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SYNTHESIS OF POLY A-RICH RNA IN EMBRYOS OF RYE DURING IMBIBITION AND EARLY GERMINATION

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Key Word Index—*Secale cereale*; Gramineae; rye; germination; biosynthesis; poly A-rich RNA.

Abstract—Poly A-rich RNA is synthesised in rye embryos as soon as they imbibe water. Three properties of newly-synthesised poly A-rich RNA have been examined at different stages of imbibition and germination: its base composition, the electrophoretic mobility of the entire molecule in polyacrylamide gels and the mobility of the poly A segment. The poly A-rich RNA synthesised during the first hr of imbibition appears to be stable for several hr *in vivo*. Evidence is presented which suggests that the poly A-rich RNA extracted in most experiments is not aggregated.

INTRODUCTION

It is thought that dry seeds contain a store of long-lived mRNA [1,2] and there is good evidence that it directs the synthesis of proteins in imbibing embryos of wheat [3]. This early protein synthesis appears to be a pre-requisite of germination because seeds imbibed in inhibitors of protein synthesis fail to germinate [4,5]. At some stage in germination, the role of long-lived mRNA must be effectively taken over by newly-synthesised mRNA. Unfortunately, the initiation of mRNA synthesis in germinating seed cannot be studied at the moment by the direct approach of template activity because the activity of newly-synthesised mRNA cannot be readily distinguished from that of long-lived mRNA. In contrast the indirect approach of the synthesis of poly A-rich RNA can be studied with comparative ease, although one has to assume that polyadenylated RNA consists mainly of mRNA.

In this communication, I show that poly A-rich RNA is synthesised *de novo* in rye embryos as soon as they become hydrated. Some properties of these molecules are also described.

RESULTS

Embryos germinated for 32 hr, pulsed with ortho-phosphate-[^{32}P] between the 20th and 21st hr, incorporated label into RNA. Some of this RNA became bound to a column of oligo dT-cellulose at high ionic strength and could be eluted when the column was washed with Tris–HCl, indicating the presence of radioactively-labelled poly A-rich RNA. When this fraction was subjected to electrophoresis in 2.6% polyacrylamide gels, it migrated heterogeneously (Fig. 1), its mean mobility being intermediate between that of 25S and 18S rRNA. A similar fraction was obtained when the experiment was repeated with embryos imbibed for 1, 3 and 6 hr and pulsed for the final 1 hr. In all these experiments, the radioactive RNA which did not bind to oligo dT-cellulose at high ionic strength was shown by polyacrylamide-gel electrophoresis to consist of a mixture of polydisperse, ribosomal precursor, ribosomal and tRNA species as expected from a previous communication [6].

Base composition analysis of the putative poly A-rich fraction showed that it was indeed rich in adenine (A) (Table 1). The amount of A varied from one experiment

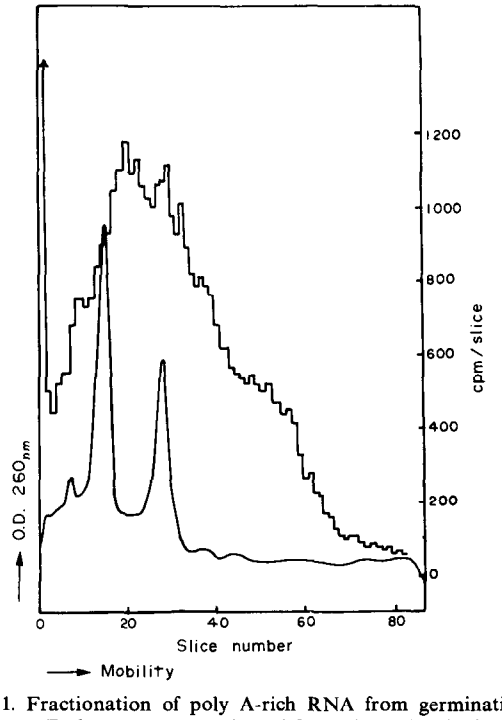

Fig. 1. Fractionation of poly A-rich RNA from germinating embryos. Embryos were germinated for 21 hr and pulsed with orthophosphate-[^{32}P] between 20 and 21 hr. The poly A-rich RNA, isolated by oligo dT-celluose chromatography, was supplemented with 40 μg of unlabelled total nucleic acid from dry rye embryos and precipitated with EtOH. The RNA was fractionated in 2.6% polyacrylamide gels for 2 hr (50 V; 5 mA/tube). After electrophoresis the gels were scanned at 260 nm, frozen in solid CO_2 and sliced into 1 mm segments. Each segment was estimated for radioactivity by Cerenkov counting.

Table 1. Nucleotide compositions of RNA fractions synthesised in imbibition and early germination

Sample	germination (hr)	Period pulsed ^{32}P (hr)	Composition (moles %)				No. of determs
			Adenine	Uracil	Cytosine	Guanine	
Total RNA with poly A-rich RNA removed	21	20–21	24.0	19.2	23.3	33.5	9
Poly A-rich RNA	21	20–21	46.6	15.0	19.3	19.0	3
Poly A-rich RNA	1	0–1	45.0	16.1	20.2	18.6	3
Poly A-rich RNA	3	2–3	40.4	18.4	21.1	20.2	3
Poly A-rich RNA	6	5–6	43.2	16.6	20.0	20.2	3
Poly A segment	3	0–3	99.0	—	0.9	0.1	3

to another, from 40 to 47%. In contrast, the relative amounts of the other bases stayed much the same at all stages of imbibition and germination tested: the C (cytosine) and G (guanine) contents were similar (*ca* 20%) whereas the U (uracil) content was consistently the lowest of the 4 bases. The base composition of unbound RNA was quite different from that of poly A-rich RNA (Table 1), being high in G and C and characteristic of rRNA [7,8].

Evidence that we have been observing the synthesis of authentic poly A-rich RNA comes from experiments which show that the fraction does contain segments of poly A. Digestion of the putative poly A-rich RNA fraction synthesised between 0 and 3 hr of germination with enzymes that specifically cleave RNA after C, G and U, but not A, leaves a resistant polynucleotide core. This undigested material has a high affinity for oligo dT-cellulose and base composition analysis confirms that it is poly A (Table 1). On electrophoresis in 12.5% polyacrylamide gels, it migrates in a heterogeneous manner (Fig. 2) though most of it has a slower mobility than 5S RNA. The poly A has a mean nucleotide length of *ca* 160 residues (MW 51000). Similar results were obtained when embryos were pulsed with orthophosphate-[^{32}P] between 0 and 1 hr of imbibition.

The longevity of poly A-rich RNA synthesised in imbibing embryos was next examined. Two lots of embryos were pulsed with orthophosphate-[^{32}P] between 0 and 1 hr of imbibition. One lot was then extracted for RNA (1 hr-pulsed embryos) whereas the other was incubated for a further 4 hr in fresh medium (1 hr-pulsed, 4 hr-chased embryos). The germination medium in the 4 hr chase period was supplemented with a 240000-fold excess of unlabelled KH_2PO_4 neutralised to pH 7 with KOH. The specific radioactivities of the poly A-rich RNA fractions from each set of embryos were of the same order, though that from the pulse-chased embryos was higher, by 35%. In comparative experiments, the specific radioactivity of poly A-rich RNA isolated from 5 hr-pulsed embryos was some 5-fold that of poly A-rich RNA from 1 hr-pulsed embryos. This indicates that 1 hr-pulsed, 4 hr-chased embryos synthesised only relatively small amounts of radioactive poly A-rich RNA in the chase period. The mobilities of the poly A-rich RNA isolated from 1 hr-pulsed and 1 hr-pulsed, 4 hr-chased embryos were identical in 2.6% polyacrylamide gels (not shown, but similar to Fig. 1). After double enzymic digestion of the two samples with pancreatic A and T_1 ribonucleases, the poly A segments similarly had identical mobilities in 12.5% polyacrylamide gels (not shown, similar to Fig. 2).

It has been reported that poly A-rich RNA extracted from plant tissue tends to aggregate [9]. Aggregation was tested for here by heating poly A-rich RNA fractions in dilute solution and then rapidly cooling them. The heating breaks the hydrogen bonds and the rapid cooling in dilute solution, whilst permitting the formation of intramolecular hydrogen bonding, should prevent bonding between molecules. Such treatment releases 5.8S RNA from 25S RNA [10] and reveals hidden breaks in partially degraded rRNA [11]. The mobility of most samples of poly A-rich RNA tested was not affected by heating and cooling, indicating that aggregation had not occurred. However in a few experiments the mobility of poly A-rich RNA differed somewhat from that shown in Fig. 1; significant levels of radioactivity were detected towards the top of the gel and peaks of radioactivity were found to be specifically associated with the unlabelled rRNA markers (Fig. 3). Heat treatment in these instances caused a reversion to the normal polydisperse profile (Fig. 3) indicating aggregation between poly A-rich RNA molecules and between poly A-rich RNA and rRNA. This aggregation only happened in occasional experiments and it could not be equated with any slight modification of the experimental procedure. It is known that excessive drying of ethanol precipitates prior to dissolving in electrophoresis buffer increases the probability of aggregation.

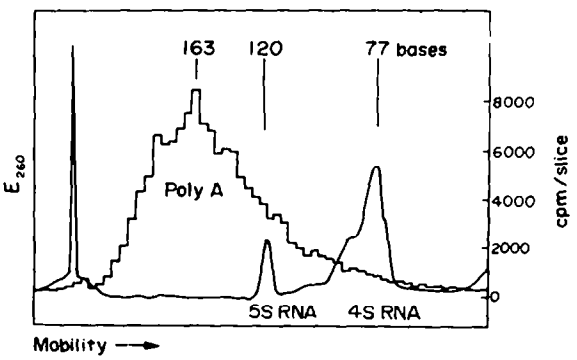

Fig. 2. Fractionation of the poly A segment of poly A-rich RNA from imbibed embryos. Embryos were soaked for 3 hr in germination medium containing orthophosphate-[^{32}P]. The poly A, isolated as described in Experimental, was supplemented with 50 μg of unlabelled low-MW RNA prepared from ungerminated embryos and then fractionated in 12.5% polyacrylamide gels for 16 hr (80 V; 3 mA/tube). The sharp inflection of the solid line on the electrophoretogram marks the junction between the 2.6% spacer gel and the 12.5% gel.

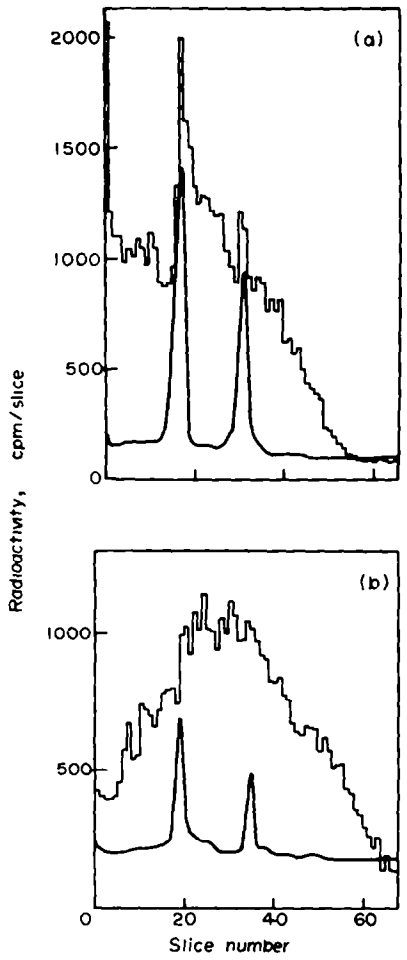

Fig. 3. Effect of heating and cooling aggregated poly A-rich RNA; (a) Aggregated poly A-rich RNA before treatment (b) the same sample after heat treatment. Aggregated, poly A-rich RNA at 1 μg/ml and in 2 ml of 0.5% (w/v) SDS, 0.15 M NaOAc was incubated at 70° for 10 min. It was then immediately plunged into liquid N_2 until it almost reached its freezing point, when it was warmed to room temp. Unlabelled RNA (40 μg) was added, followed by two vols of EtOH. Heated and unheated samples were then fractionated in 2.6% polyacrylamide gels as described in the legend of Fig. 1.

DISCUSSION

In this communication, it has been unequivocally demonstrated that poly A-rich RNA is synthesised *de novo* at all stages of imbibition and germination tested. Neither its base composition, mobility of the entire molecule on electrophoresis in polyacrylamide gels or mobility of the poly A segment appear to change as germination progresses. Furthermore, with the exception of the base composition data, which vary according to species, the poly A-rich RNA has similar properities to the corresponding RNA from fully hydrated cells of mature plants [12–14].

The actual participation of newly-synthesised poly A-rich RNA in protein synthesis has not been demonstrated in this communication. However, it has been shown in the same tissue that after 2 hr of imbibition the bulk of the newly-synthesised poly A-rich RNA is associated with a ribosome fraction (S. Sen and P. I. Payne, unpublished data). Other evidence comes from wheat embryos [15]. Radioactive RNA, synthesised between 0.5 and 1.5 hr of germination was specifically associated with polyribosomes rather than ribosome monomers. About 25% of the RNA extracted from the polyribosome fractions becane bound to filters charged with poly U, indicating the presence of poly A-rich RNA.

Very little is known of the half-life of poly A-rich RNA in plant cells. In this paper, the results of a pulse-chase experiment with rye embryos suggest that the poly A-rich RNA synthesised between 0 and 1 hr of imbibition is stable for at least a further 4 hr *in vivo*. It was further shown that the mobilities of the entire molecule and the poly A segment did not change during the chase period. This indicates that poly A-rich RNA is not substantially modified in any way as it ages for this short period *in vivo*. In contrast, a slow cleavage of the poly A segment in polyadenylated RNA has been reported in other systems [16].

The continuous synthesis of poly A-rich RNA during early germination that is stable for several hr *in vivo* would lead to a build-up of newly- synthesised molecules, presumably in response to the increasing demand for protein synthesis [17] that cannot be met by long-lived mRNA.

EXPERIMENTAL

Ribonuclease T_1 was obtained from Calbiochem Ltd. and pancreatic A ribonuclease from Cambrian Chemicals Ltd. Carrier-free orthophosphate-[^{32}P] was obtained from the Radiochemical Centre, Amersham. Rye embryos were isolated from rye grain (*Secale cereale* var. Lovaszpatonai) by the method of ref. [18]. The embryos (200) were germinated at 25° in 700 μl of germination medium which consisted of 2% (w/v) sucrose and chloramphenicol at 10 μg/ml. After various times from the start of imbibition, the embryos were blotted dry and 400 μl of fresh germination medium was added containing 5 mCi of orthophosphate-[^{32}P]. RNA was extracted from embryos by the method of ref. [19]. The method employs SDS buffered at pH 9 with Tris–HCl and redist pOH for deproteinisation. Poly A-rich RNA was isolated from this total RNA prep by chromatography on 4 cm × 1 cm columns of oligo dT-cellulose (Collaborative Research Inc., type T3) by the general method of ref. [20]. Experimental detail has been published in ref. [2]. RNA samples were fractionated in 2.6% and 12.5% polyacrylamide gels as described in ref. [21]. To prepare poly A segments, poly A-rich RNA was digested with a mixture of T_1 and pancreatic A ribonucleases in high salt [22]. Poly A was isolated from the digested fragments by oligo dT-cellulose chromatography. RNA samples for base composition analysis were incubated with 0.2 N NaOH at 37° for 16 hr. The nucleotides formed were fractionated by high-voltage paper electrophoresis at pH 3.5 [23] and detected by autoradiography. Radioactive spots were cut out and estimated by liquid scintillation spectrometry.

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REFERENCES

1. Marcus, A. and Feeley, J. (1966) *Proc. Nat. Acad. Sci. U.S.* **56**, 1770.
2. Gordon, M. E. and Payne, P. I. (1976) *Planta* **130**, 269.
3. Spiegel, S. and Marcus, A. (1975) *Nature* **256**, 228.
4. Marcus, A., Spiegel, S. and Brooker, J. D. (1975) in *Control mechanisms in development* (Meints, R. H. and Davies, E., eds). p. 1. Plenum Publishing Corp., New York.

5. Hecker, M. and Bernhardt, D. (1976) *Phytochemistry* **15**, 1105.
6. Sen, S., Payne, P. I. and Osborne, D. J. (1975) *Biochem J.* **148**, 381.
7. Leaver, C. J. and Key, J. L. (1970) *J. Mol. Biol.* **49**, 671.
8. Chakravorty, A. K. (1969) *Biochim. Biophys. Acta* **179**, 83.
9. Covey, S. N. and Grierson, D. (1976) *European J. Biochem.* **17**, 116.
10. Payne, P. I. and Dyer, T. A. (1972) *Nature, New Biol.* **235**, 145.
11. Roberts, B. E., Payne, P. I. and Osborne, D. J. (1973) *Biochem. J.* **131**, 275.
12. Higgins, T. J. V., Mercer, J. F. B. and Goodwin, P. B. (1973) *Nature, New Biol.* **246**, 68.
13. Key, J. L. and Silflow, C. (1975) *Plant Physiol.* **56**, 364.
14. Manahan, C. O., App, A. A. and Still, C. C. (1973) *Biochem. Biophys. Res. Commun.* **53**, 588.
15. Spiegel, S., Obendorf, R. L. and Marcus, A. (1975) *Plant Physiol.* **56**, 502.
16. Sheiness, D. and Darnell, J. E. (1973) *Nature, New Biol.* **241**, 265.
17. Chen, D., Sarid, S. and Katchalski, E. (1968) *Agrochimica* **12**, 389.
18. Johnston, F. B. and Stern, H. (1957) *Nature* **179**, 160.
19. Brawerman, G. (1974) *Methods Enzymol.* **30**, Part F, 605.
20. Aviv, H. and Leder, P. (1972) *Proc. Nat. Acad. Sci. U.S.* **69**, 1408.
21. Loening, U. E. (1969) *Biochem J.* **113**, 131.
22. Philipson, L., Wall, R., Glickman, G. and Darnell, J. E. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 2806.
23. Sanger, F. and Brownlee, G. G. (1967) *Methods Enzymol.* **12**, Part A, 361.