

Two Immunological Approaches to the Detection of Ribulose-1,5-Bisphosphate Carboxylase in Guard Cell Chloroplasts

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ABSTRACT

Two immunological approaches were used to determine if ribulose biphosphate carboxylase oxygenase (RuBisCo) is present in guard cell chloroplasts. Immunocytochemistry on thin plastic sections using tissue samples that were processed using traditional glutaraldehyde/osmium fixation and then restored to antigenicity with metaperiodate treatment, resulted in labeling over wild-type mesophyll and guard cell plastids of several green and white variegated *Pelargonium* chimeras. The density of immunogold labeling in guard cell chloroplasts was only about one-seventh of that noted in mesophyll chloroplasts on a square micron basis. Because guard cell chloroplasts are much smaller than mesophyll chloroplasts, and occur at lower quantities/cell, the relative differences in RuBisCo concentration between the cell types indicate that guard cells have only 0.48% of the RuBisCo of mesophyll cells. No reaction was noted over 70S ribosomeless plastids of these chimeras even though adjacent green chloroplasts were heavily stained, indicating the high specificity of the reaction for RuBisCo. Spurr's resin gave the most successful colloidal gold labeling in terms of low background staining and structural detail but L. R. White's resin appeared to be superior for antigen retention. In the white leaf edges of the white and green *Pelargonium* chimeras, the only green, functional chloroplasts are in the guard cells. When either whole tissue or plastid enriched extracts from this white tissue were electrophoresed, blotted, and probed with anti-RuBisCo a large subunit band was detected, identical to that in the green tissue. These data indicate that a low, but detectable, level of RuBisCo is present in guard cell chloroplasts.

The presence of RuBisCo¹ or any of the other enzymes in the photosynthetic carbon reduction pathway in guard cell chloroplasts has been a matter of considerable debate among stomatal physiologists (6–8). For example, although Outlaw *et al.* (7) did not observe any immunologically detectable RuBisCo in purified guard cell preparations of *Vicia faba* using rocket immunoelectrophoresis, Zemel and Gepstein (17) detected RuBisCo in this species using both immunofluorescence microscopy and in Western blots. Madhavan and Smith (6) detected RuBisCo in some species of plants by immunofluorescence but not in all. The reports of the absence of RuBisCo in at least some guard cell chloroplasts (6–8) are paradoxical in that both photosystem I and II activity are present in guard cells (12, 15). Thus, the reduction potential for CO₂ fixation does exist. Various other cellular functions for this photosynthetically-generated reduction poten-

tial other than CO₂ reduction have been proposed, such as the active ion transport required for guard cell movement.

In this report, two new approaches to determine if RuBisCo is present in guard cell chloroplasts are utilized. Electron microscopic cytochemistry on thin sections using colloidal gold (1) has the advantage over immunofluorescence microscopy in that the high endogenous fluorescence of the chloroplasts (14) does not have to be masked and the problems associated with penetration of the immunocytochemical reagents (14) are eliminated. This approach allows unambiguous detection of the protein as well as allowing for a high degree of structural clarity. In addition, a semiquantitative measure of protein levels may be obtained by morphometric analysis of the density of the colloidal gold labeling (1). As an internal control, some *Pelargonium* chimeras that have genetically wild-type chloroplasts in the epidermis and some of the mesophyll tissue in the center of the leaf, but nonfunctional 70S ribosomeless plastids (and lacking RuBisCo) in the mesophyll tissue adjacent to the epidermis and throughout the leaf edge (2) were used in the immunocytochemistry experiments. With this tissue, biochemical analysis (electrophoresis and Western blotting) can be performed on the white tissues at the edge of the leaf without the danger of contaminating functional (and RuBisCo-containing) mesophyll chloroplasts. With these two immunological methods applied to the same plant tissue, it is possible to demonstrate the presence of RuBisCo in guard cell plastids. However, the immunodetection indicates about one-seventh of the labeling of the mesophyll cell plastids and, considering the small size of guard cell chloroplasts, it is unlikely that they contribute significantly to carbon fixation.

MATERIALS AND METHODS

Plant Material. Two *Pelargonium* × *hortorum* green and white chimeras cv 'Mrs. Parker' and cv 'Flower of Spring (which lack 70S ribosomes in the white plastids) and the yellow and green chimera cv 'Mrs. Pollock' were maintained in growth chambers at 300 μmol m⁻² s⁻¹ PAR at 22°C.

Microscopy. Small leaf pieces (1 mm²) were fixed in 3% (v/v) glutaraldehyde in 0.10 M cacodylate buffer (pH 7.2) for 2 h at 4°C, washed in two changes of cacodylate buffer (15 min, each), and postfixed for 2 h in 2% (w/v) OsO₄ in cacodylate buffer. The specimens were dehydrated through ethanol for L. R. White's resin or acetone for Spurr's resin. Thin sections (pale gold-silver reflectance) of the specimens were cut with DuPont diamond knife with a Reichert Ultracut² ultramicrotome and were mounted on gold or nickel grids for immunocytochemistry and copper grids for traditional electron microscopy.

¹ Abbreviations: RuBisCo, ribulose biphosphate carboxylase/oxygenase; PBS, 50 mM sodium phosphate (pH 7.2) with 0.85 (w/v)% sodium chloride.

² Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

To restore antigenicity, grids were floated, specimen side down, on drops of 12% (w/v) sodium *m*-periodate for 1 h. The grids were washed with water from a spray bottle, blotted with filter paper, and floated on 1% (w/v) BSA in 50 mM sodium phosphate (pH 7.4) with 0.85% sodium chloride (= BSA-PBS) for 30 min to block nonspecific antibody sticking. The grids were then transferred to a drop of anti-RuBisCo (5) diluted 1:10 (v/v) in BSA-PBS for 60 min. (A preliminary study, using various primary antisera dilutions from 1:5 to 1:200 [v/v] indicated that the 1:10 [v/v] dilution was the highest antisera concentration that gave consistent immunolabeling without nonspecific background sticking. Colloidal gold labeling increased linearly with primary antisera dilutions from 1:5 to 1:50.) After transferring the grids through four drops of BSA-PBS (to remove nonspecifically bound antibody), the grids were floated on a 1:20 (v/v) dilution of protein A-colloidal gold (15 nm gold, EY Laboratories, San Mateo, CA) in BSA-PBS for 30 min. Removal of nonspecifically bound gold was achieved by transferring the grids through four drops of PBS and finally washing with H₂O from a squirt bottle. The grids were post-stained 15 min each in 2% (w/v) uranyl acetate and Reynold's lead citrate. Controls included deletion of the primary antiserum, use of preimmune sera, and use of antisera that had been preadsorbed by reaction with leaf protein immobilized to nitrocellulose. Standard electron microscopy was carried out on serial sections of the blocks used for immunocytochemistry and were stained with uranyl acetate and lead citrate as for the immunocytochemical experiments. Specimens were observed with a Zeiss EM10CR electron microscope operating at an accelerating voltage of 60 kV.

Fluorescence micrographs were obtained with a Zeiss Universal microscope in the epifluorescence mode. Photographs were taken at 3200 and the negatives developed in Diafine developer.

Specificity of the Antisera and Immunoreactions. (a) Reaction of the antitobacco RuBisCo sera (5) with extracts of *Pelargonium* or *Nicotiana tabacum* resulted in a single precipitin line and the *Nicotiana* and *Pelargonium* extracts fused completely, indicating immunological identity. Use of the same sera on Western blots of Laemmli gels recognize a single protein band at 55 kD (see Fig. 7 for an example in this report), the large subunit of RuBisCo. Antisera raised to the holoenzyme generally react only with the large subunit (3). (b) Immunostaining of the C₄ plants *Sorghum halapense* and *Eleusine indica* revealed no staining in the mesophyll chloroplasts although the bundle sheath and guard cell chloroplasts were labeled in each species. (c) Use of two other anti-RuBisCo sera (anti-spinach RuBisCo obtained from Manzanita Immunochemicals, Oracle, AZ and anti-soybean RuBisCo obtained from Dr. R. Wells, Stoneville, MS) resulted in similar staining both using the colloidal gold grid staining and Western blotting. Thus, it is unlikely that the guard cell reactions involved an anomalous behavior of the primary serum.

Morphometric Analysis. Sections through the green central area of the leaf tissue of the two green and white chimeras contain functional chloroplasts, mutant 70S ribosomeless chloroplasts, and functional green guard cell chloroplasts. Thin sections from this region were used to make the colloidal gold density comparisons to minimize grid to grid staining differences. The area of each chloroplast profile was determined by making prints at 2 times the magnification of the negatives, cutting the chloroplast from the print, and then weighing the print piece. A piece of print paper corresponding to 1 μm^2 was weighed to determine the area of the particular chloroplast profile and the gold particles on each profile were counted manually; 121 mesophyll chloroplasts, 86 guard cell chloroplasts, and 53 white mutant plastids were examined. Yellow plastids (56 total) from cv 'Mrs. Pollock' were from separate leaf sections although green mesophyll plastid staining in these chimeras was comparable to those in cvs 'Flower of Spring' and 'Mrs Parker.'

Electrophoresis and Western Blotting. Three g of tissue were ground with a chilled mortar and pestle in 20 ml of 4°C grinding

media (9). An equal weight of insoluble PVPP was added to prevent reactions of polyphenols. The brei was filtered through Miracloth into chilled centrifuge tubes and centrifuged at 150g for 5 min to remove cells and starch and at 6,000g for 15 min to isolate plastids. These plastid-enriched pellets were then solubilized in stacking gel buffer (4) supplemented with 5% (w/v) sucrose, 10% (v/v) glycerol, 0.1% (v/v) β -mercaptoethanol, and 6% (w/v) lithium dodecyl sulfate. White and green leaf tissue was also ground directly in stacking gel buffer (4) supplemented with an equal weight volume of PVPP. The brei was passed through Miracloth into chilled centrifuge tubes and centrifuged at 13,000g for 15 min. An aliquot of this material was mixed with an equal aliquot of the chloroplast solubilization media. Both the solubilized chloroplast and the soluble leaf protein extracts were centrifuged at 20,000g after solubilization to clarify the extract. A 20 μl aliquot of each was applied to 7.5% (w/v) acrylamide gels prepared according to Laemmli (4).

Passive capillary blotting and other procedures were as described previously (11). The same anti-RuBisCo serum used for immunostaining (description in Ref. 5) was also used on Western blots.

RESULTS AND DISCUSSION

Three methods were used to demonstrate that the guard cells of the variegated *Pelargonium* chimeras contain functional chloroplasts. Electron microscopic observation of the guard cells of these chimeras reveal normal appearing guard cell chloroplasts with small grana stacks, peripheral reticulum, and prominent starch grains (Fig. 1A). These are structures typical of guard cell chloroplasts from many species (*e.g.* 12). In white sectors of leaves observed with fluorescence microscopy, the guard cells are the only cell type which contain red-fluorescing chloroplasts (Fig. 1B). Third, occasional periclinal divisions of the epidermis (so-called displacements, Ref. 10) give rise to normal green sectors (Fig. 1C) indicating that the epidermal cell layer is genetically wild type. Thus, by these three criteria, the guard cells of these green and white chimeras can be assumed to represent normal guard cell chloroplasts.

Immunogold labeling of chloroplasts of tissue sections that had been processed using traditional glutaraldehyde-osmium fixation procedures but restored to antigenicity with *m*-periodate offered a high level of subcellular resolution as well as a high level of labeling. Clear resolution of all details of chloroplast ultrastructure was retained, although the *m*-periodate treatment did render the membranes less well defined (Figs. 2–6). Immunogold labeling of green wild-type mesophyll chloroplasts of *Pelargonium* was heavy and restricted to areas of the stroma (Fig. 2). Very heavy labeling occurred in detached areas of stroma lamellae near the chloroplast envelope and around peripheral reticula (Fig. 3, A and B). No label was found when no primary antisera were used (not shown), nonimmune sera (not shown), or antisera preadsorbed with leaf extracts containing RuBisCo (Fig. 3C) were used in place of the anti-RuBisCo sera. Likewise, no reactions were noted in the mutant white, 70S ribosomeless plastids of the second histogen of 'Mrs. Parker' or 'Flower of Spring' (Fig. 4) even though yellow, nonfunctional mutant plastids (that are P700 Chl *a* protein-deficient and contain plastid ribosomes) are heavily gold labeled (Fig. 5A). Guard cell chloroplasts are also labeled with colloidal gold (Fig. 6, A and B), even in this white tissue in which the white mesophyll plastids are not (Fig. 4). Besides the labeling found in the guard cells of these *Pelargonium* chimeras, guard cells of *Eleusine indica*, *Nicotiana tabacum*, *Spinacia oleracea*, and *Sorghum halapense* were also labeled using these procedures and antisera (not shown).

Although colloidal gold labeling can give only relative rather

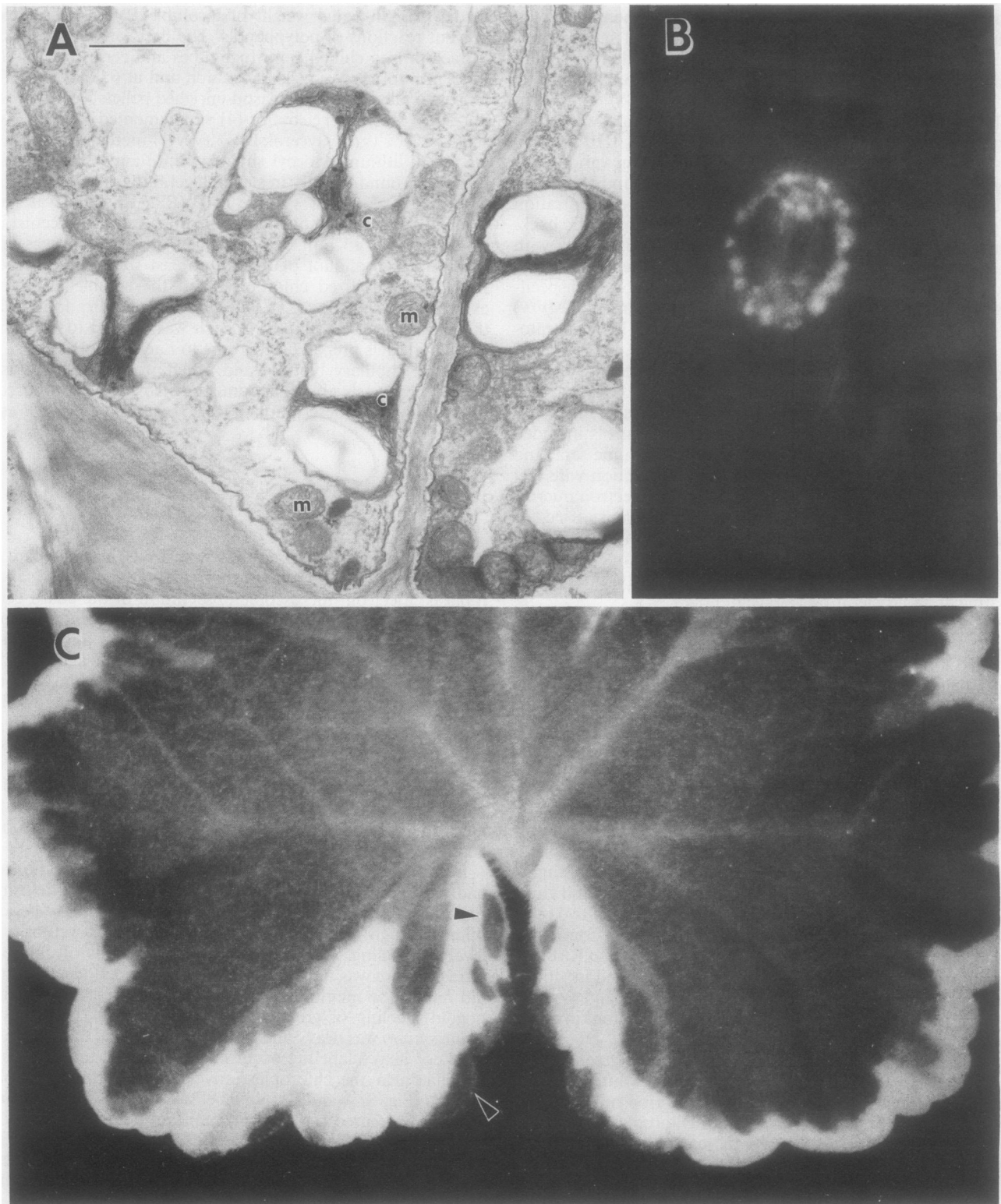


FIG. 1. Three demonstrations of the presence of normal chloroplasts in the guard cells of green-and-white variegated *Pelargonium* chimeras. A, Transmission electron micrograph of guard cells show normal guard cell chloroplasts (c) with starch grains and small grana stacks. m, Mitochondrion; bar = 5.0 μm . B, Fluorescence microscopy of leaf sections through white areas of the variegated leaves reveals strongly red fluorescent chloroplasts. $\times 800$. C, Leaf of *Pelargonium* \times *hortorum* cv 'Flower of Spring' with several green displacements events in which tissue from the first histogen has divided periclinally to produce small wedges of green tissue (arrows).

than absolute quantitative data, the comparison of the green, white, and yellow mesophyll plastids with the green guard cell plastids indicates that, although the density of labeling is less than the ribosome-containing mesophyll plastids, a large amount

of specific labeling (about one-seventh the density of green mesophyll chloroplasts) is associated with the guard cells (Table I). The size of guard cell chloroplasts (mean length about 3.5 μm versus about 6.5 μm for mesophyll chloroplasts and mean width

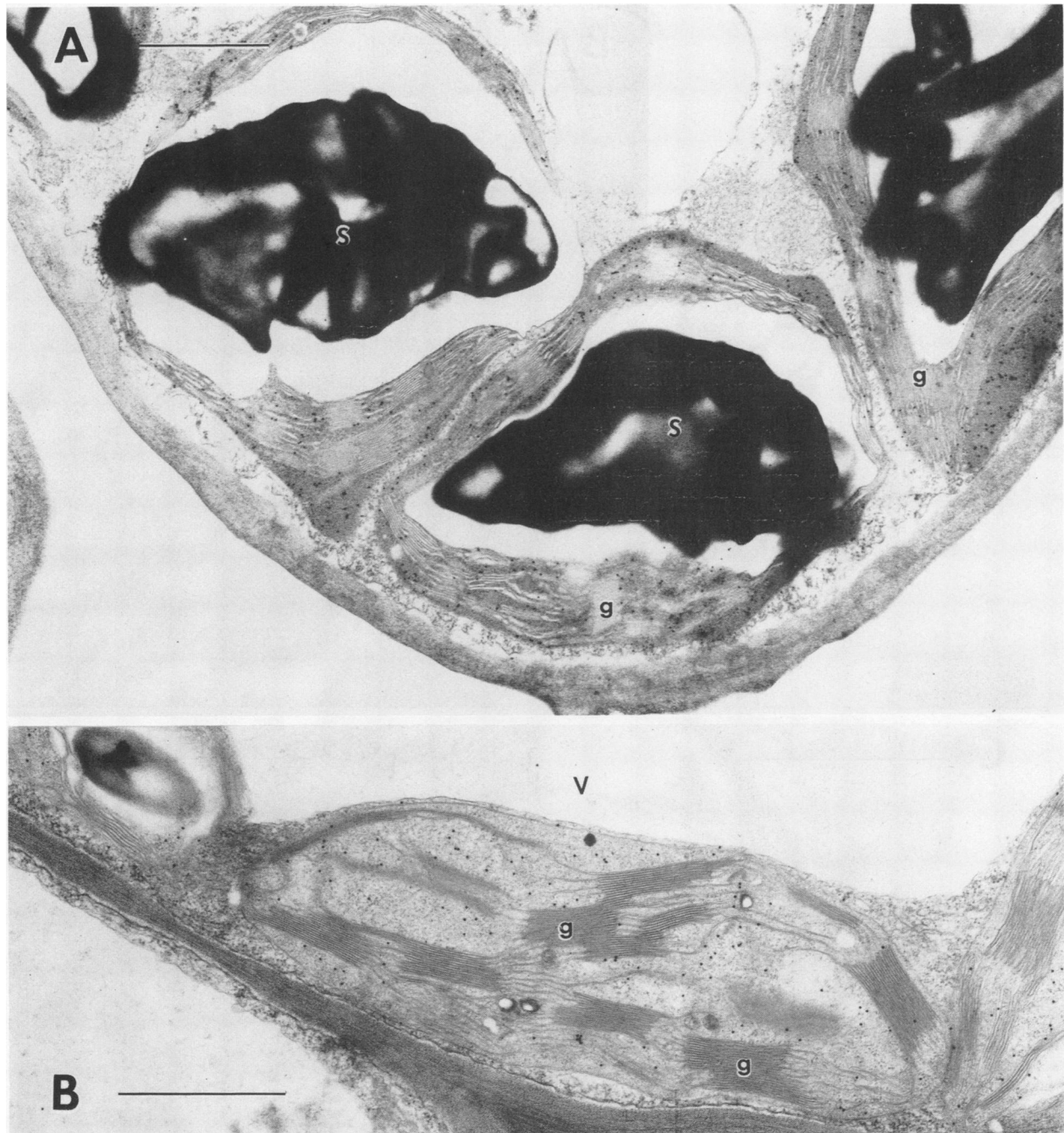


FIG. 2. Immunocytochemical detection of RuBisCo in mesophyll chloroplasts of *Pelargonium × hortorum* embedded in Spurr's resin. A, Low magnification survey micrograph reveals immunolabeling throughout the stroma but absent from the stroma or grana (g) thylakoids, starch grains (s) or other cell components; B, higher magnification micrograph revealing strict stromal deposition of colloidal gold deposits. v, Vacuole; bar = 1.0 μm .

(1.0 μm versus 3.0 μm for mesophyll chloroplasts) and their relative abundance compared) to mesophyll chloroplasts (7.5/guard cell versus 20–60/mesophyll cell) makes the contribution of the guard cell RuBisCo relatively insignificant both in terms of the chloroplast and the whole leaf, i.e. $(5.52/34.72) \times (3.5/6.5) \times (1/3) (7.5/40) = 0.48\%$ of the mesophyll cell value. Considering the variability of the colloidal gold labeling alone, guard cells contain from 0.41 to 0.68% of the mesophyll cell RuBisCo. A similar low level of RuBisCo (up to 0.20%) has been noted in comparing the protein profiles of mesophyll and guard cell extracts (MC Tarcynski, WH Outlaw, unpublished data).

Tissue dehydrated in ethanol and embedded in L. R. White's

resin retains its antigenicity much better than tissue dehydrated in acetone and embedded in Spurr's resin. For example, the yellow chloroplasts of the *Pelargonium* mutant 'Mrs. Pollock' have about 10 times the labeling of the chloroplast than the tissue embedded in Spurr's resin from the same glutaraldehyde-osmium fixation (Table 1; Fig. 5). These yellow chloroplasts were chosen for this comparison because they have very little internal membranes so that the density of labeling is not affected by the occurrence of thylakoids or starch grains and thus, the labeling is very constant on a μm^2 basis from plastid to plastid. Despite this enhanced labeling in L. R. White's resin, there was also an enhancement of nonspecific adherence of the antisera to the

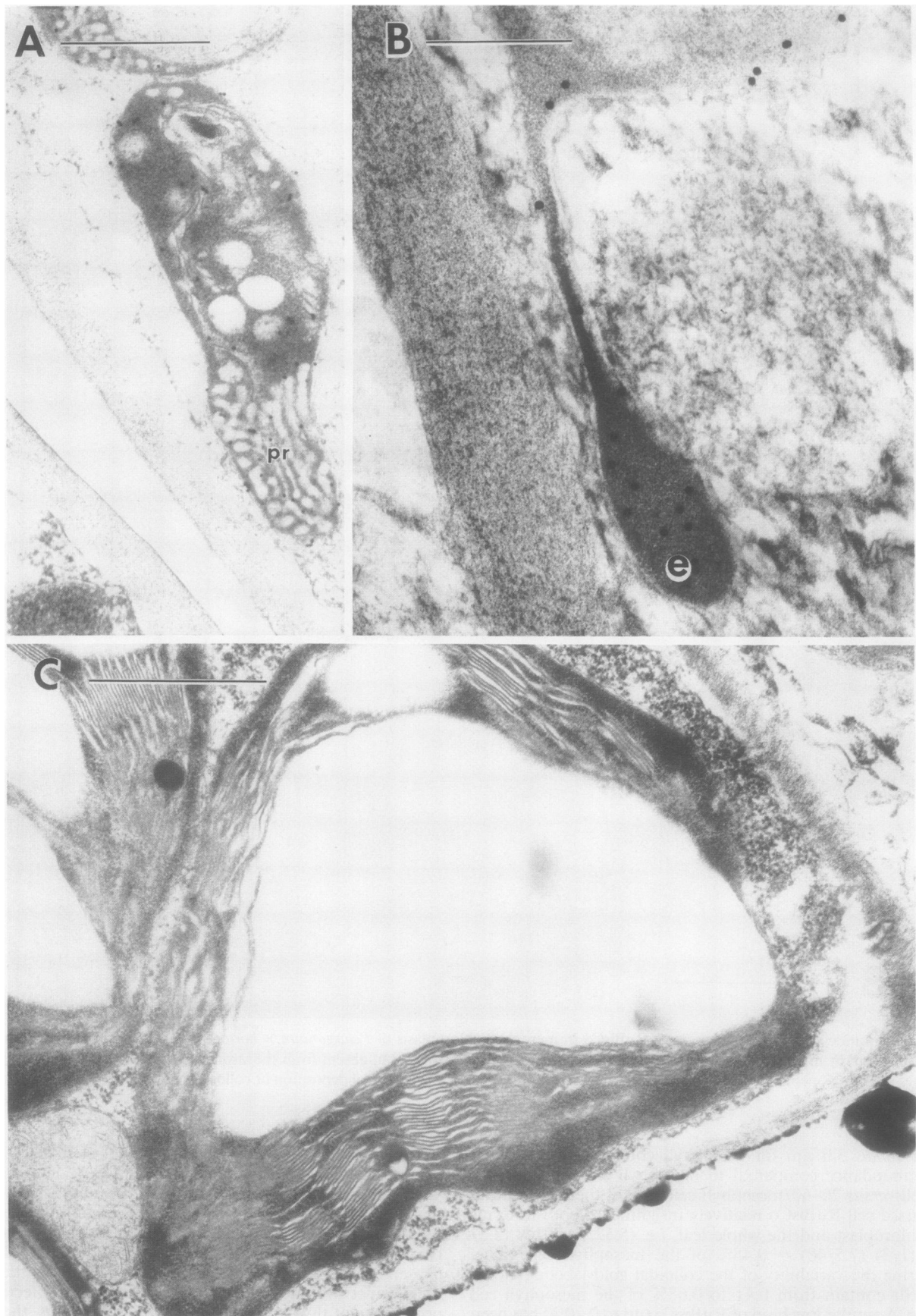


FIG. 3. Strong immunostaining of the peripheral reticulum (pr) (A) and extensions (e) of the chloroplast (B) with colloidal gold. By replacing the immune sera with sera that had been preadsorbed to leaf protein absorbed to nitrocellulose, no reactions are noted (C). Bar = 0.5 μm in A, 0.25 μm in B, 1.0 μm in C.

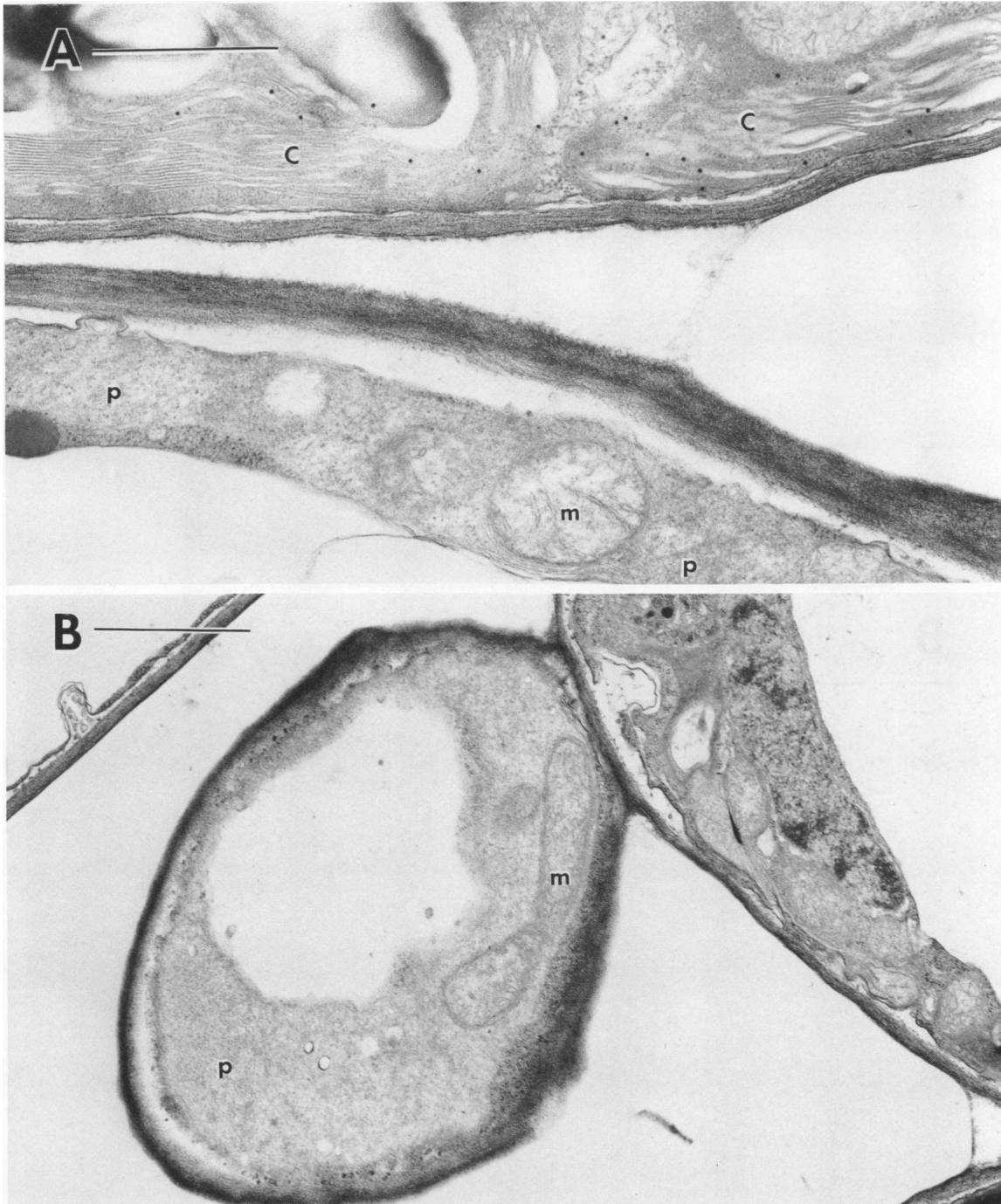


FIG. 4. Immunostaining for RuBisCo in white mutant chloroplasts of cultivar 'Mrs. Parker' (A) and 'Flower of Spring' (B). Note that wild-type chloroplasts (c) in Fig. 4A are colloidal gold stained even though adjacent mutant plastids (p) are unlabeled. m, Mitochondria; bar = 1.0 μm .

tissue section in this material (e.g. Fig. 5B). Because the presence of RuBisCo in guard cell chloroplasts is somewhat controvertible, we utilized Spurr's resin because of its very low background staining and high structural clarity. Detection of proteins that are less abundant (or more sensitive to the denaturing effects of acetone dehydration and embedding in Spurr's resin) may be more effectively immunolabeled in L. R. White's resin.

Western blots of extracts of both green leaves and white leaves or leaf areas (in which the only functional plastids are guard cell plastids) of the variegated *Pelargonium* 'Mrs. Parker' reacted sequentially with anti-RuBisCo, goat anti-rabbit labeled with peroxidase, and a peroxidase incubation media resulted in strong

immunostaining of the large subunit of RuBisCo (Fig. 7). Like other anti-RuBisCo sera prepared to the holoenzyme (3), this anti-tobacco RuBisCo (5) recognizes only the large subunit. Although Zemel and Gepstein (17) found that protease inhibitors were required to preserve guard cell RuBisCo, the relatively simple homogenization media used in this report preserved an apparently unaltered large subunit of RuBisCo. The differences may be due to the direct homogenization procedures used herein as compared with the relatively long guard cell protoplast isolation utilized by Zemel and Gepstein (17). Bands of comparable staining intensity were obtained when the green central area leaf extract was diluted 5-fold, as shown in Figure 7. The green tissue

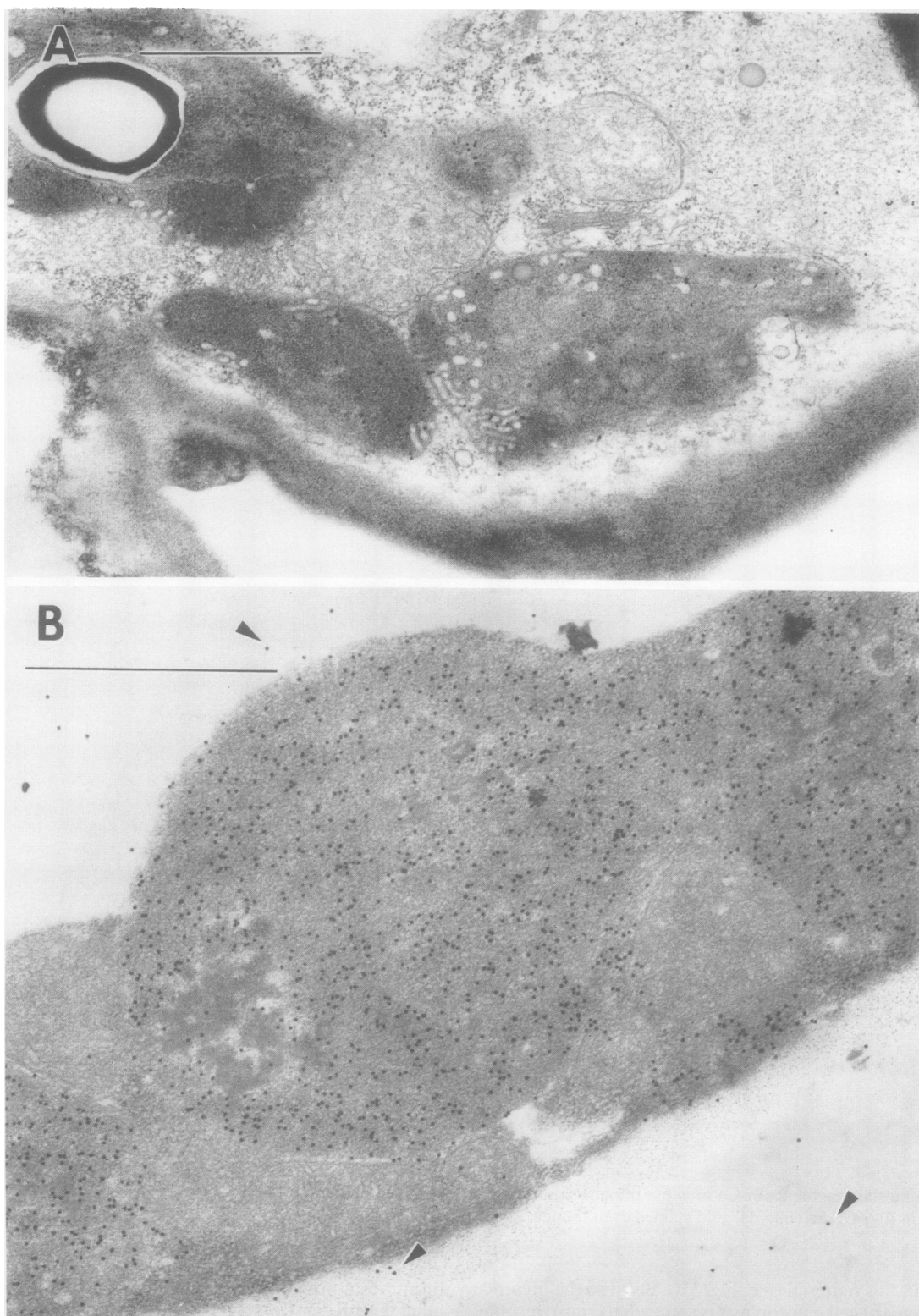


FIG. 5. Comparison of immunolabeling of yellow mutant plastids of *Pelargonium* cultivar 'Mrs. Pollock' after embedding in Spurr's (A) or L. R. White's (B) resin. Although more labeling is noted in the L. R. White's resin some background staining of structures other than chloroplasts is noted (arrows). Bar = 1.0 μm .

from the central area of the leaf is a combination of both white and green cells and thus is expected to be relatively less different from the guard cells than when comparing the immunogold labeling of green chloroplasts and guard cell chloroplasts (which showed a 7-fold difference).

This study presents two less equivocal approaches for the detection of RuBisCo in guard cell chloroplasts than some of the other approaches. Guard cell chloroplasts, like all chloroplasts, exhibit a high autofluorescence due to chlorophyll (*e.g.* Fig. 1B). Some other green autofluorescent compounds are also present,

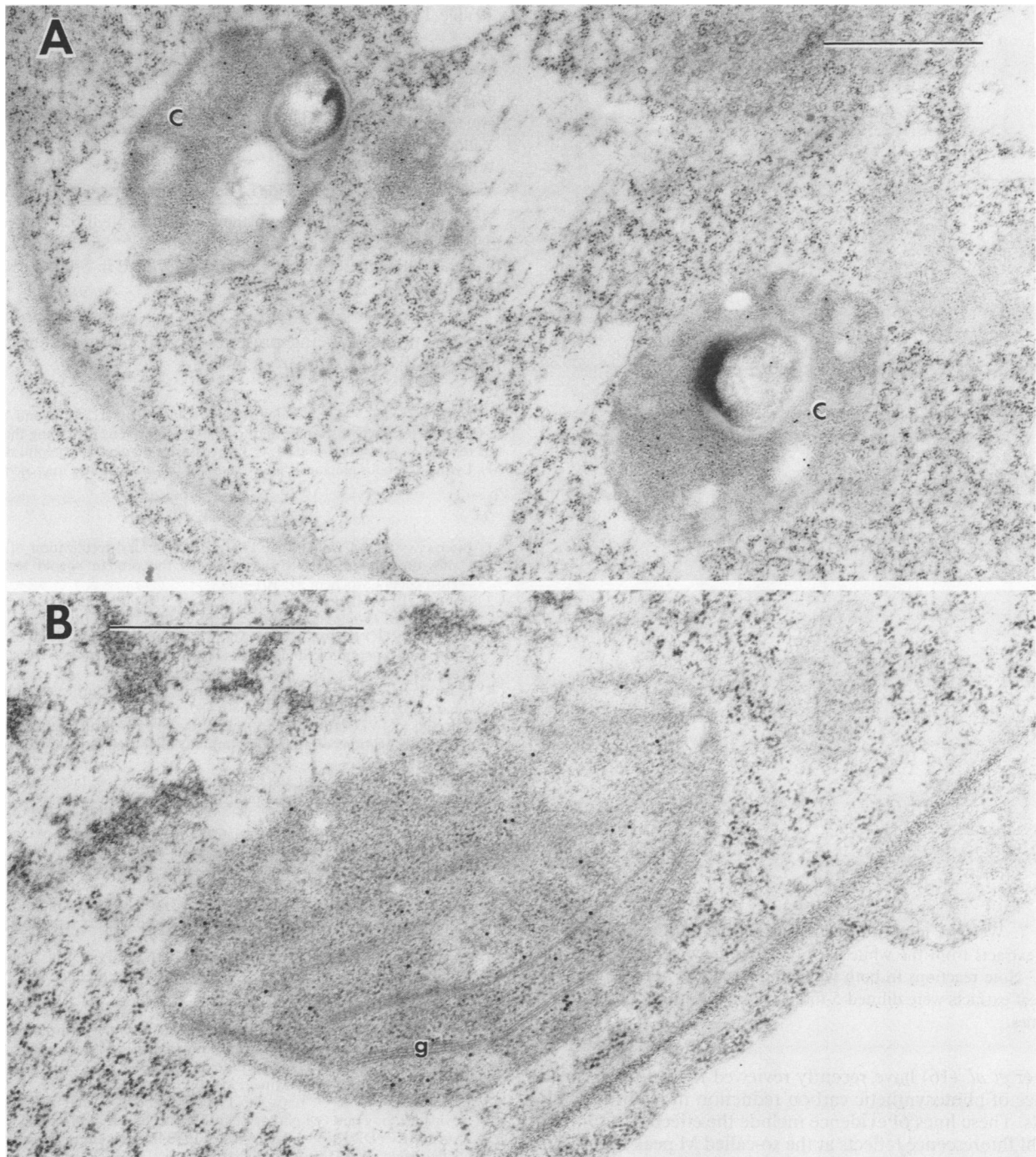


FIG. 6. Guard cells of variegated *Pelargonium* chiméras, immunogold staining for RuBisCo. A, Several chloroplasts (c) of cv 'Mrs. Parker;' B, guard cell chloroplast of cv 'Flower of Spring.' g, Grana; bar = 1.0 μ m.

probably in the vacuole (14). In addition, immunofluorescence procedures require cells to be permeabilized to allow penetration of the reagents into the guard cells (6); no problems of penetration are encountered with the grid staining procedures described herein. Both of these characteristics of guard cells make immunofluorescence a less desirable method of detection than the immunogold procedures used in this report. Moreover, this study allowed for important positive and negative controls on the same tissue slice; 70S ribosomeless plastids that lacked RuBisCo could be found in the same tissue section with positively stained green mesophyll and guard cell plastids (Fig. 4A). The use of chlorophyll chimeras to investigate guard cells was also exploited by

Zeiger *et al.* (15) in a study to determine if PSII was present in guard cell chloroplasts. By performing fluorescence measurements on tissue with albino plastids in the second histogen, the guard cells were the only cells that contained Chl, allowing unambiguous determinations of guard cell PSII activity. Unfortunately, that particular chimera is of no use in studies of RuBisCo as the white mutant plastids contain abundant chloroplast ribosomes and RuBisCo (13). However, the 70S ribosomeless *Pelargonium* chimeras lack all proteins encoded by chloroplast DNA (2), including the large subunit of RuBisCo, making these chimeras ideal material for investigation of the presence of other chloroplast-encoded proteins.

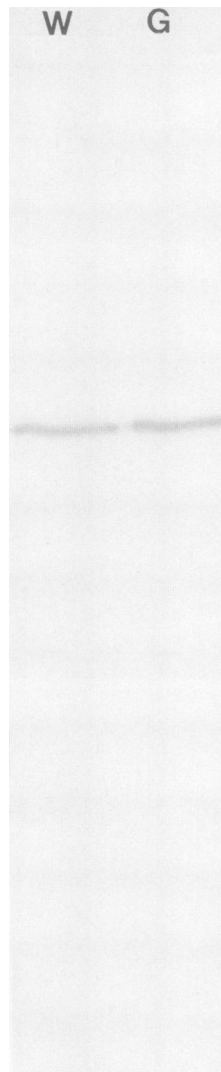


FIG. 7. Western blots immunostained for RuBisCo of plastid-enriched extracts from the white edge (W) and green center (G) of 'Mrs. Parker.' Note reactions in both white and green leaf homogenates. The green leaf extracts were diluted 5-fold to obtain relatively equal staining intensities.

Zeiger *et al.* (16) have recently reviewed the evidence for the presence of photosynthetic carbon reduction in guard cell chloroplasts. These lines of evidence include the effects of CO₂ on *in vivo* Chl fluorescence (effects at the so-called M peak due to CO₂ fixation), alkalization of the media after incubation of guard cell protoplasts, and the detection of large and small subunits of RuBisCo by immunofluorescence. Failure for others to detect RuBisCo or other Calvin cycle enzymes may be due to the high endogenous protease levels in guard cells (17), unusual conditions required for maximal activity, or techniques not sensitive enough to detect the relatively low levels of RuBisCo present in the guard cells. Data presented in this report indicate that the level of RuBisCo in a guard cell is from 0.41 to 0.68% of that found in a mesophyll cell and is consistent with the concept of guard cell

Table I. Distribution of Colloidal Gold Immunolabeling in Various Plastid Types in *Pelargonium*

Plastid Type	Gold Particles/ μm^2
Guard cell chloroplast	5.52 \pm 0.72*
Green mesophyll cell chloroplast	34.72 \pm 4.10
Yellow mesophyll cell plastid	24.94 \pm 2.91*
White mesophyll cell plastid	0.00 \pm 0.00*
Yellow mesophyll cell plastid (L. R. White's resin)	218.19 \pm 27.6**

* Significantly different from the green mesophyll cell chloroplast at the P = 0.05 level using a student's *t* test. ** Significantly different than the yellow mesophyll cell plastid embedded in Spurr's resin at the P = 0.05 level.

photosynthesis as being present only to maintain intercellular needs for fixed CO₂ (16).

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LITERATURE CITED

- BENDAYAN M, M ZOLLINGER 1983 Ultrastructural localization of antigenic sites on osmium-fixed tissues applying the protein A-gold technique. *J Histochem Cytochem* 31: 101-109
- BORNER T, F HERRMANN, R HAGEMANN 1971 Plastid ribosome deficient mutants of *Pelargonium zonale*. *FEBS Lett* 37: 117-119
- GRAY JC, RGO KEKWICK 1974 An immunological investigation of the structure and function of ribulose 1,5-bisphosphate carboxylase. *Eur J Biochem* 44: 481-489
- LAEMMLI UK 1970 Cleavage of structural proteins during assembly of the bacteriophage T₄. *Nature* 227: 680-685
- LAX AR, KC VAUGHN, SO DUKE, J ENDRIZZI 1987 Structural and physiological investigations of a plastome mutant of cotton with slow sorting out. *J Hered.* In press
- MADHAVAN S, BN SMITH 1982 Localization of ribulose bisphosphate carboxylase in the guard cells by an indirect, immunofluorescence technique. *Plant Physiol* 69: 273-277
- OUTLAW WH JR, J MANCHESTER, CA DICAMELLI, DD RANDALL, B RAPP, GM VEITH 1979 Photosynthetic carbon reduction pathway is absent in chloroplasts of *Vicia faba* guard cells. *Proc Natl Acad Sci USA* 76: 6371-6375
- OUTLAW WH JR, MC TARCYNKI, LC ANDERSON 1982 Taxonomic survey for the presence of ribulose-1,5-bisphosphate carboxylase activity in guard cells. *Plant Physiol* 70: 1218-1220
- STEINBACK KE, L MCINTOSH, L BOGORAD, CJ ARNTZEN 1981 Identification of the triazine receptor protein as a chloroplast gene product. *Proc Natl Acad Sci USA* 78: 7463-7467
- STEWART RN, P SEMENIUK, H DERMEN 1975 Competition and accommodation between apical layers and their derivatives in the ontogeny of chimeral shoots of *Pelargonium* \times *hortorum*. *Am J Bot* 61: 54-67
- VAUGHN KC, SO DUKE 1984 Tentoxin stops the processing of polyphenol oxidase into an active enzyme. *Physiol Plant* 60: 257-261
- VAUGHN KC, WH OUTLAW JR 1983 Cytochemical and cytofluorometric evidence for guard cell photosystems. *Plant Physiol* 71: 420-424
- VAUGHN KC, DL KIMPEL, KG WILSON 1980 Investigations of the plastome of *Chlorophytum*. *J Hered* 71: 154-157
- ZEIGER E 1983 Novel approaches to the biology of stomatal guard cells: protoplast and fluorescence studies. In PG Jarvis, TA Mansfield, eds, *Stomatal Physiology*. Cambridge University Press, New York, pp 103-117
- ZEIGER E, P ARMOND, A MELIS 1981 Fluorescence properties of guard cell chloroplasts: evidence for linear electron transport and pigments of PSI and PSII. *Plant Physiol* 67: 17-20
- ZEIGER E, K GOTOW, B MAWSON, S TAYLOR 1986 The guard cell chloroplast: properties and function. In J Biggins, ed, *Proc Int Photosynthesis Cong*, 7th. M Nijhoff/Dr W Junk, The Hague. In press
- ZEMEL E, S GEPSTEIN 1985 Immunological evidence for the presence of ribulose bisphosphate carboxylase in guard cell chloroplasts. *Plant Physiol* 78: 586-590