Preformed and Newly Synthesized Messenger RNA in Germinating Wheat Embryos

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Abstract. In 6 h germinated wheat (Triticum aestivum L. cv. Cama) embryos, more than half of the messenger RNAs are actively involved in translation. Neither preformed nor newly synthesized poly A⁺-RNA is translated preferentially. Germination in the presence of cordycepin showed that the half-life of the templates is about 2 h and that the newly synthesized messengers are essential to support protein synthesis in the embryo from the first hours of germination. Most of the messenger RNAs in 6 h germinated embryos are newly synthesized. The polypeptides coded for by either the endogenous messenger ribonucleoproteins or purified poly A⁺-RNA from both dry and germinated embryos are qualitatively identical; minor quantitative differences can however be observed.

Key words: Embryo (mRNA) – Germination (embryo) – Messenger ribonucleoprotein – mRNA – Ribonucleoprotein (messenger) – Translation – Triticum.

Introduction
The occurrence of preformed messenger RNA in dry seeds has been demonstrated for several plant species (Payne, 1976). Although these templates may be functional during germination of wheat embryos (Spiegel and Marcus, 1975), synthesis of poly A⁺-RNA starts immediately upon imbibition (Spiegel et al., 1975; Ajtkhozhin et al., 1973; Doshchanov et al., 1975). At the present, however, little is known about the relative importance (quantitatively and qualitatively) of both preformed and newly synthesized messengers. The results of earlier experiments with actinomycin D, which pointed towards an important role of the preformed messengers have to be interpreted with care and are not conclusive at all (Payne, 1976). The availability of potent and more specific inhibitors of mRNA synthesis, such as cordycepin and α-amanitin, enabled a reexamination of this problem. This paper deals with the translation activity, including product analysis of preformed and newly synthesized poly A⁺-RNA during germination of wheat embryos. The effect of cordycepin on template content and protein synthesis was investigated. A comparison was made between the coding properties from dry and germinated embryos.

Materials and Methods

1. Material
Wheat embryos were prepared as described previously (Carlier and Peumans, 1976).

2. Germination Conditions
Embryos were germinated at 30 °C in the dark in germination medium (GM) containing 10 mmol l⁻¹ Tris HCl pH 7; 20 mmol l⁻¹ KCl; 10 mg/ml sucrose and 50 μg/ml chloramphenicol.

3. Separation of Post-Polysomal and Polysomal Fractions
Embryos were ground in a precooled mortar in 10 volumes of polyosme buffer (PB) containing 100 mmol l⁻¹ Tris HCl pH 9; 100 mmol l⁻¹ KCl and Mg-acetate or EDTA as indicated. The homogenates were centrifuged at 10,000 g for 4 min in an Eppendorf 3200 microcentrifuge. The supernatant (1 ml/gradient) was brought on 14-38% hyperbolic sucrose gradients with a 5 ml cushion of 50% sucrose, made up in PB. Centrifugation was carried out in a Beckman SW 27 rotor for 2.5 h at 25,000 rev min⁻¹.
(82,000 g). The gradients were analysed with an ISCO density fractionator. The post-polysomal and polysomal fraction were collected separately and precipitated with 2 vol ethanol at -20°C.

4. RNA Extraction and Oligo dT-Cellulose Chromatography

RNA was extracted from the 40,000 g min supernatant, the post-polysomal and the polysomal fractions according to Brawerman (1974). Poly A⁻RNA and poly A⁺RNA were separated on oligo dT-cellulose according to Aviv and Leder (1972).

5. Preparation of Wheat Embryo Extracts for Cell-Free Protein Synthesis

Embryos were ground in a precooled mortar and extracted with HEPES buffer (20 mmol l⁻¹ HEPES-KOH; 2 mmol l⁻¹ Mg-acetate; 120 mmol l⁻¹ KCl; 6 mmol l⁻¹ mercaptoethanol). When the endogenous template activity was assayed, extraction was done at pH 7.8; for assays of exogenous RNA, extraction was done at pH 6.25 in order to lower the endogenous template activity (Peumans et al., 1978). Further preparation of the extracts was according to Peumans et al. (1978).

6. Cell-Free Amino Acid Incorporation and Product Analysis

Cell-free protein synthesis was performed as described previously (Peumans and Carlier, 1977). The ³⁵S-labelled polypeptides were electrophoresed on 15% SDS-acrylamide slab gels. Fluorography was done according to Laskey and Mills (1975).

7. Protein Synthesis in Vivo

Embryos germinated in the presence of [³H]leucine (112 Ci mmol⁻¹) were homogenized in PB with 2 mmol l⁻¹ Mg-acetate. The homogenate was centrifuged at 40,000 g min and the hot TCA insoluble radioactivity in the supernatant was determined in three aliquots of 50 µl.

Table 1. Distribution of RNA in extracts from 6h germinated wheat embryos over post-polysomal and polysomal fractions in the presence of 2 mmol l⁻¹ Mg²⁺

<table>
<thead>
<tr>
<th>Fraction</th>
<th>RNA (µg)</th>
<th>CT min⁻¹</th>
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<tbody>
<tr>
<td>Total RNA</td>
<td>3.1 mg</td>
<td>689,400</td>
</tr>
<tr>
<td>Post-polysomal RNA</td>
<td>1.1 mg</td>
<td>318,000</td>
</tr>
<tr>
<td>Poly A⁻RNA</td>
<td>3 mg</td>
<td>636,048</td>
</tr>
<tr>
<td>Poly A⁺RNA</td>
<td>17 µg</td>
<td>23,392</td>
</tr>
</tbody>
</table>

Results

1. Activity of Messengers in 6 h Germinated Embryos

In germinating wheat embryos, RNA synthesis starts immediately upon imbibition. Moreover a considerable fraction of the newly synthesized RNA represents poly A⁺RNA and thus may be considered as mRNA. In order to know how much of the newly synthesized mRNA is actively involved in protein synthesis, homogenates from 6 h germinated embryos were separated on sucrose gradients and RNA was extracted from the polysomal and post-polysomal fractions. Both RNA preparations were separated into poly A⁺RNA and poly A⁻RNA on oligo dT-cellulose columns. Table 1 shows the distribution of the RNA in the polysomal and post-polysomal fraction. Some 54% of the total poly A⁺messengers, present in the embryos, are found in the polysomal fraction. However, this fraction is likely to be contaminated with large mRNPs (Dobrzenska et al., 1973). In order to distinguish these free mRNPs from the polysomal mRNPs, an analogous homogenate was separated on sucrose gradients containing EDTA. The poly A⁺-RNA, obtained from the > 80 S fraction represented only 4 to 6% of the total messenger content so that still about 50% of the messengers in the embryo may be considered as polysomal. This could be a minimal estimation since some messengers complexed with ribosomal subunits or with monosomes (and thus involved in protein synthesis) could be present in the post-polysomal fraction. These experiments show that some more than half of the total poly A⁺-RNA in germinating embryos is active in the translation process. Since the specific activity of the messengers in both fractions is similar, it is likely that there is no selective use of either preformed or newly synthesized mRNA in the translation process.

2. Effect of Cordycepin on mRNA Synthesis and Protein Synthesis

As shown in Table 2, cordycepin strongly inhibits the RNA synthesis during germination. Control experiments demonstrated that cordycepin does not affect the uptake of [³H]adenosine, so the inhibitory effect of the drug is not due to an inhibited uptake of the tracer. Especially the synthesis of poly A⁺-RNA is inhibited when the drug is supplied from the onset of germination, although the inhibition is not complete. For determination of the life-time of the newly synthesized messengers, we added an excess of cold adenosine (1 mmol l⁻¹) together with cordycepin. As shown in Fig. 1, the half-life of poly A⁺-RNA is about 2 h. After 6 h chase, only 20% of the radioactivity is retained on oligo dT-cellulose. Non messenger RNA is much more stable.