Short Communications

Caution in the Interpretation of Plant Ribosome Studies

By CHRISTOPHER J. LEAVER and JOHN A. DYER
Department of Botany, University of Edinburgh, Mayfield Road,
Edinburgh EH9 3JH, U.K.

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Published methods of ribosome isolation from plant tissues result in a partial and selective recovery of the total ribosome population. The enrichment of polyribosomes obtained may give a false impression of the change in translational capacity of the tissue under study.

It has become common practice to purify ribosome preparations from both plant and animal tissues by sedimentation through sucrose cushions as originally described by Wettstein *et al.* (1963). As well as elimination of protein impurities and other contaminants of lower density, a concentration of the ribosome population is obtained. This is of particular advantage with preparations from plant tissue, which contain relatively low concentrations of ribosomes in a highly vacuolated cell.

A number of recent reports utilizing such techniques in conjunction with gradient analysis have purported to show rapid changes in the proportion of the total ribosome population sedimenting as polyribosomes. Such changes are interpreted as a change in the translational capacity of the tissue, which may be part of the molecular mechanism utilized by plant cells to modulate their rate of growth (see, e.g., Beevers & Poulson, 1972; Davies & Larkins, 1972; Fountain & Bewley, 1973; Verma & Marcus, 1974; Travis *et al.*, 1973).

The significance which may be attached to these changes is obviously dependent on the recovery of a representative sample of the total ribosome population and the demonstration that there is no preferential loss of any fraction of the population.

In comparing our own recoveries of ribosomes and the proportion present in polyribosomes with those of other workers (Leaver & Key, 1967; Leaver & Lovett, 1974), we have observed that under the extraction conditions normally employed a substantial proportion of the total ribosome population is not recovered. Further, a selective recovery of polyribosomes is obtained, which may result in a misleading representation of the ribosome distribution and cellular capacity for protein synthesis of the tissue under study.

Materials and methods

Peas (*Pisum sativum* var. Feltham First) were grown in sterile vermiculite for 6 days at 20°C in

complete darkness. Apical 1 cm sections of stem tissue were excised and used as a source of ribosomes.

The sections were homogenized in 5 vol. of 0.25M-sucrose containing 200mM-Tris-HCl, pH 8.5, at 2°C, 50mM-KCl and 15mM-MgCl₂ (buffer 1) with a Willems Polytron for 3 s at a speed setting of 8. The homogenate was filtered through Mirocloth (Chicopee Mills Inc., New York, U.S.A.), and Triton X-100 was added to a final concentration of 2% (v/v). The filtrate was centrifuged at 10000g for 10 min at 2°C. Ribosomes were recovered from the supernatant by layering over a 3 ml cushion of 1.0M-sucrose in 50mM-Tris-HCl, pH 8.5, 50mM-KCl and 10mM-MgCl₂ (buffer 2), and then centrifugation at 105400g_{av.} for increasing periods of time in a Spinco type 40 rotor at 0–2°C. Ribosome pellets were gently resuspended in 1–2 ml of buffer 2 and samples were removed for absorbance measurements between 220 and 320 nm and density-gradient analysis.

Samples (0.1–0.2 ml) containing 1–2 E_{260} units of ribosomes were layered over 5 ml linear 10–60% (w/v) sucrose gradients in buffer 2 and centrifuged for 45 min at 149000g_{av.} in a Spinco SW50.1 rotor at 0–2°C. Gradients were fractionated by pumping through an LKB Uvicord I monitor with a cell of path length 3 mm and recording the E_{254} profile. The % of polyribosomes was calculated from the area under the sucrose-gradient profiles of the subunits, monoribosome and polyribosome regions.

RNA was extracted from both total tissue and the 'post-ribosomal supernatants' (105400g_{av.} supernatants) and fractionated by polyacrylamide-gel electrophoresis as previously described (Leaver & Key, 1970).

Results and discussion

We have previously shown that the addition of a low concentration of Triton X-100 to plant tissue homogenates will allow the recovery of more than 95% of the total ribosome population from the supernatant obtained after an initial centrifugation of 10000g for 10 min (Leaver & Key, 1970). A variable

Fig. 1. Recovery of ribosomes after increasing periods of centrifugation through 1M-sucrose cushions

Equal samples of a post-mitochondrial supernatant, prepared from pea apices as described in the text, were layered over 3ml cushions of 1M-sucrose. The ribosome pellets obtained after increasing periods of centrifugation at $105400g_{av}$, were resuspended in buffer 2 and the E_{260} was determined (●). RNA was extracted from the corresponding post-ribosomal ($105400g$) supernatants and fractionated by polyacrylamide-gel electrophoresis. Ribosomal RNA as a percentage of the total RNA recovered from each supernatant sample was calculated (□).

proportion of the cellular ribosomes may bind to the endoplasmic reticulum and, at least in part, sediment along with nuclei and mitochondria in the initial low-speed-centrifugation step. The addition of Triton X-100 solubilizes the endoplasmic reticulum and releases the bound ribosomes.

To monitor the subsequent recovery of ribosomes during sedimentation through a sucrose cushion, replicate samples (10ml) of the low-speed supernatant were layered over 3ml cushions of 1M-sucrose in buffer 2 and centrifuged for increasing periods of time at $105400g_{av}$. The ribosome pellet obtained in each case was resuspended in buffer 2 and the absorbance between 220 and 320nm was determined, thus giving a measure of the recovery of ribosomal material and also of its relative purity.

The recovery of ribosomes in the $105400g$ pellet increases with time of centrifugation to a plateau value at 7h (Fig. 1). However, the purity of the recovered ribosomes as judged by the ratio of $E_{260}/$

E_{280} and E_{260}/E_{235} remained constant at 1.77 ± 0.02 and 1.59 ± 0.02 respectively.

Polyacrylamide-gel analysis of the RNA extracted from the corresponding post-ribosomal supernatant fractions confirmed that loss of rRNA from the supernatant coincided with the acquisition of rRNA in the $105400g$ pellet. Ribosomal RNA as a percentage of the total RNA in the post-ribosomal supernatant fell from an initial value of 78% to less than 5% after 7h of centrifugation (Fig. 1). The RNA remaining in the supernatant was tRNA with a molecular weight of 25000.

These observations suggest that a minimum of $4.4 \times 10^7 g\text{-min}$ is required to sediment the native ribosome population through a 3ml cushion of 1M-sucrose in a Spinco type 40 rotor. This value is considerably in excess of published values used by other workers in investigations of ribosome metabolism in plants. For example, in studying polyribosome metabolism during normal and hormonally induced growth of pea stems, Davies & Larkins (1972) sedimented ribosomes through a 4ml cushion of 1.5M-sucrose in a Spinco type 40 rotor for 90min at $100000g$ ($0.9 \times 10^7 g\text{-min}$). Verma & Marcus (1974) in a study of polyribosome formation in plant tissue culture sedimented ribosomes through 2.5ml of 1M-sucrose for 90min at $105000g$ ($0.95 \times 10^7 g\text{-min}$).

Sucrose-density-gradient analysis of the ribosomes recovered after increasing periods of centrifugation through a sucrose cushion shows a progressive decrease in the percentage of ribosomes sedimenting as polyribosomes (Fig. 2a). A comparison of profiles from sucrose-density-gradient analysis of ribosomes recovered after centrifugation for 1.5h (Fig. 2b) and 6h (Fig. 2c) through a sucrose cushion suggests that the enrichment of large polyribosomes after the shorter period of centrifugation is due to the selective loss of smaller polyribosomes, monoribosomes and ribosomal subunits in the high-speed supernatant. The longer period of centrifugation allows a complete recovery of ribosomes from the supernatant and a more representative distribution of monoribosomes and polyribosomes.

It was possible that the decrease in the percentage of polyribosomes was due to the action of ribonuclease in the ribosomal pellet during the longer periods of centrifugation. To rule out this possibility centrifuge tubes containing both ribosomal pellets and supernatants from the shorter periods of centrifugation were stored at the temperature of centrifugation (2°C) until the last (7h) time-sample was removed from the centrifuge. The ribosome pellets and supernatants were then processed as described above, and essentially similar results were obtained to those reported above.

We have obtained similar results, both in terms of recovery of ribosomes and representative analysis of the ribosome population, from a variety of non-green

Fig. 2. Effect of increasing periods of centrifugation through 1M-sucrose cushions on the distribution of ribosomes on sucrose density gradients

Ribosome pellets, obtained after increasing periods of centrifugation at $105\,400g_{av}$, as described in Fig. 1, were resuspended in buffer 2 and fractionated on 10–60% (w/v) sucrose density gradients as described in the text. (a) The percentage of ribosomes present as polyribosomes in the ribosomal pellet recovered after increasing periods of centrifugation was calculated from the absorbance profiles (●). Representative sucrose-density-gradient profiles of the ribosomes recovered after 1.5h (b) and 6h (c) centrifugation through a 1M-sucrose cushion are also shown.

plant tissues grown under different environmental conditions. However, the quantitative recovery of chloroplast ribosomes (70S) and polyribosomes from green plant tissue requires a minimum centrifugation of $4.7 \times 10^7 g\text{-min}$ when using the conditions of purification described above.

From the observations reported in the present paper we suggest that a certain degree of caution should be applied to the interpretation of changes in the ratio of monoribosomes to polyribosomes, in terms of modulation of translational capacity, in studies of plant development. The quantitative recovery of the cellular ribosome population by sedimentation through a sucrose cushion is obviously dependent on such variables as the volume and density of the sucrose in the cushion, the angle of the tube in the centrifuge rotor and both the speed and time of centrifugation. In all studies in which the total ribosome population is purified and concentrated by sedimentation through a sucrose cushion, strict monitoring of the recovery of ribosomes in the high-speed pellet, coupled with an analysis of the rRNA remaining in the supernatant,

should allow the investigator to recover at least a representative portion of the total ribosome population.

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