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Short Communication

Enclosure of Mitochondria by Chloroplasts[1]

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ABSTRACT

In *Panicum* species of the *Laxa* group, some of which have characteristics intermediate to C_3 and C_4 photosynthesis species, some mitochondria in leaf bundle sheath cells are surrounded by chloroplasts when viewed in profile. Serial sectioning of leaves of one *Laxa* species, *Panicum schenckii* Hack, shows that these mitochondria are enclosed by chloroplasts. Complete enclosure rather than invagination also is indicated by absence of two concentric chloroplast membranes surrounding the mitochondrial profiles.

Species in the *Laxa* group of the *Panicum* genus, notably *Panicum milioides* Nees ex Trin., have been characterized as intermediate with respect to C_3 and C_4 photosynthesis based on CO_2 exchange, activities of photosynthetic enzymes, especially phosphoenolpyruvate carboxylase, and leaf anatomy (1, 3, 6, 7). In reference to ultrastructural similarities of *P. milioides* to C_4 species, close association of chloroplasts, mitochondria, and peroxisomes in vascular bundle sheath cells has been noted (4, 5). In a recent study (2) of seven *Panicum* species, in or closely related to the *Laxa* group, a high frequency of mitochondrial profiles surrounded by chloroplasts was observed in bundle sheath cells of three C_3/C_4 intermediate species, *P. milioides*, *Panicum decipiens* Nees ex Trin., and *Panicum schenckii* Hack., and also in two C_3 species, *Panicum laxum* Sw. and *Panicum hylaeicum* Mez. From these observations, the question arose: are the surrounded mitochondria invaginated in the chloroplasts or enclosed by them? This report demonstrates by serial sectioning and by membrane configuration that some mitochondria in bundle sheath cells are enclosed by chloroplasts.

MATERIALS AND METHODS

Embedded tissue of *P. schenckii* used in experiment 2 of the previous study (2) was chosen because of the high frequency of mitochondria surrounded by chloroplasts in bundle sheath cells. Transverse sections were cut at 0.09 to 0.14 μm thickness using a diamond knife. Serial sections were picked up on Formvar-coated loops, transferred to copper grids with 1 × 2 mm slots, and stained for 20 min in aqueous uranyl acetate (saturated) and subsequently for 4 min in lead citrate. The sections were stabilized with an evaporated carbon coating and were viewed in a transmission electron microscope at 100 kv. Only bundle sheath cells were studied because the frequency of mitochondrial profiles surrounded by chloroplasts was very low in mesophyll cells (2).

RESULTS AND DISCUSSION

The photographs in Figure 1 show serial sections cut through a bundle sheath chloroplast. The total thickness of tissue represented in Figure 1 is approximately 1.0 μm. In that thickness, the size of the chloroplast profile changes very little, but some mitochondrial diameters increase to a maximum or decrease to zero in successive sections. In Figure 2, eight out of 12 serial sections are shown for a single mitochondrion. Chloroplastic tissue appears in sections 1 and 12 in positions occupied by the mitochondrion in intervening sections. The existence of chloroplastic tissue in the positions where mitochondrial profiles appear in successive sections or disappear in preceding sections shows that the mitochondria are not invaginated in the longitudinal plane (profiles in Figs. 1 and 2 are in transverse plane). The occurrence of chloroplastic membranes around mitochondrial profiles in all sections in Figures 1 and 2 in all sections of other series (not presented) also show no invaginations in the transverse plane.

If mitochondria were invaginated rather than completely enclosed by chloroplasts, then cross-sections of an intruded mitochondrion would be surrounded by two concentric chloroplastic membranes. Enlargement of a mitochondrial profile shows, however, that a single chloroplastic membrane encircles the mitochondrion (Fig. 3).

Sections shown in Figures 1 to 3 demonstrate that some of the mitochondria in bundle sheath cells of *P. schenckii* are completely enclosed by chloroplasts and not simply appressed or invaginated. This enclosure of mitochondria raises many questions about their origin and function. Do these mitochondria form inside chloroplasts? If so, is the formation concurrent with or after plastid formation? Do enclosed mitochondria perform the same metabolic role as those outside the chloroplast?

It might be expected that chloroplastic and mitochondrial metabolites would move more freely between the organelles when the mitochondrion is inside the chloroplast, although Figure 3 shows that both organelles have intact membranes separating them. There is also a 'cleared' zone surrounding the mitochondrial profiles observed in Figures 1 to 3 and in other micrographs of *Panicum* species (2, 4, 5) and occasionally large sacs are formed at the periphery of chloroplasts which in profile appear empty of organelles or nearly so (Figs. 1 and 3). Nevertheless, mitochondria enclosed in chloroplasts must be in a more favorable position for metabolite exchange than those exterior to the chloroplast.

The complete enclosure of mitochondria by chloroplasts has not been reported previously, although similar profiles to those in Figures 1 to 3 have been observed in senescing leaf cells (10, 11).

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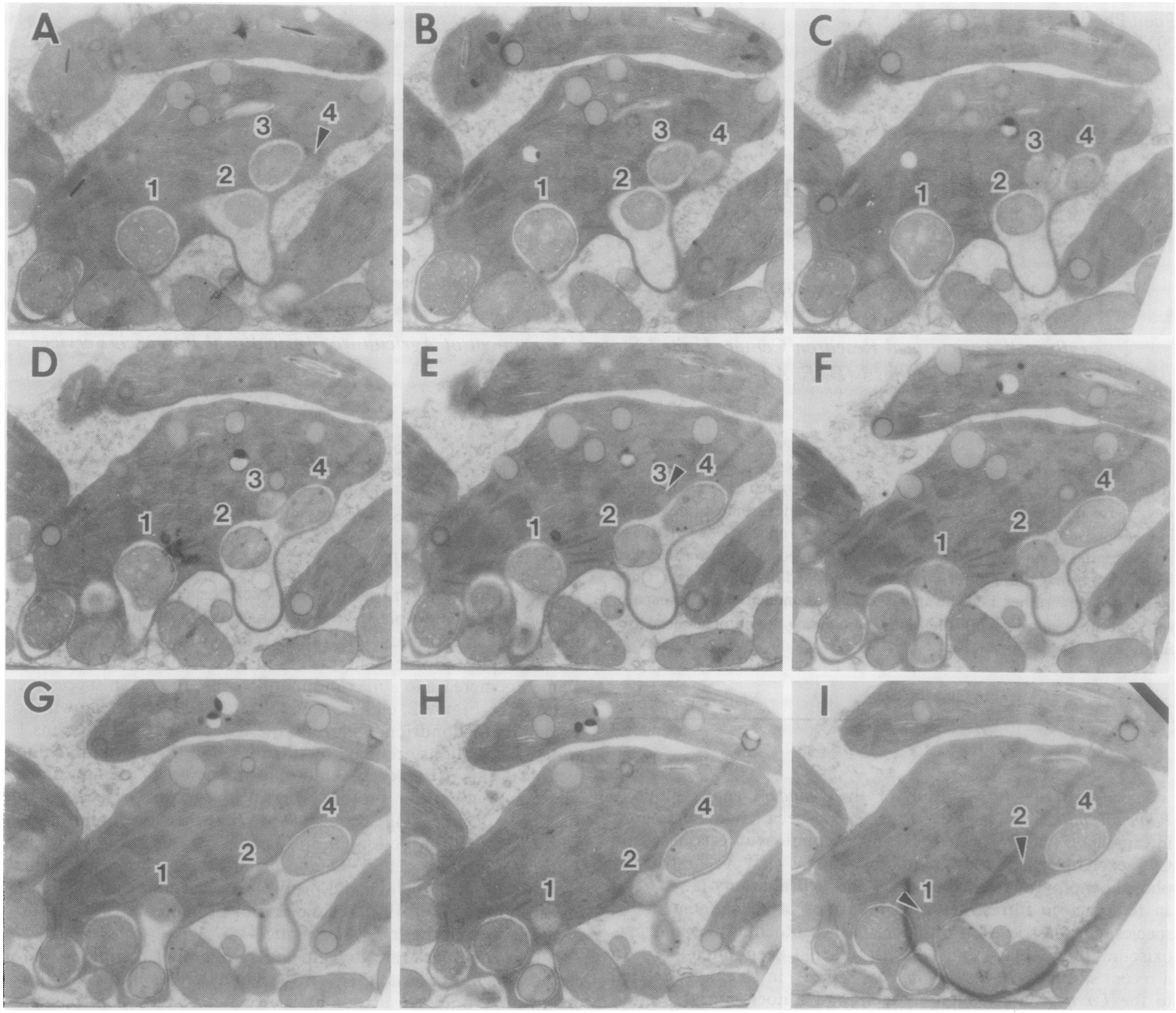

FIG. 1. Electron micrographs of serial transverse sections (A–I) of a vascular bundle sheath cell of *P. schenckii* showing several mitochondria enclosed in a single chloroplast. Numbers identify single mitochondria which appear or disappear in the series. Arrows identify chloroplastic tissue in the same position as a mitochondrion before its appearance or after its disappearance in the series. (× 11,200)

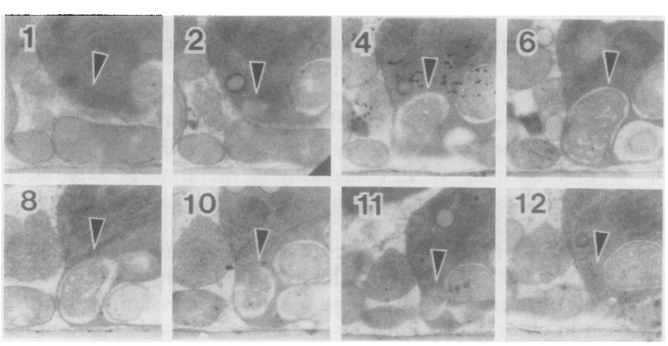

FIG. 2. Electron micrographs of a series of transverse sections of a vascular bundle sheath cell of *P. schenckii* in which a single mitochondrion (arrow) is observed to appear and disappear. Note that sections 3, 5, 7, and 9 are omitted from the sequence. (× 10,000)

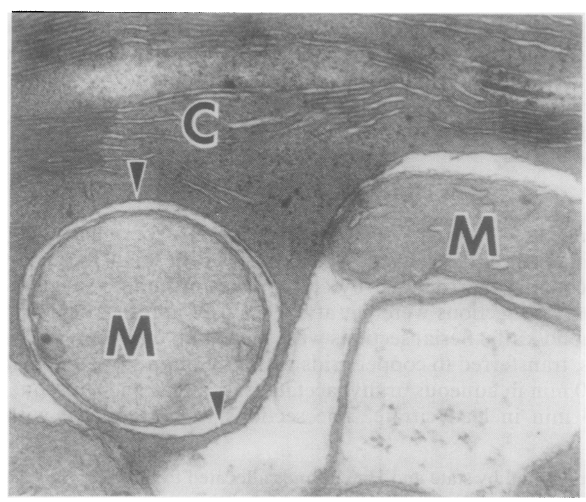

FIG. 3. Electron micrograph of a mitochondrion (M) surrounded by chloroplast tissue. Note single chloroplast membrane surrounding mitochondrial profile (arrow). (× 34,500)

Whether the enclosure of mitochondria (and other organelles; Ref. 2) by chloroplasts is a feature of leaf senescence is not known, but leaves used in this study and a previous one (2) were the youngest fully expanded leaves on elongating stems and showed no external signs of aging. In Doohan's work with *P. milioides* and *Panicum hians* Ell. (4) where mitochondrial profiles were observed to be surrounded by chloroplasts, young expanding leaves were used. Thus, for species of the *Laxa* group, a close association exists between chloroplasts and mitochondria of vascular bundle sheath cells, including complete enclosure of some mitochondria.

Whether this feature is related to the low photorespiratory loss of CO_2 in some species of the *Laxa* group is not known, but it also occurs in *P. laxum* and *P. hylaeicum* (2) which are characterized as C_3 species (8, 9). Further studies of this association between organelles should be carried out to determine its biochemical and physiological significance in leaf metabolism, especially photosynthesis.

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