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Changes in Chloroplast Structure During Differentiation of Phloem Sieve Elements and Xylem Vessels from Green Callus Tissue of *Streptanthus Tortuosus*

Richard D. Sjölund
Dept. of Botany, University of Iowa, Iowa City, Iowa 52242, U.S.A.

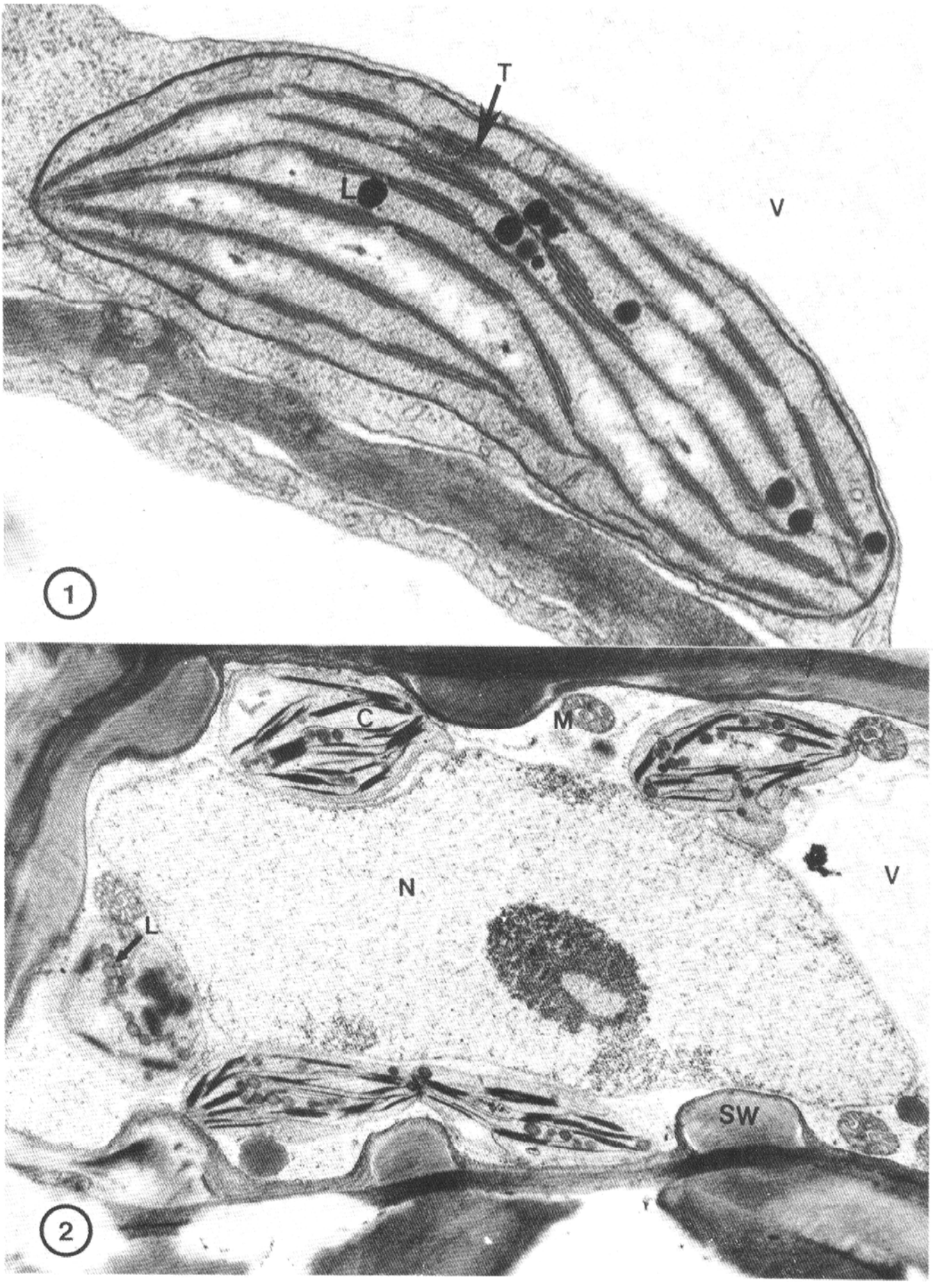
ABSTRACT

When grown in the light, the callus parenchyma cells of ***Streptanthus tortuosus*** (Brassicaceae) contain green chloroplasts with thylakoid membranes organized into grana stacks. These callus parenchyma cells can differentiate into either xylem elements or phloem elements in tissue culture media containing an auxin and a cytokinin. When the green cells become xylem vessel elements, they retain their chloroplasts and grana thylakoids until the time of cell death. When the same green cells develop into phloem, however, only companion cells retain their chloroplasts and grana thylakoids. Chloroplasts in the developing phloem sieve elements lose their thylakoid membranes and synthesize a unique form of starch. The conversion of a chloroplast into a sieve element plastid occurs early in phloem development; the plastids persist in the mature sieve elements after development.

INTRODUCTION

The development of plastids into etioplasts, chloroplasts, amyloplasts or chromoplasts is regulated by light, the role of which has been investigated extensively [1]. In addition to factors in the external environment, however, plastid development is also influenced by the developmental state of the specific cell in which the plastid is located. Plastids in light-grown leaves, for example, develop into chloroplasts in mesophyll cells but remain as pro-plastids in epidermal cells, except for guard cells which also become green. Green chloroplasts of many fruits are converted into chromoplasts during stages of fruit ripening when chlorophyll is degraded and replaced with carotenoids. Because the plastid contains its own DNA and ribosomes, the differentation of plastids represents a complex interaction between the nuclear genome, the cytoplasmic ribosomes and the plastid itself. Specific proteins associated with chromoplasts are synthesized during chloroplast to chromoplast conversions, for example, but the site of translation of these proteins and whether their transcription is from nuclear or plastid DNA remain to be established [3].

If the developmental state of a chloroplast is a function of the differentiation of the cell in which it resides, then it should be possible to influence plastid development by re-programing cells and altering their developmental states. Behnke and Schultz [5] showed that such a re-programming of plastids does occur when amyloplast-containing cells of stem parenchyma cells are re-programmed to develop as phloem sieve elements following wounding. In this case the amyloplasts are converted into the specific type of plastid found only in sieve elements. In contrast, however, the re-programming of chloroplasts does not seem to occur when green mesophyll cells of ***Zinnia*** are isolated and induced to develop as xylem ele-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ments. The chloroplasts present in the original mesophyll cells appear to remain largely intact during xylogenesis, finally breaking down at the time of cell death [6, 7] .

In this paper I will examine the re-programming of green, chloroplast-containing callus parenchyma cells to develop as either xylem or phloem elements using plant tissue culture techniques in order to observe the effect that this re-programming has on chloroplast development.

MATERIALS AND METHODS

Callus tissue of *Streptanthus tortuosus* (Brassicaceae) originated from cotyledons was maintained on a plant tissue culture medium containing 0.1 to 0.5 mg/L napthalene acetic acid (NAA) and 1.0 mg/L kinetin. The callus tissue was grown under 1100 ft-c of fluorescent illumination at 22ºC and was transferred to fresh medium at 3 week intervals. The growth of the callus and the fixation and processing for electron microscopy followed procedures described earlier [11] .

RESULTS

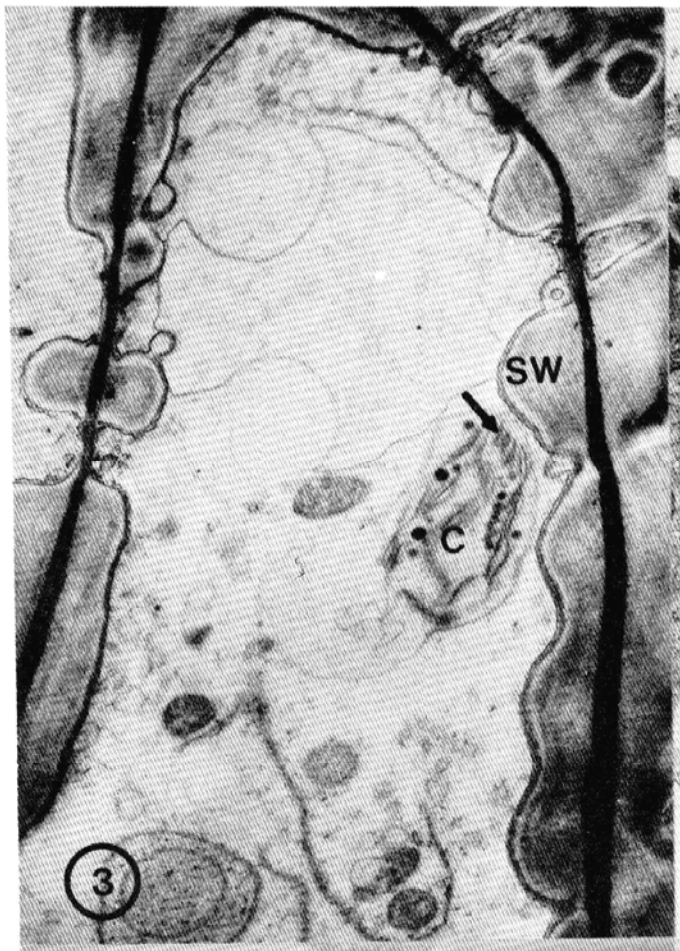
The *Streptanthus tortuosus* (Brassicaceae) callus used in this study has been used previously to investigate plastid development [12]. The photosynthetic activities of these cultures have been investigated and their carbon fixation has been characterized as the C-3 type [9, 13] . A chloroplast from a callus parenchyma cell is shown in Fig. 1. The thylakoid (T) membranes are organized into small grana (arrow) and a few lipid droplets (L) can be seen. The parenchyma cell has a large central vacuole (V).

Parenchyma cells such as that seen in Fig. 1 spontaneously differentiate into xylem vessels on media containing NAA and kinetin. A cell that contains green chloroplasts is shown in an early stage of xylogenesis in Fig. 2. The secondary cell wall (SW) characteristic of xylem vessels is being synthesized in this cell, but the nucleus (N) and the central vacuole (V) are still present. It is clear that the conversion of the parenchyma cell into a xylem element has not resulted in the loss of grana in the chloroplasts (C) of this cell although there does appear to be an increase in the amount of lipid droplets (L) present. Mitochondria also are present at this stage of xylogenesis and appear to be unchanged. A later stage of xylogenesis is shown in Fig. 3. The cytoplasmic contents of this cell are disrupted; the vacuole is absent and only remnants of the tonoplast can be seen. The secondary cell wall (SW) is fully developed and the primary cell wall appears as a dark band arround the cell. A chloroplast is visible in this cell and although it is also degenerating, thylakoid membranes organized into grana can still be seen (arrow).

The differentiation of phloem cells in the *S. tortuosus* callus is seen in Fig. 4. A companion cell (cc) and a sieve element (SE) are connected by a cell wall that contains a large plasmodesmata. The companion cell is vacuolated and the sieve element has lost its nucleus and vacuole. A chloroplast present in the companion cell contains thylakoid membranes which are organized into grana stacks but the chloroplasts in the sieve element have been converted into sieve element plastids that contain starch but lack thylakoids or grana membranes. An intermediate stage in the conversion of a chloroplast from a callus parenchyma cells into a sieve element plastid in a phloem cell is shown at a higher magnification in Fig. 5. Remnants of thylakoid membranes (T) can still be seen at this stage and a small grana stack also remains. The specific form of starch characteristic of sieve element plastids in the Brassicaceae is seen to be developing in this plastid. The starch grains are electron dense and have small

Fig. 1. A chloroplast in a callus parenchyma cell from a light-grown callus culture. The thylakoid membranes (T) are organized into grana stacks (arrow). Several small lipid droplets (L) are visible. The parenchyma cell contains a large central vacuole (V). X61,000.

Fig. 2. A differentiating xylem vessel element from a light-grown callus culture. Note that the deposition of secondary wall material (SW) is well advanced but that the cell still contains a nucleus (N), mitochondria (M) and large vacuole (V). The chloroplasts (C) have retained their thylakoid membranes which are still stacked into grana. Several lipid droplets (L) are present in each chloroplast. X12,000.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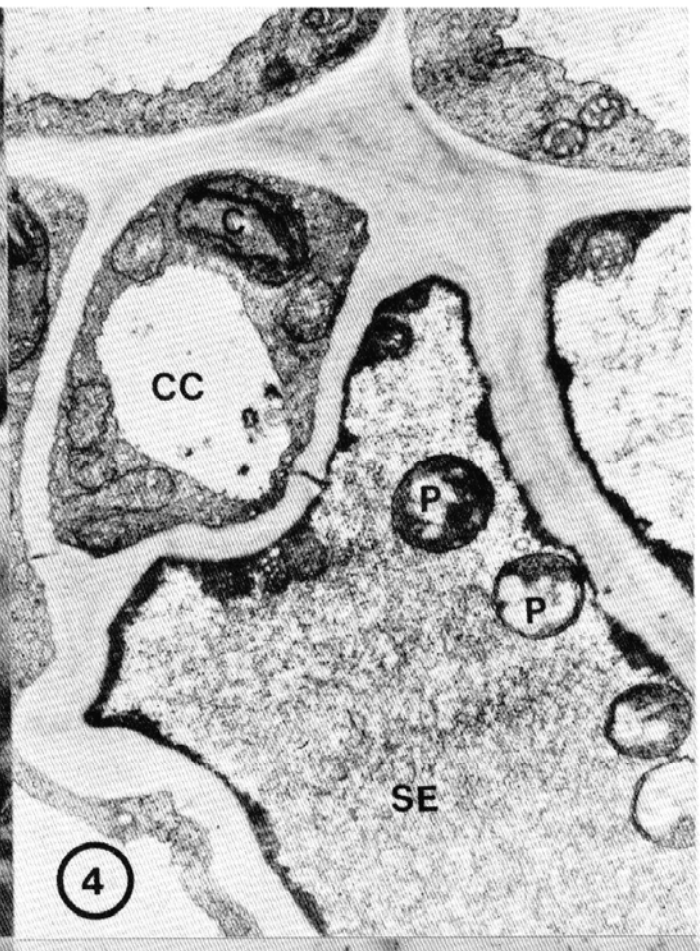
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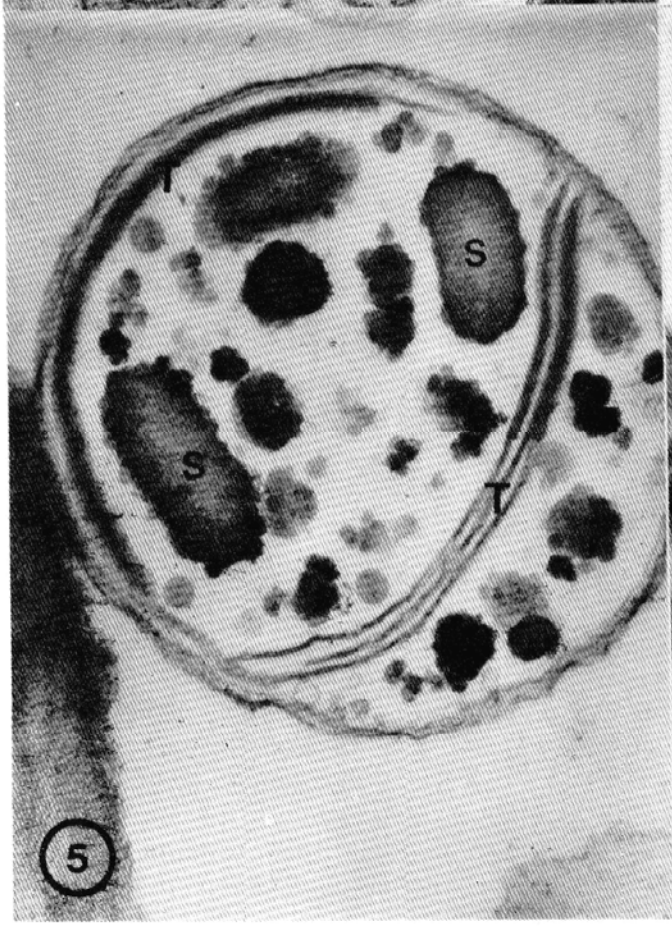
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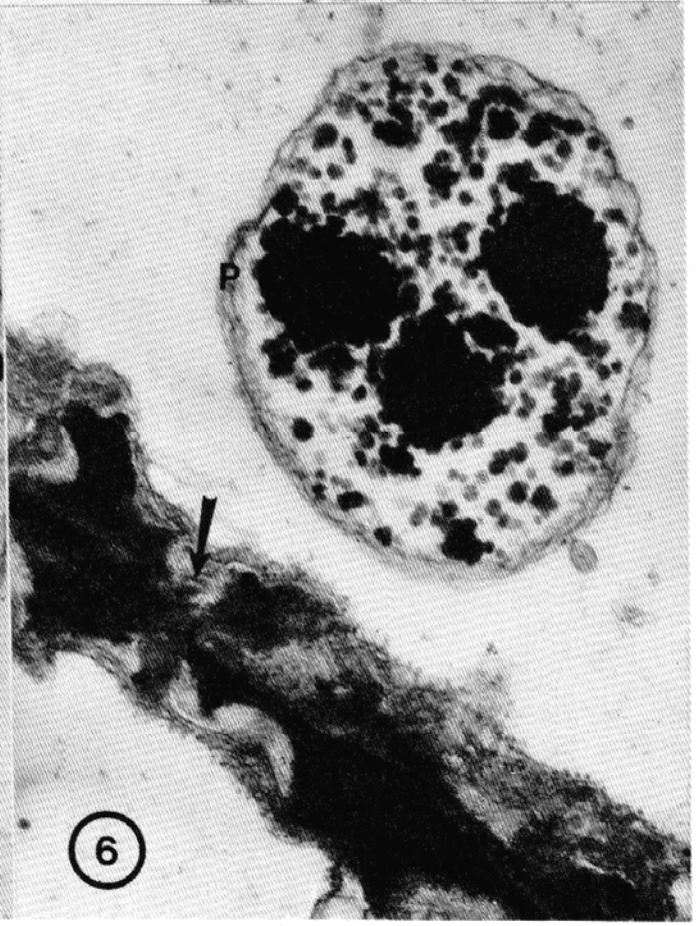
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peripheral particles associated with the large grains and scattered through the plastid. The final stage of plastid re-differentiation found in a mature sieve element is shown in Fig. 6. This sieve element has a mature sieve pore (arrow) connecting it to the next sieve element and the plastid contains no thylakoid membranes or grana. The starch present in this mature sieve element plastid is organized into large grains and many small particles.

DISCUSSION

The results of this study indicate that mature chloroplasts present in callus parenchyma cells of light-grown *S. tortuosus* tissue cultures are re-programmed to form sieve element plastids when the parenchyma cells differentiate into phloem, but that they remain as chloroplasts when the same parenchyma cells differentiate as xylem vessels. The only changes seen in chloroplast structure during xylogenesis appear to those associated with cell death and the be breakdown of cytoplasmic organelles. This is similar to the results seen in the experimental induction of xylogenesis from green leaf mesophyll cells of *Zinnia* by Burgess and Linstead [6] and Fukuda and Komamine [7]. The *Zinnia* mesophyll cells retained their chloroplasts which contained grana thylakoids during early stages of xylogenesis and secondary cell wall deposition, suggesting that the signal to develop as a xylem element did not specifically target plastids for involvement.

In the case of phloem differentiation from green callus cells of *S. tortuosus*, however, a very specific involvement of the plastids in the re-differentiation process is evident. Unlike the development of a xylem vessel from a callus parenchyma cell, the development of phloem elements involves one or more rounds of cell division. The final division results in the formation of a companion cell and a sieve element; the companion cell retains green chloroplasts but the sieve element has plastids that selectively decompose their thylakoid membranes, lose all traces of grana organization, and then synthesize a unique form of starch. The conversion of chloroplasts into sieve element plastids seen here is similar to the conversion of amyloplasts in stem parenchyma cells into sieve element plastids studied during the formation of wound phloem by Behnke and Schultz [5]. Their study showed that the original form of starch that was present in the amyloplasts was degraded and replaced with sieve element starch. Thykaloid membranes present in the amyloplasts were also degraded during the conversion. In the case of *S. tortuosus* callus tissue, no starch is present in the chloroplasts of the callus parenchyma cells, but sieve element starch is synthesized when the cells become sieve elements. Grana thylakoid membranes are degraded, but the inner and outer membranes of the plastid envelope are retained.

The significance or function of sieve element starch is unknown, but the type of plastid found in sieve elements is highly characteristic of individual plant groups and has been used as a taxonomic character (Behnke, 1981). The presence of starch-free, grana-containing chloroplasts in companion cells and sieve element plastids with specific forms of starch but no thykaloids is significant, especially since the two cells are the products of a common cell division. The formation of starch in the sieve element but not in the compa-

Fig. 3: A xylem vessel element near the end of development. The secondary cell wall (SW) is fully formed; the primary cell wall has become electron dense, possibly due to lignification. At this late stage of development the cytoplasmic contents of the cell are degenerating. A chloroplast present in the cell still has thylakoid membranes and grana stacks (arrow). X8,500.

Fig. 4. A developing phloem sieve element (SE) with and associated companion cell (cc) separated by a cell wall with a large plasmodesmata connection. The companion cell has a central vacuole and a chloroplast (C) with thylakoids organized into grana stacks. The adjacent sieve element lacks chloroplasts but has rounded, starch-filled sieve element plastids that lack thylakoids or grana. X11,000.

Fig. 5. A single chloroplast undergoing conversion into a sieve element plastid. Note that a few remnants of thylakoid membranes and grana stacks (T) are visible and that the characteristic sieve element starch (S) is being synthesized. X59,000.

Fig. 6. A mature sieve element showing a sieve pore (arrow) connection to an adjacent sieve element. The pore is lined with callose (light; area at arrow). A sieve element plastid (P) is seen to lack thylakoid membranes or grana; the sieve element starch is electron dense and particulate. X 43,000.

nion cell is probably not the result of the presence of high levels of sugars in the sieve element, because the level of solutes also is high in the companion cell as well [10] . The companion cell and the sieve element remain in cytoplasmic continuity and are connected by enlarged plasmodesmata, at least in members of the Brassicaceae and other plants belonging to the closed type of minor veins [8] where companion cells often contain large vacuoles and chloroplasts with grana thylakoids. The signal to the callus parenchyma cell to differentiate as a sieve element also contains a signal to the chloroplast to differentiate as a specialized sieve element plastid. It seems likely that a phloem-specific enzyme or enzymes is associated with the formation of the unique type of starch found only in sieve elements, but whether the genetic information for that enzyme is located in the nuclear or plastid genome remains to be determined. Since the sieve element loses its nucleus and its ribosomes early in differentiation, the conversion of a chloroplast into a sieve element plastid must take place early in the cell differentiation process.

The formation of sieve element starch in *S. tortuosus* callus tissue is so specific that we routinely use the presence of starch in a cell as a marker for phloem development. Squash preparations of callus tissue examined under the light microscope reveal starch in the sieve elements, especially when the cells are crushed and the plastids are broken. The presence of starch grains in these phloem cells is a common feature of sieve elements and has been noted in previous microscopic studies of phloem [2].

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