

# ULTRASTRUCTURE OF SPECIALIZED PARENCHYMA CELLS IN THE LEAF BLADES OF THE SENSITIVE PLANT *MIMOSA PUDICA* L.

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## Summary

The ultrastructure of specialized parenchyma cells which are believed to conduct an electrical stimulus as a membrane action potential is described for *M. pudica*.

In general morphology these cells resemble "transfer cells" of leaves described by Gunning, Pate, and Briarty (1968) which are thought to play a role in the translocation of assimilates. An additional feature of the specialized parenchyma of *M. pudica* is the presence of an unusual type of plastid in which the grana lamellae may either display an "open" conformation, i.e. thylakoid membranes have moved apart, exhibiting electron-translucent areas between them, or a "closed" conformation, i.e. thylakoid membranes are stacked in an orthodox fashion.

A possible relation is suggested between the conformational changes of the plastids and the rapid transmission of stimuli.

## I. INTRODUCTION

Sibaoka (1962, 1966) observed that certain small elongated parenchyma cells in the leaf petioles of the sensitive plant *Mimosa pudica* L. have a resting potential between the cell interior and the external medium which is in excess of that of all other cells. These parenchyma cells also have the ability to conduct an electrical stimulus as a membrane action potential, the change in potential being towards the direction of depolarization, a feature similar to that encountered in the nerve axon, muscle fibre, and characean internode. It was inferred that the excitable cells play a role in the transmission of mechanical stimuli to the secondary and main pulvini, causing the successive closure of the leaflets and the lowering of leaves along the stem. The excitable cells were described as being uniform in size, 10  $\mu\text{m}$  in diameter, and 120  $\mu\text{m}$  long. They were believed to be located in the protoxylem or sometimes in the phloem, but microscopic observation did not lead to their positive identification in the phloem. No detail of their internal structure was given.

The present investigation was initiated to provide a description at the ultrastructural level of these specialized parenchyma cells.

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## II. MATERIALS AND METHODS

Leaf blade tissue of *M. pudica* was used in preference to petiole and stem tissue as the transmission of stimuli is known to occur in both and because it was anticipated that the ramifications of the excitable cells would be more easily recognized amongst leaf mesophyll parenchyma cells than amongst the vascular elements of the normal conductive tissues of the stem and petiole.

Leaflets were cut into small pieces in a drop of ice-cold fixative [2%  $\text{KMnO}_4$  in 0.1M veronal acetate buffer (pH 7.2) or 4% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2)]. The pieces of tissue were transferred to a larger volume of fixative and fixed for 0.5 hr in  $\text{KMnO}_4$  or 1–1.5 hr in glutaraldehyde. After a number of rinses in buffer the glutaraldehyde-fixed tissue was post-fixed in 2%  $\text{OsO}_4$  in 0.1M cacodylate buffer, pH 7.2, for 1–2 hr. Tissues were dehydrated using an acetone series, embedded in Araldite, sectioned in lead citrate (15 and 45 min for  $\text{KMnO}_4$ -fixed and glutaraldehyde-fixed material respectively), and viewed with a Siemens Elmiskop I or IA electron microscope at 60kV.

## III. OBSERVATIONS

Figure 1 shows a cross-section at low magnification of the combination of cells which was repeatedly observed. It consists of two, or occasionally four, small, generally empty cells approximately  $3\text{ }\mu\text{m}$  in diameter, surrounded by five larger cells approximately  $8\text{ }\mu\text{m}$  in diameter with dense cytoplasmic contents. The larger cells often exhibit inwardly directed protrusions of the cell wall similar to the knobby thickenings of the cell wall described by Wooding and Northcote (1965) and by Gunning, Pate, and Briarty (1968). Usually, two small tracheary elements can be observed alongside this specialized group of cells. The total complex of cells in cross-section is similar in size to any one of the surrounding spongy parenchyma cells. In longitudinal section all cells of the group appear elongate (Fig. 5), the lengths of the cells being difficult to define by electron microscopy because of the limitations of chance orientation and thinness of sections. This characteristic group of cells can always be immediately recognized from the generally much larger groups of vascular elements of the leaf veins. The larger cells of the group are further characterized by the presence of a special type of plastid similar to those described by Scala, Schwab, and Simmons (1968) for the trigger hair of the Venus fly trap. The stacked internal lamellae have moved apart revealing completely electron-transparent "vacuole" spaces between and immediately outside the lamellae (Fig. 2), and in this "open" conformation form a distinct contrast to normal chloroplasts in the adjacent parenchyma cells (Fig. 3).

Sometimes the lamellae of plastids in these special cells were observed to be in the "closed" conformation and then appeared as normal stacks of thylakoid membranes (Fig. 4). On one occasion both the closed and open conformation were observed in the same bundle of cells (Fig. 5). Little difference was observed in the structural characteristics of the plastids between tissues fixed in glutaraldehyde or in permanganate (Figs. 6 and 7 respectively).

The cells are further characterized by the presence of a large nucleus and many mitochondria, which, together with the plastids, take up a large proportion of the total cell volume. This feature becomes especially apparent when cells are viewed in longitudinal section revealing mitochondria of exceptional length ( $20\text{ }\mu\text{m}$ , Fig. 8). Cells were observed in which practically the whole length (up to  $100\text{ }\mu\text{m}$ ) of the cell wall was covered by mitochondria which often seemed to be anchored by the inward protrusions of the wall (Fig. 9).

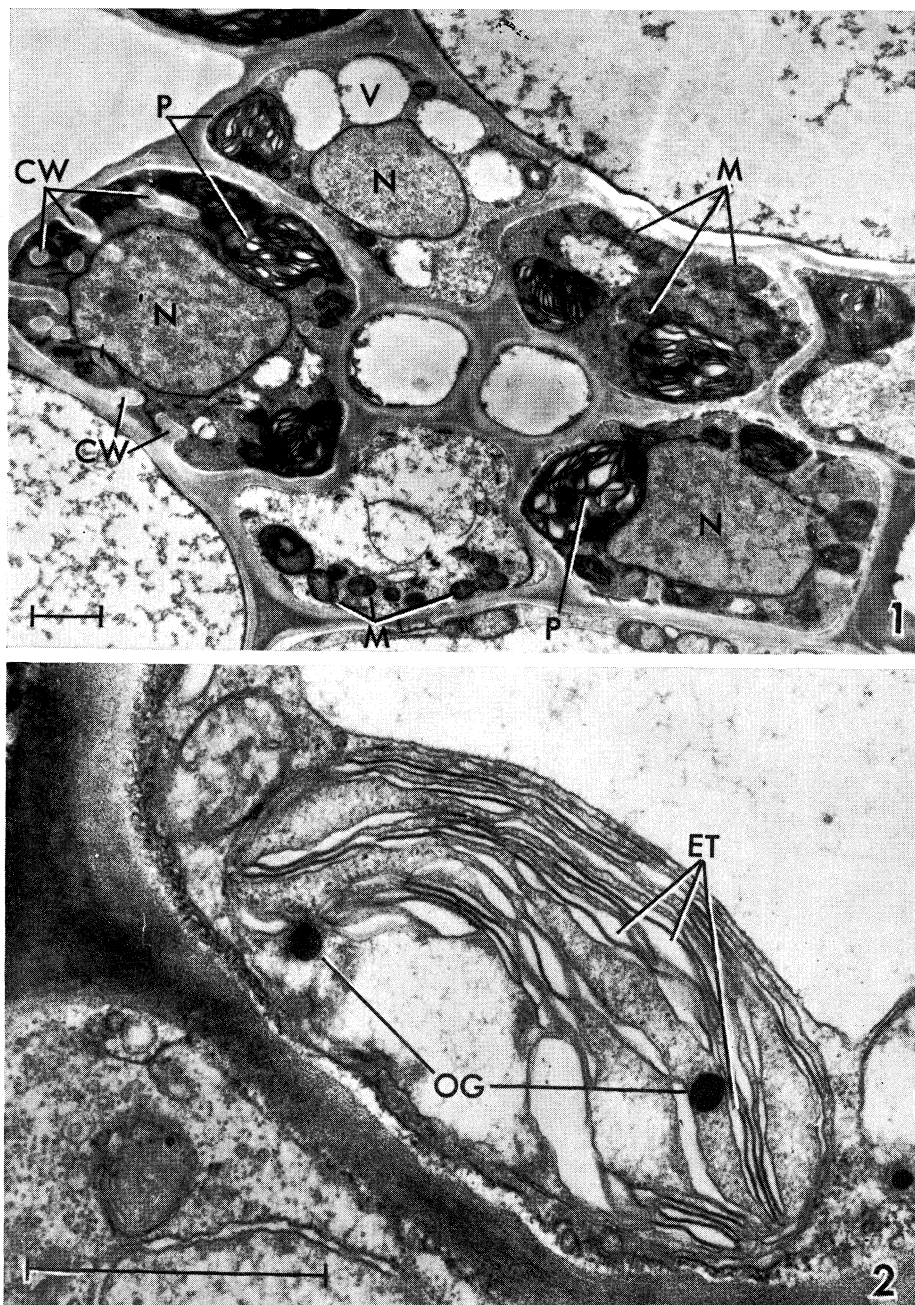


Fig. 1.—Transverse section through specialized cells in the spongy parenchyma of *M. pudica* leaf showing a group of five small parenchyma cells surrounding two smaller empty cells. Prominent features are large nuclei (*N*), a number of inwardly directed cell wall protrusions (*CW*), plastids with an expanded system of thylakoid lamellae (*P*), mitochondria (*M*), and relatively small vacuoles (*V*).  $\text{KMnO}_4$  fixed.  $\times 8,100$ . On all figures, the length of the bar represents  $1\ \mu\text{m}$ .

Fig. 2.—Detail of plastid in which the stacked thylakoid lamellae have moved apart showing electron-transparent areas (*ET*) between the lamellae. Osmiophilic globules (*OG*) are also shown. Glutaraldehyde- $\text{OsO}_4$  fixed.  $\times 39,600$ .

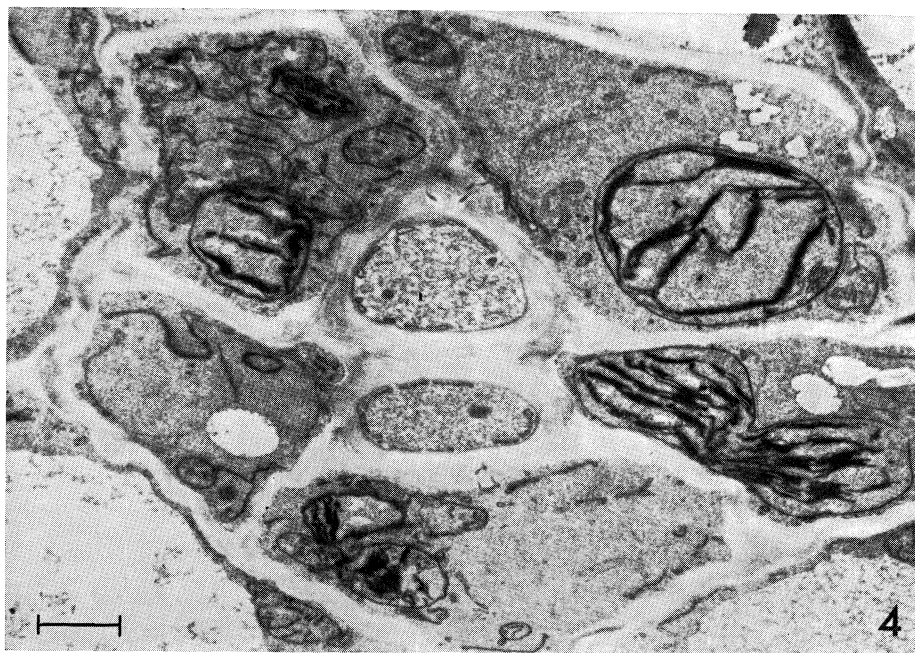
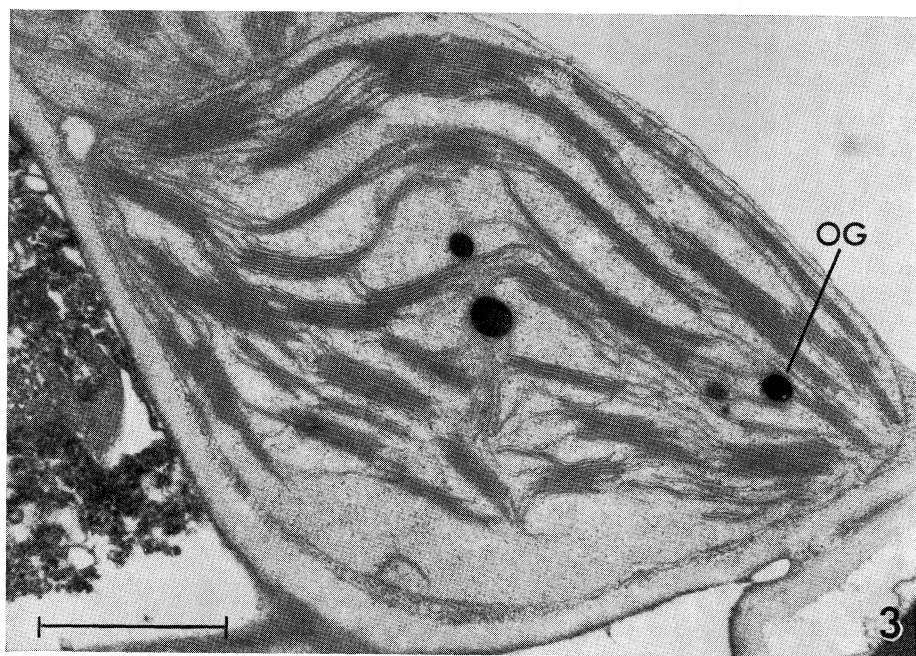


Fig. 3.—Detail of a normal chloroplast from an adjoining spongy parenchyma cell. Glutaraldehyde- $\text{OsO}_4$  fixed.  $\times 24,000$ .

Fig. 4.—Transverse section through a group of cells similar to those shown in Figure 1, but with lamellae in the closed conformation.  $\text{KMnO}_4$  fixed.  $\times 9,800$ .

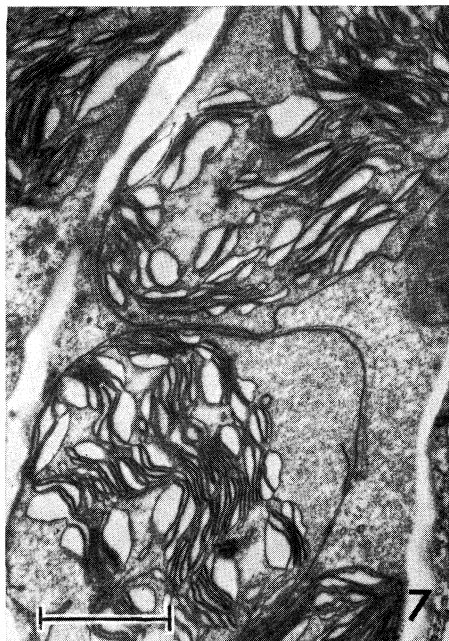
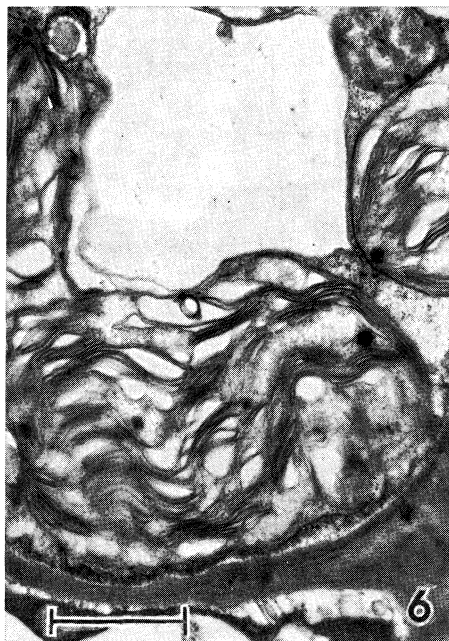
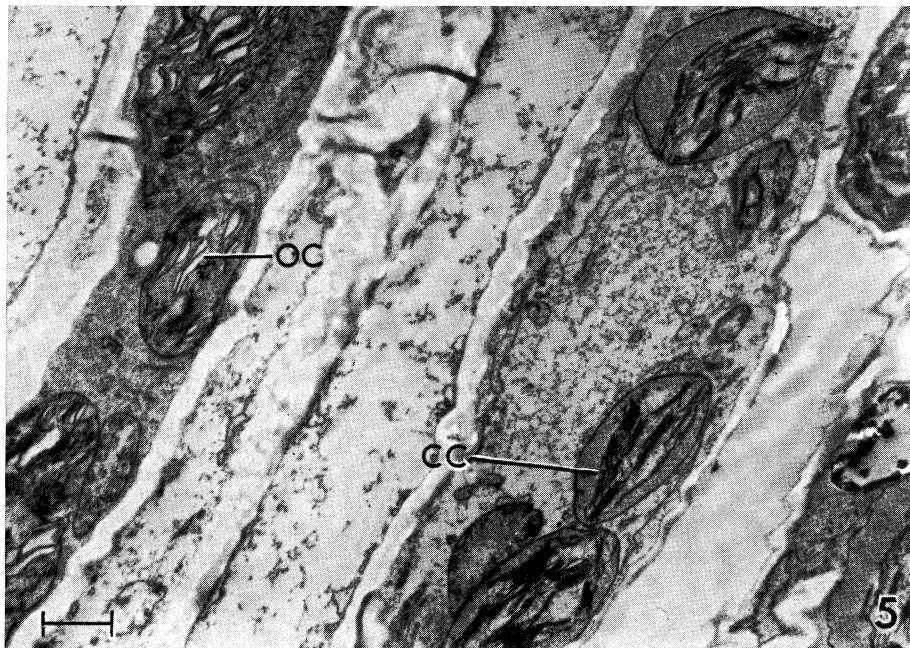


Fig. 5.—Longitudinal section through a group of specialized cells showing the two small central cells flanked by one small parenchyma cell with lamellae in the open conformation (OC) and one with lamellae in the closed conformation (CC).  $\text{KMnO}_4$  fixed.  $\times 8,500$ .

Figs. 6 and 7.—Plastid with lamellae in open conformation fixed in glutaraldehyde- $\text{OsO}_4$  (Fig. 6) and in  $\text{KMnO}_4$  (Fig. 7) respectively. Note similarity in conformation.  $\times 17,000$  and  $16,500$  respectively.



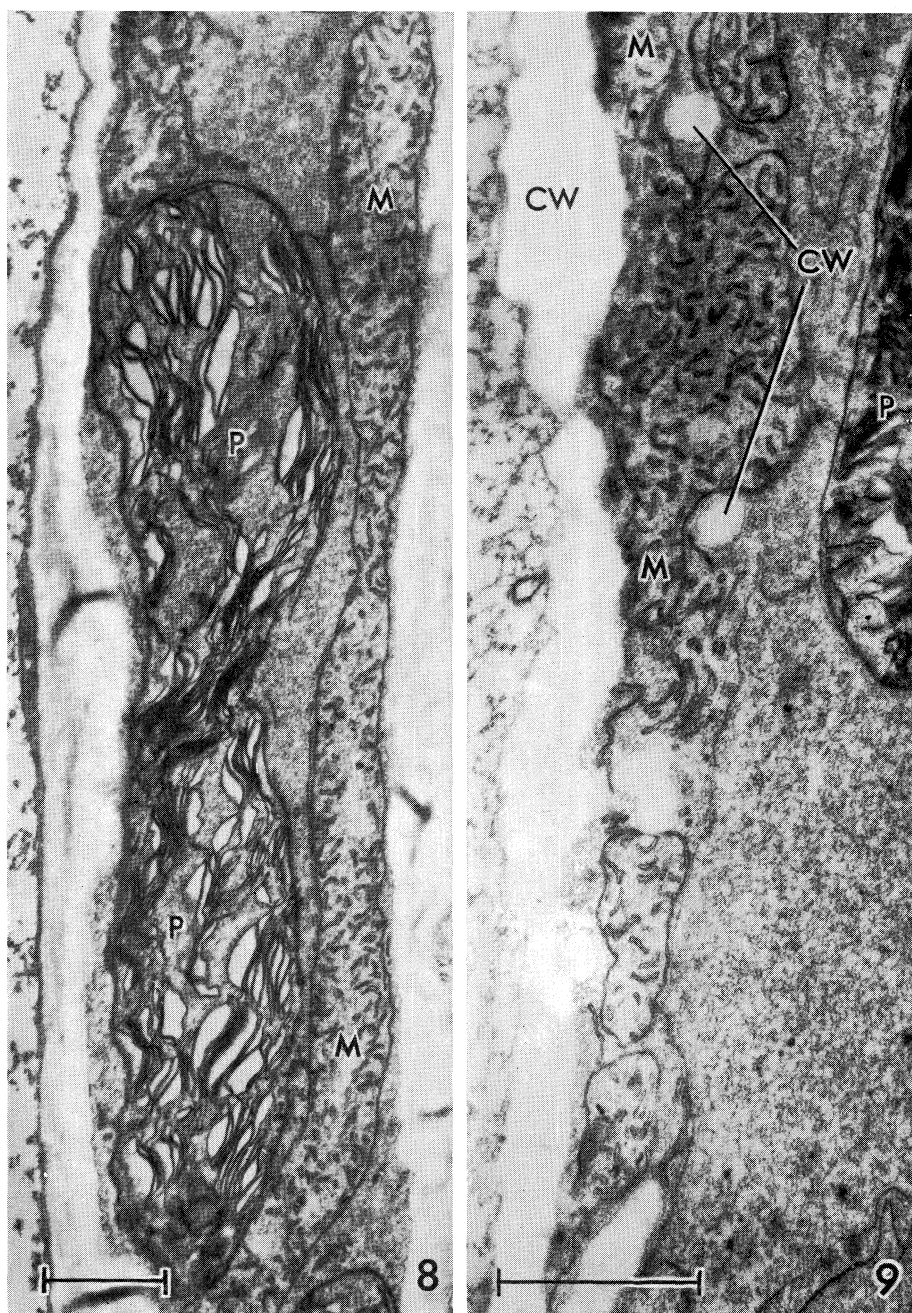


Fig. 8.—Longitudinal section of a specialized parenchyma cell showing a mitochondrion of exceptional length.  $\text{KMnO}_4$  fixed.  $\times 16,300$ .

Fig. 9.—Longitudinal section showing mitochondria “anchored” by wall protrusions.  $\text{KMnO}_4$  fixed.  $\times 26,400$ .

## IV. DISCUSSION

Although at present based on circumstantial evidence only, it is suggested that the characteristic group of elongated parenchyma cells observed in the leaflets of *M. pudica* have a function similar to the elongated cells observed with the light microscope by Sibaoka in *Mimosa* petioles. They are the only elongate cells, apart from the vascular elements of the leaf veins, and their morphological features suggest that they may play a role in the transmission of stimuli as observed by Sibaoka (1962, 1966).

It is as yet premature to ascribe a role to the two usually empty cells in the centre of the group but the larger cells immediately surrounding them are very similar in appearance to the specialized "transfer cells" described by Gunning, Pate, and Briarty (1968). Wooding and Northcote (1965) and Gunning, Pate, and Briarty (1968) suggested a translocatory role for the transfer cells, but a further degree of specialization is now indicated by the open and closed conformations of the grana lamellae of the plastids.

It was possible that the open conformation of the grana, with electron-transparent spaces between the thylakoid membranes, was due to an artefact of fixation. But, this seems to be rather unlikely since practically no difference in structural detail of the open conformation was observed using different fixatives and furthermore the normal-type chloroplasts in the adjacent parenchyma cells did not show any evidence of structural abnormalities.

Although the open conformation was observed more frequently than the closed conformation, it is as yet difficult to achieve a definite correlation between one state or the other and the transmission of a stimulus. At present, it may seem a technical impossibility to fix the specialized parenchyma cells in an unexcited state, but following a recovery period after the initial excision of leaflets it might be possible to preserve some of the tissue in an unexcited state, provided fixation is extremely rapid. Hence further experiments are necessary.

The observation of a closed and open conformation of the thylakoid lamellae in these special plastids might be related to the frequently observed light-induced volume changes of the grana lamellae (cf. Itoh, Izawa, and Shibata 1963; Deamer and Packer 1967; Murakami and Packer 1969; Nobel *et al.* 1969). A large proportion of the volume in the specialized parenchyma cells is taken up by the plastids and mitochondria which are in close proximity to each other and it might be suggested that they play a role in the creation of proton gradients (Mitchell 1967; Schwartz 1968) during an energizing phase corresponding to recovery from the excited state, and the dissipation of the gradients created during excitation. However, any such speculation awaits the confirmation that the closed and open conformations of the plastids truly represent an energized and non-energized state respectively.

## V. ACKNOWLEDGMENTS

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