Distinctive Characteristics of RNA and Protein Synthesis in Pea Cotyledons at Early Stages of Germination

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Abstract. The RNA and protein synthesis in pea cotyledons at early imbibition were studied. The results obtained show that RNA and protein synthesis are reactivated at the beginning of imbibition. The initial transcription is independent of protein synthesis, after 8 h synthesis RNA depends on translation by 44–52%. α-Amanitin (5 μg ml−1) inhibits 3H-uridine incorporation by 75%. It indicates the predominance of mRNA synthesis in early transcription.

The protein synthesis during first hours of imbibition depends significantly (by 50%) on RNA synthesis. It means newly-made mRNA is involved in the translation. Protein synthesis, which is independent of transcription, is provided by preformed mRNA. This fraction of translation decreases rapidly during imbibition.

Thus, protein synthesis in pea cotyledons depends mainly on newly-synthesized mRNA.

The in vivo synthesized polypeptides were fractionated by SDS-gel electrophoresis and visualized by autoradiography. The pattern of protein synthesis after 4 h of imbibition is very complex. Newly-synthesized polypeptides vary in m.m. (from 10 000 to 100 000), subcellular localization and solubility. 14C-labelled polypeptides do not belong to subunits of storage proteins.

The patterns of proteins, synthesized at different germination time (from 2 to 24 h), have much in common, but definite quantitative and qualitative changes in messenger population can be observed. During maturation of seed, the pattern of protein synthesis in pea cotyledons is changed rather quantitatively than qualitatively. Drastic changes in population of mRNA are observed in late embryogenesis. Patterns of proteins synthesized in late embryogenesis and early germination are similar.

The occurrence of preformed mRNA in dry seeds and reactivation of mRNA and protein synthesis in the early seed germination have been demonstrated for several species of plants (see rev. PAYNE 1977, BEWLEY and BLACK 1978, BEWLEY 1979, DELSENY et al. 1980—81). The relative importance of preformed and newly-synthesized mRNA for the protein synthesis and germination, their complexity, coding capacity and the time of translation are being studied (CHEUNG et al. 1979, CUMING and LANE 1979, DURE et al. 1980, SOPORY et al. 1980, THOMPSON and LANE 1980, SANCHEZ DE YEMENEZ et al. 1981). Little is known about the early translation and transcription in the storage tissues of seed embryos, many characteristics of these processes in cotyledons of legume seeds still remain obscure.

In this investigation an attempt was made to clarify the starting time and relationship of the RNA and protein synthesis and to characterize the products of the in vitro translation in pea cotyledons during the first hours of imbibition.

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MATERIAL AND METHODS

Pea seeds *Pisum sativum* (cv. Pobeditel) were surface sterilized by 5% hypochlorite Ca for 2 min; washed with cold water for 1 h. Then the seed coat was removed and the embryos were germinated in darkness at 28 °C with or without antibiotics (actinomycin D — 30 μg ml⁻¹; α-amanitin — 5 μg ml⁻¹; cordicepin — 200 μg ml⁻¹; cycloheximide — 50 μg ml⁻¹) in sterilized distilled water containing 50 μg ml⁻¹ of chloramphenicol. Mixture of 14C-amino acids 0.75 MBq ml⁻¹ (20 μCi ml⁻¹) “Amersham” U.K.; CFB—104, specific activity 2.1 MBq per mAtom C (57 mCi mM⁻¹) or “Chemapol” CSSR, specific activity 1.29 MBq per mAtom C (35 mCi mM⁻¹) or 3H-uridine 1.85 MBq ml⁻¹ (50 μCi ml⁻¹) USSR, specific activity 9.25 MBq per mM (26 Ci mM⁻¹) were added at zero time along with water or at indicated time of imbibition. The incubated embryos were washed with cold water, cotyledons were removed, frozen and homogenized in the presence of buffer (2 ml/1 cot.) containing 200 mM Tris-HCl, pH 8.4; 60 mM KCl; 30 mM Mg-acetate; 250 mM sucrose and 50 μg ml⁻¹ cycloheximide. The homogenate was used for determination of radioactivity in TCA-soluble and insoluble materials, to obtain subcellular fractions and samples of protein for electrophoresis. The disc assay of Mans and Novelli (1961) was used to measure incorporation of 14C-amino acids into proteins. The discs were washed with hot and cold 7% TCA, mixture of ethanol : ether (1 : 1), and ether. To determine the radioactivity incorporated into RNA an equal volume of 15% cold TCA was added to aliquots of cold homogenate; TCA-insoluble material was collected on Whatman paper discs, washed with cold 7% TCA, ethanol-ether mixture and ether, dried and counted in Intertechnique SL-30 scintillation counter. Radioactivity was expressed for 3H-uridine in s⁻¹ ml⁻¹ of homogenate and for 14C-amino acids in s⁻¹ mg⁻¹ of protein. Radioactivity in the TCA-soluble fraction was expressed in s⁻¹ ml⁻¹ of homogenate. Concentration of protein was determined as described (Bramhall *et al.* 1969).

Protein samples for electrophoresis were prepared as described (Maizel 1971). The samples were precipitated with cold 10% TCA, washed with TCA and acetone and solubilized in the buffer containing 62.5 mM Tris-HCl, pH 6.8; 2% SDS; 5% 2-β-mercaptoethanol; 8 M urea; heated at 100 °C, cooled and overlaid on the gel.

SDS-PAGE was carried out in the Laemmli’s system (1970), using the gel slabs with gradient of 10—20% acrylamide concentration (O’Farrel 1975). Details of the procedure were described earlier (Skazhennik *et al.* 1981). The labelled proteins were visualized by autoradiography.

The salt-soluble proteins were extracted by 5% K₂SO₄ and separated on albumins and globulins by Murray’s procedure (1979). Legumin and vicilin fractions were prepared by Scholz’s method (Scholz *et al.* 1974).

RESULTS AND DISCUSSION

The preliminary experiments have shown that the level of incorporated radioactivity depends on the amount of the label introduced into the incubation medium, temperature and time of imbibition. All the inhibitors used: actinomycin D (Act. D), α-amanitin (α-am), cycloheximide (CH) and cordicepin (Cord) — completely inhibited germination, producing no effect on embryo imbibition and absorption of the labelled precursors. As is seen in Fig. 1A, the cotyledons absorb the precursors in parallel with water uptake.