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Polysome Formation in Light-controlled Dormancy

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ROSEMARY C. MITCHELL AND T. A. VILLIERS
Department of Plant Biology, University of Natal, Durban, South Africa

ABSTRACT

Lettuce (*Lactuca sativa*) seeds var. Grand Rapids could be maintained many weeks in the dark without germination. Following illumination with white light, a gradual increase in polyribosome population up to the time of germination was demonstrated by sucrose gradient centrifugation. Polysomes could not be detected in imbibed seeds maintained continuously in the dark. Thus, polysome formation and therefore the capacity for a high rate of protein synthesis required for germination and growth, is not associated with the process of imbibition, but is dependent upon the seeds having received the dormancy-breaking stimulus of illumination.

It has been shown that in dry seeds the ribosomes are present largely or entirely as monosomes and that during imbibition and subsequent germination these aggregate to form polysomes (4, 8). It is considered that polysome formation is a prerequisite for the high rate of protein synthesis associated with germination and the establishment of normal growth by the seedling. Polysome formation requires the presence of mRNA, and therefore control of the production or availability of mRNA would be a convenient mechanism for the control of germination.

Many seeds, particularly those of cultivated plants, require only water at a temperature suitable for normal growth in order to commence germination, and are termed "quiescent." Many wild plants, however, have special germination requirements and remain dormant, even though fully imbibed, until this special condition is fulfilled. The possession of dormancy characteristics is of biological advantage and selection for the imposition and control of dormancy would be expected.

It is known that water stress in growing tissue is accompanied by the dissociation of polysomes to monosomes (7), and it is therefore of importance to establish whether the dissociation of ribosomes in the maturing seed is caused solely by water loss and is followed by their reaggregation immediately upon imbibition, or whether polysome formation is a phenomenon connected specifically with the germination process proper. For this purpose it is essential to choose a seed type showing true dormancy characteristics and to withhold the dormancy-releasing stimulus until after imbibition is completed.

Lettuce seeds var. Grand Rapids remain dormant unless exposed to light. Srivastava and Paulson (9) reported changes in fine structure which they observed by electron microscopy during imbibition and germination of Grand Rapids lettuce. They found that ribosome-like particles present in the dry seed apparantly became associated into groups upon imbibition.

As Srivastava and Paulson (9) did not separate the process of germination from that of imbibition in these seeds, the present experiments are reported in order to show that even after full imbibition of the seeds, polysome formation is delayed until the dormancy-breaking stimulus of light is received.

MATERIALS AND METHODS

Germination of Seeds. *Lactuca sativa* seeds, var. Grand Rapids were obtained as a gift from J. Ohlsens Enke, Copenhagen, and stored in glass bottles at 4 C. These were placed in batches of 100 on two layers of filter paper moistened with 4.5 ml of distilled water, in 9-cm Petri dishes. This was carried out under a green safelight. The dishes were then wrapped in aluminum foil and placed in an incubator at 25 C. After 24 hr any germinated seeds (about 4%) were discarded, the remainder were exposed to white light 20 cm from a daylight fluorescent tube for 20 min, and then replaced in the incubator. Ninety-six per cent of the seeds exposed to light germinated within 24 hr, whereas only approximately 4 to 10% germinated when kept in continuous darkness, even over a period of 12 weeks.

Ribosome Isolation. At different stages of imbibition and germination, 100 embryos were removed from their seed coverings. During excision the embryos were maintained at 0 C and immediately homogenized in a glass tissue grinder in 1.25 ml of extraction medium. This medium consisted of tris buffer (50 mM tris HCl, pH 8) containing 250 mM sucrose (RNase-free), 15 mM KCl, and 20 mM magnesium acetate. The homogenate was then centrifuged at 8000*g* for 20 min (Spinco SW 50 rotor) to remove cell debris. The pellet was discarded, and the supernatant was treated with 2% deoxycholate and then centrifuged for a further 20 min at 8000*g* (Spinco SW 50 rotor).

Nineteen ml linear sucrose gradients (10–34%) containing 50 mM tris–HCl, pH 8, 15 mM KCl, and 5 mM magnesium acetate, were formed over 5-ml cushions of 68% sucrose. One ml of the ribosomal supernatant was layered over the gradient and centrifuged at 90,000*g* for 120 min in a Spinco SW 25.1 rotor.

After centrifugation, the distribution of ribosomes in the gradient was determined by collection with continuous UV[1] recording in an Isco Model D density gradient fractionator, 17 fractions being collected from the top of the tube. A syringe speed of 2.5 ml/min was used and the absorbance profiles were determined at 254 nM. All steps (homogenization, centrifugation, and density gradient fractionation) were carried out between 0 to 5 C.

Samples for viewing in the electron microscope were prepared as follows from the fractions collected. One drop of sample was placed on a collodion-coated copper grid and left

[1] Abbreviation: UV: ultraviolet.

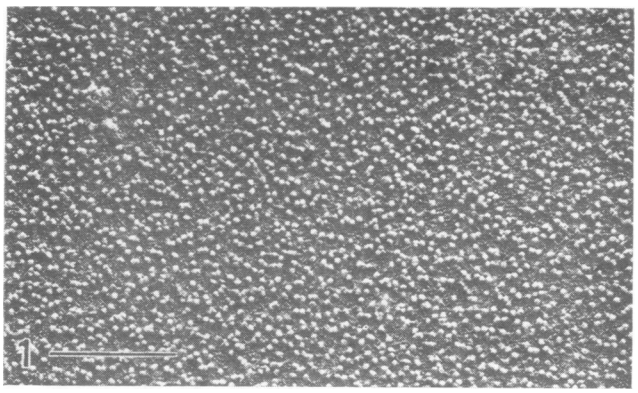

FIG. 1. Ribosomes isolated by density-gradient centrifugation from dark-imbibed lettuce embryos. Shadowed with gold-palladium. Scale line equivalent to 0.5 μM.

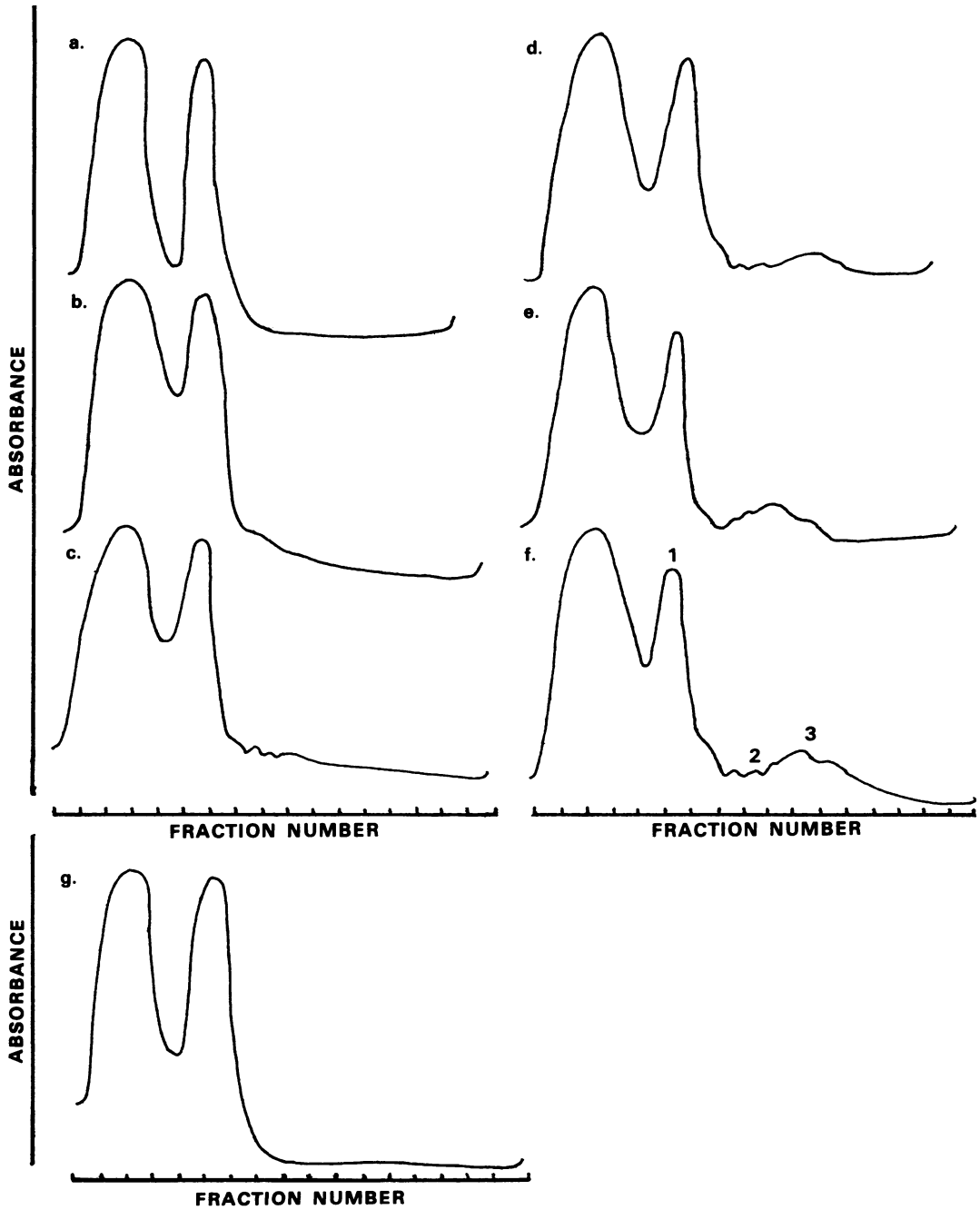

FIG. 2. Changes in density gradient profiles of ribosomes isolated from lettuce embryos during early stages of germination. a: Dormant embryos imbibed for 24 hr in the dark. b–f: Ribosome profiles from embryos imbibed for 24 hr in the dark, illuminated for 20 min, and returned to the dark for: b: 4 hr; d: 12 hr; e: 18 hr; f: 24 hr (after illumination). g: Dormant embryos imbibed 48 hr continuously in the dark.

for 5 min before draining. The grids were then floated face down on 10% formalin solution for a further 5 min and then washed in distilled water. When dry, the grids were shadowed with gold-palladium and viewed in a Philips EM 200.

RESULTS

Lettuce seeds var. Grand Rapids could be maintained fully imbibed in the dark at 25 C for at least 12 weeks, as long as the experiment was continued. Extracted ribosomal preparations when shadowed and examined in the electron microscope appeared to contain only single units (Fig. 1). When such a preparation was passed through a sucrose gradient and analyzed in a continuous recording UV analyzer, no polysomal peaks were obtained (Fig. 2a).

Following 24 hr imbibition in the dark, exposure to light resulted in 96% germination within a further 24-hr period. The UV absorption profiles of ribosomes extracted after exposure to light and subjected to sucrose gradient centrifugation changed to show the appearance and gradual increase in area of absorption peaks corresponding to polysomes of varying sizes (Fig. 2, b–f).

In each illustration of Figure 2, the first UV absorbing peak on the left of each profile was shown to consist of green-pigmented, amorphous material. Attempted purification caused a considerable loss of ribosomal material from the preparations and was not therefore routinely used. Ribosomal subunit peaks, if present, were probably masked by this pigment.

Samples taken from the sucrose gradient and metal-shadowed for electron microscopy, showed a range of polysomes of increasing sizes. Figures 3 A and 3 B are photographs of the fractions labeled 2 and 3 in the sucrose gradient profile shown as Figure 2f.

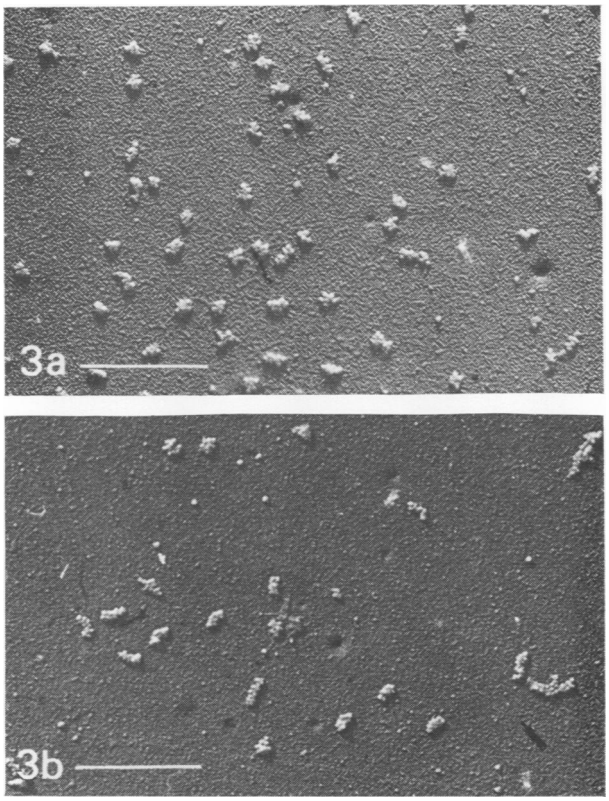

FIG. 3. Samples taken from fractions labeled 2 and 3 in Figure 2f. Shadowed with gold-palladium. A: Small polysomes from fraction 2; B: larger polysomes from fraction 3. Scale line equivalent to 0.5 μM.

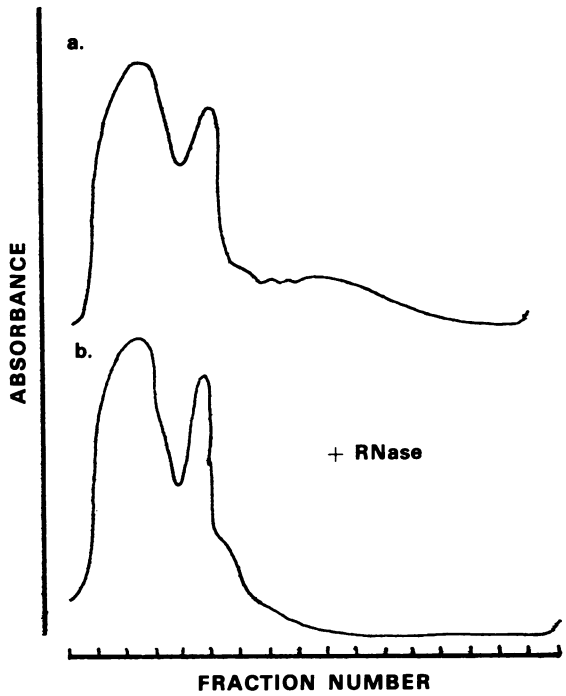

FIG. 4. Effect of RNase on density gradient profiles of ribosomal fractions isolated from embryos 20 hr after the light stimulus. a: Control; b: RNase treated (1 μg/ml, 4 min at 37 C).

However, if the seeds were not illuminated but imbibed continuously in the dark, no polysomes could be detected in sucrose gradient profiles of the ribosomal extracts (Fig. 2g).

Treatment of the ribosomal suspension from illuminated seeds with ribonuclease (1 μg/ml, 4 min at 37 C) caused the disappearance of the majority of the polysomes and increased the areas under the dimer and monosome peaks (Fig. 4), providing evidence that the ribosomal aggregates were linked by RNA and were probably therefore polysomes.

DISCUSSION

The results presented here show that in light-requiring lettuce seed, the formation of at least the major fraction of polysomes does not take place immediately upon imbibition, but is dependent upon the seeds having received the dormancy-breaking stimulus of illumination. Seeds have been kept fully imbibed in the dark for many weeks without germinating.

Previous workers have not been greatly concerned to separate the process of imbibition from that of dormancy-breaking proper, and frequently describe seeds as dormant when in fact they are merely quiescent. If it is required to investigate the germination process itself, and especially if the mode of action of the dormany-breaking stimulus is being investigated, it is helpful to be able to separate those processes which can take place independently of the application of the stimulus itself when normal conditions for growth prevail. Thus Villiers (10) has shown that in cold-requiring seeds, organelles such as plastids and mitochondria can differentiate, other organelle systems such as endoplasmic reticulum, golgi bodies and microbodies can be produced and multiply, and that food interconversions can take place during deep dormancy following imbibition, when there is no possibility of germination taking place.

Previous reports have connected polysome formation with the imbibition process. Thus in peanut cotyledons (5), wheat

embryos (6), pea cotyledons (1), cotton embryos (11), and red pine embryos (8), polysome formation was shown to take place following imbibition. As stated above, it has been shown that when normal tissues are placed under water stress, the polysomes dissociate into monosomal units (7). The breakdown of polysomes in maturing seed may similarly be a result of desiccation, but their reaggregation is delayed after uptake of water by the seed, until the dormancy-breaking stimulus is received.

This raises the question of the nature of the block preventing polysome formation. This may be due to the masking or inactivation of the ribosomes, or because mRNA is masked or absent.

Efron *et al.* (2) have shown that ribosomes extracted from dry or imbibed Grand Rapids lettuce seeds maintained in the dark are able to support protein synthesis if supplied with synthetic messenger in an *in vitro* amino acid-incorporating system. It therefore appears that the ribosomes from dry and from dormant seeds are functional.

Frankland *et al.* (3) showed that the nucleotide base analogue 6-methylpurine completely prevented the germination of Grand Rapids lettuce seeds after illumination, but that this was only completely effective if applied early during the imbibition period. Application of 6-methylpurine after the first 6 hr of imbibition allowed a high germination count to be achieved. Illumination was provided early in the imbibition process and the "escape" to germination of a large proportion of the seeds when allowed to commence imbibition before the addition of the RNA-synthesis inhibitor, was interpreted as a requirement for RNA synthesis early in the imbibition process.

Therefore, as the ribosomes themselves do not appear to be inactivated (2) and as a nucleotide base analogue completely prevents germination when administered before illumination (3), it would appear that the block to polysome formation is possibly due to the absence of at least a part of the mRNA fraction.

Frankland *et al.* (3) could not demonstrate changes in the rate of RNA synthesis in lettuce seeds until about 12 hr after illumination, when radicle growth had already commenced. It is, however, possible that the production of a relatively small fraction of newly synthesised mRNA could initiate germination. This would be possible, for example, if it directed the formation of an enzyme or enzymes controlling the release of sequestered mRNA making it available to the ribosomal protein-synthesizing system.

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