Isolation of Ribosomes from Cell-Wall Preparations of Barley (*Hordeum vulgare*)

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Ribosomes can be released from highly purified preparations of the cell walls of barley shoots by passing the suspension of cell walls through a French press. The sedimentation coefficients and base composition of the cell-wall ribosomes are identical with those of cytoplasmic ribosomes. It is suggested that the ribosomes isolated from the walls were originally incorporated into the wall structure during formation of the primary wall.

Plant cell walls that have been exhaustively washed to remove cytoplasm and strands of endoplasmic reticulum contain up to 6mg of RNA/g dry wt. of walls, and the base composition of the wall RNA is identical with that of cytoplasmic rRNA (Phethean, Jervis & Hallaway, 1968). The present paper describes the release and characterization of ribosomes from barley shoot cell walls. A preliminary account of some of this work has been published (Jervis & Hallaway, 1969).

MATERIALS AND METHODS

Materials. Barley seeds (*Hordeum vulgare* var. Impala and var. English Proctor) were obtained from Carter's Seeds Ltd., London S.W.20, U.K. After a preliminary soak in running water for 8 h the seeds were grown in the dark at 15-18°C, either on damp vermiculite if shoots were required or on damp absorbent paper if whole seedlings were needed.

Preparation of the cell walls. Shoots from 5-7-day-old plants were macerated either in an Ultra-Turrax (Janke und Kunkel K.G., Staufen i. Br., Germany) or in a Kenwood Chef Liquidizer at 4-5°C, in 50 mM-tris-HCl buffer, pH 7.8, containing 1 mM-MgCl₂ (10 ml/g of tissue) for 10 min in ten 1 min periods. Between each maceration the suspension was filtered on a sintered-glass Buchner funnel (no. 1 porosity) and the residue was resuspended in fresh buffer. The preparation was then similarly macerated five times in 50 mM-tris-HCl buffer, pH 7.8, containing 1 mM-MgCl₂, 0.1 M-KCl and 0.2% sodium deoxycholate. Some preparations were macerated in 67 mM-potassium phosphate buffer, pH 6.0, as described in Phethean *et al.* (1968).

Isolation of cytoplasmic ribosomes. A 10 g batch of 2-3-day-old barley seedlings was ground in a chilled mortar with 10 g of acid-washed sand and 20 ml of solution A (0.4 M-sucrose-10 mM-tris-1 mM-MgCl₂-0.2% sodium deoxycholate, adjusted to pH 7.8 with 1 M-HCl) at 0°C. The ground seedlings were filtered through two layers of muslin and the filtrate was centrifuged at 30000g for 30 min at 0°C. The supernatant was layered on to 2 ml of solution B (2 M-sucrose-10 mM-tris-1 mM-MgCl₂ adjusted to pH 7.8) in 13.5 ml centrifuge tubes and centrifuged at 100000g for 2 h at 0°C in the 40 rotor of a Spinco model L centrifuge (Jackmezyk & Cherry, 1968). The sediment was rinsed twice in solution C (0.4 M-sucrose-10 mM-tris-1 mM-MgCl₂ adjusted to pH 7.8) and finally resuspended in 1.5 ml of solution C. The suspension was clarified by centrifugation at 10000g for 10 min.

Isolation of cell-wall ribosomes. Cell walls from 150 g of barley shoots were suspended in 60 ml of solution A at 0°C. The suspension was passed through a French press at 5000 kg/cm², and then filtered through a glass sinter (no. 3 porosity). The filtrate was centrifuged at 30000g for 30 min at 0°C and the supernatant was layered on to 2 ml of solution B and centrifuged at 100000g for 2 h at 0°C. The sedimented material was rinsed twice in solution C, and then finally resuspended in 1.5 ml of solution C and shaken gently for 2-3 h. The suspension was centrifuged at 10000g for 10 min at 0°C.

Determination of cell-wall RNA. The amount of RNA in each cell-wall sample was measured by incubating the walls with 0.3 M-KOH for 17 h at 37°C, then purifying the nucleotides and assaying them as described by Phethean *et al.* (1968).

Determination of the sedimentation coefficient of the ribosomes. Ribosomal preparations in solution C were centrifuged at a maximum speed of 39460 rev./min in the 12 mm cell (D rotor) of the Spinco model E analytical ultracentrifuge. Photographs were taken at 4 min intervals, the schlieren optical system being used. The results are given as observed sedimentation coefficient, s_{obs} , at 20°C for this solvent under the conditions described.

Assay of protein. The total nitrogen of preparations was measured by the micro-Kjeldahl method in the Markham

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apparatus. The N content, adjusted for the contribution by RNA, was multiplied by 6.25 to give the protein content.

Measurement of base ratios. This was done by cation-exchange chromatography (Katz & Comb, 1963).

RESULTS

Characteristics of the RNA released from cell-wall preparations by the French press. A preparation of cell walls in 67 mM-phosphate buffer, pH 6.0, was passed through a French press at 8000 kg/cm² and the finely divided cell walls were collected by filtration on a sintered-glass funnel (no. 1 porosity). The filtrate, containing material released by the procedure, was freeze-dried. This treatment decreased the RNA content of one sample of cell walls from 4.32 to 2.76 mg/g dry wt. (i.e. by 36%) and of another from 3.84 to 2.58 mg/g dry wt. (by 33%). Passing the cell-wall preparations through the French press a second time did not appreciably increase the amount of RNA released. The dried filtrate contained 3.2% RNA and 4.4% protein, and over 90% of the material absorbing at 256 nm was eluted from a column of Sephadex G-10 immediately after the void volume, indicating that the molecular weights of the components were above 10000 (Braun, 1967). A sample of the filtrate from a cell-wall preparation in 67 mM-phosphate buffer, pH 6.0, that had been passed through the French press was centrifuged, first at 30000g for 30 min to remove small cell-wall fragments and then at 100000g for 2 h in the 40 rotor of the Spinco model L centrifuge. The resuspended pellet showed no absorption maximum at 260 nm but a broad shoulder from 250–290 nm. The pellet contained 38% RNA and 62% protein (w/w) and accounted for 94% of the RNA released from the cell-wall preparation. It seemed probable from the composition of the pellet and its absorption in the ultraviolet that the RNA was being released from the wall as ribosomes contaminated with protein. When cell walls were

treated as described in the Materials and Methods section to release and purify ribosomes the final preparation contained 54% protein and 46% RNA (w/w) and absorbed strongly in the ultraviolet with a maximum at 260 nm and a minimum at 242 nm (Fig. 1). The $E_{\max.}/E_{\min.}$ ratio was 1.24. For comparison, barley cytoplasmic ribosomes also contained 54% protein and 46% RNA (w/w), absorbed strongly at 260 nm and the $E_{\max.}/E_{\min.}$ ratio was 1.41.

Table 1. *Properties of cell-wall and cytoplasmic ribosomes*

Property	Cell-wall ribosomes	Cytoplasmic ribosomes
Base composition (%)		
AMP	21.21	21.07
CMP	23.46	23.53
GMP	33.87	33.93
UMP	21.51	21.45
Sedimentation coefficients ($s_{\text{obs.}}$)		
Dimer	108S	104S
Monomer	80S	81S
Large subunit	54S	54S
Small subunit	34S	33S

Fig. 1. Absorption spectrum of purified cell-wall ribosomes in solution C. The concentration of ribosomes was 0.9 mg/ml.

Fig. 2. Sedimentation pattern of cell-wall ribosomes after 12 min at 39460 rev./min. The concentration of ribosomes was 0.9 mg/ml, suspended in solution C; the temperature was 20°C.

The base compositions of the cell-wall and cytoplasmic ribosomes were identical (Table 1).

Ultracentrifugation of cell-wall and cytoplasmic ribosomes. The sedimentation patterns for barley cell-wall and cytoplasmic ribosomes and for a mixture of the two were identical (Fig. 2), and the sedimentation coefficients of the components of the preparations agreed closely (Table 1).

DISCUSSION

These data show that ribosomes identical with cytoplasmic ribosomes can be obtained from cell-wall preparations. These ribosomes could be derived from contaminating cytoplasm, or from endoplasmic reticulum held within the wall. Since the walls have been exhaustively washed with buffers, saline and deoxycholate solutions and contain less than 1% nitrogen it is unlikely that more than a trace of cytoplasm is present (see discussion by Phethean *et al.* 1968). On the other hand, evidence from electron micrographs shows that endoplasmic reticulum is associated with the formation of cell walls (Whaley, Kephart & Mollenhauer, 1959; Porter & Machado, 1960). Whaley, Dauwalder & Kephart (1966) in a study of cell-plate formation observed that numerous profiles of endoplasmic reticulum lie close to and pass through the developing cell plate. They suggest that some profiles may fuse with the plate and contribute to the formation

of the primary wall; in addition the region of the developing plate is rich in free ribosome-sized particles.

We suggest that the ribosomes present in highly purified cell-wall preparations were incorporated into the wall structure, either as free ribosomes or attached to elements of endoplasmic reticulum, while the wall was being laid down. Whether these ribosomes retain activity and whether they have a function within the wall remains to be established.

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