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RNA POLYMERASE ACTIVITY IN ISOLATED *TRITICUM AESTIVUM* EMBRYOS DURING GERMINATION

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Abstract—A gradual decrease in the total activity of DNA-dependent RNA polymerase in isolated wheat embryos began 6 hr after germination and continued for up to 48 hr. DEAE-cellulose column chromatography indicated the presence of two RNA polymerase fractions (major and minor) in the resting embryos, only one of which (major) could be detected in the embryos germinated for 48 hr. The major RNA polymerase fraction was tentatively identified as nucleoplasmic (RNA polymerase II).

INTRODUCTION

RECENT studies on the activation of RNA synthesis during seed germination have indicated that the synthesis of various cellular RNA species is triggered in a sequential manner. The sequence seems to begin with the initiation of nuclear heterogeneous[1,2] or messenger[3] RNA synthesis, followed by activation of cytoplasmic ribosomal,[2-4] transfer[2] and organellar ribosomal[5] RNA synthesis, in that order. Such a sequential activation of the genome transcription indicates that different RNA polymerases may appear during germination in a similar manner. RNA polymerase isoenzymes have been found in many eukaryotes[6-10] and the patterns vary during development.[11-17] In particular, Gong and Van Etten[12] have demonstrated that germinated spores of *Rhizopus stolonifer* contain three DNA-dependent RNA polymerases and that only two of them are present in the resting spores. Our previous investigations[18] have indicated that resting whole wheat grains have a high level of RNA polymerase activity which increases 2-fold during the 48 hr-germination period.

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The purpose of the present investigation was to establish whether isolated wheat embryos develop new RNA polymerase activity on germination. To answer this question, the following experimental approaches were applied: measurements of the total activity in embryos germinated for various periods (0–48 hr); comparison of catalytic properties of the enzyme preparations isolated from dry and germinated embryos; and fractionation of the enzyme preparations by DEAE-cellulose column chromatography. The fact that DNA-dependent RNA polymerase (ribonucleoside triphosphate: RNA nucleotidyltransferase, E.C. 2.7.7.6) can be easily extracted from cereal seedlings[18–20] has facilitated such investigations.

RESULTS

Quantitative Changes

To determine changes in the level of total activity of RNA polymerase, the extraction and purification procedure summarized in Table 1 was employed. The last purification step was omitted from the routine determinations as it resulted in the low final yield. The preparation purified to step 3 was free of interfering enzymes (RNase, polynucleotide phosphorylase, phosphodiesterase and phosphatase activities could not be detected) and little, if any, of the RNA polymerase activity was lost with the discarded fractions.

TABLE 1. SUMMARY OF ENZYME PURIFICATION

Purification step and fraction	Total protein (mg)	Specific activity (pmol UMP incorporated per mg protein/10 min)	Yield (%)
1 Crude extract	131·2	10·29	100
2 $(NH_4)_2SO_4$ precipitate	19·4	46·50	67
3 Sephadex G-25	14·8	72·40	60
4 DEAE-cellulose	2·16	118·50	19

These values were obtained for a 3 g sample of wheat embryos germinated for 24 hr (see Experimental for further details).

The results given in Table 2 indicate that dry wheat embryos had a high RNA-synthesizing ability. No noticeable increase in this ability could be observed after the isolated embryos were exposed to the optimal germination conditions. On the contrary, a gradual decrease of RNA polymerase activity occurred throughout the investigated period (0–48 hr). The drop was slight but significant, particularly if the results were expressed as total activity per embryo (last column of Table 2). The decrease was observed in spite of the fact that from the 12th hr of germination new RNA polymerase molecules were probably synthesized by the isolated embryos. This was evident from the significant *in vivo* incorporation of ^{14}C-leucine into the protein fraction isolated from the embryos as the finally purified (including the DEAE-cellulose column step) RNA polymerase preparation.

Properties of the Enzyme Preparation

To compare the catalytic properties of RNA polymerase present in resting and germinating embryos, the enzyme was extracted and purified to step 3 from both sources. The pre-

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parations isolated from the dry and 48 hr-germinated embryos had similar requirements, and all components necessary for their maximal activities were included in the standard incubation mixture. No significant differences in their response to divalent cations and speci-

TABLE 2. CHANGES IN SPECIFIC AND TOTAL ACTIVITY OF RNA POLYMERASE IN *Triticum aestivum* EMBRYO DURING GERMINATION

Germination time (hr)	Preparation yield (mg of protein)	pmol of UMP incorporated per 10 min per mg protein	per embryo
0	5·9	96·4	2·84
1	5·3	103·6	2·76
2	4·8	122·4	2·93
4	4·4	107·8	2·37
6	4·3	83·9	1·80
12	4·2	89·1	1·87
24	3·1	72·3	1·12
48	3·0	53·6	0·80

The enzyme was purified to step 3 and assayed as described under Experimental.

fic RNA polymerase inhibitors could be observed (Table 3). The only difference concerned the optimal pH values. These were 8·4 and 8·0 for the preparations isolated from dry and germinated embryos, respectively. The change from standard to optimal pH value resulted in

TABLE 3. EFFECTS OF METAL IONS, INHIBITORS AND pH ON ACTIVITY OF RNA POLYMERASE PREPARATIONS ISOLATED FROM RESTING AND GERMINATING *Triticum aestivum* EMBRYOS

Assay system	Enzyme source: Dry embryos (relative activity)	Enzyme source: Germinated embryos (relative activity)
'Semi-complete'	5	4
+Mg^{2+}, 5 mM	15	15
+Mg^{2+}, 25 mM	32	26
+Mn^{2+}, 2 mM	71	85
+Mn^{2+}, 10 mM	81	71
'Complete'	100*	100†
+Mg^{2+}, 25 mM	105	105
+α-Amanitin, 0·1 μg/ml	33	30
+α-Amanitin, 10 μg/ml	28	25
+ Rifampicin, 10 μg/ml	102	102
+ Actinomycin-D, 10 μg/ml	38	38
+ PPi, 5 mM	3	8
Complete, pH 8·0	88	124
Complete, pH 8·4	112	75

The enzyme was purified to step 3 from both dry and 48 hr-germinated embryos. 'Complete' assay contained all components of the standard incubation mixture, including Mn^{2+} ion (5 mM, see Experimental), which was omitted from the 'semi-complete' assay. Results given in each column are expressed as % of value obtained for the complete assay. Differences are significant when higher than 5 and 10% for vertical and horizontal comparison, respectively.

* Value actually found was 90 pmolUMP incorporated/mg protein/min.

† Value actually found was 55 pmol UMP incorporated/mg protein/min.

10–25% higher activity of the preparations tested. Further experiments have shown that the shift in the optimal pH value occurs between the 12th- and 24th-hr of germination.

Elution Profiles

To determine the possible presence of multiple polymerases, the enzyme preparation purified to step 3 from 48 hr-germinated embryos was subjected to DEAE-cellulose chromatography. The enzyme was eluted from the column in one major peak of activity at a salt concentration of 0·6. However, the elution profile for a similar preparation obtained from dry embryos indicated the presence of two peaks of activity; a major peak followed by a minor peak, or 0·5 and 0·6 M ammonium sulphate respectively. Due to its lability, RNA polymerase of the minor peak has not been subjected to a detailed characterization. Activity of the major peak could be classified in both cases tentatively as RNA polymerase II. This polymerase was inhibited by α-amanitin (90% at 1·0 μg/ml), preferred Mn^{2+} to Mg^{2+} as the divalent cation, and required a relatively high salt concentration (0·3 M) for optimal activity.

DISCUSSION

Unlike fungal spores,[12] the wheat embryo does not develop new RNA polymerase activity during early germination. On the contrary, the resting embryo seems to be richer in total RNA polymerase activity than the germinating one. Moreover, only one of two activities present in the dry embryo may be detected in embryos germinated for 48 hr. The enzyme seems to behave in a manner typical for storage proteins, being degraded gradually with the germination time, even though synthesis of the new enzyme protein may occur, as indicated by *in vivo* ^{14}C-leucine incorporation. The stored activity seems to be sufficient to catalyse the synthesis of all RNA species during early germination. Nevertheless, the slight shift in the optimal pH value (8·4–8·0) coinciding in time with the beginning of extensive ^{14}C-leucine incorporation into the RNA polymerase fraction does indicate that further attempts to distinguish between the stored and the newly-synthesized RNA polymerase may be still desirable. Probably whole wheat grain, where a moderate increase in total activity of RNA polymerase during early germination was observed,[18] would be more suitable for such a study than the isolated embryo. Different patterns of activation of RNA polymerase in the embryo and endosperm moiety of germinating onion seeds have been recently demonstrated with the use of autoradiographic techniques.[15]

EXPERIMENTAL

Germination. Embryos were isolated from dry grains of wheat (*Triticum aestivum*, cv. Kutnowska) according to the method of Jonston and Stern.[21] Samples of 200 embryos, weighing on average 150 mg, were germinated in 0·5% glucose solution under sterile conditions at 22° for periods varying from 0 to 48 hr, as specified for each experiment.

Enzyme extraction. Unless stated otherwise a sample of 200 embryos was ground and homogenized with 4 ml ice-cold 0·05 M Tris-HCl buffer, pH 8·0 containing 0·25 M saccharose and 0·1 mM 2-mercaptoethanol (TSM-buffer). The homogenate was centrifuged at 15 000 *g* for 10 min at 4° (step 1). RNA polymerase fraction was precipitated from the supernatant with $(NH_4)_2SO_4$ at 50% saturation (step 2). The precipitate was collected by centrifugation, dissolved in the same buffer and desalted on a Sephadex G25 column (step 3). The enzyme was then assayed and characterized, or subjected to DEAE-cellulose column chromatogrsphy.

DEAE-cellulose chromatography. The enzyme preparation (10–15 mg) obtained as described above, except that larger lots (3–5 g) of the embryos were used as the starting material, was applied to a DEAE-cellulose column (1·2 × 10 cm). The column was washed with 5 ml of TSM-buffer and then eluted with a linear

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$(NH_4)_2SO_4$ gradient (0–1·0 M, 100 ml total). 1 ml fractions were collected and 50 μl aliquots assayed for polymerase activity.

Enzyme assay. The standard assay contained in a final vol. of 0·3 ml: Tris-HCl buffer, 50 μmol (pH 8·2); heat-denatured calf thymus DNA, 100 μg; 0·2 μmol each of ATP, CTP and GTP, 5-^{3}H UTP, 2·5 μCi (1·2 Ci/mmol); $MnCl_2$, 1·3 μmol; spermidine, 1·2 μmol; 2-mercaptoethanol, 0·03 μmol and enzyme preparation (*ca.* 100 μg of protein, when purified to the step 3). The mixture was incubated at 30° for 10 min. The acid-precipitable material was then collected on filter paper disk and used for the measurement of radioactivity. These conditions for the extraction, purification and determination of RNA polymerase activity were adopted from Stout and Mans[19] and Burgess.[23]

Interfering activities. Ribonuclease and phosphatase were tested as described previously.[18,22] Absence of polynucleotide phosphorylase activity was indicated by the observation that Pi (5 mM) did not inhibit the precursor incorporation. Phosphodiesterase activity was tested according to the method of Sierakowska and Shugar.[24]

In vivo ^{14}C-*leucine incorporation.* The embryos were germinated and harvested as described above except that 1-^{14}C leucine was added to the germinating medium (5 μCi, 7·8 mCi/mmol) and the non-labelled amino acid (1 mM) was present in the buffer used for homogenization. Control embryos were grown in parallel in the absence of ^{14}C-leucine, and the precursor (0·5 μCi) was added to the homogenate. RNA polymerase was then purified to step 4 from both incubated and control embryos. Significant incorporation means that radioactivity of the preparation isolated from the incubated embryos was at least ten-fold higher than that of the control embryos.

Other determinations. Protein was estimated according to the method of Lowry *et al.*[25] Radioactivity was measured in Packard liquid scintillator counter.

Chemicals. All reagents, except α-amanitin, were of commercial origin.

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