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Continuity of the Chloroplast Membrane Systems in Zea mays L.[1]

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Abstract. **Ultrastructural studies of the chloroplasts of the normal, yellow-green, and pale green phenotypes of *Zea mays* L. indicate that the internal membrane system is continuous with the plastid envelop. The intramembraneous spaces, loculi, and fret channels are also continuous with inner component of the plastid envelop. High energy compounds or other photosynthates, formed in the grana or frets are thus separated from both stroma and cytoplasm by a single membrane, either the fret membrane or the outer component of the plastid envelop. Since this type of plastid ultrastructure is apparently found only in plants exhibiting the Hatch and Slack pathways of photosynthesis there may be a relation between plastid ultrastructure and the pathways of photosynthetic carbon fixation.**

The ultrastructure of the typical chloroplast is interpreted as being constituted by a membranous portion and a stromatic portion (1). The membranous portion consists of an external double membrane, which forms the envelope, and an internal system in which the grana and frets are found (4). The grana and frets are continuous and in surface views the system has the appearance of a reticulum. In side views the membranes appear as stacks of loculi forming the grana with interconnecting fret membranes. The internal and external membranous portions have been interpreted as discontinuous except in the early stages of development of the plastids during which invaginations of the inner membrane of the envelope appear to give origin to the inner membrane system (5). It has been considered that these connections between the 2 systems are absent during the later stages of development of the plastid. Our current studies on the nature of the corn chloroplast indicate that the internal membrane system and the envelope are connected. This proposition opens up new possibilities concerning the probable movement of ATP within the plastid and possibly to its exterior, and a possible access route for inorganic phosphate.

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Materials and Methods

Fully developed leaves of greenhouse grown *Zea mays* L. plants approximately 2 weeks old were used. The plants used were of normal, yellow-green and pale-green genetic stock. The material was sectioned and killed in 6 % phosphate-buffered (pH 7.2, 0.15 M) glutaraldehyde for 2 hours followed by 2-hour treatments with a 2 % solution of $KMnO_4$ or a 1 % phosphate-buffered (pH 7.2, 0.15 M) solution of OsO_4. The sections were then dehydrated in an alcohol series and embedded in Maraglas or Araldite. The thin sections were post-stained by floating sections on a drop of barium permanganate for 20 minutes or of lead citrate at high pH (Reynolds, 1963) for 20 minutes.

Observations. The ultrastructure of the normal corn chloroplast has been previously reported by Hodge, McLean, and Mercer (2). Paolillo and Falk (3) have published a detailed study of the grana-fretwork arrangement. Our present studies agree with Paolillo and Falk's reported observations. A detailed study of the peripheral region of the plastid, however, reveals the presence of numerous connections between the envelope and the internal membrane system not previously reported. These connections can be seen clearly in surface and side views of the plastids.

The typical side view of a plastid, such as figure 1, indicates the presence of the typical grana-fret

arrangement of the inner membrane system. In the peripheral area multiple invaginations of the inner membrane of the envelope can be seen. Also, short isolated double membrane portions are present producing the general effect of a reticulum. In figures 2 and 3 we can see these invaginations in further detail. It can be noticed that some of them are rather short but others can be rather extensive. Frequently, as seen in figures 4 and 5, some of these invaginations run into what can be recognized as frets and eventually into the loculi.

In figure 6 we have a surface view of a typical plastid. In this section the internal membrane system appears as a reticulum in which the grana and frets can be easily recognized. The peripheral region of this reticulum appears devoid of grana and the membranes are finely subdivided forming a very fine reticulum more tubular in nature than the internal fretwork which seems to be membranous. This fine peripheral reticulum extends into the region of the envelope fusing with its internal membrane. Sections whose angle is such that both surface and side views of the plastid can be seen, such as figures 7 and 8, indicate that tangential surface views of the plastid present the peripheral area as a fine reticulum while side views present it as formed by invaginations of the inner membrane of the envelope some of which connect with the frets and loculi. Also, very small isolated portions of the frets are present. It will seem, then, that the peripheral infoldings and projections of the envelope as well as the isolated membrane portions seen in side views correspond to the fine peripheral reticulum seen in face views. Furthermore, these connections are frequently present in the normal as well as the yellow-green and pale-green mutants.

Discussion

Surface and side views of the corn chloroplast indicate that its external and internal membrane systems are continuous. Surface views demonstrate the presence of a peripheral reticulum between the envelope and the internal membrane system. Sections with side views and those with side and surface views indicate that this peripheral reticulum seems to be formed by tubular double membranes which are continuous with the inner membrane system and with the envelope. As the grana and frets form a continuous system it can be said that the inner membrane system is continuous with the peripheral reticulum and the inner membrane of the chloroplast envelope. Since the internal and external membrane systems are continuous the inner space that is found between the membranes is also continuous. Thus, the intramembranous space of the envelope is continuous with the corresponding space within the peripheral reticulum and within the frets and grana. The internal surfaces of those membranes thus lead into a common channel. This common channel is separated from the stroma by the fret membrane and from the cytoplasm by the external membrane of the envelope. It seems reasonable to assume that the continuity of the membranes and their corresponding intramembranous spaces would provide a continuous active region within the plastid.

It can be assumed that the reactions which take place within the internal membrane system are related to the reactions occurring in the stroma. That is, the energy trapping and transfer that take place within the membranes themselves or within the loculi are related to the utilization of that energy in the dark reactions which occur in the stromatic region. That the trapping of energy occurs within the internal membrane system should be clear.

It seems logical to postulate the existence of a shuttle system involving transfer of energy from the granal membranes and spaces to the stroma, and possibly also to the cytoplasm. A continuous intramembranous space occurs in corn plastids and presumably in plastids of other plants exhibiting the Hatch and Slack (1966) carbon fixation pathway. The internal membranes of plastids with the Calvin cycle are not connected to the plastid envelope. May not the more direct connection between the loculi, fret channels and the inner component of the plastid envelope play a part in facilitating the movement of materials involved in photosynthesis in the corn plastid?

FIGS. 1 to 5. Electron micrographs of chloroplasts from normal and chlorophyll mutants of *Zea mays*.

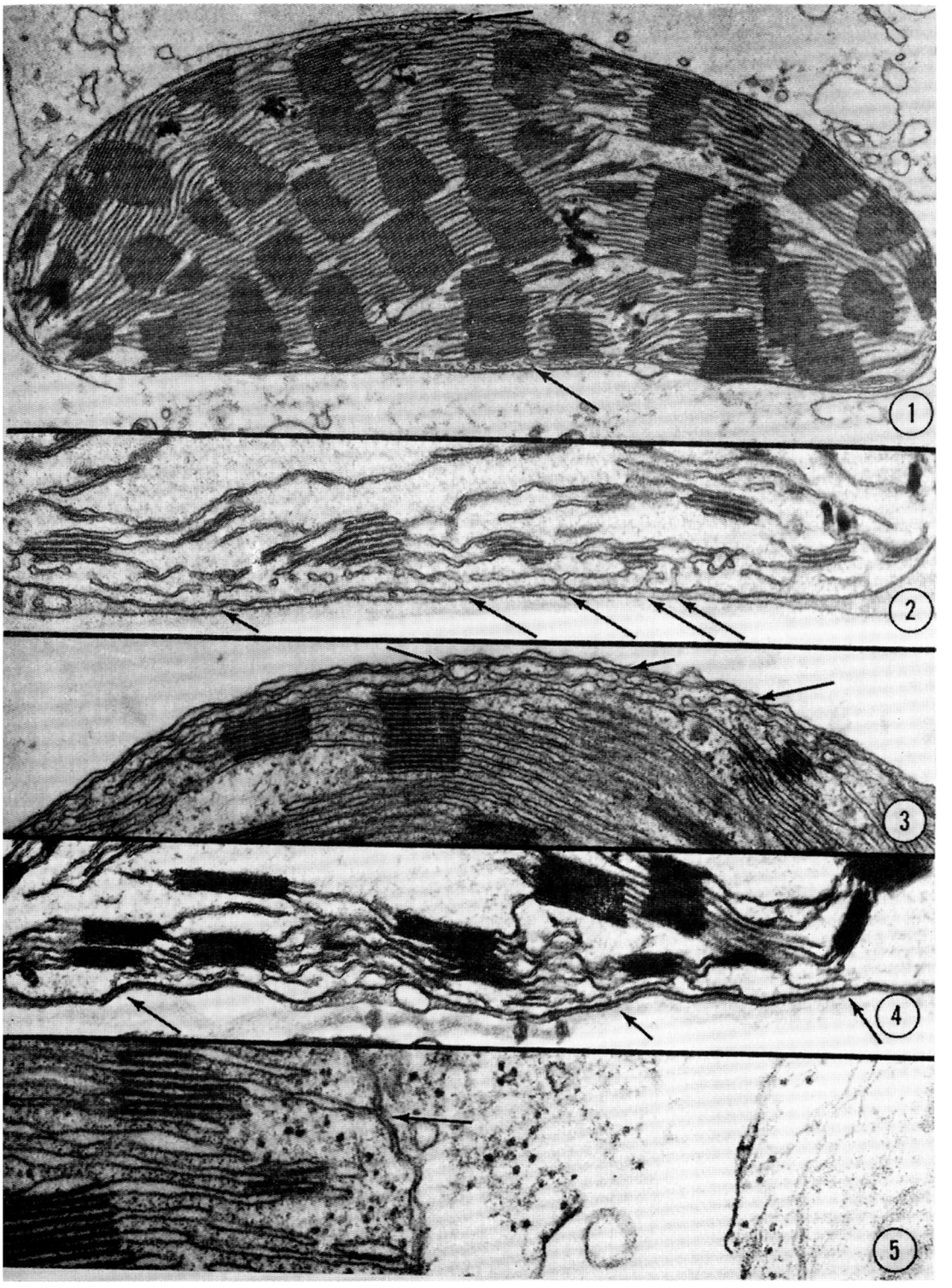
FIG. 1. Profile of a chloroplast from a normal plant showing (arrows) a peripheral reticulum between the internal membrane system and the plastid envelope. × 18,200.

FIG. 2. Portion of a chloroplast from a pale green mutant showing examples (arrows) of the continuity of the internal component of the plastid envelope with the internal membrane system of the plastid. $KmnO_4$. × 30,000.

FIG. 3. Portion of a chloroplast from a normal plant showing examples (arrows) of the continuity of the internal component of the plastid envelope with the internal membrane system of the plastid. Glutaraldehyde, OsO_4. × 32,000.

FIG. 4. Portion of a chloroplast from the yellow-green mutant in which invaginations (arrows) of the internal component of the envelope connects directly with frets and grana. $KmnO_4$. × 30,000.

FIG. 5. Portion of a chloroplast from a normal plant illustrating membrane continuity between the grana, frets and plastid envelope. Glutaraldehyde, OsO_4. × 64,000.



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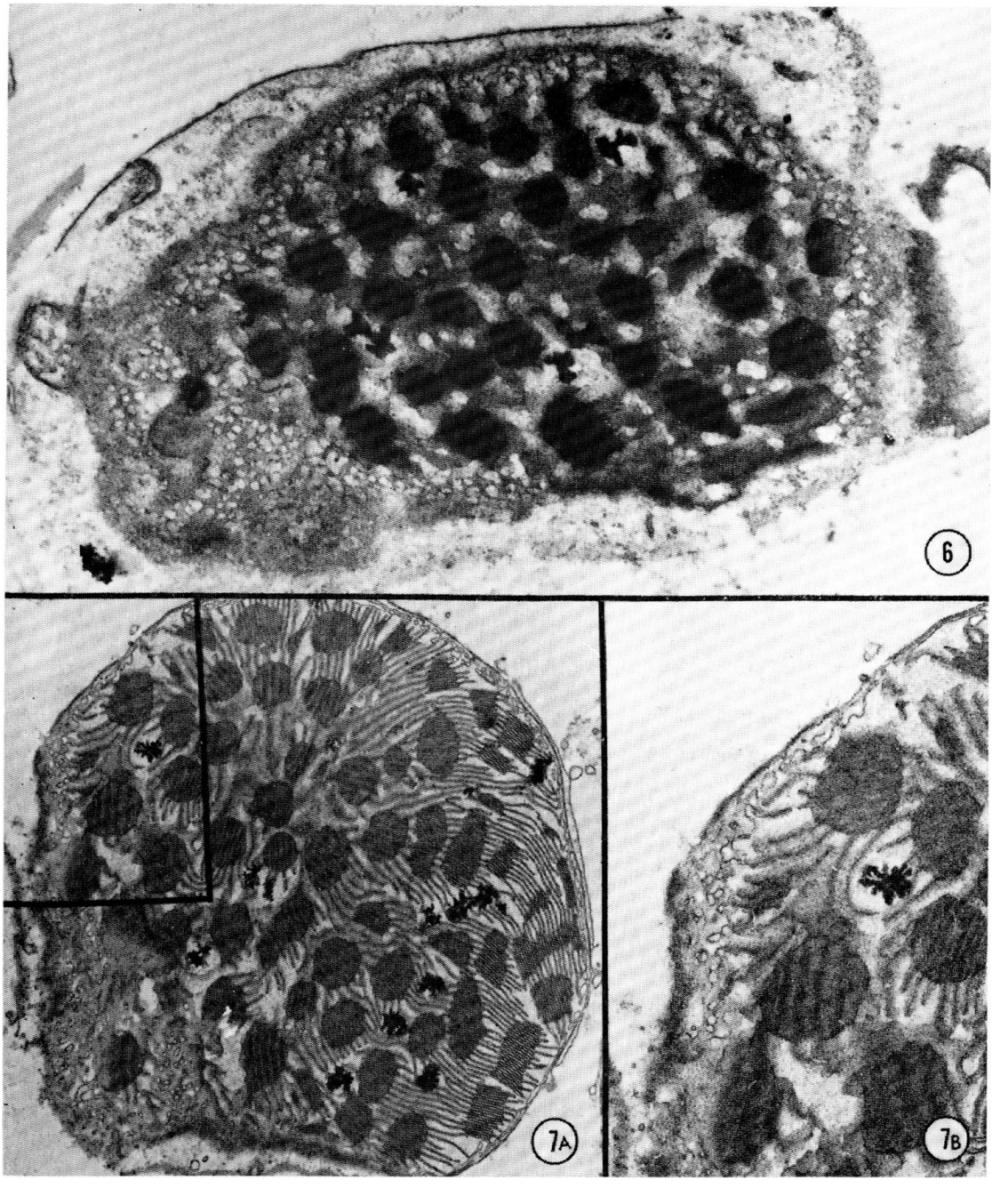

FIGS. 6 to 7. Electron micrographs of normal chloroplasts of *Zea mays*.

FIG. 6. Surface view showing the peripheral reticulum and its continuity with the more coarsely anastomosing fret system. $KmnO_4$. × 20,000.

FIG. 7. Oblique section through a plastid showing profiles of grana on the lower right and close to face views of the grana at the upper left. Figure 7B is an enlarged portion of the upper left area from figure 7A. The continuity between the envelope, peripheral reticulum, frets, and grana is distinctly illustrated.

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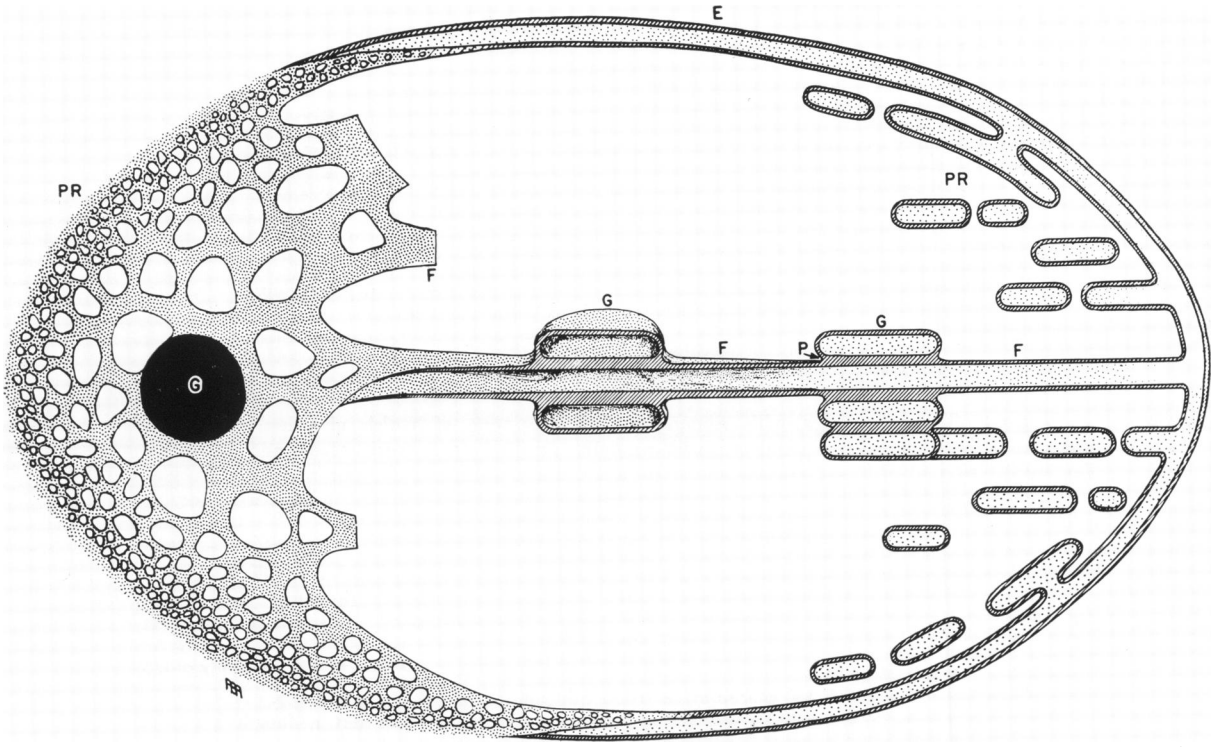

FIG. 8. Diagram showing the transition and continuity between plastid membranes of a profile and face view sections. G) grana, F) frets, PR) peripheral reticulum, E) envelope, and P) portion.

Acknowledgments

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