Planta 150, 249–254 (1980)

Planta

Polysomes and Intracisternal Accumulations in Enucleate Sieve Elements of Rice (*Oryza sativa* L.)

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Abstract. Polysomes in sieve elements of rice (*Oryza sativa* L.) were studied with the electron microscope. The polysomes were found on the rough endoplasmic reticulum (ER) present in immature sieve elements and also on the cisternae of aggregated ER in the parietal layer of mature, enucleate sieve elements. In the immature sieve elements the ER cisternae existed as narrow profiles while in the mature sieve elements the ER cisternae were considerably dilated and contained a fibrillar material and, occasionally, electron-opaque inclusions. In addition to the aggregated ER, single profiles of ER were found applied to the lateral walls and also the sieve plates. These cisternae also bore ribosomes and were separated from the plasmalemma by a narrow, dense space. In the mature sieve elements much of the surface of the ER membranes was covered with polysomes. The dimensions of the polysomes are described and the possibility that they contribute to the formation of the fibrillar material in the intracisternal space is discussed.

Key words: Endoplasmic reticulum – *Oryza* – Polysomes – Sieve elements.

Introduction

The endoplasmic reticulum (ER) found within mature sieve elements in higher plants has been shown to exist in a smooth form in the parietal layer. This ER may be present as stacks in which the cisternae are aligned parallel to one another or it may occur as an anastomosing network (Srivastava 1975 and references therein). Occasionally both arrangements of ER may be found within a single sieve element (Esau 1978).

In immature sieve elements the ER is usually present as rough cisternae distributed throughout the cytoplasm and ribosomes on this ER may be arranged as polysomes (Esau 1969, 1972). As the rough ER begins to aggregate into stacks, however, the ribosomes are shed from the ER membranes and an electron-opaque material accumulates between adjacent cisternae. Occasionally, ribosomes are retained on those cisternae of a stack which face the cytoplasm although these ribosomes, also, are eventually shed from the ER membranes (Esau and Gill 1973; Melaragno and Walsh 1976). Commonly, the ER loses its ribosomes to become stacked while the sieve element still has a vacuole and an intact nucleus. Thus, by the time the sieve-element nucleus has degenerated the ER is smooth.

This paper, which forms part of a study of translocation in the rice plant, describes enucleate sieve elements in roots of rice in which polysomes are retained on the membranes of aggregated ER in the parietal layer. The possibility is discussed that the polysomes function in protein synthesis and that they contribute to the formation of a fibrillar material within dilated ER cisternae.

Abbreviations: ER = endoplasmic reticulum

Materials and Methods

Grains of rice (*Oryza sativa* L.) were obtained from the International Rice Research Institute (IRRI), Los Baños, Philippines (IRRI accession numbers IR26 and IR480-5-9-3). The grains were surface sterilised in 0.1% mercuric chloride, washed in distilled water, and germinated on moist filter paper at room temperature. For electron microscopy the main roots of 10-day old seedlings were excised into 2% glutaraldehyde, buffered with 0.1 M sodium cacodylate at pH 7.0 and fixed for 1 h. The roots were then postfixed in 1% aqueous osmium tetroxide for 90 min, dehydrated through a graded acetone series and embedded in Spurr's low viscosity resin (Spurr 1969). Sections on grids were stained with uranyl acetate followed by lead citrate and viewed in a Philips EM400 electron microscope operating at 60 kV. The microscope magnification was calibrated to within ±1.5%.

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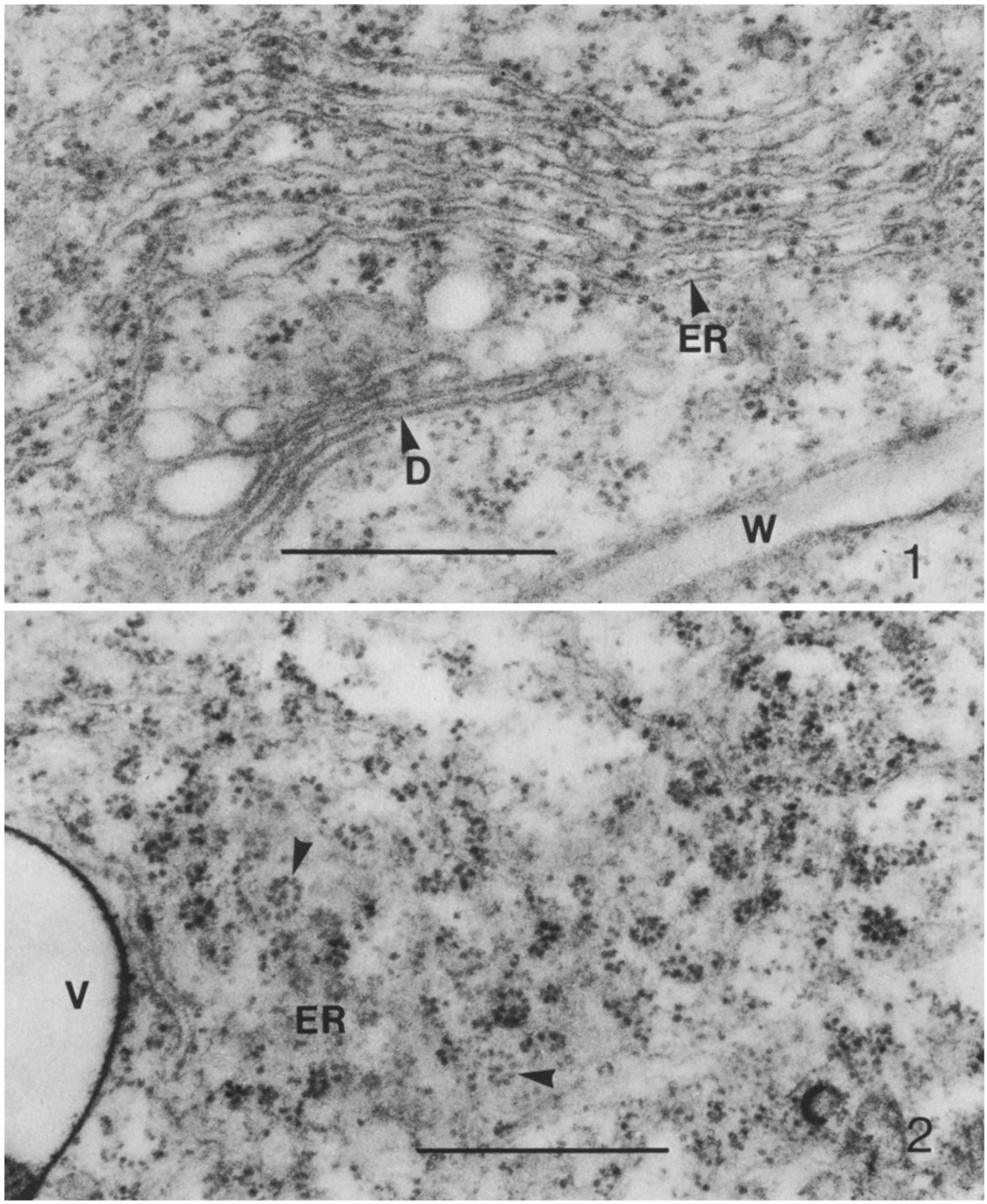

Fig. 1. Aggregated ER cisternae in an immature sieve element. Groups of ribosomes are bound to the ER membranes. ×89,000. Scale=0.5 μm, *ER*, endoplasmic reticulum; *D*, dictyosome; *W*, wall

Fig. 2. Glancing section through rough ER in an immature sieve element. Some of the polysomes are arranged as spirals (darts). The underlying ER membrane appears amorphous. ×78,000. Scale=0.5 μm, *V*, vacuole

Figs. 3–5. Aggregated ER in the parietal layer of mature, enucleate sieve elements

Fig. 3. Ribosomes remain attached to the ER membranes and appear aligned in rows. The ER cisternae are dilated and contain a fibrillar material. ×64,000. Scale=0.5 μm. *R*, ribosomes; *F*, fibrillar material

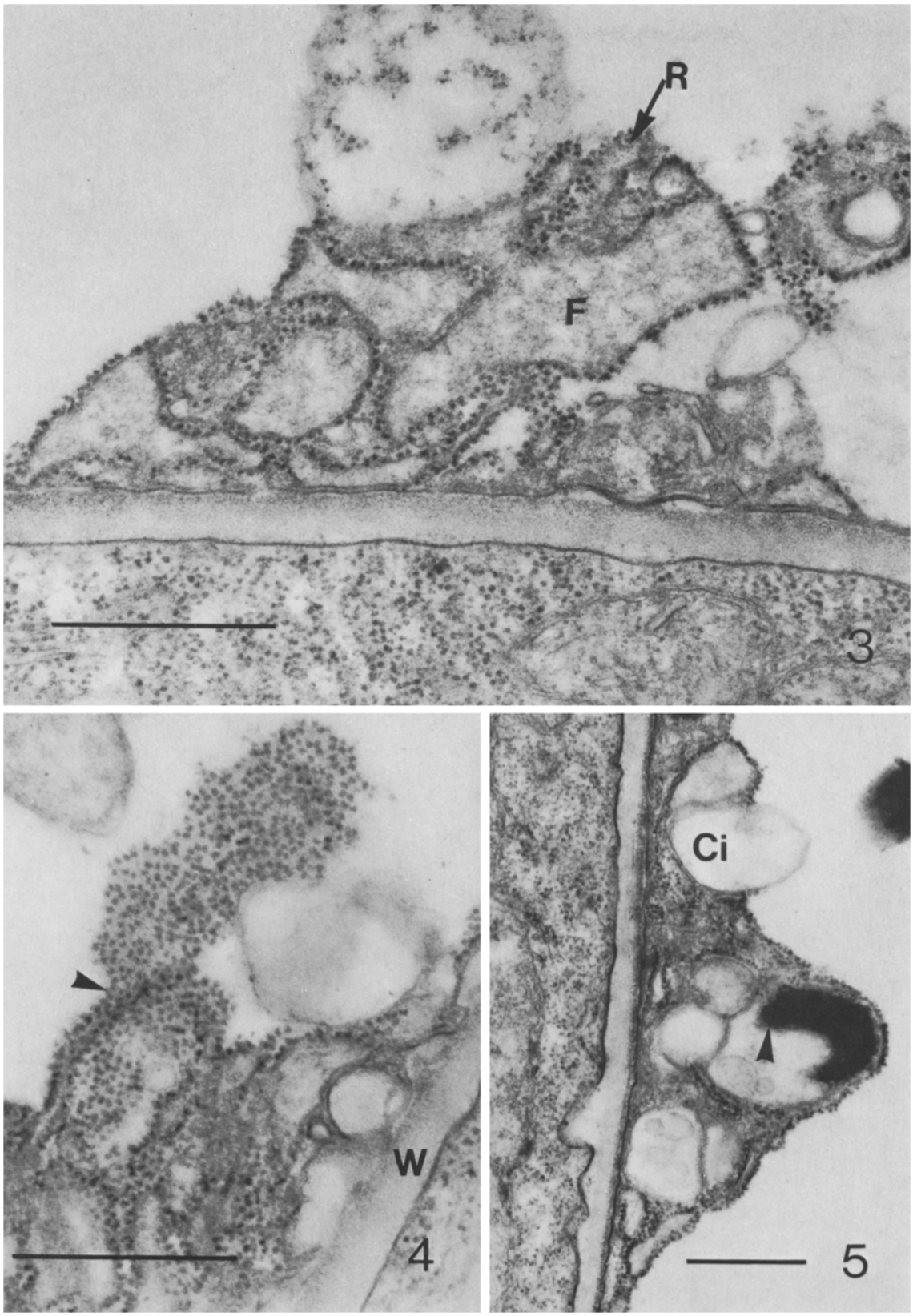

Fig. 4. The dart indicates a region where the ER membranes twist from an end-on view to a surface view. Polysomes, revealed as both chains and spirals, are seen on the surface of the membrane. ×74,000. Scale = 0.5 μm

Fig. 5. An electron-opaque inclusion (dart) within a dilated ER cisterna. Ribosomes are aligned along the ER membranes. ×39,000. Scale = 0.5 μm, *Ci*, dilated ER cisterna

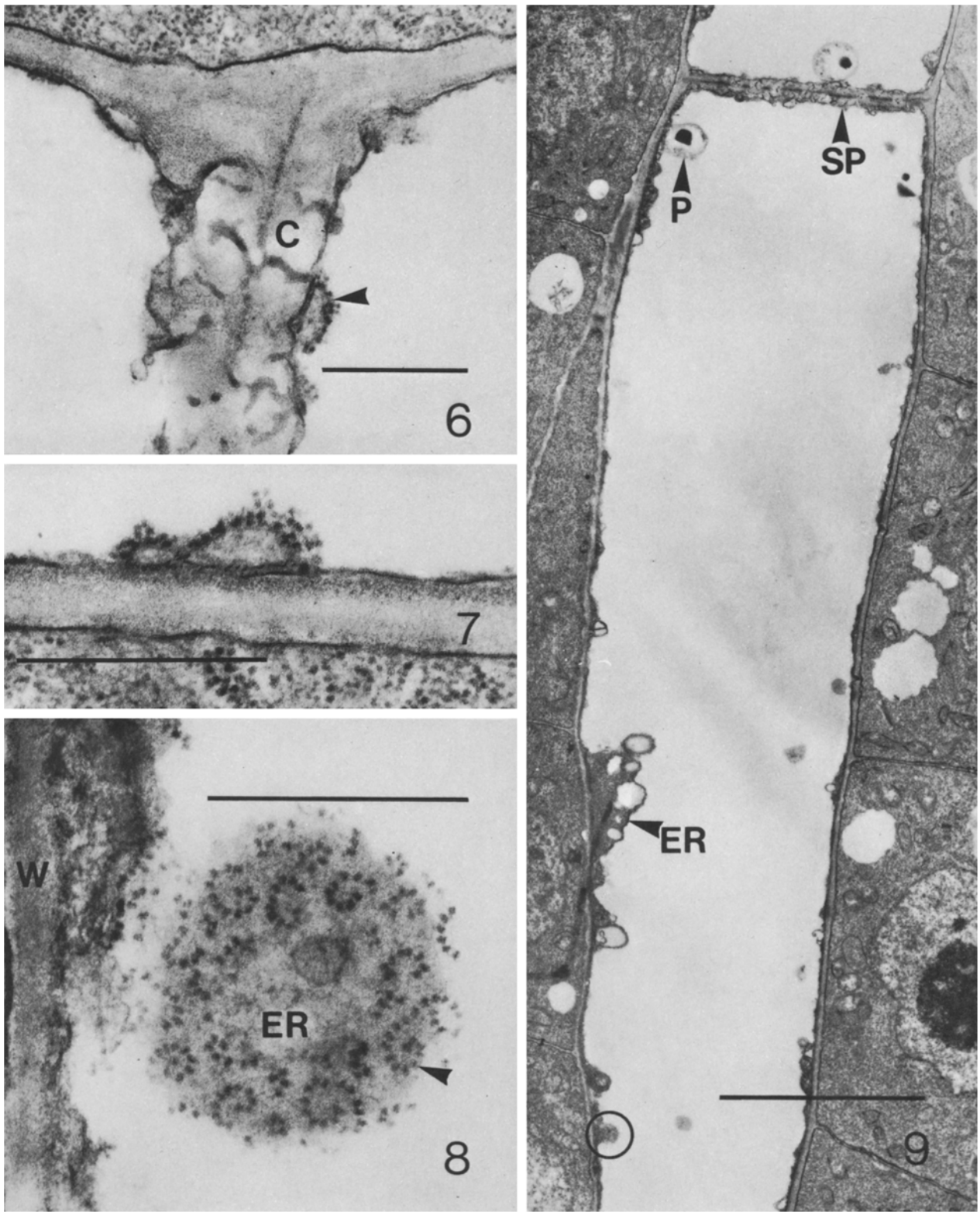

Fig. 6. Single ER profile applied to the sieve plate of a mature sieve element. The dart indicates ribosomes on the ER membrane. ×51,000. Scale=0.5 μm. *C*, callose

Fig. 7. Single, rough ER profile in the parietal layer of a mature sieve element. The cisterna is separated from the plasmalemma by a narrow, dense space. ×86,000. Scale=0.5 μm

Fig. 8. Serial section of the encircled region shown in Figure 9. The ER is seen in glancing section. Polysomes remain on the ER membrane. The dart indicates a polysome with 22 ribosomes in a double spiral. ×89,000. Scale=0.5 μm

Fig. 9. Longitudinal section of a mature sieve element. The parietal layer is composed of ER aggreagtes and plastids. The lumen of the cell is structurally empty. ×7,000. Scale=5 μm. *P*, plastid; *Sp*, sieve plate

Longitudinal and transverse sections of roots were cut from an actively elongating region about 3 mm behind the root apex. This region contained mature protophloem sieve elements as well as differentiating and mature metaphloem sieve elements. Additional sections were cut from a fully elongated region of the root about 3 cm behind the root apex.

Results

Immature sieve elements in the root had dense protoplasts and contained numerous profiles of rough ER. In older sieve elements, in which small vacuoles had developed, most of the rough ER cisternae had come together to form aggregates in the cytoplasm. Within the aggregates the cisternae were aligned parallel to one another and ribosomes remained present on the surface of the ER cisternae (Fig. 1). In glancing section the surface of the ER membranes appeared amorphous while polysomes were visible on the cisternae (Fig. 2). The polysomes were most frequently arranged as spirals although occasionally other arrangements, such as double spirals and straight chains, were seen. Cytoplasmic ribosomes were not found in polysomal configurations.

In yet older sieve elements, in which the vacuole and nucleus were absent, the ER aggregates were found only in the parietal layer of the cell. In these sieve elements also, ribosomes remained attached to the ER cisternae. The ER cisternae, however, were no longer present as narrow profiles but were dilated and often contained a fibrillar material (Fig. 3). In addition to the fibrillar material, electron-opaque inclusions were sometimes found within the dilated cisternae (Fig. 5). Sections of ER stacks which had passed through cisternae as well as intercisternal spaces showed the ribosomes to be arranged in rows along the ER membranes (Figs. 3, 5). However, glancing sections through the intercisternal spaces, parallel to the ER membrane, revealed most of the ribosomes to be arranged as polysomes. In Fig. 4 the dart indicates a region where the ER membranes twist from an end view to a surface view in the thickness of the section. Polysomes are clearly seen on the surface of the membrane. Occasionally sections were obtained which had passed parallel to the ER membrane but not through it. In these cases all of the ribosomes on the ER membrane were seen as polysomes (Fig. 8).

Some of the polysomes were measured by projecting photographic negatives onto a wall at a total magnification of $\times 396{,}000$ ($\pm 6{,}000$). The polysomes were then traced onto a card. Measurements of individual ribosomes were made with a millimetre rule while the length of the polysome spirals was estimated using a map measurer (see also Bonnet and Newcomb 1965). The polysome marked by a dart in Fig. 8 contains 22 ribosomes in a double spiral length of 660 nm. The mean width of 30 ribosomes was 12.5 nm with a spacing between the ribosomes in a spiral of about 10 nm. The region of ER shown in Fig. 8 is a serial section of the encircled region of Fig. 9. The sieve element shown in Fig. 9 was probably a mature protophloem sieve element since surrounding cells had not fully vacuolated or extended. However, polysomes were also found in structurally mature, metaphloem sieve elements located in fully elongated regions of the root.

As well as the aggregates of dilated ER single profiles of ER were often found lying parallel to the plasmalemma of the sieve element. Usually only short lengths of cisternae appeared in a section. These cisternae also bore ribosomes and were usually separated from the plasmalemma by a dense material (Fig. 7). Similar cisternae were found applied to the sieve plates in mature sieve elements (Fig. 6).

Most of the mature sieve elements examined resembled the one shown in Fig. 9. Such cells were characterised by their clear lumina and parietal layers composed of plastids, mitochondria and aggregates of dilated ER cisternae. P-protein was absent from the sieve elements. Nuclei, or remnants of nuclei, were not found in any of the mature sieve elements.

Discussion

The development of ER in root sieve elements of rice deviates from that commonly reported to occur in higher plants. In particular, the retention of polysomes in mature sieve elements is an unusual feature not previously recorded. Esau and Gill (1973) depicted ribosomes on stacked ER cisternae in mature sieve elements of *Allium* (see their Fig. 11), although they emphasised that this was an unusual feature in a mature sieve element. Buvat (1963) also reported ribosomes to occur in mature sieve elements of *Cucurbita*, although he did not show them to be arranged as polysomes.

It remains to be shown whether the membrane-bound polysomes of enucleate sieve elements of rice continue to function in protein synthesis. However, it seems likely that they do. Gunning and Steer (1975) have suggested that polysomes are "visible indications that protein molecules were being assembled at the moment when the cell was fixed". Also, long-lived mRNA molecules have been reported to occur in mature, enucleate sieve elements (Neumann and Wollgiehn 1964; but see Gietl and Ziegler 1979). The longest membrane-bound polysome shown in this paper measured 660 nm and contained 22 ribosomes in a double spiral (see Fig. 8). If one applies the calcu-

lation of Gunning and Steer (1975) then a protein with a maximum molecular weight of 80,000 could be translated by such a polysome, assuming that each codon on the mRNA molecule occupies 1 nm and that each amino acid has an average molecular weight of 120. Conceivably the fibrillar material which accumulates within dilated ER cisternae is protein and manufactured, at least in part, by polysomes on the surface of the ER membrane. This material was less evident in the aggregated ER cisternae of immature sieve elements suggesting that it was incorporated into the ER cisternae in later stages of development. Proteinaceous accumulations have now been found within dilated ER cisternae in specialised cells of a number of plants (see Jørgensen et al. 1977 and references therein) although rarely in sieve elements. Kruatrachue and Evert (1974) found dilated ER cisternae which accumulated a fibrillar material in sieve elements of *Isoetes*. These authors were able to demonstrate that the smooth ER cisternae travelled towards the plasmalemma where they then discharged their contents to the outside. I have found no evidence that the fibrillar material is discharged from the ER cisternae in sieve elements of rice, although this remains a possibility. The ultimate fate of the intracisternal material remains to be shown.

While the presence of polysomes in an enucleate sieve element is an unusual feature it does suggest that synthetic activity in the parietal layer can continue after the nucleus of the cell has degenerated. Conceivably all of the organelles in the parietal layer of mature sieve elements continue to function. In this respect it is worth note that mitochondria in mature sieve elements of rice have been shown to retain at least some of their enzyme activity (Öpik 1975).

This research was funded by the Ministry of Overseas Development. I am grateful to Professor D. Boulter, Dr N. Harris and Dr R.P.C. Johnson for informative discussions.

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Received 12 June; accepted 11 July 1980