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RNA POLYMERASE ACTIVITY IN GERMINATING ONION SEEDS

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Key Word Index—*Allium cepa*; Liliaceae; seed; frozen sections; autoradiography; RNA polymerase.

Abstract—Frozen sections of endosperm cut from dry unimbibed onion seed were immersed in an aqueous solution of tritium labelled triphosphate; nucleolar RNA polymerase (ribonucleoside triphosphate: RNA nucleotidyltransferase E.C. 2.7.7.6) activity was detected by autoradiography after soaking for 10–15 min in the solution of the radioactive nucleotide. Throughout germination, activity appears to be mainly confined to the nucleolus with chromatin incorporation being very low or non-existent. In the embryo, in contrast to the endosperm, chromatin activity is initiated after 1 hr presoaking, while the nucleolus displays a lag of several hours. No incorporation could be detected *in vivo* before 18 hr.

INTRODUCTION

A CHARACTERISTIC of germinating seeds is the regeneration of the ribosomal system[1–5] with concomitant production of enzymes like α-amylase,[6] for mobilization of reserve food. Marcus *et al.*[7] showed that protein synthesis is activated in germinating wheat embryos after 30 min inhibition; however, RNA synthesis is observed in germinating seeds only after a lag of several hr.[5,8–14] The earliest incorporation (3 hr) of a pyrimidine precursor into a seed embryo has been reported for wheat.[5] More recently Rejman[15] has detected immediate incorporation of RNA precursors in intact wheat seeds, after soaking in the precursors for 8 hr at 2°, during which time no incorporation was detected in the acid insoluble fraction. Studies[16–19] in both dormant and non-dormant tissues have shown that gibberellic acid may control early germination by gene derepression which increases the amount of DNA

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template available for transcription and subsequently RNA polymerase activity. Such increased activity however has only been apparent after several hours or even a few days germination. Barker[4] reported that the nuclear fraction of dry *Pisum* seeds was able to incorporate a low level of ATP into acid precipitable material after 1 hr incubation at 37°, suggesting the presence of RNA polymerase activity in resting seeds.

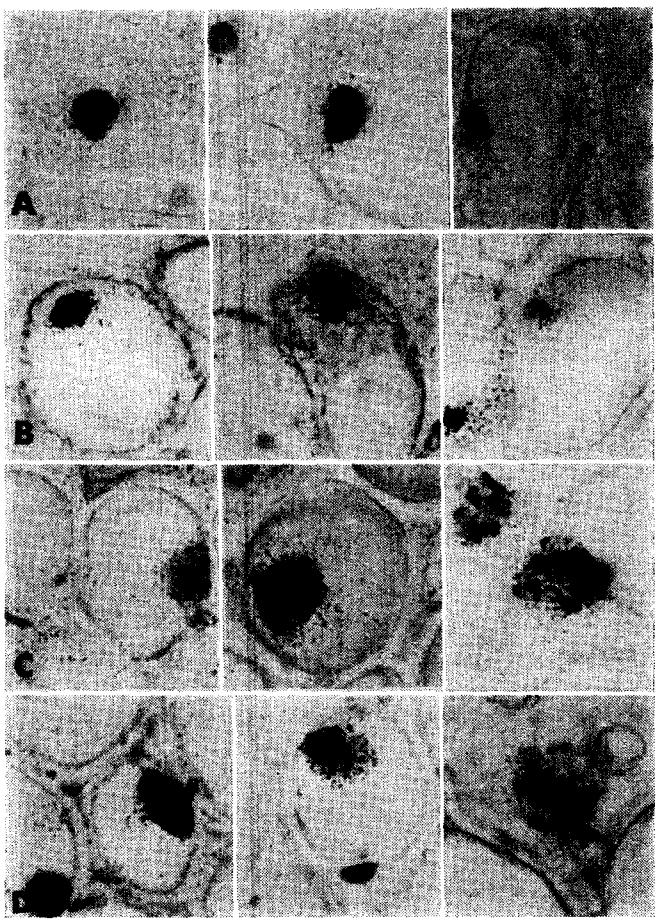

FIG. 1. TYPICAL PATTERNS OF ^{3}H-UTP INCORPORATION IN FROZEN SECTIONS OF ONION SEED ENDOSPERM CELLS AT DIFFERENNT SECTIONING PLANES AFTER A, 0·25; B, 1·0; C, 2·0; AND D, 7·0 hr HYDRATION.

A novel attempt was made to investigate RNA polymerase in different tissues of dry and hydrated onion seeds by frozen section autoradiography. Previous work[20] has characterized nucleic acid metabolism in the root apex during germination. It should be noted that tissues of the embryo cylindrical axis, which lies coiled in the endosperm, include leaf primordium, cotyledon, hypocotyl, promeristem and root cap zones.[21]

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RESULTS

Frozen sections from dry and imbibed seeds were incubated with tritium labelled uridine triphosphate (UTP) (Table 1). Incorporation is initiated very rapidly and is distinct in 10–15 min samples of dry seeds. Endosperm cells (50%) demonstrated heavy nucleolar labelling (Fig. 1) after 30 min hydration (15 min presoaking +15 min incubation in substrate). After 1 hr, 80% of the cells were labelled, a constant value (90%) being reached by 2 hr. In the endosperm all periods of incubation displayed cells with labelling predominant in the region of the nucleoli. In some instances however (<2% of the cell population), clustered or fused nucleolar masses falsely give the appearance of actual heavy chromatin labelling. No distinct nucleolar labelling could be detected in embryonic tissue after 7 hr hydration; in this period, activity was distributed over the chromatin (Fig. 2), displaying a lag of 1 hr before its initiation on presoaking. Even after 1 hr however, labelling extended throughout the whole embryonic axis. The exact time of initiation of nucleolar activity in the embryo has not been determined but samples (>90%) of frozen sections from seeds hydrated for 12, 24 and 30 hr show intense activity in the nucleoli as opposed to the chromatin. Virtually no incorporation could be demonstrated in RNase and DNase controls.

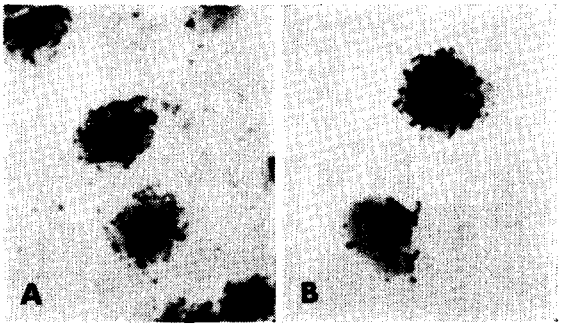

FIG. 2. TYPICAL PATTERNS OF ^{3}H-UTP INCORPORATION IN FROZEN SECTIONS OF ONION SEED EMBRYOS. (A) OVER THE CHROMATIN (2 hr HYDRATION). (B) OVER THE NUCLEOLI (12 hr HYDRATION).

Even with ^{3}H-uridine of high specific activity, no incorporation was detected *in vivo* before 18 hr continuous incubation (Table 2) in agreement with previous work on *Allium*.[20] Removal of a portion of the seed coat did not increase the rate of incorporation but did permit the early detection of activity. With an entire seed, activity is not detected until the radicle has broken the nucleoside impermeable[20] seed coat. Entire seeds incubated for periods up to 20 hr in the complete nucleotide media, of varying concentrations and specific activities, did not incorporate any precursor. This can probably be attributed to the known relative impermeability of cell membranes to such compounds.

DISCUSSION

It has been suggested[22] that mature seeds may have a complete enzyme system for immediate initiation of RNA synthesis from low MW precursors, even before protein synthesis begins. Rejman[15] demonstrated a more rapid synthesis of RNA in whole wheat

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seeds as opposed to the excised embryos used by Chen *et al.*[5,14] He suggested that this was probably due to the absence of endosperm or aleurone layer which may promote embryo biosynthetic activity in the intact seed. The reverse situation, where an embryo factor, gibberellin, increases α-amylase activity in barley aleurone has been reported.[23,24] Our results suggest that the early RNA synthesis recorded for whole wheat seeds may initially be restricted to aleurone tissue, with a lag before embryo activity commences. Also, it would be of interest to see if the enzymes reported for nucleotide synthesis in dry wheat[18] and pea seeds[8] can be demonstrated in the excised dry embryo alone.

TABLE 1. INCORPORATION OF ^{3}H-UTP BY FROZEN SECTIONS OF ONION SEED

Presoak time (hr)	Incubation time (hr)	Total hydration (hr)	% Cells labelled	
			Endosperm	Embryo and cotyledon
0·00	0·25	0·25	17 ± 1·9*	—
0·25	0·25	0·50	51 ± 3·0	—
0·00	1·00	1·00	81 ± 0·9	—
1·00	1·00	2·00	89 ± 1·1	90 ± 1·2
6·00	1·00	7·00	90 ± 1·0	92 ± 0·9
12·00	1·00†	13·00	>90	>90
24·00	1·00	25·00	>90	>90
30·00	1·00	30·00	>90	>90

The incubation mixture consisted of 0·25 ml of 0·04 M Tris-HCl buffer pH 7·4, 0·01 M $MgCl_2$, 0·15 M KCl, 0·5 mM EDTA, 1·0 mM dTT, 0·25 mM each of GTP, ATP, CTP and 100 μCi ^{3}H-UTP (spec. act. 21 Ci mM^{-1}).

* Mean and ± s.e. for approximately $10{\cdot}0 \times 10^3$ cells from a random sample of 300 sections from 10 seeds.

† Samples from 12–30 hr were also incubated for 15 min and 3 hr in separate experiments.

It is now commonly accepted[25,26] that nucleolar RNA is not derived from extra-nucleolar chromosomal activity and indeed it has been shown[27] that the multiple RNA polymerases of rat liver nuclei isolated in high and low salt concentrations have the ionic requirements for ribosomal (nucleolar) or non-ribosomal (nucleoplasmic) RNA formation. A recent autoradiographic study[28] of germinating corn suggests that early chromatin-synthesized RNA moves to the nucleolus at later stages of growth. The initiation of an intense nucleolar activity would appear to give the same results, unless labelling patterns could be critically quantitated. *Allium* hydrated for 12, 24 and 36 hr always demonstrated an intense embryo nucleolar activity in comparison to chromatin, even after incubation periods of only 15 min.

The ribosomal genes in wheat embryos are reported[29] to be located entirely within the chromosomal DNA, but nucleolar satellites have been reported for pumpkin, barley,[30]

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and wheat roots.[31] The onion demonstrates nucleolar activity in endosperm from the beginning of germination and embryo nucleolar activity after an initial lag of several hours which is characterized by predominantly chromatin labelling. A rapid activation of endosperm nucleoli with subsequent development of the enzymatic machinery for metabolism of polymeric reserves would be coincident with evidence from plant[32,33] and especially animal systems[34] that in cells, particularly those that grow rapidly or produce much protein, nucleolar labelling precedes that of the cytoplasm or the remainder of the nucleus.

TABLE 2. INITIATION OF *in vivo* RNA SYNTHESIS IN ONION SEED AS DETERMINED BY AUTORADIOGRAPHY

Precursor	% Seeds demonstrating activity in embryo and endosperm Time (hr) 0–18	18–21	21–36
^{3}H-uridine	0	70	95
^{3}H-UTP	0	0	—

Seeds were hydrated in, (a) ^{3}H-uridine (100 μCi ml^{-1}, 500 μCi ml^{-1}, 1000 μCi ml^{-1} spec. act. 22 Ci mM^{-1}), (b) ^{3}H-UTP (100 μCi ml^{-1}, 500 μCi ml^{-1}, 1000 μCi ml^{-1}, spec. act. 21 Ci mM^{-1}) 0·25–3 mM each of ATP, GTP, CTP in 3 separate experiments using progressively higher conc. of precursors. In each experiment exposure to the nucleotide mixture was continuous, or alternatively after 3, 6, etc. hr growth the seeds were pulsed for 3 hr before sampling. Only seeds from which a portion of the precursor impermeable seed coat had been removed incorporated ^{3}H-uridine at 18 hr.

It has been suggested[5] that early transcription in the wheat embryo may be repressed by a regulatory or an earlier translational step involving DNA-dependent RNA polymerase activity. Gibberellin is reported[35] to function only when a cofactor(s) (probably limiting in dry embryos) is available, by controlling the expression of preformed messages but it has also been suggested[16–19,36] that it may act primarily by changes in the transcriptional process. In onion endosperm nuclei, such enzymes as RNA polymerase, DNA modifying enzymes ('unwindases') various binding factors and hormones as may be needed for transcription appear to be preformed. Moreover a repressive factor, if present, is rapidly inactivated. Factors responsible for the lag in embryo transcription and the specific control of nucleolar and chromatin activity remain obscure. Attempts are now being made to initiate RNA-polymerase activity in the embryo by exogenous additions of various hormones or extracts prepared from seeds germinated for varying periods.

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EXPERIMENTAL

RNA polymerase activity was demonstrated by a modification of the method of Fisher.[37] Whole seeds (*Allium cepa*—white pickling variety) were cut at 8 μm in a freezing cryostat (−20°), placed on microscope slides and dried for 10 min (20 psi) in a vacuum desiccator at room temp. Slides were placed in Petri dishes lined with filter paper, soaked in 2 mM dithiothreitol and 0·25 ml of substrate was added to each slide. The substrate consisted of 0·04 M Tris-HCl buffer pH 7·4, 0·01 M $MgCl_2$, 0·15 M KCl, 0·5 mM EDTA, 1·0 mM dTT, 0·25 mM each of GTP, ATP, CTP and 100 μCi ^{3}H-UTP (spec. act. 21 Ci mM^{-1}) in 5 ml solution. The reaction was stopped by immersing slides in acid–alcohol (1:3). The slides were extracted in 5% TCA (4°) for 30 min. Radioautographs were prepared by dipping slides in NTB_2 liquid emulsion. After 14 days, the slides were developed and stained with azure B for 2 min. RNase and DNase controls were carried out according to Fisher.[37]

To investigate *in vivo* activity seeds were hydrated for varying periods in, (a) ^{3}H-uridine (100 or 1000 μCi ml^{-1} spec. act. 22 Ci mM^{-1}), (b) the complete nucleotide mixture as used for the frozen sections except conc. of cold nucleotides from 0·25–3 mM and ^{3}H-UTP from 100–1000 μCi ml^{-1} were tried in 3 separate experiments using progressively higher conc. of precursors. In each experiment a batch of seeds was continuously exposed to the nucleotide mixture or alternatively after 3, 6, etc. hr growth was pulsed for 3 hr. Seeds were incubated in the dark at 23°, either intact or after slicing off a portion of the seed coat to eliminate the seed coat nucleoside impermeability factor. Hydration was on 7 cm^2 pieces of No. 2 filter paper in Petri dishes with 1·5 ml of precursor. Flooding with a larger vol. inhibited germination. A germination test revealed >95% of the seed stock viable. In all experiments, a sample of not less than 10 seeds was taken at 3 hr intervals. From seeds fixed in acetic acid–alcohol (1:3) for several hr, 6 μm paraffin sections were processed for radioautography as above. From each 10 seed sample not less than 300 sections were examined.

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