PARTICLE ARRANGEMENTS IN PROPLASTIDS OF *TRITICUM VULGARE* L. SEEDLINGS

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ABSTRACT

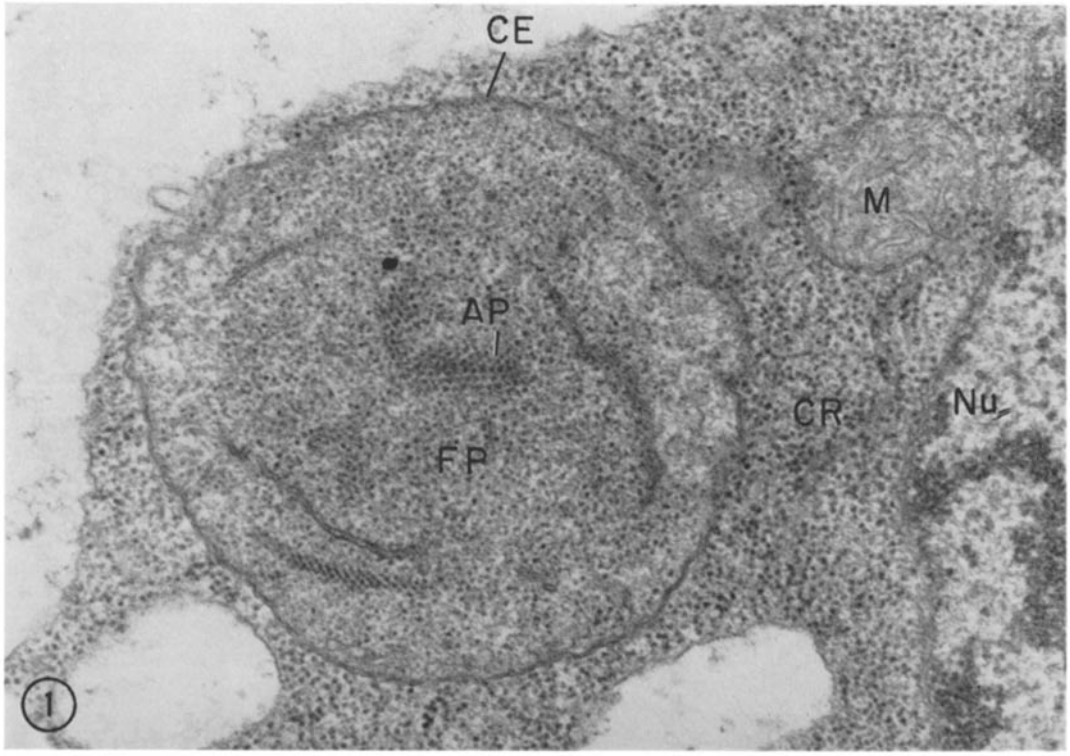
Particles having ribosome-like characteristics are described in proplastids of dark-grown wheat seedlings as the membranes of the prolamellar body become transformed, under the influence of light, into grana and fret membranes. Three arrangements of particles were noted: (1) a random distribution of discrete particles; (2) particles occurring in helices or parallel rows; and (3) particles arranged in rough squares with six to eight particles per side. It is possible that the third type of particle is a cross-section of long parallel rods. A particle ranges in size from 170 to 220 A, those of group three being somewhat smaller. The particulates vary from diamond shaped with smooth surfaces to circular with irregular surfaces. These particles have the characteristics of ribosomes as visualized by the electron microscope: they are preserved by glutaraldehyde and osmium tetroxide, they stain intensely with uranyl acetate, and are digested by RNase. Their properties do not coincide with those of viruses, smog-induced particles, stromacenter particles, or phytoferritin. They are frequently adjacent to membranes but never attached to membranes. The involvement of ribosomes in membrane development is discussed.

INTRODUCTION

RNA-containing particulates have been isolated from chloroplasts. Lyttleton (1962) isolated them from spinach chloroplasts and demonstrated that they had a sedimentation constant of 66S. Boardman, Francki, and Wildman (1965) and Spencer (1965) demonstrated that such particles isolated from tobacco and spinach chloroplasts, respectively, were able to actively incorporate labeled amino acids into chloroplast proteins.

Ribosomes as visualized by the electron microscope are preserved by glutaraldehyde and osmium tetroxide. They stain with uranyl acetate and are digested by ribonuclease. In animal cells, they range from 150–200 A in size and may be distributed at random in the cytoplasm, associated with the endoplasmic reticulum or in helices (Behnke, 1963; Cedergren and Harary, 1964). In the pleuropneumonia-like organism, Maniloff et al. (1965) reported the occurrence of ribosomes arranged in parallel rows two to four abreast. Grouped ribosomes or polysomes may be held together by a thin strand of messenger RNA. In investigations on animal cells, the relationship between isolated ribosomes and those visualized by the electron microscope is sufficiently well established so that Dallner, Siekevitz, and Palade (1966) are able to assume that all the RNA in the microsome fraction of rat hepatocytes is to be found in the ribosomes. Granick's (1963) careful reasoning on the continuity of plastids presents strong theoretical evidence for the presence of both ribosomes and DNA in chloroplasts. Jacobson, Swift, and Bogorad (1963) found 170-A particulates in plastid of *Zea mays*; these granules are

removed by treatment with ribonuclease, are preserved by formaldehyde, and stain with uranyl acetate. While these particles contain RNA, Jacobson et al. (1963) were unable to designate them as ribosomes since at that time the ability of RNA-containing particles to synthesis a polypeptide had not been demonstrated. Since this activity has now been demonstrated (Boardman et al., 1965; Spencer, 1965), the assumption seems warranted that at least some of the RNA-containing particulates in plastids are ribosomal in nature. This paper reports the presence of particles re-



Key to Labeling

Plastid envelope	*CE*	Starch	*S*
Free plastid particles	*FP*	Membranes	*Me*
Aggregates of plastid particles	*AP*	Rodlike structure	*RS*
Helically arrayed particles	*HP*	Subunits	*SU*
Cytoplasmic ribosomes	*CR*	Quasi-crystalline array	*CP*
Nucleoplasm-like region	*N*	Central core	*C*
Prolamellar body	*PB*	Nucleus	*Nu*
Mitochondrion	*M*		

FIGURES 1–10 Wheat leaf tissue was first fixed in glutaraldehyde and postfixed with Dalton's chrome-osmium fixative at 4°C. The wheat plants were grown in the dark for 5 days and exposed to 75 ft-c of light for about 10 min while the tissue was being cut into 1 mm^2 sections.

FIGURE 1 A section of a proplastid showing particles arrayed in parallel rows (*AP*), two to four abreast, and freely distributed throughout the plastid (*FP*). These particles stain to the same extent and are of the same diameter (200 A) as the cytoplasmic ribosomes (*CR*). The nucleus (*Nu*) shows the chromatin material clearly. × 37,500.

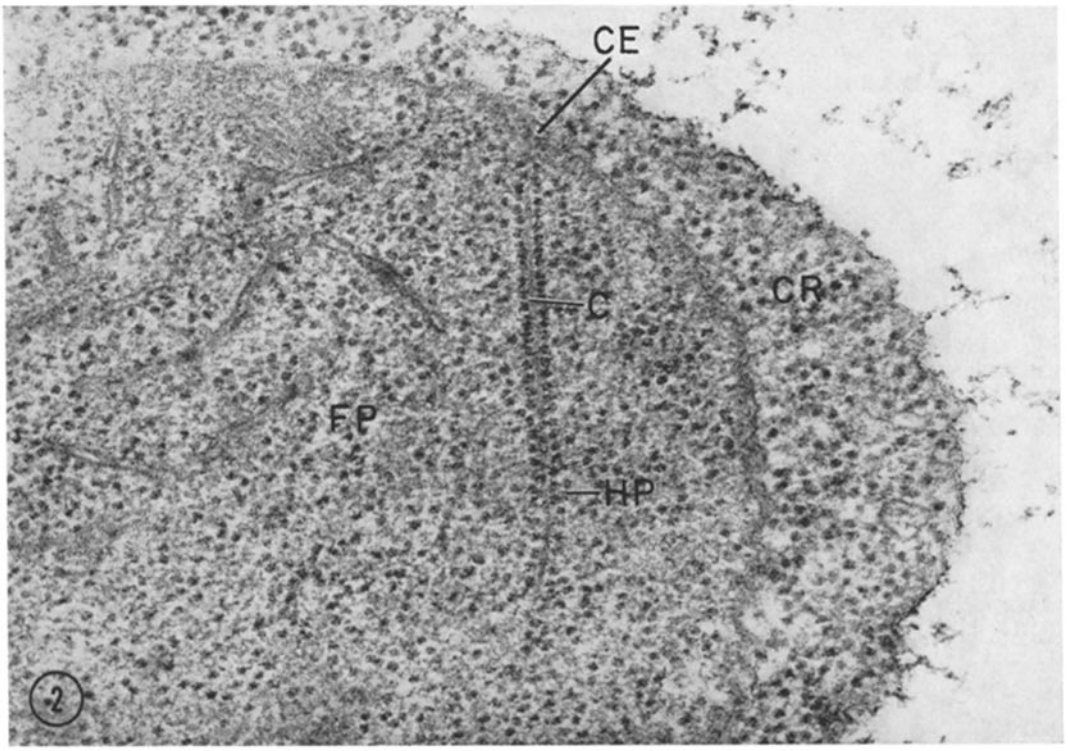


FIGURE 2 A portion of proplastid showing particles arranged in a helix (*HP*). These particles appear to surround a central core (*C*). The size and morphology of these particles are similar to those of the free plastid particles (*FP*) and cytoplasmic ribosomes (*CR*). × 75,000.

sembling ribosomes in developing plastids of wheat seedlings and presents evidence suggesting that they may be involved in membrane development.

MATERIALS AND METHODS

1-mm² pieces of leaf tissue from 5-day-old dark-grown wheat seedlings (var. Federation) were fixed with cold 6% glutaraldehyde buffered with 0.1 M phosphate, pH 7.0, for 6 hr. The pieces were washed several times, over a 2½-hr period, in 0.1 M phosphate buffer and then postfixed with Dalton's chrome-osmium fixative (Dalton, 1955) for 9 hr. The use of long postfixation times was found to be necessary in order to permit observation of the various organelle membranes. All operations of fixing and washing were completed at 4°C. The leaf tissue was exposed to about 75 ft-c of light for 8–10 min while the tissue was being cut into 1 mm² pieces in glutaraldehyde. All other operations of fixing and washing were carried out in the dark. The fixed leaf segments were dehydrated in a graded acetone series, embedded in Maraglas (Weier et al., 1965), and sectioned with glass knives on a MT-1 Porter-Blum microtome. Sections were doubly poststained with saturated aqueous uranyl acetate solution for 18 hr and lead citrate (Reynolds, 1963) at full strength for 5 min. Grids were examined with a Hitachi HU 11 electron microscope.

After glutaraldehyde fixation, some leaf sections were incubated for 3 hr at 37°C in a solution of crystalline pancreatic ribonuclease (Worthington Biochemicals Corporation, Freehold, New Jersey). The concentration of the enzyme was 1 mg/ml at pH 6.7.

RESULTS

Figs. 1–9 show three typical arrangements of particles in the proplastids of wheat seedlings. First, they (*FP*) appeared to be distributed more or less uniformly throughout the stroma; secondly, some of the particles are clustered and, depending on the plane of sectioning, appear in parallel rows two to three abreast (*AP* in Figs. 1, 4, and 4 *a*) or in a helix (*HP*) (Figs. 2 and 3); thirdly, they are aggregated into a quasi-crystalline inclusion (*CP* in

Figs. 6 and 7). The diameter of the particles, aggregated in rows or a helix or free, ranged from 170–220 A. The cytoplasmic ribosomes (*CR*) are of about the same dimensions. Smaller diameters of 140–190 A were observed for particles in the quasi-crystalline bodies (*CP* in Fig. 7).

The array of particles shown in Figs 3 and 3 *a* may be interpreted to be a double-stranded helix (*HP*) with the gyres having a pitch of about 60° with respect to the long axis. This helical arrangement (*HP*) is observed less often than the parallel rows, probably because it must be in the plane of the section to be apparent. Figs. 2 (*HP*) and 4 (*AP*) may represent sections through the outer surface of the helix, and thus the particles in this outer surface will appear as parallel rows. Since serial sections of this structure were not made, a three-dimensional interpretation is not possible.

An alternate interpretation to the helical configuration of Fig. 3 (*HP*) is that the particles are arranged, in a highly ordered manner, around a gray-staining central core (*C*), which may be seen in Fig. 2.

The particles in the helical configuration (*HP* in Fig. 3) look morphologically very much like the particles which surround the central core in Fig. 2. The core does not appear in Fig. 3, probably because the section includes only the outer surfaces of the array.

The third type of particulate arrangement is shown by the arrays at *CP* in Figs. 5–7. This is the most common type of configuration, occurring in about 80% of the micrographs examined. This array consists of roughly square clusters of particles lying in the stroma between the membranes radiating from the prolamellar body (*PB*) (Figs. 5 and 6, *CP*). These arrays are made up of six to eight particles per side and are reminiscent of virus crystals (Edwardson et al., 1966). Frequently, short or long rows of parallel rods (RS) are observed adjacent to the square arrays (*RS* in Figs. 5, 6, 8, and 9). Fig. 9 is an enlargement of the par-

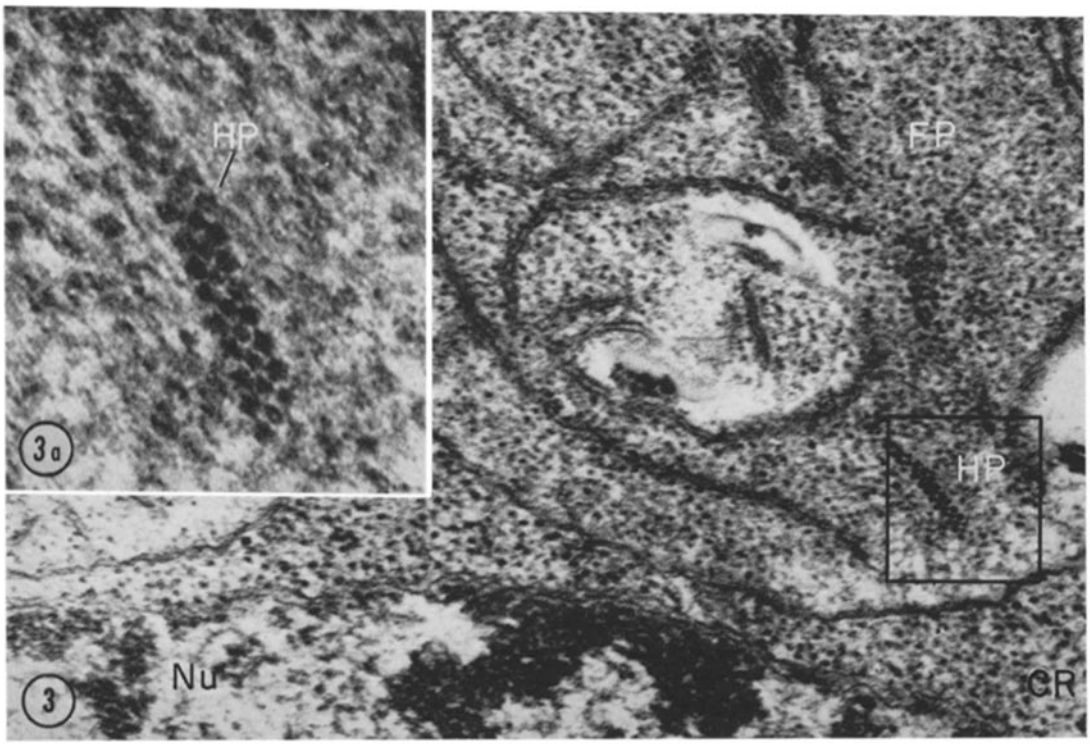


FIGURE 3 Helically arrayed particles (*HP*) in a wheat proplastid. The central core does not appear. Some of the particles appear to be connected by strands of dark-staining material. The insert (Fig. 3 *a*) shows an enlarged view of the helix (*HP*). The size and morphology of the particles appear similar to those of cytoplasmic ribosomes (*CR*) and the free plastid particles (*FP*). × 45,000. Insert, × 160,000.

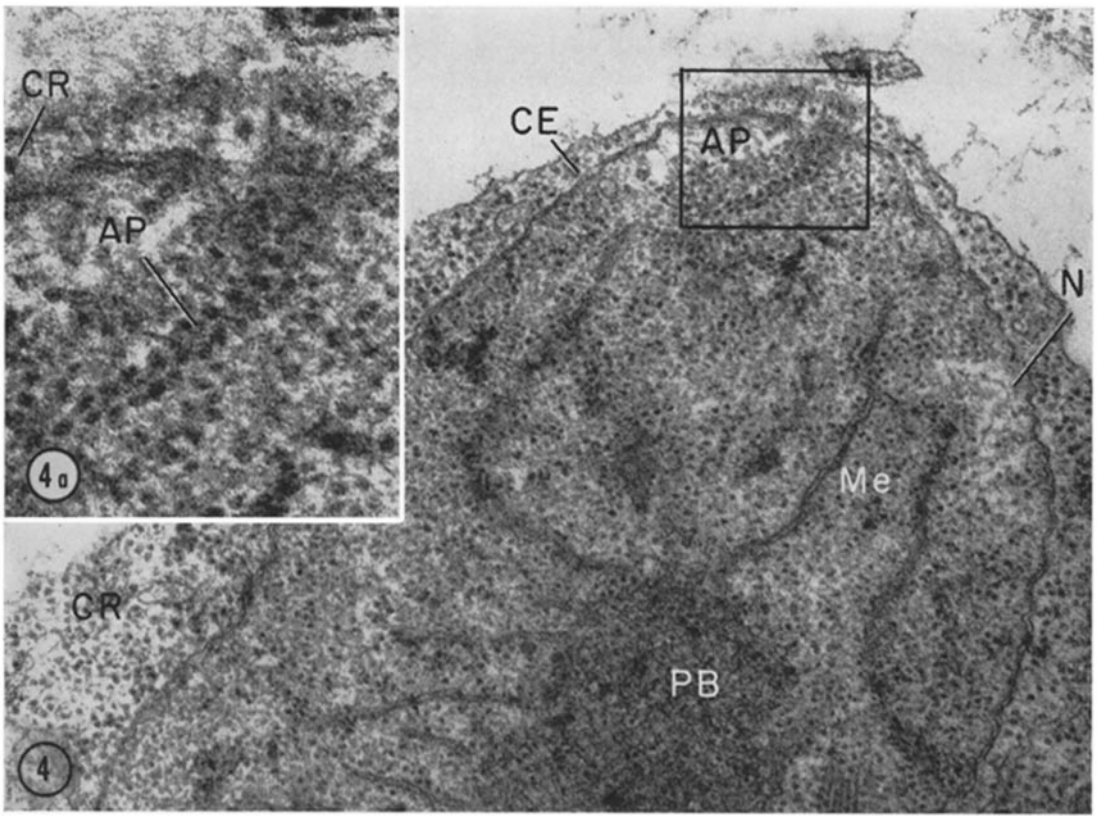


FIGURE 4 Part of a wheat proplastid with an array of particles (*AP*) extending in from the inner membrane of the plastid. Note the prolamellar body (*PB*) with extending membranes (*Me*) which was induced by light to degenerate. Nucleoplasm-like region (*N*) which may represent the DNA of the chloroplast. The insert (Fig. 4 *a*) is an enlarged view of the arrayed particles. × 45,000. Insert, × 120,000.

ticles at the end of the rods. It is possible that the square arrays are cross-sections of the parallel rods. Some micrographs (Fig. 6, *SU*) suggest that the rods may at times be formed of subunits having dimensions similar to those of the particles in the square arrays. It is tempting to speculate that the rods may be formed through a fusion of particles. Fig. 8 illustrates an exceptionally long bundle of rods that is 5 μ long and 200 A wide. This array is about three-fourths of the length of the proplastid.

The prolamellar body is of particular interest because it also contains particulates with ribosomal characteristics (compare Fig. 5, *PB* and Fig. 10, *PB*). Close examination shows that many of these particulates are surrounded by a membrane lattice (Fig. 5 *a*). Gunning (1965 *b*) has described a similar occurrence of ribosome-like particles within the prolamellar body (*Avena sativa* leaves, var. Victory). He postulated that they were trapped by the developing prolamellar body membranes and suggested that they could be responsible for the configuration of that body.

All of these particulates, the randomly distributed granules, the helical arrays, the square and parallel arrays, and the particulates within the prolamellar body are removed by treatment with RNase (Fig. 10).

DISCUSSION

While the types of particles just described presumably contain RNA, we have no direct evidence for ribosomal activity for any of them. However, active protein synthesis is taking place in developing chloroplasts. A synthesis of lipids, chlorophyll, and possibly other materials including nucleic acids is also occurring. Furthermore, these

materials are being assembled to form the chloroplast membranes. Since ribosomal activity (Boardman et al., 1965); Spencer, 1965) has been demonstrated in RNA particulates isolated from chloroplasts, it seems logical to assume that some of the RNA particulates visualized by the electron microscope may be ribosomes. Echlin (1965) has noted similar arrays of helical ribosome-like particles in the cytoplasm of developing pollen grains of *Ipomoea purpurea* and discusses the possibility of their involvement in protein synthesis in the developing pollen. The arrays described could also be virus particles or particulates induced by smog (Thomson et al., 1965) or "stromocentres" (Gunning, 1965*a*) or arrays of phytoferritin (Hyde, 1963).

The square arrays of parallel rods may not be ribosomes (Figs. 5–7). They are smaller, ranging from 120–140 A in diameter (Fig. 7), and they have a smooth surface and are hexagonal in cross-section. They may be of viral origin. Zaitlin and Boardman (1958) have suggested that chloroplasts can synthesize viruses, and Schnepf and Brandes (1962) state that viruses may be present in plants that show no signs of infection. The wheat seedlings used in the present work were grown from certified seed. Over 1,000 seedlings were grown in pots for 4 months and checked regularly by Dr. M. S. Nelson of the University of Arizona, Department of Plant Pathology, and the seedlings showed no signs of infection at any time. Shalla (1959) failed to find virus particles in the chloroplasts of plants in-

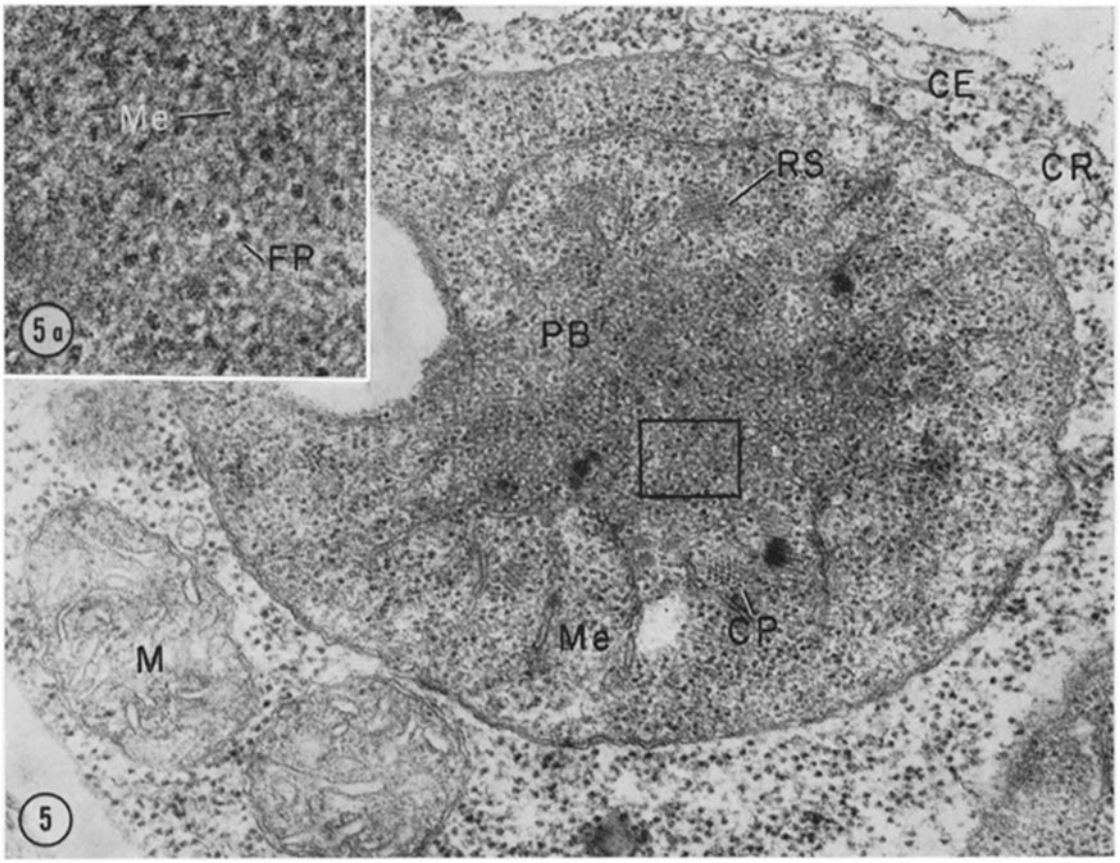


FIGURE 5 Whole proplastid from wheat leaf tissue showing prolamellar body (*PB*). Note the crystalline-like inclusion (*CP*) between the plastid membranes (*Me*). Adjacent to these inclusions are parallel rows of short rodlike structures (*RS*). The insert (Fig. 5 *a*) shows an enlarged view of prolamellar body (*PB*) with unaggregated particles (*FP*) surrounded by membranes. × 42,000. Insert, × 105,000.

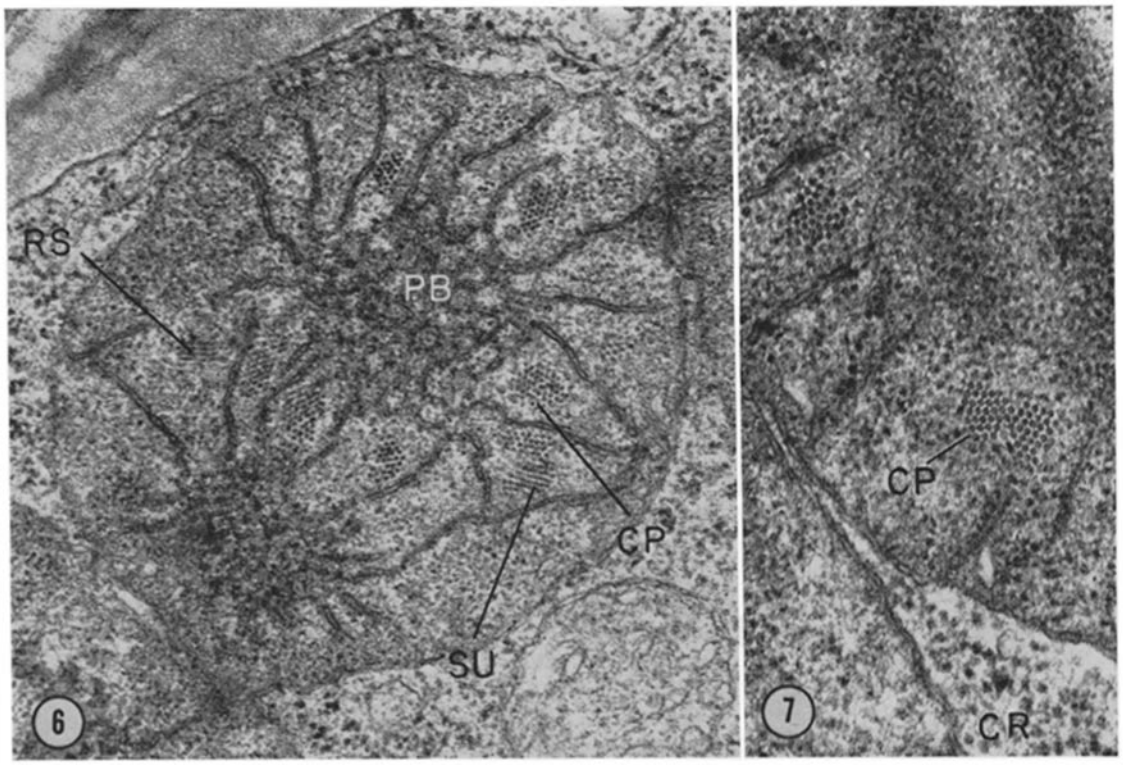


FIGURE 6 Similar to Fig. 5, the micrograph shows crystalline-like arrayed particles (*CP*) and rodlike structures (*RS*) adjacent to the prolamellar body (*PB*). The rodlike structures appear to be composed of subunits (*SU*) which are of the same size as the particles of the crystal. × 45,000.

FIGURE 7 This micrograph shows an enlarged view of a crystalline inclusion (*CP*) from another proplastid. The particles are diamond shaped, have transparent centers, and appear to be linked by thin strands of material. × 67,500.

fected with tobacco mosaic, and Lee (1965) was unable to demonstrate them in wheat plants infected with wheat streak mosaic virus.

Cronshaw, Hoefert, and Esau (1966) have described coarse arrays of particles in sugar beet plants infected with virus causing curly top. They do not find helical arrays of ribosome-like particles in any cell organelle or in the cytoplasm. They state that they do not know whether the particles in the chloroplasts are of a viral nature. It seems unlikely that the arrays of particles observed in wheat seedling chloroplasts are viruses. Further investigation seems warranted, however. Thomson, Dugger, and Palmer (1965) found 85-A particulates in chloroplasts of plants treated with chemicals obtained from smog. These particulates formed only when plants were treated in the light. The particles in wheat chloroplasts are thus larger than the smog-induced particles and form in the dark.

Gunning (1965*a*) described the presence of aggregations of fibrils about 85 A in diameter in the stroma of oat plastids and noted that they were of uncertain length. Such arrays he called "stromacentres." The particles occurring in wheat chloroplasts are larger than those found in the stromacentres of oat plastids: 200 as apposed to 85 A. Since the stromacentre fibrils do not stain densely with uranyl acetate, Gunning (1965*a*) concluded that these fibrils are largely protein and lack RNA. The stromacentre may be observed with the light microscope; it is, therefore, much larger than the array of particulates noted in wheat chloroplasts.

The quasi-crystalline arrangement of the particulates of wheat chloroplasts resemble the arrays of phytoferritin particles described by Hyde et al.

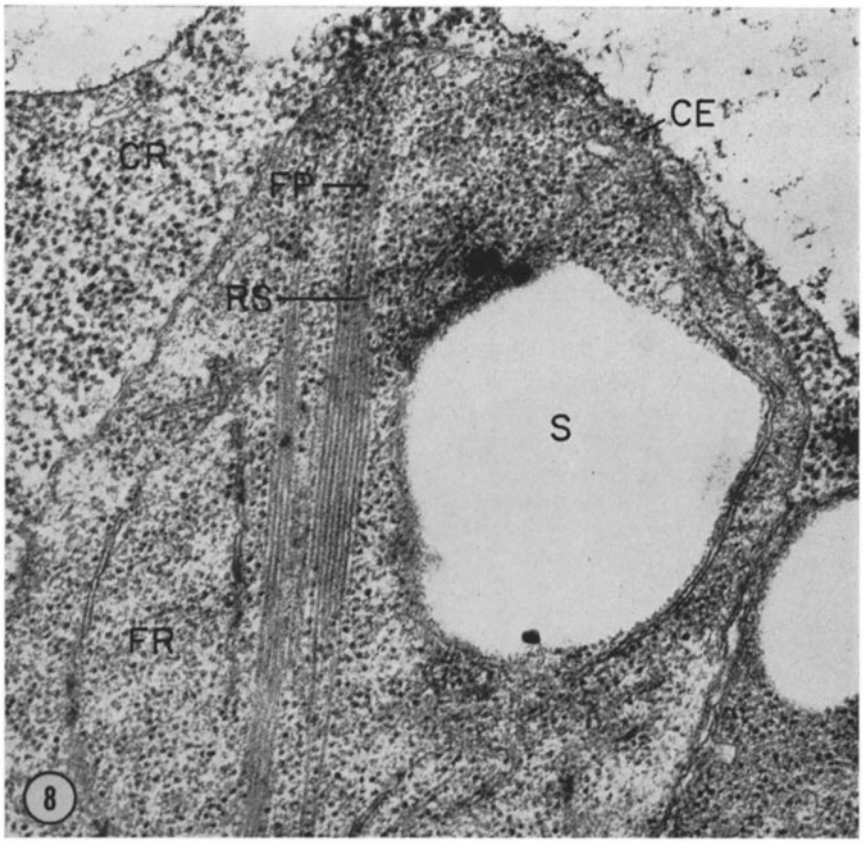


FIGURE 8 Part of a section of proplastid with long rodlike structures (*RS*) transversing about three-fourths of the plastid. At the ends of the rods are linearly arranged particles (*FP*). × 45,000.

(1963). The phytoferritin particles are only 63 A in diameter as apposed to the 200-A diameter for the particulates in the wheat plastid. The phytoferritin particles are electron opaque and so may be visualized without uranyl acetate staining.

Our observations indicate that in wheat the proplastids RNase-digestible particles which are free or arrayed as helices or in parallel rows do not conform to the requirements of virus particles, smog-induced particles, stromacentre particulates, or particles of phytoferritin.

The synthetic activity of developing chloroplasts, the isolation from plastids of particles with ribosomal activities, and the correspondence of the particulates demonstrated in Figs. 1–10 with the ribosomes in other tissue (Dallner, Siekevitz, and Palade, 1966) lead us to conclude that at least some of them possess ribosomal activities.

New membranes in developing chloroplasts are formed by the invagination of the inner component of the plastid envelope. When dark-grown, etiolated chloroplasts are placed in the light, membranes grow from the prolamellar body (Mühlethaler and Frey-Wyssling, 1963). Membrane formation may be dependent upon the presence of ribosomes in proplastids. Aminotriazole blocks the development of membranes from the proplastid stage. Ribosomes are lacking in the proplastids as visualized by the electron microscope, although they are abundant in the cytoplasm (Bartels and Weier, 1965). In the iojap mutant of corn (Rhoades, 1946) the leaf shows a white striping. Plastids in these strips do not develop beyond the prolamellar body stage. They contain areas of DNA fibrils but no ribosomes (Shumway and Weier, 1966). However, ribosomes are present in the adjacent cytoplasm. During this membrane development, proteins, lipids, pigments, and

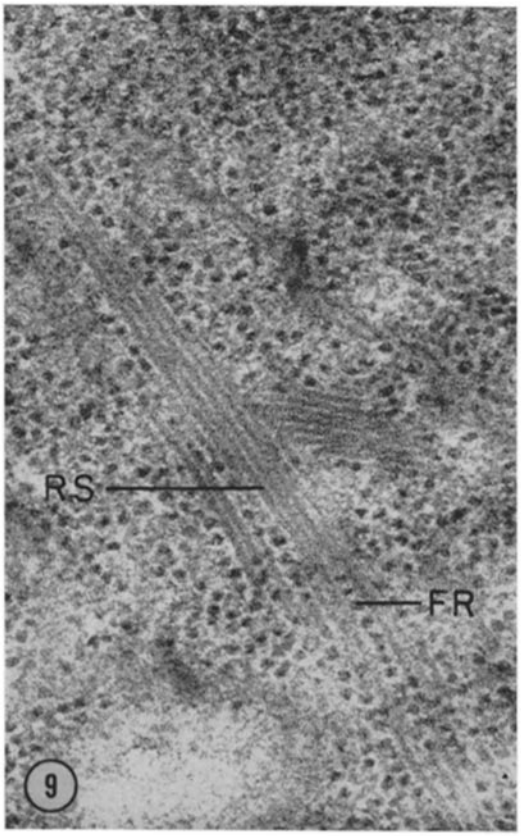


FIGURE 9 An enlarged view of the rods (*RS*) similar to those in Fig. 8, but from another proplastid. × 75,000.

other materials are required for membrane synthesis; in other words, ribosomal activity may be a prerequisite for membrane synthesis. The location of the helices and parallel rows of particles (Figs. 1–4), particularly at the peripheral regions of the proplastid in which membrane invagination is known to occur, suggests an involvement of the particles in membrane formation. Furthermore, the accumulation of RNA-containing particulates between the membranes radiating from the prolamellar body during active membrane development suggests a relationship between ribosomes and membrane formation. Gibbs (1962) has made similar observations on the relationship between ribosomes and membrane formation in chloroplasts of several algae. She has also suggested an involvement of ribosomes in membrane formation. In an initial and thorough study of membrane development in the rat hepatocyte, Dallner, Siekevitz, and Palade (1966) reach the conclusion that the protein components of the developing membranes are probably synthesized by attached ribosomes.

These observations have considerable significance. Genetic information must eventually be transmitted to cell structures. The genetic pathway of information does not always end with a soluble protein. Protein must be built into cellular structures, and the pathway that the information takes may well be by way of ribosomes to membranes. Dallner, Siekevitz, and Palade (1966) have expressed similar ideas.

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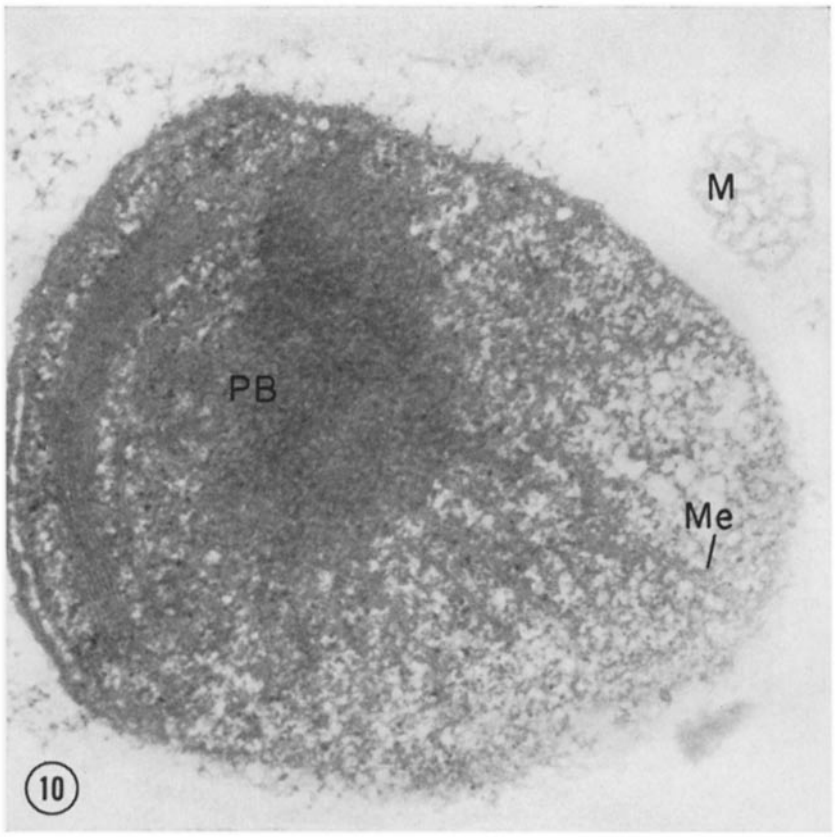

FIGURE 10 A section of RNase-treated proplastid after glutaraldehyde fixation, showing complete absence of particles previously seen in Figs. 1–9. Note the presence of mitochondria (*M*), prolamellar bodies (*PB*), and membranes (*Me*). These structures appear unaffected by the enzyme. × 37,500.

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