a phenol-cresol mixture, as described in the original method, to obtain undegraded *mF*RNA. In connection with the purposes of the present investigation, the following features of Weeks and Marcus' procedure should be emphasized: (a) It allows removal of the bulk of the cytoplasmic RNA at an early stage of fractionation. (b) The use of an alkaline buffer (20 mM $KHCO_3$) facilitates the extraction of ribonucleoproteins from the crude nuclear pellet and simultaneously prevents the degradation of RNA by plant nucleases, which are known to have a negligible activity at high pH values.[15,39,40] (c) The differential centrifugation of the nuclear extract yields the messenger fraction sedimenting in a narrow range of gravity forces (between 23 000 and 39 000 *g*), thus free of most of the other nuclear RNAs.

Analytical methods. RNA was determined spectrophotometrically assuming $E^{1\,cm,\,1\,mg\,1ml}_{260\,nm}$ as 31 and 22 for hydrolysed (0·3 N KOH, 37°, 15 hr) and undegraded RNA solutions, respectively. To separate nucleosides and nucleotides resulting from alkaline hydrolysis of RNA, the hydrolysate was neutralized with $HClO_4$ and applied to a Dowex 1 × 1 (Cl) column (2 × 10 cm). Nucleosides were eluted (H_2O) and nucleotides recovered with 200 ml of 0·05 N HCl. The nucleotide fraction was evaporated under reduced pressure and applied to paper chromatography in *n*-PrOH–NH_4OH–H_2O (6:3:1) solvent.[41] AMP, CMP, GMP and UMP thus separated were eluted with 0·1 N HCl and determined spectrophotometrically. Protein was estimated by the method of Lowry *et al.*[42] The MW of *mF*RNA was obtained from the polyacrylamide gel electrophoretic mobility as described by Loening.[43] Radioactivity was measured in Packard liquid scintillation counter.

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