DNA CONTENT OF DORMANT BARLEY LEAF NUCLEI AND THE SYNTHESIS OF RNA AND DNA DURING GERMINATION

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1. Introduction

The nature of the RNA synthesized during early germination appears to be controversial. In onion [1], lettuce [2], *Vicia faba* [3], *Phaseolus vulgaris* [4] and *Triticum vulgare* [5] RNA species synthesized include predominantly ribosomal and transfer RNA. In *Triticum durum,* Chen et al. [6] found no mRNA synthesis during the first 24 hr of germination; synthesis was triggered at a later time. On the other hand, reports on maize [7–10] suggest that earliest RNA synthesis (occurring between 4 and 8 hr of germination) involves mRNA but not ribosomal and transfer RNA. Similar results were also reported for *Triticum aestivum* [11] where it has been claimed that earliest RNA synthesis involves a discrete mRNA species of mol. wt 1.5×10^6 daltons.

We report here the DNA content in the dormant cell population of an organ of the barley embryo and the synthesis of RNA and DNA during the first 24 hr of germination. During this period the cell population does not change significantly in size since no appreciable cell division activity starts until about 22 hr after germination. The conflicting reports on the nature of the early RNA synthesis led us to consider a possible correlation between the DNA content of the dormant cell population and post dormancy RNA synthesis. A possible basis for such a correlation is discussed.

2. Materials and methods

The first leaf of barley embryo (*Hordeum vulgare,* var. Himalaya) was used in all experiments. The DNA content of nuclei was determined by two-wavelength Feulgen cytophotometry using a Zeiss UMSP 1 Universal Microspectrophotometer. Seeds were surface sterilized by 1% Na-hypochlorite for 10 min, washed and germinated on sterile filter paper in Petri dishes in the dark at 24 ± 1°C. To determine the rate of RNA and DNA synthesis part of the coleoptile was removed and the seeds were labelled for 1 hr with a solution of 20 μCi/ml [^{3}H] uridine (45 Ci/mmol) or [^{3}H] thymidine (6.7 Ci/mmol) obtained from New England Nuclear Corp., Boston, Mass. After fixation in ethanol: acetic acid, 3:1, leaves were dissected from the embryo, washed for about an hour in distilled water and homogenized. Radioactivity in the homogenate was determined by the method of Jakob and Bovey [12] except that the 5% TCA wash of the material collected on a GF-C disc was followed by sequential washes with ethanol, ethanol–ether 3:1 and finally ether. Discs were counted in 10 ml of Liquifluor (New England Nuclear) mixed with toluene. For the isolation of total RNA seeds germinated for 13.5 hr were labelled for 1 hr with a 150 μCi/ml solution of [^{3}H] uridine followed by a 4 hr incubation on a filter paper moistened with non-radioactive uridine solution in a Petri dish. The seeds were fixed and RNA was isolated from the washed leaves essentially by the method of Loening and Ingle [13]. It was treated with DNAse (Sigma, electrophoretically pure) and analyzed by polyacrylamide gel electrophoresis according to Bishop et al. [14] with a 2.4% gel. Soluble RNA was resolved into 5S and 4S components on a 5% gel. The gel was scanned in a Joyce-Loebel UV scanner, frozen on dry ice and sliced by a Mickle gel slicer. Two one-mm slices were put in a scintillation vial, digested overnight with 0.3 ml H_2O_2 at 70°C and counted in 5 ml Aquasol (New England Nuclear). A Nuclear

Chicago Isocap/300 instrument was used for all scintillation counting.

3. Results

DNA content determined in a sample of 100 nuclei from each of 8 different dormant leaves revealed that approximately 98.6% of the nuclei had 2C, 0.5% 4C and 0.8% intermediate DNA values.

Fig.1 shows the rates of RNA and DNA synthesis during germination. Both exhibit clear maxima. Rapid DNA synthesis starts at 12 hr and increases steadily to a peak at 18 hr. We have autoradiographic data (not presented here) which indicate that this pattern is caused by a cell population replicating their genome in partial synchrony. The pattern of RNA synthesis is quite similar but the peak is at 14 hr. The mol. wt of heavy and light rRNA were taken as 1.3×10^6 and 0.7×10^6, respectively since co-electrophoresis of barley RNA and wheat rRNA, for which the molecular weights of these fractions have been established [5] showed no differences in mobility. Mol wts of RNA species resolved on 2.4% gel were estimated using the heavy and light rRNA's as markers. Absorbancy and radioactivity profiles of the gel are shown in fig.2. Labelled RNA species included precursor rRNA (mol. wt 2.3×10^6), heavy and light rRNA, 5S and 4S RNA. Polydisperse radioactive peaks are not conspicuously present but some polydispersity in the radioactivity may represent heterogenous and/or mRNA.

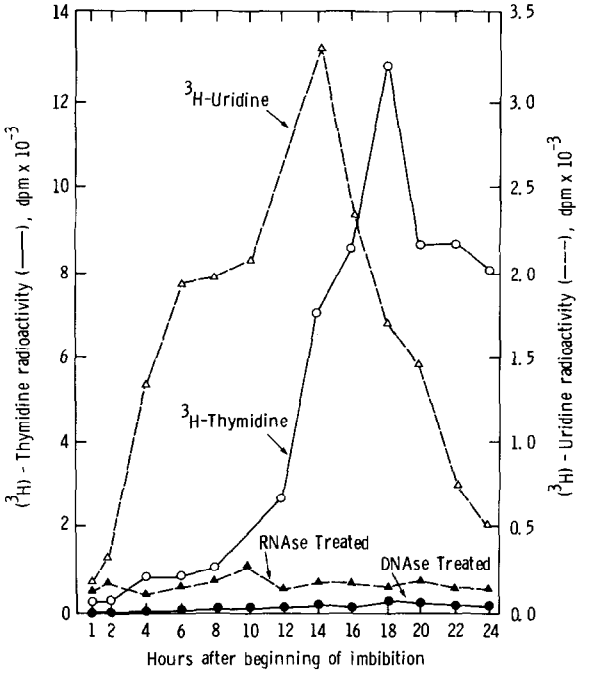

Fig.1. Rate of RNA and DNA synthesis in the first leaf of the germinating barley embryo. At different times during germination seeds were labelled for 1 hr with [^{3}H]uridine or [^{3}H]thymidine. For each experimental point 16 leaves were homogenized in 0.9 ml buffer and the homogenate was divided into two equal parts, one of which was treated with RNAse (uridine label) or DNAse (thymidine label). Radioactivity in the TCA precipitable material was determined.

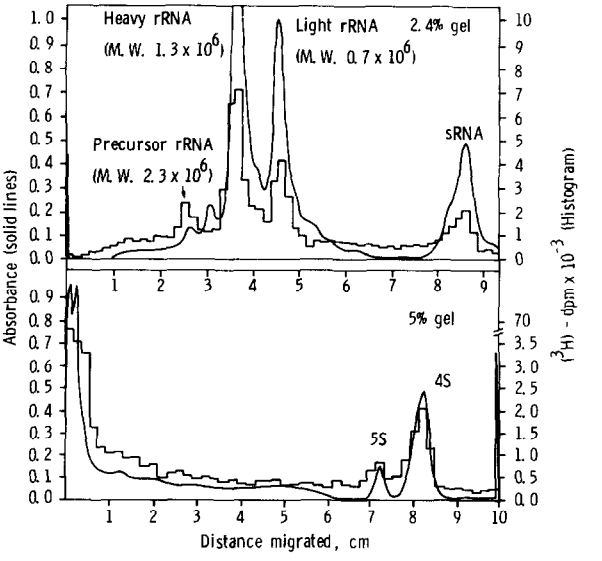

Fig.2. Absorbancy and radioactivity profiles of barley total RNA analyzed in 2.4% (top) and 5% (bottom) gel of diameter 0.65 cm. RNA was dissolved in Electrophoresis buffer containing 0.2% SDS and 15% sucrose. 75 µl RNA solution containing approximately 1.5 A_{260} unit (assuming $E_{260}^{1\,cm}$ 1 mg/ml as 20) was layered on the gel and electrophoresed for 3 hr at 5 mA per gel.

4. Discussion

Available information on the state of the dormant genome in relation to DNA replication indicates that in onion [15], lettuce [16], *Vicia faba* [12,17],

Triticum durum [18] and barley [19,20] the genome of the dormant cells is in the pre-DNA replicative state, while in maize [21] it is in the post-DNA replicative state. On reviewing the nature of early RNA synthesis one finds that embryos of the first group predominantly synthesize rRNA and very little or no mRNA while those of the second responds to germination by synthesizing RNA species other than ribosomal and transfer RNA, presumably mRNA, and the synthesis of rRNA is delayed until a later time. Our results indicate that dormant genomes in barley leaf are in the pre-DNA replicative state and RNA synthesized prior to the DNA synthetic peak consists primarily of ribosomal and transfer RNA.

The reason for the apparent correlation between the replication state of the dormant genome and the nature of the early RNA synthesis can only be conjectured. One likely possibility, however, is that cells facing the task of replicating the genome during early germination may need to reinforce the protein synthesizing machinery in order to ensure a normal genome replication. In embryos where cells enter early germination with the genome already replicated during embryo development such a response may be delayed. There are very convincing demonstrations that early protein synthesis in the germinating embryo is supported by pre-formed mRNA (for discussion see Walbot [4] and Walbot et al. [22] and that the entire apparatus for protein synthesis is present in the dormant embryo [23,24]. However, the possibility that the DNA replication apparatus, particularly its protein component, is unable to function optimally cannot be ruled out. Thus, transcription of rRNA genes may be the earliest detectable event in cells needing the machinery to replicate the genome. This would explain the results in onion [1], lettuce [2], *Vicia faba* [3], *Phaseolus vulgaris* [4], *Triticum vulgare* [5], and barley (present report) while in maize [7–10] which has cells entering germination with an already replicated genome the earliest detectable transcription does not involve rRNA genes. Possibily the situation in *Triticum aestivum* [11] is similar to that in maize although data on the state of the dormant genome of this species are lacking. If rRNA genes are the earliest to be transcribed then mRNA, even if synthesized simultaneously, would not be detectable by the techniques which have been used because of the large excess of rRNA. On the other hand, a delay in ribosomal gene transcription would render any mRNA synthesis detectable. Therefore, the question is not which RNA is synthesized first but rather when is rRNA synthesized.

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