Production of Messenger RNA During Seed Germination *

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Previous reports have shown a threefold increase in the level of RNA in peanut cotyledons during germination (Marcus and Feeley, 1962; Cherry, 1963a). Another study (Cherry, 1963b) showed with germination of peanut seeds a number of enzyme systems are initiated, as can be concluded from an increase in enzyme activities. The activity pattern of the enzymes studied closely resembles the pattern for RNA content per cotyledon during germination. Data from these studies show that DPNH cytochrome C reductase, glucose-6-phosphate dehydrogenase and isocitritase reach maximum activities after approximately 6 to 8 days of germination. During the latter period of germination (7 to 14 days), the activities of these enzymes and the level of RNA decrease while RNAase activity increases. It would be expected that accompaning changes in enzyme activity patterns, one would observe changes in messenger RNAs. We have shown (Cherry et al., 1965) that the amount of messenger RNA synthesized by peanut cotyledons decreases with age. It would be expected that a preferential synthesis of the constitutive enzymes in the early part of germination and of RNAase and other enzymes

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associated with the final stage of senescence, would enable one to detect qualitative changes in the mRNA synthesized in cotyledons at various stages of germination.

We have compared the messenger RNA population in peanut cotyledons by a double labeling technique of Kano-Sueoka and Spiegelman (1961) and show quantitative and qualitative differences in mRNAs at various stages of germination.

Methods Cotyledons were excised from etiolated peanut seedlings after 2, 7 and 14 days of germination as described previously (Cherry et al., 1965). Ten gram samples of sliced cotyledons were incubated for 4.5 hours in 15 ml of a medium (1% sucrose, 10^{-4} M ammonium citrate buffer, pH 6.0) at 30°. Peanut cotyledons grown for 2- and 7- days were sliced and incubated with 1 mc of uridine-H^3, while cotyledons of 2-, and 14-day old peanuts were each incubated with 0.02 mc of uridine-2-C^{14}. After incubation, the cotyledons were washed with water and the nucleic acid was isolated by a method previously described (Cherry et al., 1965), with the exception that the concentration of dupanol was 2% with respect to the aqueous phase.

In order to compare the labeling patterns of nucleic acid with age, two samples, one labeled with H^3 and the other with C^{14} were chromatographed on methylated albumin (MAK) columns prepared according to Mandell and Hershey (1960). Nucleic acids were eluted by means of a linear gradient of sodium chloride from 0.4 M to 1.2 M NaCl in 0.05 M sodium phosphate, pH 6.7 and the amount in consecutive 5 ml fractions of effluent determined by U.V. absorbancy at 260 mu. Four ml aliquots of each fraction of the effluent were mixed with 0.5 mg carrier DNA and the nucleic acid was then precipitated in 5% TCA. The precipitate was collected on a millipore filter and washed with TCA. The dried filter was placed in a vial containing 0.4% PPO and 0.1% POPOP in toluene, and total radioactivity from H^3 and C^{14} nucleic acids was determined with a Packard Tri-Carb Scintillation Spectrophotometer.

Results The nucleic acids isolated from peanut cotyledons was fractionated

on MAK columns into six fractions. In this paper the main concern deals with changes in the ribosomal and messenger RNAs. These nucleic acid fractions have been partially characterized by Cherry (1964), Cherry et al. (1965), and Chroboczek (1965). According to the results of Kano-Sueoka and Spiegelman (1962) differences in RNA extracted from a bacteria and bacteriophage can be resolved if the nucleic acid of each source is labeled with a different isotope, but with the same precursor and then chromatographed on a MAK column. A difference in the two radioactive profiles would indicate a change in the RNA synthesized. To test this technique with peanut cotyledon tissue, we compared chromatographic patterns of nucleic acids from cotyledons of the same age, but labeled with different isotopes. Figure 1A shows that the elution profiles of 2-day old peanut cotyledon nucleic acids labeled with both uridine-H^3 and uridine-2-C^{14} are similar, but not identical in the ribosomal and messenger RNA region. While the H^3/C^{14} ratio indicates a slightly larger synthesis of H^3 labeled mRNA, it seems that the synthesis of mRNA by these two samples of peanut cotyledon slices of the same age are nearly identical. The slight difference indicates the amount of variation one may expect from two samples that are supposedly identical.

When nucleic acids from 2-, and 7-day old cotyledons are chromatographed together on MAK columns, the bulk of the messenger RNA produced by the 7-day old peanuts is eluted at a higher NaCl concentration than that of the 2-day old peanuts (figure 1B). It is also noted there is a relatively greater amount of mRNA synthesized by 7-day old peanut cotyledons than by 2-day old cotyledons. The difference in the elution pattern of mRNA extracted from 2-, and 14-day old peanuts is even more distinct (figure 1C). The pattern of mRNA between 7-day and 14-day old cotyledons is also different as shown in figure 1D. A comparison of the H^3/C^{14} or C^{14}/H^3 ratios reveal a relatively larger synthesis of mRNA in older peanut cotyledons as compared to younger cotyledons. The fact that the ratio of mRNA synthesized by older cotyledons to mRNA synthesized by younger cotyledons increases but does not decrease (figure 1C and 1D)

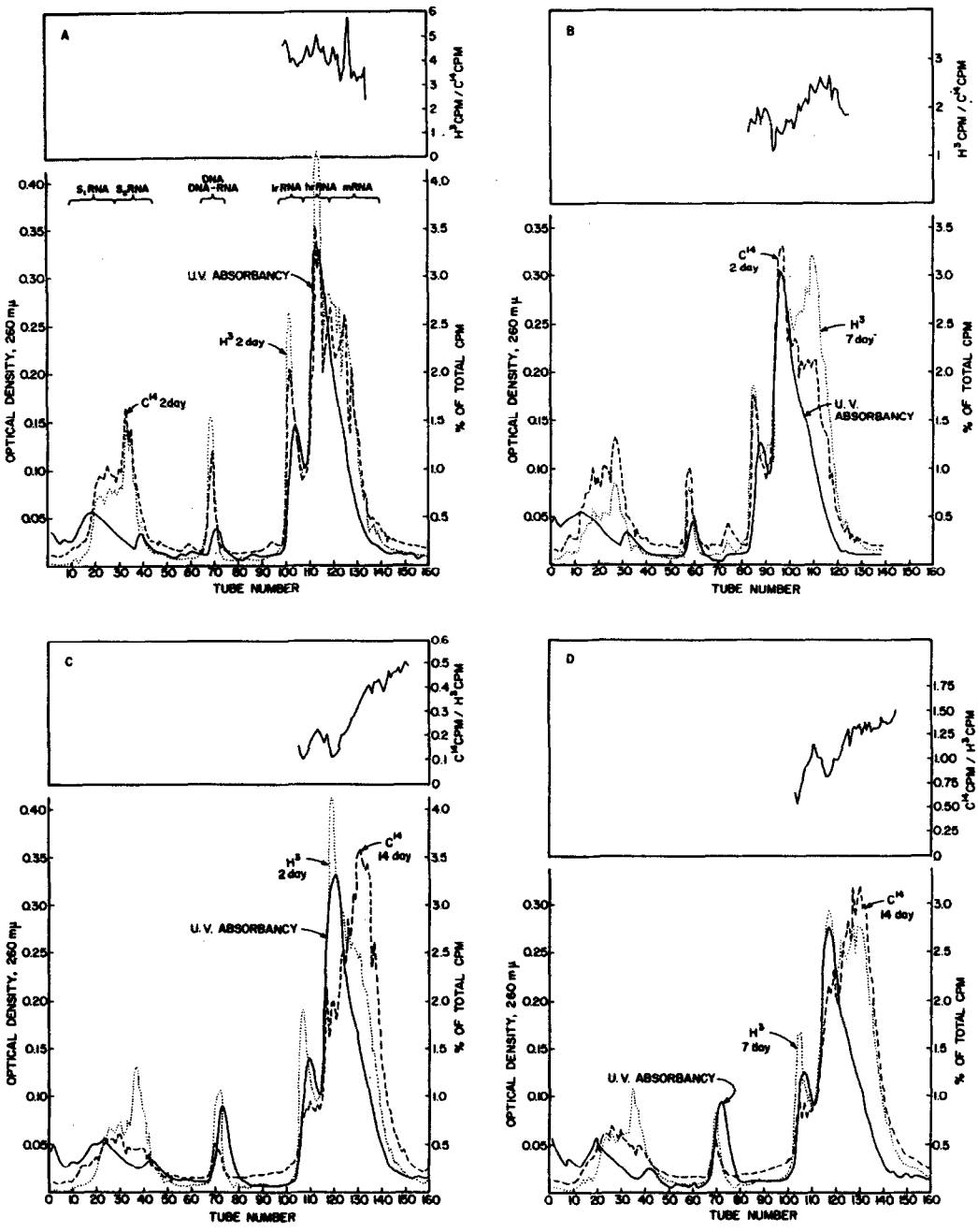

Figure 1. Comparison of MAK elution patterns of nucleic acids isolated from peanut cotyledons of various ages. Peanut cotyledons were removed from seedlings at 2, 7 and 14 days after planting the seed and labeled with either uridine-H^3 or uridine-2-C^{14}. Combination of two nucleic acid samples in which one was labeled with H^3 and the other with C^{14} was placed on a MAK column. The nucleic acid was eluted with a linear gradient of NaCl from 0.4 M to 1.2 M and the six following fractions were obtained: soluble (S_1) RNA, soluble (S_2) RNA, DNA-RNA, light ribosomal (lr) RNA, heavy ribosomal (hr) RNA, and messenger (m) RNA. Two day H^3-nucleic acid and 2 day C^{14}-nucleic acid are compared in A. Two day C^{14}-nucleic acid and 7 day H^3-nucleic acid are compared in B. Two day H^3-nucleic acid and 14 day C^{14}-nucleic acid are compared in C. Seven day H^3-nucleic acid and 14 day C^{14}-nucleic acid are compared in D.

indicates that the mRNA synthesized by 14-day old peanut cotyledons is qualitatively different from that synthesized by 2-day and 7-day old cotyledons.

Sueoko and Cheng (1962), reported that the MAK column fractionates nucleic acid on the basis of their nucleotide composition and their molecular weight in which both contribute to the total molecular charge. Similar findings were reported for our system by Chroboczek (1965), i.e. nucleic acids of peanut cotyledons eluted from the MAK column at higher NaCl concentrations have a higher content of adenylic acid and uridylic acid. Therefore, the observed change in the elution pattern of the mRNA in peanut cotyledons of different ages is evidence of a change in the base composition of the average mRNA during germination.

The data presented here show that messenger RNA is preferentially synthetized during prolonged incubation of peanut cotyledon slices obtained from seeds germinated for 7 and 14 days. After 7 and 14 days of germination, the storage materials (lipids, proteins, etc.) of the cotyledons are depleted by 50% and 70%, respectively (Cherry, 1963a). Slices of 2-day old cotyledons incubated under the same conditions synthesize relatively little mRNA. The depletion of storage materials may cause a proportional decrease in RNA and protein synthesis during incubation. This observed phenomenon appears to be similar to the "step-down" transition of bacterial cells. Hayashi and Spiegelman (1962) have shown that bacteria preferentially synthesize mRNA, when transferred to a culture medium deficient in a particular nutrient. It is possible that some kind of repression mechanism exists in cells which retards the synthesis of sRNA and ribosomal RNAs while the synthesis of messenger RNA is not affected.

Cherry *et al.* (1965) reported that aged peanut cotyledons synthesize less mRNA than those in the early stages of germination. Seeds used in the experiments given in this paper were smaller and the time of incubation was longer than in the previous report. These two factors might lead to a greater depletion of storage materials during incubation, thus causing a "step-down" type of effect.

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