Plant Physiol. (1971) 48, 73-81

Nucleic Acid Metabolism in Germinating Onion

I. CHANGES IN ROOT TIP NUCLEIC ACID DURING GERMINATION

Received for publication January 21, 1971

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ABSTRACT

Nucleic acid synthesis in the G_1 cell population of the 1-millimeter apex of the *Allium cepa* embryo was studied during the initial 73 hours of germination. Quantitative data indicate that the total amount of RNA per cell began to increase after 18 hours of germination while the initial DNA per cell increase did not occur until some 20 hours later. Polyacrylamide gel electrophoresis patterns of ^{3}H-uridine-labeled total nucleic acid samples indicated that synthesis of all detectable RNA fractions present in the pre-emergent 1-millimeter apex (*i.e.*, cytoplasmic and "chloroplast-like" RNA) began at approximately the same time (18 hours). Synthesis of the various cytoplasmic RNA fractions continued throughout the germination period. Data indicating synthesis of the "chloroplast-like" RNA were obtained only for the initial 36 hours of germination. Specific radioactivity of ^{3}H-uridine-labeled total nucleic acid increased during the first 41.5 hours of germination but then decreased while the accumulation of RNA per cell continued to increase throughout the 73-hour period. In addition, a method is described which reduced the bacterial contamination of *Allium* seed to a level not detectable by incorporation of radioactive precursors into bacterial ribosomal RNA.

Although many studies concerning nucleic acid synthesis during germination have been reported (5, 16, 27, 28, 40), little consideration has been given to the cellular configuration of the samples studied (*i.e.*, are the cells G_1 [2C] or G_2 [4C], or are they a mixed population?). Work which has taken this into account has been of a cytochemical nature (3, 4, 6, 15, 37) and few if any biochemical determinations have been made. The *Allium* root tip offers a unique opportunity to study a population of higher plant cells which, while in the early stages of germination, are in an extended G_1 phase (3).

This report presents the results of experiments designed to map the pattern of nucleic acid synthesis in the 1 mm apex of the *Allium* root tip during the initial 73 hr of germination.

The apical 1 mm of the *Allium* embryo contains several tissues including the leaf primordium, cotyledon, hypocotyl, promeristem, provascular tissue, and root cap (11). These tissues are associated with the apical 1 mm of the axis throughout germination until the emergent axis is approximately 5 mm in length, by which time the leaf primordium and cotyledon have been displaced out of the 1-mm apex by cell elongation and division (Mallery and Melera, unpublished observations; Bryant, personal communication, and [11]). The samples used in this and the following paper were all 1-mm apices taken at different stages of germination and referred to as "root tips." It should be noted that the word root is used as a matter of convenience only and is not intended as an anatomical or functional term.

MATERIALS AND METHODS

Germination and Harvesting of the Seeds. Seeds of *Allium cepa* var. Evergreen Long White Bunching (Burpee Seed Co., Philadelphia, Pa.) were sown on moist filter paper in covered glass Petri dishes and placed in a dark incubator at 24 C. At the appropriate time seeds were removed and taken to a 24 C darkroom where they were harvested under dim green light. Care was taken to keep the seeds in the dark at all times prior to harvesting. All samples were sown to be harvested between the hours of 8 and 10 AM.

Seeds were sown in the manner described. At 36 hr the lengths of all emergent roots were estimated to the nearest 0.1 mm. All those roots between 0.8 mm and 1.2 mm in lengths were transferred to new dishes and measured at subsequent 24-hr intervals. The mean length and standard deviation for each root was recorded and the data used to plot a standard 1-mm growth curve.

The pre-emergent intervals analyzed were defined by the length of time the seeds were exposed to germinative conditions and included 4-, 12-, 18-, and 24-hr seed. Postemergent intervals included 1-, 2-, 5-, and 10-mm roots, all of which were harvested at the optimal time designated by the growth curve. In all cases the apical 1 mm of the root was used as sample. After being subjected to the bacterial decontamination step outlined below, the pre-emergent samples were harvested by gently squashing the seeds with a spatula (thereby forcing the embryo from the seed at the micropylar end), and then excising the apical 1 mm of the root with a scalpel. The postemergent samples were treated with the decontamination procedure, and the apical 1 mm of the root was excised directly.

Extraction of the Tips for TNA[3] and Subsequent Prepara-

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[3] Abbreviations: TNA: total nucleic acid; SDS: sodium dodecyl sulfate; butyl PBD: 2-(4't-butylphenyl)-5-(4'-biphenyl)-1,3,4-oxidiazole; PBBO: 2-(4'-biphenyl)-6-phenyl-benzoxazole; rDNA: template for the *in vivo* synthesis of rRNA by DNA-dependent RNA polymerase; tot RNA: total RNA (*i.e.*, 1.3m, 1.07m, 0.80m, 0.70m, 0.59m, 0.43m, 4S, and 5S RNA); sol RNA: 4S and 5S RNA; chl RNA: "chloroplast-like" RNA (*i.e.*, 1.07m, 0.59m, 0.43m, and 0.34m RNA); UV: ultraviolet. All molecular weights of RNA are referred to in millions, *i.e.*, 1.07m RNA = RNA with molecular weight 1.07×10^6.

tion of rRNA. The three step phenol-SDS-bentonite procedure of Tester and Dure (36) was used to isolate RNA from the *Allium* root tips. Slight modifications included homogenization of the tissue in a phenol reagent, alteration in the concentration of bentonite, and a change in the manner in which traces of phenol were removed from the final preparations.

The apical 1-mm portions of 100 roots were homogenized in 0.75 ml of 0.1 M sodium acetate, pH 6.0, 1 mM Na_2EDTA, 2% SDS, and 2 mg/ml of bentonite, to which had been added 0.5 ml of redistilled phenol containing 10% redistilled *m*-cresol and 1% 8-hydroxyquinolinol (17). The SDS used was obtained by recrystallization of a commercial grade or in specially purified form from British Drug Houses Ltd. Bentonite was prepared by the method of Fraenkel-Conrat *et al.* (9). The rest of the extraction procedure was as described by Tester and Dure (36).

The resulting ethanol-precipitated nucleic acids were collected by centrifugation, drained of excess liquid, and then washed in cold 73% (v/v) ethanol. The washed nucleic acids were then recentrifuged and, depending upon the experiment to be performed, dissolved in the appropriate buffer.

The purity of the nucleic acids thus obtained was determined by their UV absorption spectra. All spectrophotometric determinations were carried out in a Beckman DB-G recording spectrophotometer. Ribosomal RNA was prepared by 1 M NaCl precipitation from the TNA (36).

The ability of the extraction procedure to release nucleic acids from the *Allium* root tips was judged by subjecting the final interface layer obtained after extraction of 800 36 hr (1 mm) root tips to boiling 2 M NaCl. A UV spectrum was taken on the released ethanol precipitable material.

Fractionation of TNA. Fractionation of the TNA preparations was achieved by polyacrylamide gel electrophoresis. The technique used was essentially that of Loening (20). Polyacrylamide gels cross-linked with bis-acrylamide were prepared as described by Loening and Ingle (23). Because minimal interference of base composition on RNA molecular weight determinations were desired, the buffer system contained 36 mM tris, 0.30 mM NaH_2PO_4, 1 mM Na_2EDTA, and 0.2% SDS (22). The use of glycerol in the gels was as described by Weinburg *et al.* (41). Electrophoresis was carried out at room temperature in 3% gels cast in 0.6- × 9.2-cm Plexiglas tubes. All runs were made at 5 ma/gel; all tubes were prerun at room temperature at 5 ma/gel for 1 hr before application of the sample.

After the run, gels were soaked for 1 hr in distilled water before being scanned at 265 nm with a Joyce-Loebl chromoscan and then frozen on powdered Dry Ice and sliced at 1-mm intervals. The slices were placed in scintillation vials, and 0.5 ml of 30% peroxide was added (19). The vials were tightly capped and held at 70 C for 4 to 5 hr, at which time the acrylamide had completely dissolved. The vials were then cooled to room temperature and 10 ml of a toluene-based scintillation fluid containing 8 g/l butyl PBD, 0.5 g/l PBBO, and 200 ml/l Beckman BBS-3 Biosolv were added. Vials were counted in a Packard Model 3320 scintillation spectrometer. Counting efficiency was determined by the method of Herberg (10).

The approximate Svedberg values and molecular weights of the *Allium* RNA were determined by applying the techniques of Loening and Ingle (23) and of Bishop *et al.* (2). Bacterial rRNA prepared from *E. coli* KB, (kindly supplied by Dr. Norman Sansing, University of Georgia) was used as standards and was assumed to have Svedberg values of 23S and 16S (23) with molecular weights of 1.07m and 0.56m respectively (21).

Quantitation of Nucleic Acids. The amount of TNA extracted at each interval was calculated from spectrophotometric data using the conversion factor 25 A_{260} units/ml = 1 mg/ml TNA. Quantitation of the amounts of RNA and DNA in each TNA sample was accomplished by integrating the areas under the various RNA peaks and the DNA peak of the scanned gels and then calculating these as percentages of the total integrated area (12). Because the amount of "chloroplast-like" RNA represents a small percentage of the total cellular RNA in the *Allium* root tip, the use of a correction factor similar to the one described by Ingle (12) for radish cotyledon RNA which assumed the breakdown of 1.1m RNA into 0.70m and 0.40m RNA was not attempted in this study. Instead, the amounts of 1.07m, 0.59m, 0.43m, and 0.34m RNA were combined and referred to as chl RNA. Therefore, the per cell levels of chl RNA noted on this report must be considered as minimal values. Any contribution to the observed amount of 0.70m RNA due to cleavage of 1.07m RNA would not significantly affect the results or conclusions of this report.

A near-linear response of the Joyce-Loebl chromoscan UV optics to 10-μg samples of rRNA was indicated when it was found that electrophoresis of 10 μg of soybean hypocotyl rRNA gave gels which when scanned and integrated produced 25S/18S ratios of 1.8 to 1.9 (14). Similar results were obtained using *Allium* rRNA, with 10-μg samples giving 25S/18S ratios of approximately 2.00 (29). Ten micrograms of rRNA with a 25S/18S ratio of 2.00 contains about 6.7 μg of 25S RNA and 3.3 μg of 18S RNA; it was therefore assumed that the chromoscan could respond in a reasonably linear fashion to nucleic acid bands containing, at most, 6.7 μg of nucleic acid. The samples used in this study contained 14 to 16 μg of total nucleic acid, which was distributed among a minimum of four distinct bands (Fig. 8d).

Quantitation of the amount of RNA and DNA per cell was accomplished by making cell counts of 1-mm apices from each interval and then carrying out the appropriate calculations using the RNA and DNA values obtained above. Cell counts were made from Feulgen stained squashes of 1-mm apices. Slides were scored by counting nuclei at a magnification of 1000 diameters with a Zeiss GFL microscope fitted with a Wild projection head. Late telophase figures were considered to represent two nuclei.

Specific activity of RNA was determined from ^{3}H-uridine-labeled TNA. In an effort to determine that the counts incorporated were actually in RNA, the labeled TNA was suspended in buffer, an aliquot was removed for counting, and the remainder was subjected to alkaline hydrolysis in 0.3 N KOH at 37 C for 18 hr. The preparation was then neutralized with HCl and 2.5 volumes of cold ethanol were added. The solution was held at −20 C overnight and the resulting precipitate was collected by centrifugation. The pellet was drained of excess liquid, washed with cold 73% ethanol, suspended in buffer, and counted.

Control of Bacterial Contamination Levels. A sequence of sterile water washes was used to reduce the bacterial contamination of the *Allium* seed to levels not detectable by radioactive precursor incorporation into bacterial RNA. Figure 1 outlines the procedure and also indicates how labeling of the *Allium* samples was achieved. A discussion of the bacterial contamination problem is presented in the "Appendix."

RESULTS

Growth Analysis. Thirty-six hours after sowing, 32.8% of the seeds had germinated; of these 41.4% had roots in the 0.8-mm to 1.2-mm range. The predominance of 0.8-mm to 1.2-mm roots was clear. All postemergent intervals were col-

lected at the time prescribed by the standard 1-mm growth curve (Fig. 2); *i.e.*, 1.0-mm roots at 36 hr, 2.0-mm roots at 41.5 hr. The percentage viability of the seeds used in these studies was greater than 96%. For a more detailed discussion of the growth characteristics of the *Allium* seed see Bryant (3).

Effectiveness of the 3-Step Phenol Procedure in Extracting TNA from *Allium* Root Tips. The purity of the TNA preparations was determined by their UV absorption spectra. Typical spectra had 260/280 and 260/230 absorbance ratios of at least 2.00 with 260/220 ratios considerably greater than 1.00. The lack of any significant 270 absorption indicated that no appreciable amount of phenol was carried through the 73% ethanol wash.

The quality of the isolated nucleic acid was judged by the absorbance profiles obtained by gel electrophoresis. Figure 8c shows the profile of 5-mm root tip TNA. No degradation was detectable in the gels by absorption at 265 nm. Electrophoresis of a 10-μg sample of rRNA revealed a ratio of 2.05:1 for the 25S and 18S rRNA, respectively (29).

The UV absorption spectrum of the material released by hot salt extraction of the final interface layer was not at all typical of nucleic acid (29). This, together with the need to extract 800 root tips in order to obtain sufficient material to analyze, indicated that the 3-step phenol-SDS-bentonite extraction procedure was releasing essentially all of the nucleic acids recognizable by their UV absorption spectrum.

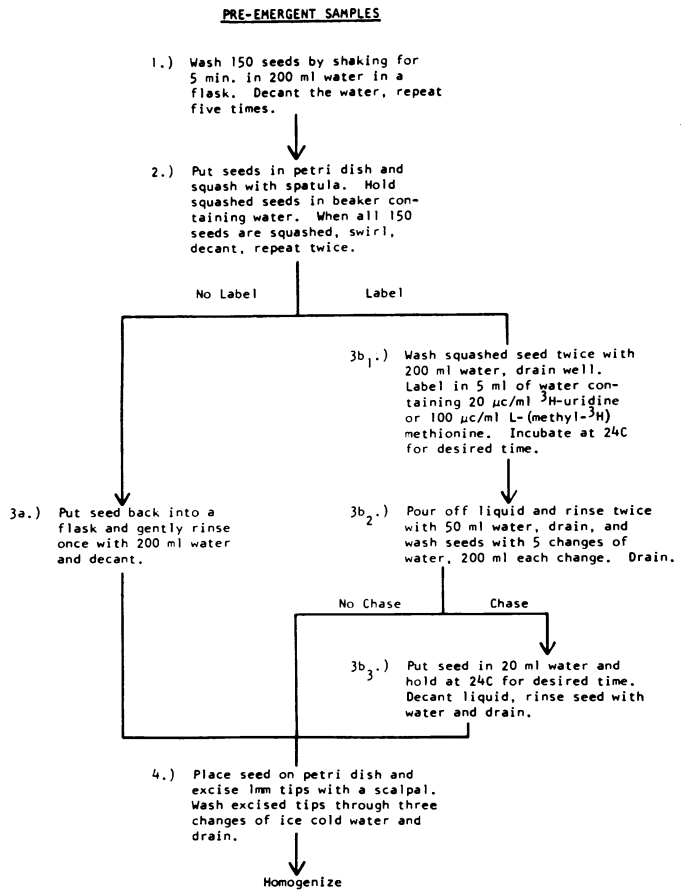

FIG. 1. Sterile water washing procedure. Sterile technique was used throughout. Entire procedure was carried out in a dark room under dim green light at 24 C. Postemergent samples were treated as in step 1, then if no labeling was required they were taken directly to step 4; if labeling was required then postemergent samples were taken directly to step $3b_1$.

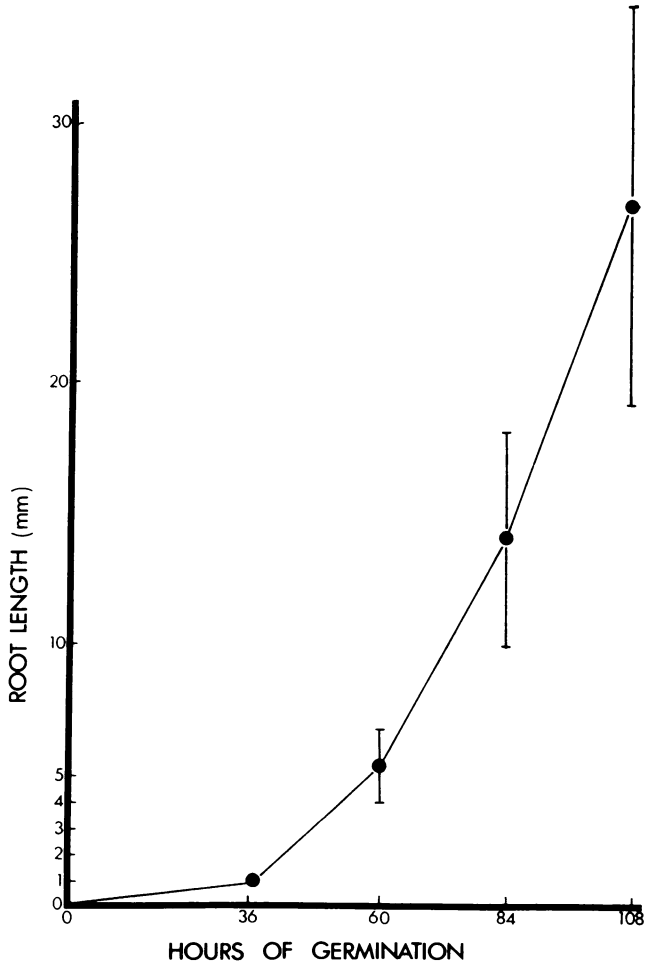

FIG. 2. Standard 1 mm growth curve.

Quantitation and Specific Radioactivity of Nucleic Acid during Germination. The amount of tot RNA per root decreased during the first 24 hr of germination and remained essentially constant from 23 hr through 73 hr (10 mm) (Fig. 3). The DNA content per root followed the tot RNA decrease during the first 24 hr but continued to decrease slowly to 58 hr (5 mm), after which it too remained essentially constant (Fig. 3). Determination of the number of cells present in the 1-mm apices at each interval showed that as germination progressed the cell number decreased (Fig. 4). Appropriate calculations then indicated that the total RNA content per cell increased during the first 24 hr of germination and, during the remaining 49 hr, accumulated to a value 3-fold higher than the 4-hr figure (Fig. 5). Furthermore, the DNA content per cell remained constant during the first 36 hr (1 mm) but then began a slow increase resulting in a 73-hr (10 mm) figure which was approximately 62% higher than the pre-emergent value (Fig. 5).

The specific radioactivity determinations are shown in Figure 6. During the first 41.5 hr (2 mm) of germination, the specific radioactivity of the TNA increased more than 10-fold. However, after 41.5 hr (2 mm) the specific radioactivity began to decrease even though the accumulation of tot RNA per cell continued (Fig. 5). Data indicating that the ^{3}H-uridine counts incorporated into TNA were in RNA is presented in Table I. At least 98% of the total counts incorporated into TNA were alkali labile, suggesting their presence in RNA.

Changes in RNA during Germination. Electrophoresis of TNA from root tips of germinating onion indicated the pres-

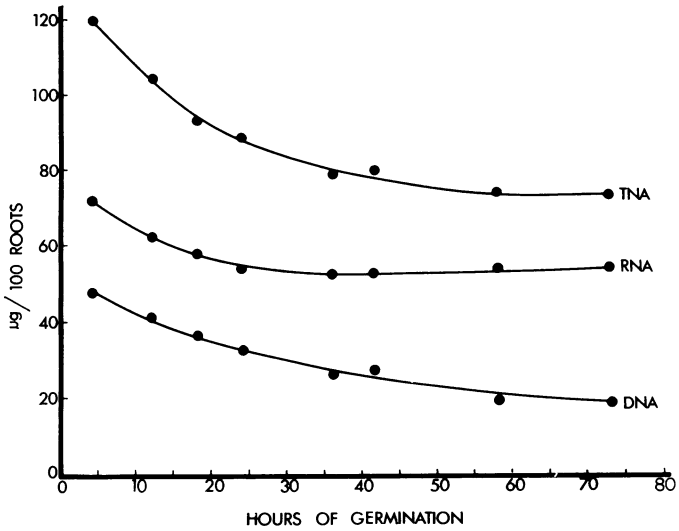

FIG. 3. Determination of the amount of TNA, DNA, and RNA (*i.e.*, tot RNA) per 1-mm apex. Quantitative measurements were made as described in "Materials and Methods."

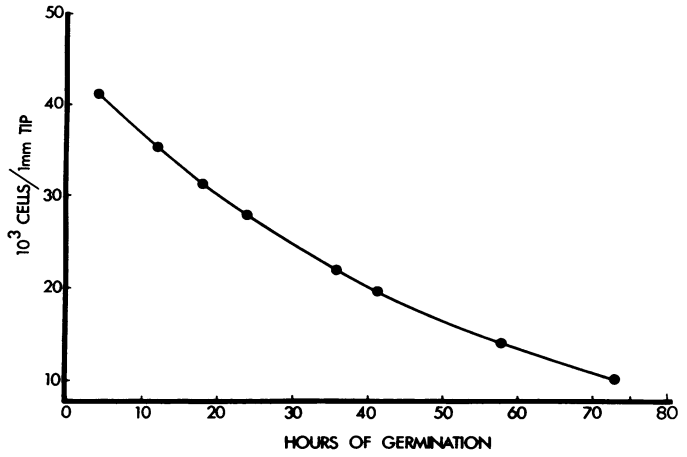

FIG. 4. Number of Feulgen stained nuclei per 1-mm apex.

ence of several minor species of RNA, most of which were similar on a molecular weight basis to ones found in the chloroplasts and proplastids of higher plants (12, 23). The data of Figure 7 and Figure 8, a and b, show these species (*i.e.*, 1.07m, 0.59m, 0.43m, and 0.34m), to be present at all stages of pre-emergence and throughout the initial stages of postemergence. They were not present to any appreciable extent in the apices of 5-mm (58 hr) roots and were undetectable after 10 mm (73 hr) of germination (Fig. 8, c and d).

The incorporation of ^{3}H-uridine into RNA measured as radioactivity banding during electrophoresis was initially detected after 18 hr of germination. At this time significant incorporation was found to be associated with the 1.3m, 1.07m, 0.70m and 0.59m RNA fractions as well as with the 4S to 5S region of the gel (Fig. 7c). Incorporation into these fractions continued, reaching a peak at 2 mm (41.5 hr), then decreasing through 10 mm (73 hr) of germination (Fig. 8). No significant incorporation was found associated with the 0.80m fraction at any time studied.

In both pre-emergent and post-emergent intervals the presence of a radioactivity peak not coincident with an absorbance peak was evident. Its activity essentially followed that of the other uridine-labeled components, being low in the pre-

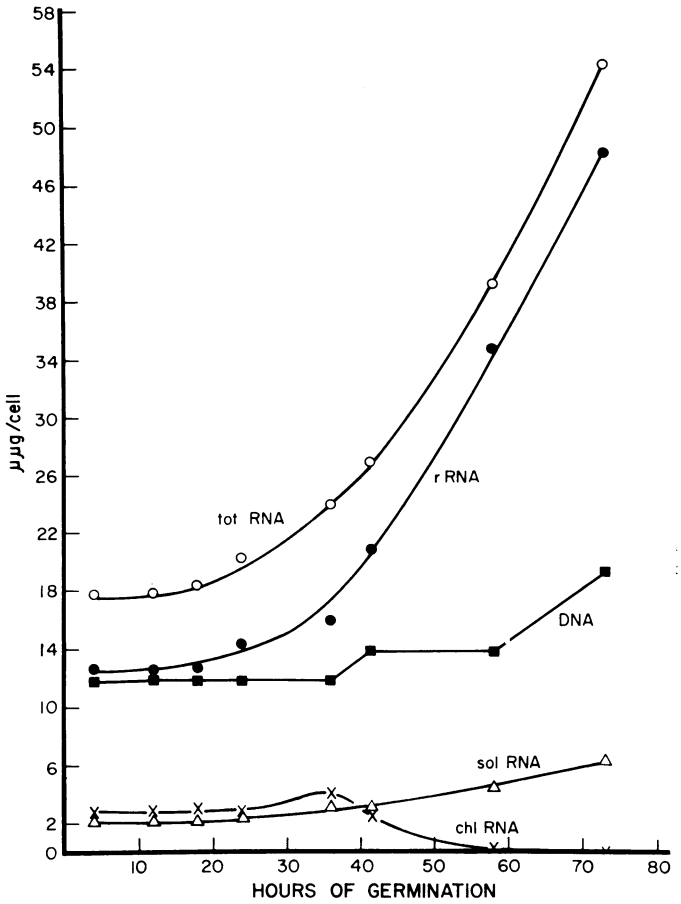

FIG. 5. Changes in the per cell amounts ($\mu\mu$g[pg]/cell) of the various nucleic acid fractions during germination.

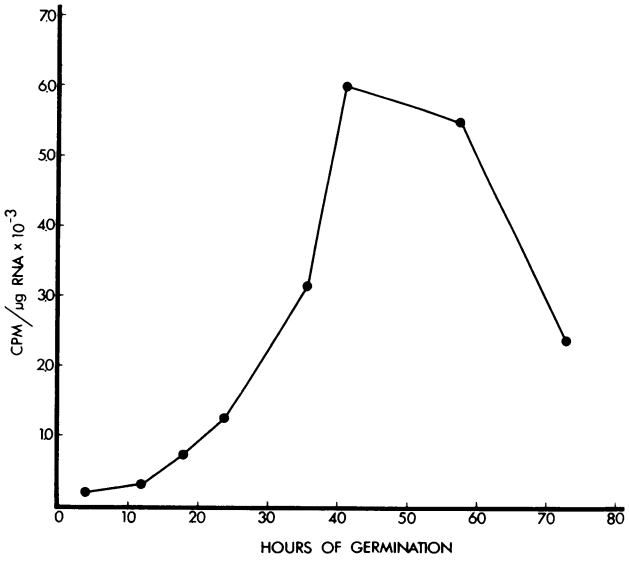

FIG. 6. Specific activity (cpm/μg) of TNA after a 1-hr incubation of the root tips in 20 μc/ml ^{3}H-uridine. Alkaline hydrolysis data (Table I) indicated at least 98% of the radioactivity was in RNA.

emergent samples (Fig. 7, c and d), peaking at 2 mm (41.5 hr) and then decreasing through 10 mm (73 hr) of germination (Fig. 8). The peak had an estimated Svedberg value of

30S to 32S with an approximate molecular weight of 2.2m to 2.4m. A 2.5 hr chase of the label incorporated by 1 mm (36 hr) root tips suggested that the turnover rate of the 2.4m fraction was greater than either that of the 1.3m or 0.70m fraction. The results of the experiment are shown in Figure 9. Comparison of the 1-hr labeling profile of Figure 8a with the profile of Figure 9 indicates an activity accumulation of approximately 150% in both the 1.3m and 0.70m species as a result of the chase, while there was no significant accumulation of activity in the 2.4m fraction.

These results indicate that the 2.4m molecule may be similar to the 2.3m molecule, which has been shown to be the initial precursor in the maturation sequence of higher plant rRNA (18, 24, 33). It must be pointed out here that under the conditions used for electrophoresis in this initial study the counting of 1-mm slices of gel did not provide radioactivity profiles containing the same degree of resolution found in the absorbance scans. Consequently, the coincidence of the radioactivity peaks with the absorbance peaks was not as precise as it might have been had longer running times been used or smaller gel slices counted. For these reasons the 1.4m precursor RNA molecule also reported (18, 24, 33) to be present in higher plants, if present in the *Allium* root tip, could not have been adequately resolved from the 1.3m rRNA.

Table I. *Percentage of Alkali-labile Radioactivity in TNA*

TNA samples were prepared from root tips labeled for 1 hr in ^{3}H-uridine. An aliquot was removed for counting, and the remainder of the sample was subjected to 0.3 KOH for 18 hr at 37 C.

Sample	Total cpm Incorporated	cpm After Alkaline Hydrolysis	Alkali Labile cpm
			%
18 hr	4.2×10^4	6×10^2	98
24 hr	8.6×10^4	8×10^2	99
36 hr (1 mm)	1.7×10^5	2.4×10^3	98

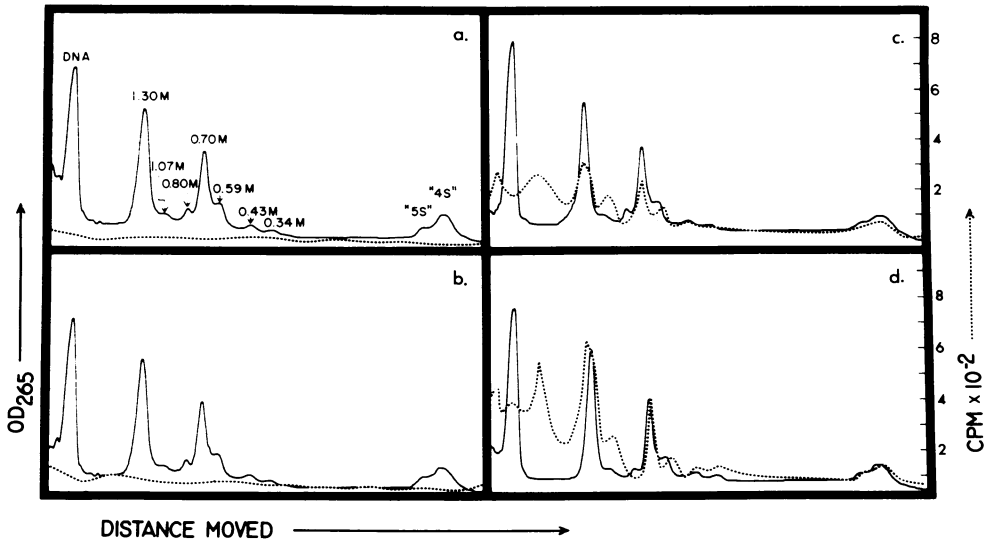

FIG. 7. Electrophoresis of pre-emergent samples. Root tips were labeled for 1 hr in ^{3}H-uridine and the RNA extracted. Three per cent gels were run for 105 min at room temperature. A_{265} : ——; cpm $\times 10^{-2}$: -----. a: 4 hr; b: 12 hr; c: 18 hr; d: 24 hr.

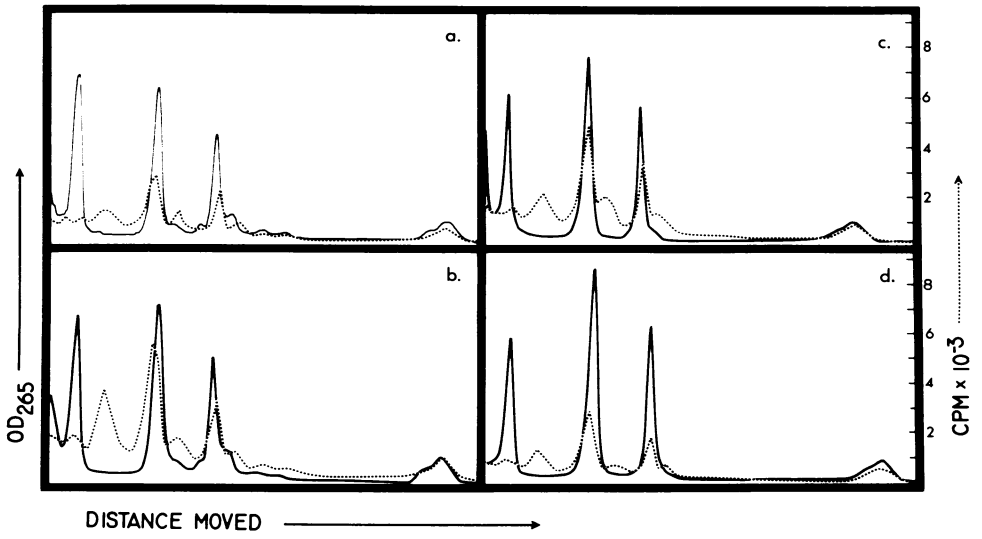

FIG. 8. Electrophoresis of postemergent samples. Conditions were the same as in Figure 7. Note the change in cpm scale. a: 1 mm (36 hr); b: 2 mm (41.5 hr); c: 5 mm (58 hr); d: 10 mm (73 hr).

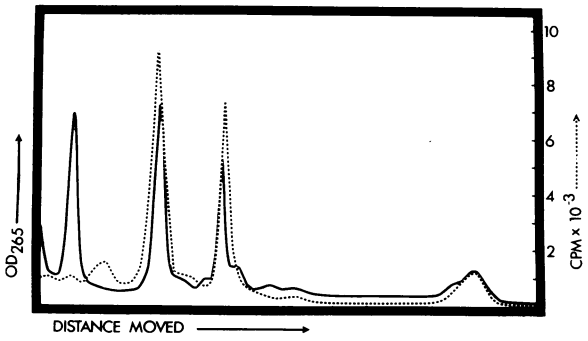

FIG. 9. Thirty-six-hour (1 mm) roots were labeled for 1 hr in ^{3}H-uridine and chased for 2.5 hr in the manner previously described (Fig. 1). Electrophoresis was carried out as indicated in the legend to Figure 7.

Evidence suggesting that the observed incorporation of ^{3}H-uridine into the various RNA fractions reflects both *de novo* and net synthesis of those fractions is given in Figure 5. The data clearly show that per cell levels of rRNA and soluble RNA increase throughout the first 73 hr (10 mm) of germination. Similarly the per cell amount of chl RNA also increased during the first 36 hr (1 mm) of germination but then decreased at 58 hr (5 mm), to a level undectable by UV scanning.

DISCUSSION

This study has reported the results of the first experiments designed to map the patterns of nucleic acid synthesis during the first 73 hr of germination in seeds of *Allium cepa*. It has been concerned with some of the quantitative and qualitative changes in the RNA and DNA associated with the 1-mm apex of the *Allium* embryo as it undergoes its initial post-quiescent stages of development.

The data presented in Figure 7 combined with that of Bryant (3, 4) clearly show that the onset of RNA synthesis in germinating *Allium cepa* precedes the initial uptake of ^{3}H-thymidine into nuclear DNA and most probably nuclear DNA synthesis, by a minimum of 18 to 20 hr. The per cell amount of DNA remains constant during the pre-emergent stages of germination with the initial per cell increases beginning sometime during the period in which the root increases from 1 mm (36 hr) to 2 mm (41.5 hr) in length. These data confirm the earlier reports by Bryant, who showed cytochemically that the initial uptake of ^{3}H-thymidine (3) and the initial increase in DNA content per cell (4) occur when the root is 1.4 mm to 2.0 mm in length. The reliability of the electrophoretically determined DNA content per cell can be demonstrated in the following manner. It has been shown that by 10 mm (73 hr) of germination the cells of the 1-mm apex have reached a stable mitotic index with a cycle time of 12.8 hr during which the cells are in a G_2 (4C) configuration approximately 50% of the time (4). Using the G_1 (2C) value of 11.8 pg DNA per cell (Fig. 5), one can calculate that at 10 mm (73 hr) the expected DNA content per cell should be 17.7 pg, a value slightly lower than the observed value of 19.04 pg (Fig. 5). Although the discrepancy is less than 10%, an explanation can be found in the fact that the 10-mm (73 hr) root tip contains a number of apparently nondividing 4C cells (Bryant, personal communication), the presence of which would tend to increase the observed per cell DNA values.

The data of Figure 5 also indicate that the 3-fold increase of total RNA per cell during the 10 mm (73 hr) of germination is the combined result of a 3-fold increase in sol RNA, a 4-fold increase in rRNA, and a depletion of chl RNA and 0.80m RNA. Interestingly, the sol RNA comprises approximately the same percentage of the total RNA at 4 hr and at 73 hr (10 mm) of germination (*i.e.*, 11–12%). If the sol RNA is calculated as a percentage of the rRNA, only small fluctuations are noted (*i.e.*, 14% at 4 hr, 16% at 41.5 hr (2 mm) and 11% at 73 hr (10 mm)). It appears, therefore, that drastic changes in the relative amounts of sol RNA and rRNA per cell do not occur during the germination period. Perhaps the increase in amounts of tot RNA per cell simply reflect the cells' increasing capacity for protein synthesis, as suggested by the data of Mallery (26), while the relatively constant sol RNA-rRNA ratio indicates optimal conditions for *in vivo* translation.

The loss of chl RNA from the samples after 58 hr (5 mm) of germination is easily explained by the displacement of cells containing these species out of the 1-mm apex by cell enlargement and cell division. The unpublished observations of Mallery and Melera, and Bryant have shown that the 1-mm tip of a 5-mm *Allium* root does not contain the leaf primordium or, due to their relative positions in the embryo (11) any cotyledonary tissue. A definite statement concerning the distribution of hypocotyl (a tissue shown to contain chloroplast-like rRNA [14]) at this point in germination cannot be made, however, and it is quite possible that after 58 hr (5 mm) of germination the 1-mm apex still contains some hypocotyl tissue.

The radioactivity which remains associated with the 23S and 16S regions of the gel after the chl RNA absorbance is no longer present is somewhat difficult to interpret. The possibility of bacterial contamination cannot be ruled out here as it was in the pre-emergent intervals (see "Appendix"), in fact with the bacterial contamination constant (*i.e.*, $1–2 \times 10^4$ bacteria per 100 root tips), and the amount of chl RNA decreasing (Fig. 5), any bacterial contribution to the 23S and 16S radioactivity becomes more significant as germination time increases. If, however, the considerable labeling seen in the 5 mm root 23S and 16S RNA (Fig. 8c) is due to bacterial contamination, the cause of the low level of incorporation seen in the 10-mm root 23S and 16S RNA (Fig. 8d) is not clear. It seems more reasonable to conclude that the 10 mm labeling represents the level of the bacterial contribution, if any, and that the 23S and 16S labeling in the 5-mm roots is primarily due to the synthesis of plant 23S and 16S RNA.

The function of the 0.80m RNA is unknown. This RNA is not synthesized during the time studied in this work as indicated by its lack of ^{3}H-uridine incorporation. The loss of the 0.80m RNA from the cells of the 1-mm apex coincides in time with the loss of the chl RNA species, suggesting that both these types of RNA are in the same cells. There is a 30% increase in the total RNA per cell through the initial 36 hr of germination (Fig. 5), yet preliminary results (Melera, unpublished observations) suggest that the relative percentage of the 0.80m RNA remains essentially constant (*i.e.*, 3–4% of the total RNA) during this time. These data suggest that the 0.80m RNA is synthesized from existing unlabeled precursors. a process difficult to visualize in light of the other data presented here concerning RNA synthesis in the apex. One possibility is that the 0.80m molecule may be a degradation product of existing and therefore unlabeled 1.07m RNA. Under the conditions of extraction used here the extent of degradation caused by exonuclease is minimized and it seems unlikely that one fourth (0.27m) of a 1.07m molecule would be degraded. Since no evidence for a 0.27m molecule has been found to date in the *Allium* root tip, it cannot be concluded that the 0.80m molecule is a product of an endonucleolytic attack on the 1.07m molecule.

At the time of the initial detection of ^{3}H-uridine incorporation into electrophoretically banded RNA (*i.e.*, at 18 hr of germination), both the cytoplasmic (*i.e.*, 1.3m and 0.70m) and "chloroplast-like" (*i.e.*, 1.07m and 0.59m) RNA became labeled. This observation is not in agreement with that of Ingle (12) who reported a lag of 24 hr between the initial ^{32}P labeling of cytoplasmic rRNA and chloroplast rRNA in the cotyledons of germinating radishes. Several recent papers (12, 13, 32) have presented data which suggest that chloroplast rRNA is synthesized in the chloroplast, and that light enhances the synthesis of chloroplast rRNA over that of cytoplasmic rRNA. That light is not an absolute requirement for proplastic rRNA synthesis was suggested by Vedel (38) in work with cucumber seedlings and by Ingle (13) using radish cotyledons. Assuming the 1.07m and 0.59m RNA of the *Allium* root tip to be proplastic, the data of Figure 7 and Figure 5 support the observation that synthesis of proplastic RNA does not require light.

The apparent initiation of synthesis of both the "chloroplast-like" and cytoplasmic RNA components of the *Allium* root tip at the same time of germination might be explained in several ways. Three of the more obvious possibilities are (*a*) the mechanism(s) responsible for the initiation of synthesis of cytoplasmic rRNA (*i.e.*, rRNA transcribed from genes located in the nucleus) and the mechanism(s) responsible for the initiation of the synthesis of proplastic rRNA (genes for which, presumably, are in the proplastid, in a manner similar to the location of chloroplast rRNA genes in the chloroplast), are both triggered at the same point during the germination process; (*b*) nuclear RNA synthesis is initiated at 18 hr of germination and that at this early stage nuclear 23S and 16S rRNA genes are being transcribed in the nucleus, or (*c*) the data are not valid and the synthesis of 23S and 16S rRNA in *Chlamydomonas* are in the chloroplasts (35), data the "Appendix" of this paper and the quantitative data of Figure 5 indicate that bacterial contaminants did not contribute significantly to these observations. The available information does not allow a choice between (*a*) and (*b*), nor are they mutually exclusive. It should be pointed out that although data have been published which indicate that the only functional genes for the production of 23S and 16S rRNA in *Chlamydomonas* are in the chloroplasts (35), data have not been forthcoming showing this to be the case in higher plant cells. In fact, recent evidence indicates that nuclear DNA isolated from several different higher plant species contains sequences complementary to both chloroplastic and cytoplasmic rRNA (28). Therefore, the possibility for the existence of functional 23S and 16S rRNA genes in the nucleus of the higher plant cell cannot, at this time, be ruled out.

It is apparent that the G_1 (2C) cells of the *Allium* root apex undergo changes in RNA metabolism as they enter the initial postgerminative cell cycle (*i.e.*, S phase at about 1.4 cm [3]). The accumulation of RNA per cell begins to increase rapidly at this time and the possible effect of gene-dosage must be considered as the rDNA is presumably duplicated. A comparison of the early data from 4 hr to 41.5 hr (2 mm) with the data obtained at 73 hr (10 mm) shows that while there is both an increase in specific radioactivity of RNA and in the amount of RNA per cell during the early period, there is a large decrease in specific radioactivity of the RNA with a continual accumulation of RNA per cell during the latter period. Obviously, pool-size changes cannot be ruled out, and their effect on the specific activity of the RNA could be drastic; however, the data may indicate a gradual switch from the predominant synthesis of rapidly turning over RNA (*i.e.*, high specific radioactivity, but relatively little accumulation) during the earlier stages of germination to the predominant synthesis of slowly turning over RNA (*i.e.*, relatively low specific radioactivity, but large accumulation) during the latter stages of germination.

Studies on animal (1) and plant cells (42) have indicated that the metabolism of the G_1 (2C) cell is different from that of the G_2 (4C) cell. Furthermore, the synthesis of RNA has been shown to change as the animal cell passes from G_1 phase to the G_2 phase of the cell cycle (7, 8, 30, 31). It has also been indicated that the control responsible for this change cannot be explained by a simple gene-dosage phenomenon alone (8). From these observations it seems reasonable to assume that control mechanisms influencing G_1 cells differ from those influencing G_2 cells. If this is the case the picture becomes more complex when the apparent overall controls involved with the entrance of cells into, and the exit of cells from, quiescence or dormancy are considered. Some simplification of these conditions can be obtained with the use of naturally G_1 or G_2 cell populations and the *Allium* root tip may prove to be a useful tool in the study of eucaryotic cellular control mechanisms.

APPENDIX

The problem of bacterial contamination in studies of plant nucleic acid metabolism has been considered by several authors (12, 25, 34, 36). In most cases the use of antibiotics has been effective in reducing the bacterial populations to presumably insignificant levels. In others (12) the tissue used was significantly bacteria free so that only aseptic handling was necessary to avoid bacterial contamination problems.

The seeds of *Allium* were found to be highly contaminated with bacteria, primarily *Pseudomonas*. All attempts at surface sterilization (including the use of various concentrations of hypochlorite or mercuric chloride or washes with SDS) either inhibited germination or disturbed the growth curve or both. The use of antibiotics in the germination and incubation media was ruled out due to reports that drugs such as chloramphenicol and streptomycin inhibit the synthesis of chloroplast RNA (13). This, together with concern that such antibiotics might affect the mitotic characteristics of the root tips, indicated that the sterile water wash procedure was the best way to reduce the bacterial contamination in this system.

Initial autoradiographic experiments had shown pre-emergent seeds of *Allium* to be impermeable to labeled precursors such as nucleosides and amino acids (Melera, unpublished observations; Bryant, personal communication). Other studies ([26, 29], and Mallery and Melera, unpublished observations) had established the interior areas of the *Allium* seeds as essentially bacteria free while showing that the primary, if not sole source of bacterial contamination, was the outer layer of the seed coat.

The impermeability of the seed coat to labeled precursors allowed a quantitative estimation of the amount of label incorporation being carried on by the contaminating bacteria. Seeds were germinated as described and a pre-emergent sample incubated for 1 hr in ^{3}H-uridine (20 μc/ml, 4 c/mmole, Schwartz) or L-(methyl-^{3}H)methionine (100 μc/ml, 5.2 c/mmole, Schwartz). After labeling the seeds were rinsed well with distilled water, squashed, and the apical 1 mm of the root harvested. The nucleic acids were then extracted and fractionated. A second experiment with a similar sample was performed except that the seeds were subjected to the sterile water wash sequence outlined in Figure 1.

To check the effectiveness of the wash procedure on root tips, seeds were germinated for 24 hr, at which time they were collected, squashed, and incubated for 1 hr in L-(methyl-^{3}H)methionine. At the end of the incubation time the squashed

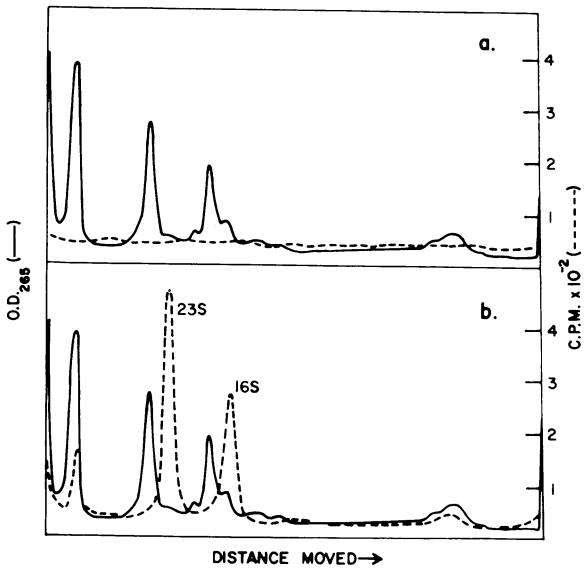

FIG. 10. Electrophoresis of 18-hr unsquashed seed TNA labeled with ^{3}H-uridine. Electrophoresis was as in Figure 7. a: Washed seed; b: non-washed seed.

seeds were rinsed with distilled water, the root tips were excised, and the nucleic acids were extracted and fractionated. In another experiment the 24-hr seeds were squashed, and the exposed root tips were washed and labeled as described in Figure 1.

Figure 10 shows the results of a 1-hr incubation of 18-hr seed in ^{3}H-uridine. The bulk of the incorporation is seen to be in the 23S and 16S regions of the gel with some incorporation coincident with the plant DNA. That this incorporation is not into the plant 23S and 16S RNA or plant DNA is indicated by Figure 10a, which shows a complete loss of the incorporation if the seeds are carried through the sterile water washing procedure. Identical results were obtained for 4-, 12-, and 24-hr seed. Incubation of the seed in L-(methyl-^{3}H)-methionine gave similar results.

The experiment designed to check the effectiveness of the wash procedure on exposed root tips gave results similar to those for the pre-emergent seed shown in Figure 10. Twenty-four-hour seeds of *Allium* will not incorporate radioactive precursors due to the impermeability of the seed coat. However, if one squashes the seed and then incubates in radioactive solutions, the exposed root tip does incorporate ^{3}H-uridine (Fig. 7d) and ^{3}H-amino acids (26), but does not incorporate methyl label from L-(methyl-^{3}H)methionine in a 1-hr incubation at a concentration of 100 μc/ml. Methyl group incorporation into the DNA and 23S and 16S regions of the nucleic acid profiles of 24-hr nonwashed root tips was eliminated if the root tips were subjected to the sterile water wash procedure.

As a final check on the bacterial contamination, root tips from each interval were aseptically homogenized after the sterile water washing and small aliquots plated (29). It was found that none of the samples were bacteria free, and that the average number of bacteria per 100 root tips at any given interval was 1 to 2×10^4.

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