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Transcription of Ribosomal and Messenger RNAs in Early Wheat Embryo Germination[1]

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ABSTRACT

Germinating wheat embryos (*Triticum aestivum* L). synthesize both ribosomal and messenger RNA at the earliest times after the onset of germination. The rates of synthesis of these two RNAs are determined at various stages in germination by an analysis of newly synthesized radioactive RNA on oligo(dT)-cellulose. The rate of messenger RNA synthesis is essentially constant throughout 18 hours of germination, while that of ribosomal RNA synthesis increases steadily, particularly after the onset of cell expansion (6 hours), reaching at 16 to 18 hours, a rate of synthesis between 5- and 20-fold greater than that observed at the earliest stages. The net effect is a relative decrease in the fraction of transcribed high molecular weight RNA that is mRNA. Throughout the first 7 hours of germination, mRNA is 25 to 30% of the transcribed fraction, whereas by 16 to 18 hours it has declined to a level of 4 to 8%.

Protein synthesis has been clearly established as an obligatory early component of seed germination (7, 19, 32, 33). The situation with regard to RNA synthesis is less certain. To what extent are the various types of RNA synthesized at different stages of early germination, and to what degree are such syntheses obligatory for germination? On the first of these points, experiments with dissected axes of *Phaseolus* showed a steadily increasing rate of RNA synthesis during the first 12 hr of germination with an abrupt further increase upon the onset of cell expansion (30). Comparable studies with isolated embryos of wheat and rye (2, 3, 10) indicated a more drastic change during early germination, with essentially no RNA synthesis occurring prior to the onset of cell expansion. In an attempt to clarify this situation, particularly with regard to the germinating wheat embryo, we have analyzed the synthesis of high mol wt RNA at several stages early in embryo germination. We conclude that both mRNA and rRNA are synthesized at the earliest times. The rate of mRNA synthesis is essentially constant throughout the first 18 hr. At the same time, the rate of ribosomal RNA synthesis increases primarily after the onset of cell expansion and accounts for more than 90% of the high mol wt RNA synthesized after 16 hr.

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MATERIALS AND METHODS

Preparation and Germination of Embryos. Embryos were prepared from wheat seed (*Triticum aestivum* L.) by the procedure of Johnston and Stern (13) using either CCl_4-cyclohexane flotation (20) or sucrose flotation to separate the embryos from the endosperm. The embryos obtained by the sucrose flotation procedure are referred to as "sucrose" embryos. Germination was carried out as previously described (23), except that the embryos were agitated only during periods of radioactive labeling. After such labeling periods, the embryos were rinsed with 30 to 40 ml of H_2O, dried by suction filtration, and stored in dry ice. [5,6-^{3}H]-Uridine (45 Ci/mmole), [8-^{14}C]adenosine (50.4 mCi/mmole), and [^{14}C-methyl]methionine (45.7 mCi/mmole) were obtained from New England Nuclear Corp.

Ribosome Isolation. Two 125-mg portions of embryos frozen in dry ice were converted separately into "dry ice powders" (22) and then combined for homogenization in 7 ml of isolation media (0.25 M sucrose, 20 mM KCl, 100 mM tris-HCl [pH 7.6], 5 mM magnesium acetate, 5 mM mercaptoethanol) in a Duall conical glass homogenizer. The homogenate was cleared by centrifugation (23,000*g* for 10 min) and layered over 2 ml of a cushion of 1.8 M sucrose, 20 mM KCl, 5 mM magnesium acetate, 100 mM tris-HCl (pH 7.6), 5 mM mercaptoethanol, and centrifuged for 90 min at 150,000*g* in a Spinco Ti50 rotor. The resulting pellet was resuspended in 0.6 ml of 20 mM KCl, 5 mM magnesium acetate, 50 mM tris-HCl (pH 7.6), 1 mM dithiothreitol and clarified by centrifugation for 10 min at 23,000*g*. The ribosome content was determined by measuring the absorbance at 260 nm of an aliquot (10 A_{260} = 1 mg of ribosomes). For fractionation, a volume containing 340 μg of ribosomes was layered on 4.5 ml of a 15 to 38% linear sucrose gradients over 0.5 ml of a 1.5 M sucrose cushion (both gradient and cushion solutions contained 20 mM KCl, 5 mM magnesium acetate, 50 mM tris-HCl [pH 7.6], 1 mM dithiothreitol) and centrifuged for 45 min at 150,000*g* in a SW50.1 rotor. In the EDTA release experiment, a volume containing 540 μg of ribosomes was layered on 5 ml of a 5 to 21% linear sucrose gradient containing 50 mM KCl, 50 mM tris-HCl (pH 7.6), 10 mM potassium EDTA (pH 7.4), and centrifuged for 80 min at 150,000*g* in a SW50.1 rotor. The gradients were fractionated with continual monitoring of absorbance at 254 nm; the fractions were precipitated in the cold with 5% trichloroacetic acid, collected on glass fiber filters (Whatman GF/C), dried and counted. Where appropriate, aliquots of the initial postribosomal supernatant were spotted directly on glass fiber filters to determine total uptake of radioactivity.

Extraction of High Molecular Weight RNA. The dry ice powder of a 125-mg embryo sample was suspended in 3 ml of extraction solution (0.1 M sodium acetate, 0.1 M NaCl, 50 mM tris-HCl [pH 8.2], 10 mM sodium EDTA [pH 7.2]), made to 0.5% with SDS and mixed with 5 ml of phenol containing 0.1% hydroxyquinoline (saturated with 10 mM tris-HCl [pH 8.2], 0.1 M NaCl, 1 mM EDTA): chloroform (1:1, v/v) (24). The mixture was

homogenized for 2 min in a Teflon motor-driven homogenizer and centrifuged at 23,000g for 10 min. The aqueous phase was removed and kept on ice, while the phenol phase and the interphase were re-extracted for 5 min with 3 ml of extraction solution in a 50 C H_2O bath. Following centrifugation, the aqueous phase and interphase were combined with the previous aqueous phase and re-extracted with 5 ml of the phenol-chloroform mixture for 10 min on a rotary shaker at room temperature. After centrifugation, the interphase was discarded, and the aqueous phase was again extracted with 5 ml of phenol-chloroform. Aliquots were taken from the final aqueous phase and spotted on glass fiber paper for determination of the uptake of radioactivity, and the RNA was precipitated overnight at −20 C with 2 volumes of ethanol. The precipitate was washed twice with 3 ml of 3.3 M sodium acetate (pH 6.0) (15), once with 70% ethanol, 0.1 M sodium acetate, vacuum-dried and dissolved in 1 ml of 50 mM NaCl, 10 mM tris-HCl (pH 8.2), 10 mM sodium EDTA (pH 7.2). The absorbance at 260 nm was determined, and the acid-insoluble radioactivity was measured by precipitating an aliquot with 5% trichloroacetic acid. Where RNA was extracted from ribosomes, the same procedure was applied by suspending the ribosome pellet in the extraction solution. Sucrose gradient analyses of RNA were as previously described (29).

Analysis of RNA for Poly(A+) and Poly(A−) Fractions. RNA was fractionated into poly(A+) and poly(A−) classes on the basis of binding to oligo(dT)-cellulose (1). Columns containing 200 mg of oligo(dT)-cellulose mixed with 200 mg of cellulose were kept at room temperature in 20 mM sodium azide, 1 M NaCl, and were prewashed just prior to use with 0.5 M KCl, 0.01 tris-HCl (pH 7.6). A 0.5-ml sample containing 250 μg of RNA in the 0.5 M KCl, 0.01 M tris-HCl (pH 7.6) solution was applied, and the column was washed with the same solution until 4 ml were collected. This 0.5 M salt eluate is referred to as the poly(A−) fraction. After discarding a further 4-ml wash of the 0.5 M KCl solution, the column was eluted with 0.01 M tris-HCl (pH 7.6), and 4 ml of eluate were collected as the poly(A+) fraction. Each fraction was precipitated with 5% trichloroacetic acid, collected on glass fiber filters and counted.

Analysis of Amino Acid Incorporation. The dry ice powder of a 50-mg embryo sample was transferred to 4.2 ml of 5% trichloroacetic acid, 3 mM leucine and homogenized for 2 min in a Teflon motor-driven homogenizer. Aliquots of the homogenate were spotted on glass fiber filters for determination of isotope uptake, and the remainder of the homogenate was kept on ice for 10 min and then centrifuged. The supernatant was discarded, and the pellet was resuspended in 4.1 ml of 5% trichloroacetic acid, 1.5 mM leucine, kept at 90 C for 15 min, and centrifuged. The pellet was suspended in 0.5 ml of 0.2 N NaOH and incubated at 30 C for 10 min. Aliquots were precipitated with 5% trichloroacetic acid, 1.5 mM leucine, collected on glass fiber filters, dried, and counted for radioactivity.

RESULTS

Synthesis of Ribosomal and meRNA in Early Germination. The incorporation of $^{14}CH_3$-methionine into high mol wt RNA allows the selective determination of the synthesis of rRNA (8, 17, 35). In initial experiments attempting to utilize this specificity, we found 4 to 8 times more $^{14}CH_3$-methionine radioactivity incorporated into RNA of ribosomes from embryos incubated during the period between 6 and 7 hr, as compared to embryos incubated between 1 and 2 hr. Subsequent experiments indicated that the major difference between the two time periods was a longer lag in the movement of transcribed RNA into ribosomes during the early time period. Table I, experiment 1, shows data from an experiment in which embryos were germinated directly in $^{14}CH_3$-methionine and ribosomes were isolated after 1.5 hr and at hourly intervals thereafter. While the level of soluble radioactivity in the embryos shows only a small change throughout this period, the radioactivity in the ribosomes increases strikingly with time of exposure. In other experiments (data not shown) we found that the incorporation of $^{14}CH_3$-methionine into total RNA is essentially linear with time through the 0.5- to 3.5-hr period. Thus the rate-limiting step in the incorporation into RNA of ribosomes (in short term experiments) appears to be the processing of the nuclear rRNA presursor into rRNA. A similar conclusion derives from the results of experiment 2, in which the radioactivity incorporated into ribosomes in a 2 hr incubation is compared with that obtained in two 1-hr incubations spanning the same period of germination. Again the major difference in the two situations is the time lag involved in the movement of radioactive RNA from the rRNA precursor into ribosomes.

Table I. *Accumulation of ^{14}C-Methyl-labeled RNA into Ribosome Fraction*

Embryo samples (250 mg, var. Polk 1969) were incubated with 4 μCi of $^{14}CH_3$-methionine during the periods indicated. Ribosomes were harvested from the embryos; RNA was extracted from the ribosome fraction, and acid-insoluble radioactivity in RNA was determined.

Experiment	Germination Period (hr)			
	0–1.5	0–2.5	0–3.5	0–4.5
1. ^{14}C-methionine added at 0 time				
^{14}C uptake (cpm × 10^{-5})	6.02	6.57	7.32	8.22
^{14}C in ribosomes (cpm/mg RNA)	105	699	1842	3343
	0.5–1.5	1.5–2.5	0.5–2.5	
2. ^{14}C-methionine added during period indicated				
^{14}C in ribosomes (cpm/mg RNA)	80	169	579	

Direct evidence that rRNA is indeed synthesized in this early period of germination is shown in the sucrose gradient analysis of RNA isolated from ribosomes during a 2-hr incubation between 0.5 and 2.5 hr of germination. The radioactivity incorporated both from $^{14}CH_3$-methionine and ^3H-uridine is clearly rRNA (Fig. 1). When shorter labeling periods are employed, synthesis of mRNA becomes apparent. Figure 2A shows a sucrose gradient profile of ribosomes from embryos labeled with ^3H-uridine for a 1-hr period between 0.5 and 1.5 hr. The radioactivity is found predominantly in the polyribosome region. When the ribosomes are dissociated with EDTA (Fig. 2B), the radioactivity profile no longer tracks with the subunit absorbance, but rather sediments heterogeneously in a manner characteristic of mRNA (14, 27). Confirmation that a significant part of the radioactivity incorporated is mRNA, was obtained by analysis (of RNA extracted from the ribosomes after a 1-hr ^3H-uridine label) of adsorption to poly(U) filters (26). In a number of such experiments, 15 to 30% of the extracted RNA was retained by the poly(U) filters.

Relative Rates of RNA Synthesis at Various Times after Germination. Having established that both rRNA and mRNA are synthesized during the first hours of embryo germination, we attempted to quantitate the rates of these reactions in relation to the early developmental time course. In order to eliminate the variable introduced by a changing rate of precursor processing, total high mol wt RNA was isolated at each period of germination and fractionated on columns of oligo(dT)-cellulose into poly(A+) and poly(A−) RNAs. On the assumption that the majority of cellular mRNA is poly(A+) (6, 9, 16, 26), these

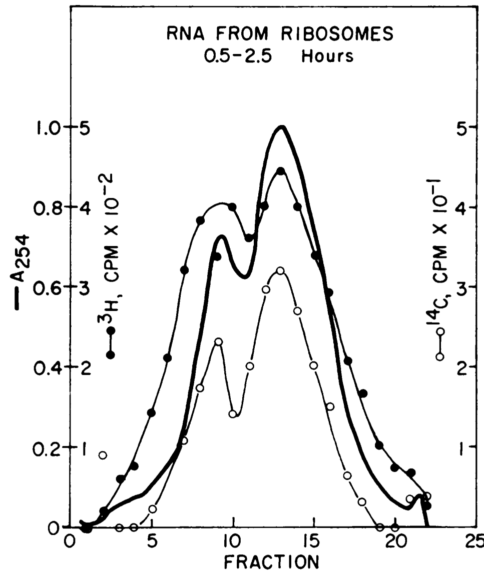

FIG. 1. Synthesis of rRNA in early germination. Embryos (250 mg, var. Polk 1969) were imbibed for 0.5 hr in H_2O and then transferred to a solution containing 30 μCi of ^{3}H-uridine and 2 μCi of $^{14}CH_3$-methionine. After an additional 2-hr incubation, ribosomes were isolated and the RNA was extracted and analyzed.

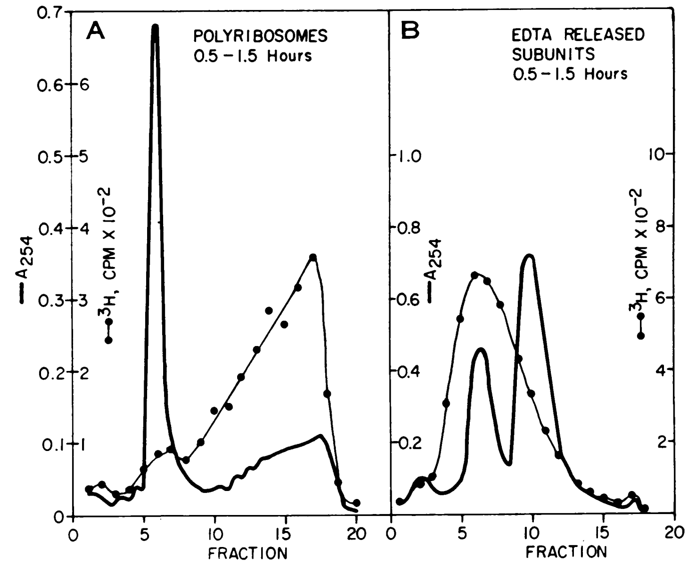

FIG. 2. Synthesis of mRNA in early germination. Embryos were imbibed as in Fig. 1 except that the labeling incubation was for 1 hr with 30 μCi of ^{3}H-uridine. Ribosomes were isolated and analyzed either directly or after EDTA dissociation.

analyses provide a measure of the relative rates of transcription of mRNA and rRNA. A determination of isotope uptake (into the soluble fraction) was also made in each case and the data for incorporation into RNA is presented in a form corrected for differences in uptake at the various stages of germination.[3]

Table II presents data from a number of embryo preparations. The results for a given embryo preparation are reproducible. In addition, in several double label experiments where embryos were incubated with ^{3}H-uridine and ^{14}C-adenosine, identical results were obtained with both precursors (see Table II, experiment with Polk (1969) embryos, for typical data). With different embryo preparations, we have encountered substantial differences and the data presented cover the range of relative activity.[4] In general, it can be seen that the rate of mRNA transcription changes little throughout the period from 1 to 18 hr, whereas

Table II. *Rates of RNA Synthesis in Early Germination*

Incubation	Uptake	Incorporation into RNA: Poly(A−) Actual	Poly(A−) Corrected[1]	Poly(A+) Actual	Poly(A+) Corrected[1]
	cpm × 10⁻⁵	*cpm/mg RNA × 10⁻³*			
Var. Polk (1969)					
^{3}H-uridine (100 μCi)					
1–2 hr	19	37		12.4	
6–7 hr	58	158	50.5	47.5	15.2
		(4.3)	(1.4)	(3.8)	(1.2)
17–18 hr	52	928	334	21	7.6
		(25)	(9.0)	(1.7)	(0.6)
^{14}C-adenosine (2.5 μCi)					
1–2 hr	3.6	5.1		2.4	
6–7 hr	9.5	27	10.1	9.2	3.4
		(5.3)	(2.0)	(3.8)	(1.4)
17–18 hr	10.9	139	45.7	7.6	2.5
		(27)	(8.9)	(3.1)	(1.0)
Var. Fortuna (1973)					
^{3}H-uridine (50 μCi)					
1–2 hr	14	32.7		11.4	
6–7 hr	20	37.4	25.4	13	8.7
		(1.1)	(0.8)	(1.1)	(0.8)
17–18 hr	22	230	141	12	7.4
		(7.0)	(4.3)	(1.0)	(0.6)
Var. Fortuna "sucrose" (1973)[2]					
^{3}H-uridine (50 μCi)					
1–2 hr	8.8	34.1		8.6	
6–7 hr	16.3	57.0	30.8	15.3	8.2
		(1.7)	(0.9)	(1.8)	(0.9)
17–18 hr	22.0	375.5	150.2	20	8.0
		(11)	(4.4)	(2.3)	(0.9)

[1] Data are corrected to the uptake level of the 1- to 2-hr period. Figures in parentheses are rates relative to a value of 1.0 for the 1- to 2-hr period.

[2] Embryos were prepared by the sucrose flotation method.

[3] The rationale for using uptake to estimate precursor specific radioactivity is based on the observation that the absolute level of ATP does not change between 1 and 18 hr of germination (23). In more recent analyses (C. P. Cheung, unpublished) this result has been confirmed for Polk (1969) embryos, while the Fortuna (1973) embryos showed an increase in ATP content of 30 and 70% at 6.5 and 17.5 hr. These latter data would increase the "corrected" relative rates (Table II) for this embryo preparation by 30 and 70% at the 6.5- and 17.5-hr periods. Finally, although the UTP precursor pool has not been determined, the observation that the relative uptake and incorporation rates for adenosine and uridine are essentially identical (see Table II and text) indicates that the relative pool size of both of these triphosphate precursors is identical, thus allowing the use of isotope uptake as a correction for relative specific radioactivity also in the uridine experiments.

[4] The experiment comparing "sucrose" embryos with standard "solvent-treated" embryos (Fortuna 1973, Table II) establishes that the preparative procedure is not responsible for the differences noted. A similar conclusion derives from another experiment in which embryo samples re-extracted with solvent for an extended period gave essentially identical results with embryo prepared from the same lot of seed by the standard solvent method. We have also examined the water uptake curve and the timing of germination for the various embryo preparations and have found only minor differences in both of these parameters. The differences between the embryo samples appears to be due to either the length of storage of the embryos after preparation, harvest conditions of the particular seed lot, or varietal variation.

Table III. *Rates of rRNA Synthesis as Determined by ^{14}C-Methyl Incorporation into Total RNA*

Embryo samples (125 mg, var. Polk 1969) were imbibed with 4 μCi of $^{14}CH_3$-methionine for the periods shown. RNA was extracted and analyzed as indicated.

Incubation	Uptake	Incorporation into RNA		Relative Rates of rRNA Synthesis[2]
		TCA-insoluble	rRNA[1]	
	cpm × 10⁻⁴	*cpm/mg RNA*		
1–2 hr	10.8	1141	536	
6–7 hr	21.6	3315	1505	2.8 (1.4)
17–18 hr	23.7	8857	6764	12.6 (5.7)

[1] Radioactivity cosedimenting in a sucrose gradient with 18S and 28S RNA.

[2] Calculated relative to a value of 1 for the 1- to 2-hr period. Figures in parentheses are normalized to the uptake level of the 1- to 2-hr sample.

the rate of rRNA synthesis increases considerably, particularly in the period after 6.5 hr, *i.e.* during cell expansion. If the correction for isotope uptake is not considered, the relative increase in the rate of rRNA synthesis varies from 7- to 30-fold over the 17-hr period. Taking the uptake correction in account, the relative rate of increase is 4- to 9-fold. Results essentially similar to these are obtained by the analysis of incorporation of $^{14}CH_3$-methionine. Table III presents data for Polk (1969) embryo showing that, compared to labeling between 1 and 2 hr, the relative rates of rRNA synthesis are 1.4 and 5.7 times greater for the 6.5- and 17.5-hr periods. The endogenous methionine level of the embryos increases about 2-fold at 17 hr of germination. With this consideration, the methionine incorporation rates would be about 2-fold and 10-fold, respectively, in reasonable agreement with the poly(A−) analysis of the Polk embryos (Table II, experiment 1).

A further point seen in the data of Table II is that in the 17-hr period there is a marked diminution of the mRNA component relative to the total transcribed high mol wt RNA. A direct calculation of this result is shown in Table IV which presents results of a number of experiments in addition to those documented in Table II. At both the 1.5- and 6.5-hr periods, about 25% of the RNA that is transcribed is mRNA whereas at 17.5 hr, mRNA is only 5% of the total RNA. To ascertain that the smaller percentage of poly(A+) RNA in the 17.5-hr samples was not due to unusual lability to isolation at this period of germination, we isolated RNA from a mixed sample containing embryos that had been incubated in ^{3}H-uridine for the 1- to 2-hr period and embryos that were incubated in ^{14}C-uridine for the 17- to 18-hr period. The fractional radioactivity in poly(A+) RNA was similar to that obtained in the individually isolated samples *i.e.* 27% [^{3}H] and 7% [^{14}C]. Thus the difference between the time periods is real. The combined data (Tables II and IV) suggest that as cellular expansion progresses, there occurs an increase in the transcription of rRNA genes independent of an effect on the mRNA genes. In procayotes the existence of such a situation is well established (22).

A further point of interest in the data of Table IV is the observation that the fractional percentage of adenosine radioactivity in mRNA is consistently greater than that of uridine. This is undoubtedly due to the greater difference in adenosine content of mRNA over rRNA relative to the uridine content. The adenosine content of mRNA is 27.3% while that of rRNA is 20.2%. The uridine content of mRNA is 26.9% while that of rRNA is 28.4% (29). Notwithstanding these considerations, the 17- to 18-hr poly(A+) fraction still appears to have an unusually high content of adenosine. This time period of germination may be characterized by the synthesis of mRNAs of shorter chain length, such that the poly(A) component of the mRNA (18, 28) would be more significant. A more likely explanation is that the increased adenosine content is a consequence of a higher rate of polyadenylation of pre-existent mRNA. Such a reaction could be linked with the recruitment of the mRNA (30), or alternatively it would simply represent a rapid turnover of the poly(A) chain (4).

RNA Synthesis at Onset of Germination. The initial plateau of H_2O uptake with isolated embryos occurs after the embryos are exposed to H_2O for 20 to 30 min (21). The 1- to 2-hr period was therefore employed as the starting point for comparison to the various periods of germination. In an attempt to get an insight into the processes occurring within the 1st hr of germination, we resorted to imbibition in the cold. Exposure of embryos for 75 min at 0 C results in a gain of fresh weight of 90% of that observed at 25 C. At the same time, incubation with ^{3}H-uridine at 0 C does not result in any incorporation of ^{3}H-uridine into RNA. We therefore utilized a cold inbibition period to assess the rates of RNA synthesis during the first minutes of germination. Two samples of embryos were imbibed for 75 min at 0 C and one sample was then transferred to 25 C for 90 min. Both samples were then incubated with ^{3}H-uridine for a 15-min period at 25 C, and the RNA was isolated and analyzed as in the earlier experiments. A comparison with a sample that had not been exposed to the cold treatment was also included. As noted in Table V there is a significant impairment as a consequence of the cold imbibition. Nevertheless, the data indicate that the early rates of both mRNA and rRNA synthesis are already established within 10 min of exposure at 25 C. The validity of this conclusion is reinforced by comparison with an identical experiment in which amino acid incorporation is measured (Table V). Again, the deleterious effect of the cold imbibition is obvious. Nevertheless, there is a clearly observed 4-fold increase in the rate of protein synthesis, a conclusion established in earlier studies (19, 21). We conclude that the data for RNA synthesis are valid and that isolated embryos synthesize both mRNA and rRNA at a significant rate at the earliest periods of germination.

DISCUSSION

Isolated wheat embryos clearly synthesize both mRNA and rRNA at the earliest stages of germination. A number of reports with both wheat embryo (5) and other seed systems (32, 34)

Table IV. *Relative Rates of Synthesis of Poly(A+) RNA at Different Stages of Germination*

Variety	Total Radioactivity in Poly(A+) Fraction					
	1–2 hr		6–7 hr		17–18 hr	
	^{3}H-uridine	^{14}C-adenosine	^{3}H-uridine	^{14}C-adenosine	^{3}H-uridine	^{14}C-adenosine
	%					
Polk 1969	17	23	19	23	3	9
	25	32	23	26	2	6
Fortuna 1970	24	29	26	28	2	4
	33		31		5	
	25		24		3	
Fortuna 1972	17	20	20	23	4	12
	26		27		11	
Fortuna 1973	30		30		5	
	27		26		5	
	26		27		7	
Fortuna 1973 "sucrose"	20		21		5	

Table V. *Rate of RNA and Protein Synthesis at Early Germination following Imbibition in Cold*

Imbibition[1]	^{3}H-uridine (100 μCi) Incubation	Uptake	Incorporation into RNA	
			Poly-(A−)	Poly-(A+)
		cpm × *10*$^{-5}$	*cpm/mg RNA* × *10*$^{-3}$	
75 min 0 C	0–15 min	8.4	6477	1773
75 min 0 C → 90 min 25 C	90–105 min	8.3	6241	1339
90 min 25 C	90–105 min	9.8	10614	3419
Imbibition	^{14}C-Leucine (5 μCi) Incubation	Uptake	Incorporation into Protein	
		cpm × *10*$^{-4}$	*cpm/50 mg embryos*	
75 min 0 C	0–15 min	4.8	4662	
75 min 0 C → 90 min 25 C	90–105 min	5.6	17980	
90 min 25 C	90–105 min	5.6	35295	

[1] Uridine incorporation into RNA: 125 mg of Fortuna (1973) embryos; leucine incorporation into protein: 50 mg of Fortuna (1973) embryos.

generally concur with this conclusion. In explanation of some of the apparent divergent data reported in other publications (2, 3, 10), we suggest a number of considerations. It is clear even from the data presented here that the synthesis of rRNA can increase as much as 10-fold at later stages of germination. In a situation in which the uptake of a radioactive precursor also increases markedly at the later stages of germination, the net result could be a difference of incorporation as high as 30-fold, leading to the erroneous conclusion that synthesis at early times of germination is negligible. Furthermore, as detailed in Table I, if analyses are made on isolated ribosomal fractions, the delayed accumulation of radioactivity into the ribosomes during early germination will again give an erroneous impression of a decreased rate of synthesis in this period. Finally, it should be noted that almost all studies of RNA synthesis in germinating seeds have been done by determining the incorporation of a radioactive precursor into trichloroacetic acid-insoluble material, rather than into extracted RNA. Such analyses would include tRNA as well as other acid-insoluble non-RNA polymers that might incorporate the precursor. In any event, it is clear that both mRNA and rRNA synthesis occurs during the earliest stages of germination.

With regard to quantitative aspects, interpretation is more difficult. Do the uptake data represent the relative specific activities of the precursor pools? A definitive answer is not possible, particularly considering the possibility of specific intracellular pools (11, 25). Nevertheless, the differences in uptake at the various times of germination are significant and must be considered in making a semiquantitative assessment. We suggest the following conclusions. (*a*) The rate of mRNA synthesis does not change markedly throughout 18 hr of germination and may vary between 0.5- and 2-fold the rate observed at the earliest stages. (*b*) The relative rate of rRNA synthesis increases steadily, particularly after the onset of cell expansion (6 hr), such that at 16 to 18 hr it is between 5- and 20-fold the rate observed at the earliest stages of germination. (*c*) The relative fraction of transcribed high mol wt RNA that is mRNA is 25 to 30% throughout the first 7 hr of germination and gradually falls thereafter to a level of 4 to 8% at 16 to 18 hr. Point *c* is a quantitative conclusion since it is independent of any variation in precursor specific radioactivity. One further consideration is involved in the above conclusions. Equating the rate of synthesis of poly(A+) RNA to mRNA synthesis presupposes that the extent of processing of the poly(A+) RNA into mRNA is similar at the various stages of germination. The data of Figure 2, showing that early synthesized mRNA does indeed move out into the cytoplasm, suggest that this assumption is probably valid.

Having established the progress of RNA synthesis in the germinating wheat embryo, it would appear appropriate to analyze the mechanisms controlling the changes in RNA synthesis, and the extent to which the synthesis of these RNAs is obligatory to other aspects of early germination. Studies of RNA polymerase of wheat embryo (12) are relevant to the first of these goals.

Note Added in Proof. S. Sen, P. I. Payne, and D. J. Osborne (1975. Biochem. J. 148, 381.) report substantial synthesis of ribosomal RNA in the earliest stages of germination of rye embryo, in good agreement with the results presented here. The unusually low rates of RNA synthesis early in germination, reported previously, were apparently due to the use of embryos kept for an unusually long time under suboptimal conditions of storage (D. J. Osborne, personal communication). Such embryos, when present in the intact seed, might be weak in germination, but this weakness would not be manifested in water uptake tests with isolated embryos.

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