Mol. Cells, Vol. 7, No. 3, pp. 352-359

Plastid Development in Disc Cells of Glandular Trichomes of *Cannabis* (Cannabaceae)

Eun-Soo Kim* and Paul G. Mahlberg[1]
Department of Biology, Konkuk University, Seoul 133-701, Korea; [1]Department of Biology, Indiana University, Bloomington, Indiana 47405, U.S.A.

(Received on January 30, 1997)

Plastids in lipophilic glandular trichomes of chemically fixed (CF) and high pressure cryofixed-cryosubstituted (HPC-CS) bracteal tissues of *Cannabis* were examined by transmission electron microscopy. In CF preparations, plastids in disc cells prior to secretory cavity formation possessed several lobed and dilated thylakoid-like features. In glands with secretory cavities, thylakoid-like features aggregated to form reticulate bodies that distended regions of the elongated plastids. Electron-gray inclusions evident on the plastid surface appeared continuous with the reticulate body. Inclusions of similar electron density also appeared in the cell cytoplasm, along the plasma membrane, between the plasma membrane and cell wall facing the cavity, and in the secretory cavity in both CF and HPC-CS preparations. The bilayer structure of membranes of the plastid envelope was evident in HPC-CS but not in CF preparations. In HPC-CS preparations, secretions were evident on the plastid surface and were continuous with those in the plastid through pores in the envelope. This study supports an interpretation that these specialized plastids, lipoplasts, synthesize secretions that are transported through the plasma membrane and cell wall to subsequently accumulate in the secretory cavity.

Lipophilic glands of *Cannabis* contain an array of terpenes, phenols, and terpenophenolic or cannabinoid compounds typically termed the essential oils (Hammond and Mahlberg, 1994; Lanyon *et al.*, 1981; Malingr *et al.*, 1975; Turner *et al.*, 1978; Hammond *et al.*, unpublished). These substances accumulate in a secretory cavity formed from a subjacent tier of disc cells containing numerous plastids interpreted to be associated with the synthesis of these compounds (Hammond and Mahlberg, 1978; Mahlberg *et al.*, 1984). The distinctive morphology of these plastids in the disc cells, unlike that of plastids in bract or leaf cells, suggests a functional role other than photosynthesis.

In studies on lipophilic glands, authors report difficulty in chemical fixation and preservation of typical membrane structure and describe membranes like those of plastids to be diffuse or not well defined (Dell and McComb, 1975; Duke and Paul, 1993; Oliveira and Pais, 1990; Schnepf, 1969; Werker and Fahn, 1981; Douce, pers. comm.). This difficulty may occur in plastids associated with the synthesis and accumulation of terpenes (Gleizes *et al.*, 1983). The inability to preserve the bilayer structure of membranes in terpene- or resin-producing cells prompted Dell and McComb (1974) to suggest that this phenomenon may be a general feature of such cells.

Secretory substances in *Cannabis* glands accumulate in a secretory cavity formed by periclinal partitioning of the outer wall of the disc cells. Secretions occur as numerous lipophilic bodies in the cavity (Hammond and Mahlberg, 1978; Kim and Mahlberg, 1991; Mahlberg and Kim, 1991). Progressive enlargement of the secretory cavity is associated with the continued formation of these bodies in the cavity. These substances are derived from secretions passed through the cell wall and accumulate as vesicle-like bodies in the secretory cavity (Mahlberg and Kim, 1992).

The appearance of plastids in lipophilic glands of *Cannabis* and the association of inclusions with plastids in the disc cells suggest that this organelle may be the source of secretions accumulated inside the secretory cavity. Initial studies on the general character of these plastids and on the development of the secretory cavity have been reported (Hammond and Mahlberg, 1978; Kim and Mahlberg, 1991; Mahlberg and Kim, 1992; Mahlberg *et al.*, 1984). In this study we examined plastid development in the disc cells of the capitate gland and interpreted the role of this organelle in the secretory process. We utilized chemically fixed tissues in this study to describe plastids in the disc cell during gland development. In addition,

* To whom correspondence should be addressed.

The abbreviations used are: CF, chemically fixed; HPC-CS, high pressure cryofixed-cryosubstituted.

we depicted plastid structures and the appearance of inclusions in disc cells in an initial examination of adult glands by high pressure cryofixation-cryosubstitution, which retains cellular features with minimal distortion (Hunziker, 1993), for comparison with chemically fixed tissues.

Materials and Methods

Glandular trichomes of different ages on bracts of pistillate *Cannabis* plants, grown under greenhouse conditions (Hammond and Mahlberg, 1973), were chemically fixed in glutaraldehyde and osmium tetroxide (CF) and embedded in resin as previously described (Kim and Mahlberg, 1991; Mahlberg and Kim, 1991). Similar bracts with glands of different ages also were fixed and processed by Balzers high pressure cryofixation-cryosubstitution (HPC-CS) procedure. Small segments of bracts were placed in brass Swiss Precision planchettes and immersed in 1-hexadecene (Sigma, St. Louis, MO) and immediately transferred to the Balzers HPM 010 instrument for cryoimmobilization under a pressure of 2,100 bar. Immediately following HPC, the samples were transferred to liquid nitrogen and stored until all samples were ready for CS. The samples were quickly transferred to sieve-dried methanol containing either 3% glutaraldehyde or 3% glutaraldehyde and 1% osmium tetroxide and were immediately placed in a Balzers FSU automated CS instrument. CS proceeded uninterrupted from −90 ℃ to −60 ℃ to −30 ℃, each for 8 h, and to 0 ℃ for 1 h. Following CS, the samples were transferred to fresh sieve-dried methanol over ice and subsequently brought to room temperature (Kim and Mahlberg, 1995). Samples were immediately embedded in Spurr's low-viscosity resin (Spurr, 1969).

Thin sections of CF and HPC-CS preparations were sectioned with diamond or glass knives on an LKB-IV Ultramicrotome and stained with 1% uranyl acetate for 10 min followed by 1% lead citrate for 10 min (Reynolds, 1963). Sections were examined and photographed with a Philips EM 300 transmission electron microscope at 60 kV. Illustrations were selected from several hundred electron micrographs derived from examination of numerous glands.

Results

CF preparations: Plastid organization during early period of gland formation

Several plastids were evident in a gland initial on the bract surface and appeared similar to those in other protodermal cells (Mahlberg and Kim, 1991). Several thylakoid-like membranes and osmophilic bodies were present in the stroma of the plastids in gland initials. An electron-dense body resembling a starch grain was also evident in sections of some plastids (figure not shown).

Upon formation of the terminal cell and its division to form the tier of disc cells, the plastids in these cells appeared oval in shape and possessed several lipophilic bodies as well as electron-gray areas in the stroma (Fig. 1). A bilayered structure for membranes of the plastid envelope was not evident. In contrast, a bilayered plasma membrane adjacent to the cell wall was evident in the cell (Fig. 1, inset). A narrow periplasmic space and small infoldings of the plasma membrane were detected at some positions along the wall.

Plastid organization in disc cells prior to secretory cavity formation

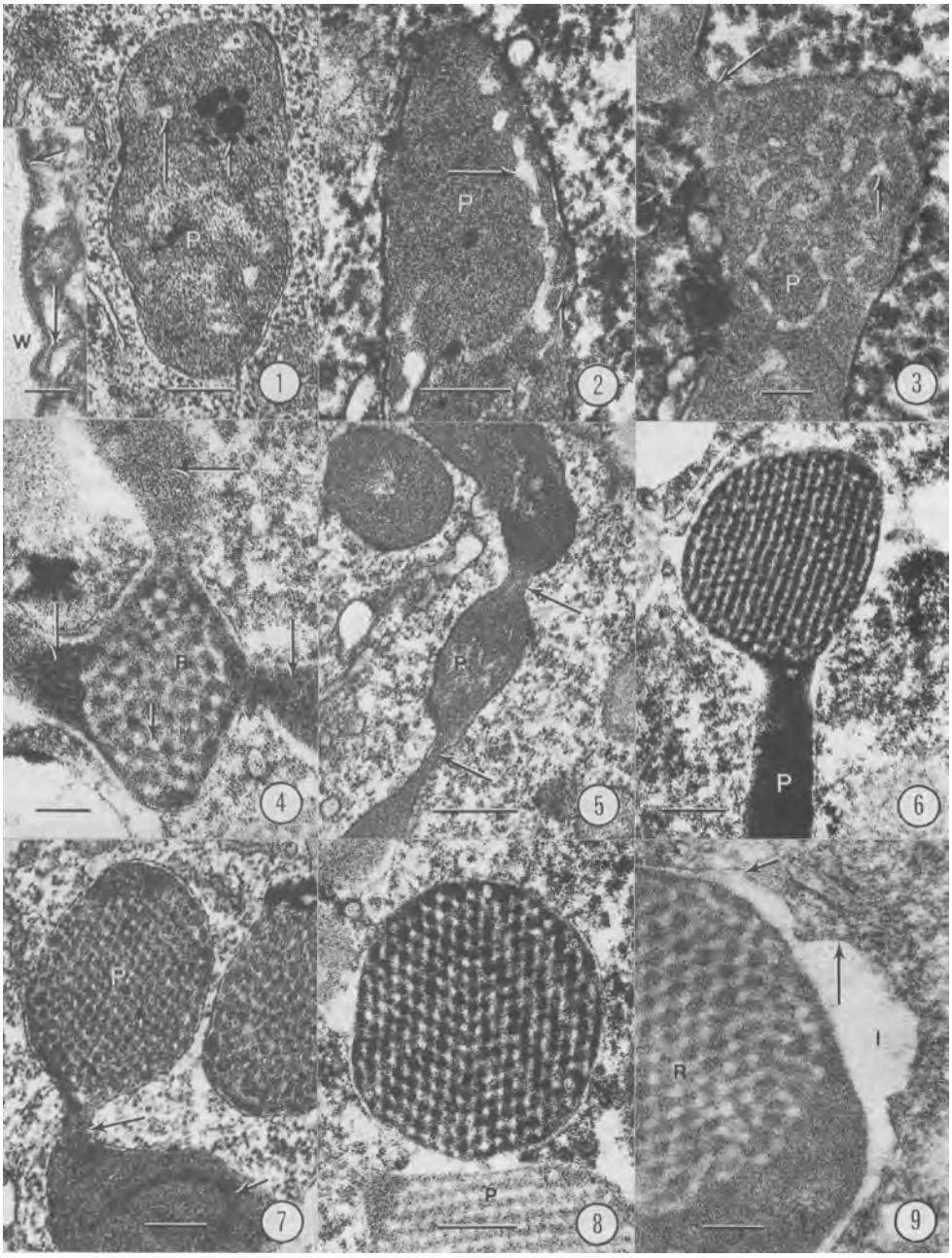
Plastids in the tier of disc cells appeared elongated, often curved, and possessed one or several osmophilic bodies; starch grains were absent, or rarely observed, in these plastids. They possessed one or more elongated electron-gray features, interpreted as thylakoids, that typically contained dilations along their length (Fig. 2). These features extended along much of the length of the plastid and at several positions were observed to extend toward the plastid envelope. Although an electron-dense boundary was present between these features and the stroma, a bilayered structure was not evident for the boundary. The plastid envelope appeared as a wide dense band rather than two membranes separated by an intermembrane space (Fig. 3). These thylakoid-like features became localized in plastids, where they appeared as curved and lobed, or branched, configurations (Fig. 3). In other plastids the branched or lobed configurations formed a reticulate network (Fig. 4).

Plastid organization upon development of secretory cavity

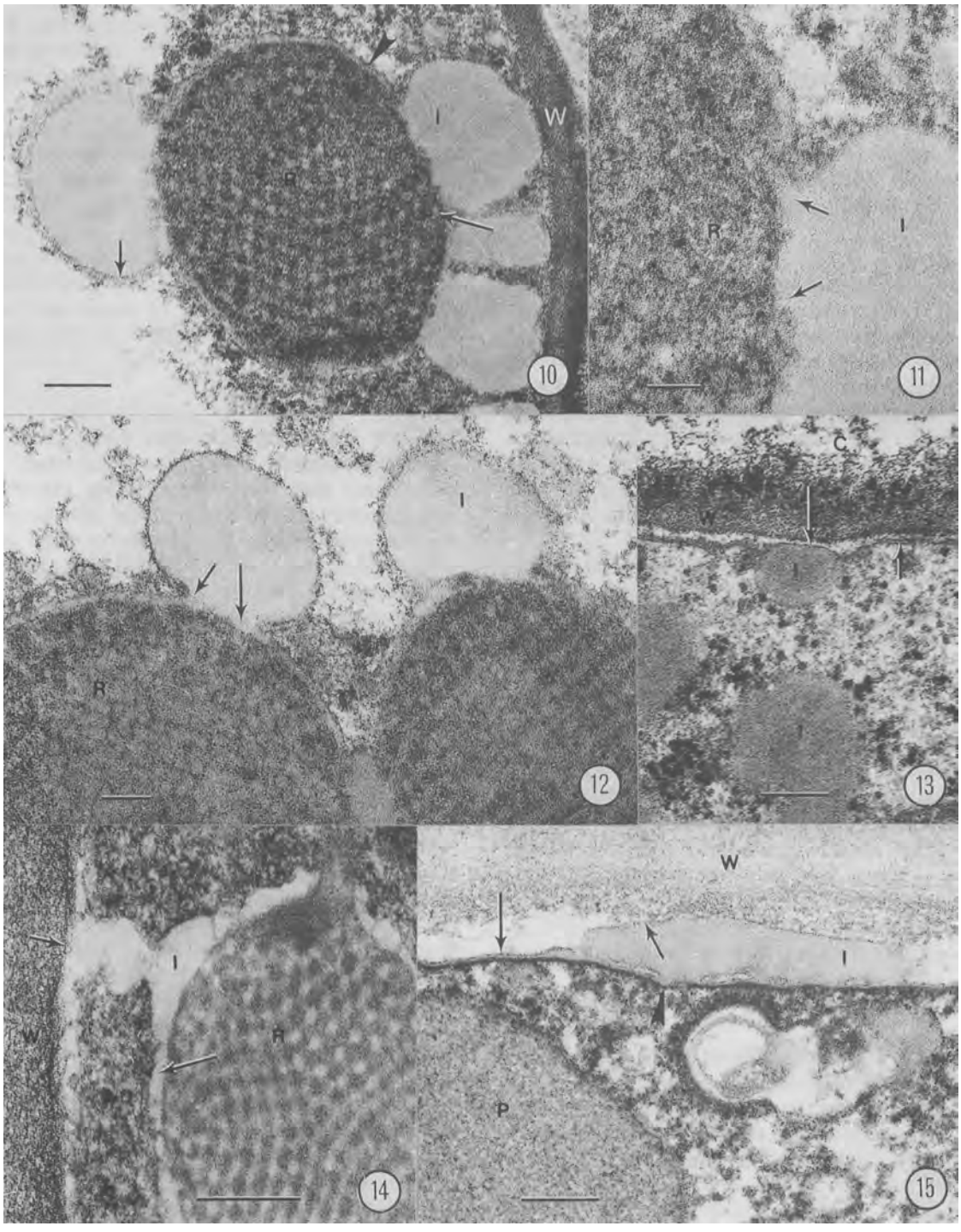
At the time of secretory cavity development, many plastids appeared as elongated organelles that often possessed one or more constrictions along their length (Fig. 5). Increases in length and formation of constrictions along some plastids resulted in oddly shaped tri- and tetralobed configurations (Fig. 4).

The reticulate network, which now appeared to be aggregated into a distinctive reticulate body, was evident in many plastids (Fig. 6). This body attained a diameter of approximately 0.8 μm in some plastids. In some sections the reticulate body appeared to occupy the entire plastid, whereas in other views it represented a distended portion of a narrow, elongated plastid. Numerous sectional views of these bodies of different sizes, as well as narrow regions of plastids, were evident in sections of disc cells. Many views of plastids showed only a large reticulate body and a small zone of stroma surrounded by a dense envelope-like feature (Fig. 7). More than one reticulate body was evident in some elongated plastids (figure not shown).

Most frequently the reticulate body possessed a single mesh lattice pattern in which the membrane-like



Figures 1-9. Plastids in disc cells of glands in CF preparations. 1) Plastid in formative cell of disc tier of developing gland. Possible portion of thylakoid (long arrow) and osmophilic body (short arrow) present in stroma. Bar=0.2 μm. Inset. Plasma membrane (short arrow) and fold along this membrane (long arrow). Bar=0.1 μm. 2) Elongated and dilated portions of thylakoid-like feature (long arrow), a portion of which extends toward plastid envelope (short arrow). Bar=0.2 μm. 3) Plastid showing localized aggregation of dilated thylakoid-like features (short arrows), some curved and branched, in stroma. A constriction with a dense band across the isthmus (long arrow) is present along length of plastid. Plastid envelope appears as a dark band (lower right). Bar=0.1 μm. 4) Plastid, possessing three lobes (long arrows), showing small thylakoid complex joined into an anastomosing network, or reticulate body. Light areas (small arrow) in reticulate body are interpreted as nexi of thylakoids. Bar=0.1 μm. 5) Elongated plastid, prior to formation of secretory cavity, showing two constrictions (arrows) along its length. Bar=0.5 μm. 6) Elongated plastid, after secretory cavity development, showing distended region containing reticulate body and a subtending long narrow portion of plastid. Reticulate body contains a single lattice pattern. Bar=0.2 μm. 7) Plastid showing constriction with a dense band across the isthmus (long arrow) and two distended regions, one containing a reticulate body. Dark band (short arrow) may represent a thylakoid-feature. Bar=0.2 μm. 8) Reticulate body occupies entire circular outline of plastid and shows different orientations of lattice patterns. Plastids in cells can show different intensities of staining. Bar=0.2 μm. 9) Plastid containing reticulate body showing accumulation of inclusion on its surface (short arrow). Inclusion is sharply demarcated from cytoplasm (long arrow). Bar=0.1 μm. (Abbreviations: C, secretory cavity; I, inclusion; P, plastid; R, reticulate body; V, secretory vesicle; W, cell wall).



Figures 10-15. Plastids in disc cells of glands in CF preparations. 10) Plastid showing voluminous accumulations of inclusions along envelope surface (arrowhead). Inclusion sharply demarcated from cytoplasm (small arrow). Bar=0.2 μm. 11) Plastid showing continuity of inclusion with the light areas (arrows) of reticulate body. Bar=0.05 μm. 12) Plastids showing association of inclusion with plastid envelope (short arrow). Diffuse appearance of the central region in each reticulate body may represent accumulated inclusions or possibly peripheral view of this body. Electron-gray area from reticulate body is continuous with inclusion (long arrow). Bar=0.1 μm. 13) Inclusion in cytoplasm shows contact (at long arrow) with plasma membrane (small arrow). Bar=0.1 μm. 14) Plastid showing inclusion extending from surface (long arrow) to the plasma membrane adjacent to cell wall (short arrow). Bar=0.2 μm. 15) Plasma membrane (long arrow) showing inclusion positioned in periplasmic space. Inclusion, delimited by a surface feature (short arrow), appears in contact with plasma membrane at several positions (as at arrowhead). Bar=0.1 μm. (Abbreviations: C, secretory cavity; I, inclusion; P, plastid; R, reticulate body; V, secretory vesicle; W, cell wall).

features were oriented approximately at the same angle to each other (Figs. 6 and 7). In other plastids, more than one lattice pattern was evident in which the lattices were oriented at different angles to each other (Fig. 8). The elongated gray areas of the reticulate body were interconnected to form the lattice pattern (compare Figs. 3, 4 and 9). The electron-dense material between the gray areas was continuous with the dark contents of the stroma (Figs. 3 and 9).

Formation of inclusions in disc cell plastids

Electron-gray inclusions were associated with plastids of glands possessing a secretory cavity. They first became evident along the plastid surface and were sharply demarcated from the cytoplasm and stroma (Fig. 9). Inclusions accumulated as voluminous masses along the plastid surface. In some views, where more than one mass are present, they approximated the profile area of a plastid (Fig. 10). For some plastids, the inclusions surrounded the organelle in sectional view (figure not shown).

Inclusions along the plastid periphery were continuous with the gray zone of the reticulate body (Figs. 11 and 12). The central area of the reticulate body, in some plastids with enlarged masses of surface inclusions, appeared diffuse and the outline of the reticulate body was indistinct (Fig. 12).

Division of disc cell plastids

Many plastids possessed one or more distinctive constrictions indicative of their division. Often, a narrow dense band traversed the constriction separating the plastid into similar or dissimilar sizes, as viewed in section (Figs. 3 and 7). Some plastids had more than one constriction along their length, or were constricted into three or four lobes (Figs. 4 and 5). Constriction of a reticulate body was not observed.

Inclusions in the cytoplasm of disc cells and secretory cavity

Electron-gray areas of different sizes were evident in the cytoplasm of disc cells (Fig. 13). Although these bodies were numerous and distributed throughout the cytoplasm, they were often observed in the vicinity of the plasma membrane adjacent to the cell wall subtending the secretory cavity (Fig. 13).

Inclusions on the surface of plastids near the cell periphery were also observed in contact with the plasma membrane (Fig. 14). Gray bodies in the cytoplasm may represent inclusions separated from the plastid, or views of inclusions attached to plastids but not in the plane of section.

Inclusions contacting the plasma membrane became confluent with it and subsequently accumulated in the periplasmic space between the plasma membrane and cell wall (Fig. 15). They appeared to penetrate directly through the plasma membrane (Fig. 16). The typical appearance of the bilayer configuration of the plasma membrane was not evident at the position where the inclusion penetrated the membrane (Fig. 16). That portion of the inclusion projecting into the periplasmic space appeared to be covered with a thin somewhat dense surface feature. The surface of the inclusion remained partially enveloped by cytoplasm adjacent to the plastid but also appeared to be surrounded by a surface feature in continuity with the plasma membrane (Fig. 16). Other inclusions were positioned entirely in the periplasmic space (Figs. 15 and 16). The bilayer structure of the plasma membrane was evident between it and the inclusion. Similar inclusions of small size and other components were also evident in the periplasmic space (Fig. 16).

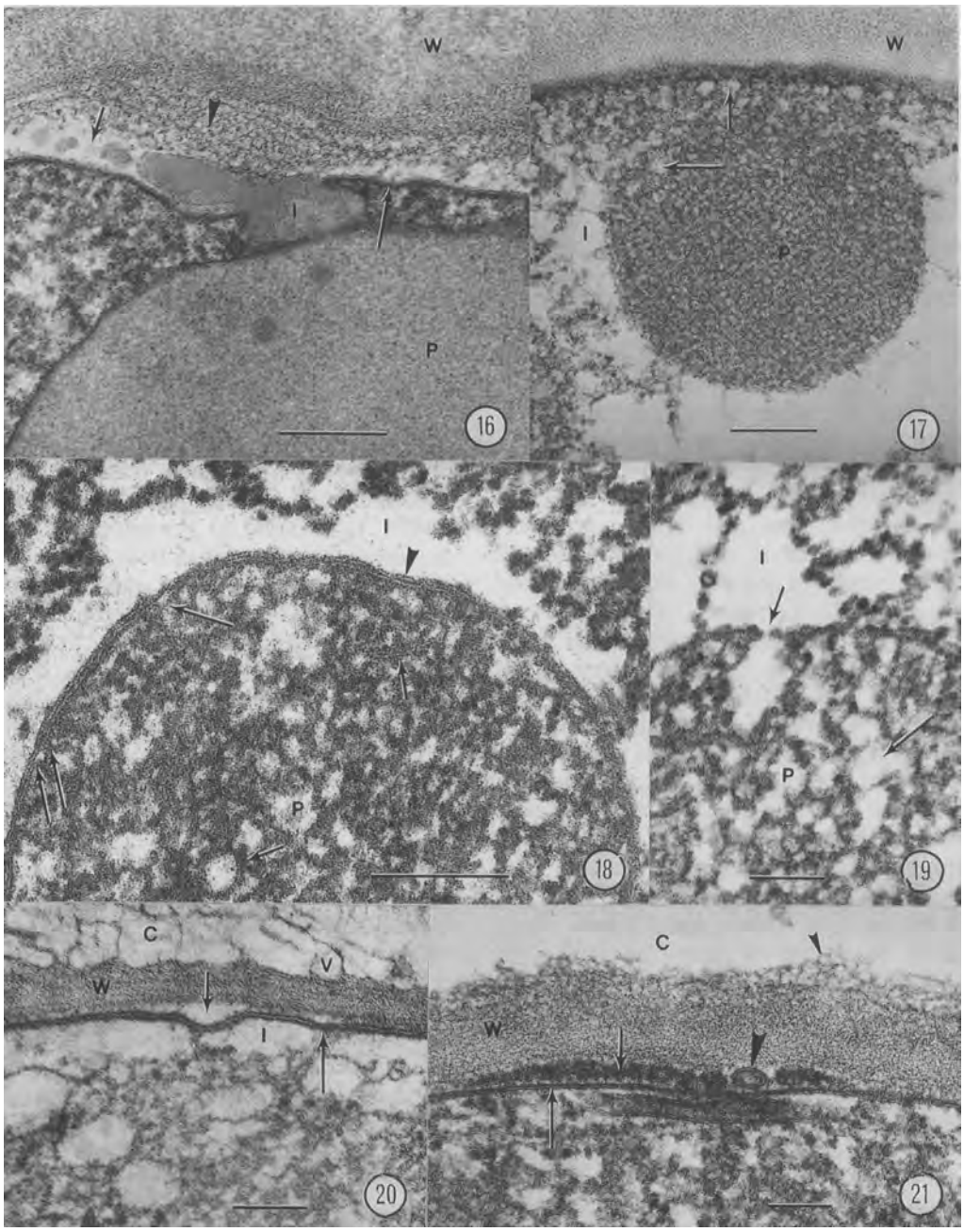
HPC-CS preparation

Where plastids in disc cells were adjacent to the plasma membrane, the region of contact appeared more dense than other regions along this membrane, and the bilayered structure of the plasma membrane was not evident (Fig. 17). Numerous small gray areas were evident between the plastid and the dense region along the plasma membrane, and similar gray areas were present in the plastid. Other large gray areas were localized along the surface of the plastid.

The plastid envelope was evident as two nearly contiguous membranes (Fig. 18). The inner membrane turned inwardly at several positions along the envelope and was associated with dense, irregular features in the plastid. At these positions along the envelope, only the outer membrane was present over an electron-gray area adjacent to the infolded inner membrane. Segments of parallel membranes, zones of electron-gray areas, and dense materials were present in the plastid. Electron-gray material was evident along the surface of the plastid. Although this material was sharply demarcated from the cytoplasm, no membrane separated it from the cytoplasm.

Pores in the plastid envelope showed continuity between electron-gray areas inside with those on the plastid surface (Fig. 19). Other gray areas, elongated and somewhat irregular in outline, were present in the plastid. Electron-gray areas were present in the cytoplasm of the disc cells adjacent to the plasma membrane (Fig. 20). Similar gray areas of different sizes were evident in the periplasmic space. One large area pressed the plasma membrane toward the cytoplasm of the disc cell. Other gray areas delimited by a surface feature were present in the secretory cavity.

The periplasmic space was also observed to contain dense materials which appeared somewhat fibrillar in character (Fig. 21). A vesicle delimited with a membrane was present in this space. A membrane-like structure in the disc cell was adjacent to this area along the plasma membrane. Electron-gray areas were evident in the cell wall, and numerous small gray areas surrounded by a surface feature were evident along the wall surface facing the secretory cavity.



Figures 16-21. Plastids and inclusions in CF and HPC-CS preparations. 16) CF of inclusion showing its penetration through plasma membrane (long arrow) and localization in periplasmic space (short arrow) along with other components including fibrillar-like material (arrowhead). Bar=0.1 μm. 17) HPC-CS of plastid adjacent to plasma membrane region showing numerous electron-gray areas in this region (short arrow) and in plastid (long arrow). Bar=0.2 μm. 18) HPC-CS of plastid showing closely oriented membranes of envelope (arrowhead). Inner membrane appears to curve inwardly (long arrows) to associate with other components in plastid. Inclusion occurs along surface of plastid envelope and is sharply demarcated from cytoplasmic components. Segments of membranes (short arrows) are evident in plastid. Bar=0.2 μm. 19) HPC-CS of plastid showing pore (short arrow) in envelope and continuity of inclusion on plastid surface with internal plastid contents. Other electron-gray contents in plastid appear as irregularly contoured features (long arrow). Bar=0.1 μm. 20) HPC-CS of disc cell region facing secretory cavity showing electron-gray inclusion in cytoplasm adjacent to plasma membrane (long arrow) and in periplasmic space (short arrow). Other small features also appear in periplasmic space. Vesicles are evident in secretory cavity. Bar=0.1 μm. 21) HPC-CS of disc cell region facing secretory cavity showing plasma membrane (long arrow) delimiting periplasmic space containing dense materials (short arrow) and a membraned vesicle (large arrowhead). Numerous electron-gray features (small arrowhead) are evident along wall surface facing secretory cavity. Unknown membrane-like structure is evident under plasma membrane (to right of long arrow). Bar=0.1 μm. (Abbreviations: C, secretory cavity; I, inclusion; P, plastid; R, reticulate body; V, secretory vesicle; W, cell wall).

Discussion

The abundant inclusions associated with plastids in disc cells of *Cannabis* glands, which accumulate monoterpenes and terpenophenols, are interpreted as lipophilic secretions (Hammond and Mahlberg, 1994; Lanyon *et al.*, 1981; Malingr *et al.*, 1975; Turner *et al.*, 1978; Hammond *et al.*, unpublished). They resembled materials reported as lipophilic and resembled terpene secretions for plastids in terpene-containing glands in other plants (Dell and McComb, 1974; Robinson, 1985). The occurrence of distinctive plastids in the disc cells associated with secretory activity, in contrast to plastids in subjacent parenchyma cells, indicate both an evolutionary and functional divergence from the chloroplast and emphasize recognition as a distinctive type of plastid, the lipoplast. Secretory activity of plastids in disc cells of *Cannabis* appears to differ from that of glandular secretory cells in other plants in which Golgi apparati or ER are reported active in secretion (Kronestedt-Robards and Robards, 1991; Robinson, 1985). However, plastid involvement in glandular secretions has been suggested, but not described, for other plants (Dell and McComb, 1974; 1975; Duke and Paul, 1993; Oliveira and Pais, 1990; Werker and Fahn, 1981).

Detection of envelope membranes for plastids in HPC-CS preparations, in contrast to CF, supports the interpretation of Dell and McComb (1974; 1975) that plastid membranes interact with the lipophilic secretions during CF to alter their organization. The width of the intermembrane space, which varies for plastids in CF preparations (Douce and Joyard, 1990), was very narrow in plastids of these disc cells. An inability to resolve plastid membrane structure in CF preparations of secretory cells of other glands has made it difficult to study the secretory process in other glands (Duke and Paul, 1993; Oliveira and Pais, 1990; Schnepf, 1969; Werker and Fahn, 1981).

Secretion accumulation along the plastid periphery and its continuity with the reticulate body supports an interpretation that this organelle is associated with the production of secretions prior to releasing them into the cytoplasm. Plastids in terpene-rich cells are reported to synthesize lipophilic monoterpenes and enzymes related to lipid synthesis are associated with the plastid envelope (Douce and Joyard, 1990; Gleizes *et al.*, 1983). The reticulate body, as observed here, appears to produce substances that migrate through pores in the plastid envelope to accumulate on the plastid surface. These secretions migrate through the plasma membrane to accumulate in the periplasmic space, whereupon they pass through the cell wall into the secretory cavity (Kim and Mahlberg, 1991; 1995; Mahlberg and Kim, 1992). While morphology shows the secretion to pass through the plasma membrane, distending the bilayered structure during its migration, the physiological processes associated with movement of the secretion are as yet unclear.

Absence of a membrane between the secretion on the plastid surface and the cytoplasm in both HPC-CS and CF preparations supports an interpretation that another form of structural feature may delimit the secretion from the cytoplasm. Secretory vesicles in glands with an enlarged secretory cavity in both HPC-CS and CF preparations are reported to possess a surface feature, but not a membrane (Kim and Mahlberg, 1991; 1995; Mahlberg and Kim, 1992). It is possible that this feature may represent a lipoprotein such as described for oil bodies in plant cells or very low density lipoprotein bodies in animal cells (Hamilton, Moorehouse and Havel, 1991; Tzen *et al.*, 1992).

The inner membrane of the plastid envelope appeared to be associated with thylakoids. These formed the reticulate body, which resembled the prolamellar body of other plastids (Gunning, 1965; Lindstedt and Liljenberg, 1990; Murakami *et al.*, 1985). In contrast to the prolamellar body, the reticulate body persisted in both the light and dark during the extended period of gland presence on the developing plant. It did not contribute to formation of grana, which were lacking in disc cell plastids.

The plastid population throughout gland development was derived from repeated fission, as evident from the presence of numerous constricted plastids in disc cells (Wellburn, 1984). Many of these plastids possessed a dense band across their isthmus comparable to that associated with dividing plastids in other tissues (Kuroiwa, 1989). The occurrence among the numerous plastids of several constrictions along their length as well as the appearance of multilobed plastids with a constriction on each lobe was unusual and different from plastid features observed in other bracteal cells.

Inclusions in the cytoplasm or associated with plastids near the plasma membrane appear to pass through the membrane, possibly through pores, to accumulate in the periplasmic space. The origin of fibril-like material and membraned vesicles in the periplasmic space is as yet unclear. These various inclusions are interpreted to pass through the thickened cell wall to accumulate in the secretory cavity (Kim and Mahlberg, 1991; 1995; Mahlberg and Kim, 1991; 1992).

The structurally unusual plastids in disc cells of *Cannabis* contribute to the production of secretions that subsequently accumulate in the secretory cavity. The HPC-CS procedure, in contrast to chemical fixation, provides improved preservation of protoplasmic components of these lipid-rich cells. Further study of these cells utilizing HPC-CS preparations, as well as chemical analyses of gland content, will provide additional insight into the morphology and physiology of the secretion process in developing glandular trichomes and, more broadly, in plant cells in general.

Acknowledgments

This study was supported with stipends from the

Korean Ministry of Education through Research Funds (ESK), and the Indiana University Faculty Research Program (PGM).

Support and technical assistance were provided in part by C. Lavin at the IMR, Madison, WI, through NIH Biomedical Research Technology Grant RR 00570. Drug Enforcement Administration registration number PI00433113 (PGM).

References

Dell, B., and McComb, A. J. (1974) *Aust. J. Bot.* **22**, 195-210.

Dell, B., and McComb, A. J. (1975) *Aust. J. Bot.* **23**, 373-390.

Douce, R., and Joyard, J. (1990) *Annu. Rev. Cell Biol.* **6**, 173-216.

Duke, S. O., and Paul, R. N. (1993) *Int. J. Plant Sci.* **154**, 107-118.

Gleizes, M., Pauly, G., Carde, J. P., Marpeau, A., and Bernard-Dagan, C. (1983) *Planta* **159**, 373-381.

Gunning, B. E. S. (1965) *Protoplasma* **60**, 111-130.

Hamilton, R. L., Moorehouse, A., and Havel, R. J. (1991) *J. Lipid Res.* **32**, 529-543.

Hammond, C. T., and Mahlberg, P. G. (1973) *Am. J. Bot.* **60**, 524-528.

Hammond, C. T., and Mahlberg, P. G. (1978) *Am. J. Bot.* **65**, 140-151.

Hammond, C. T., and Mahlberg, P. G. (1994) *Phytochemistry* **37**, 755-756.

Hunziker, E. B. (1993) *Micro. Res. Tech.* **24**, 457-464.

Kim, E. S., and Mahlberg, P. G. (1991) *Am. J. Bot.* **78**, 220-229.

Kim, E. S., and Mahlberg, P. G. (1995) *Am. J. Bot.* **82**, 1207-1214.

Kronestedt-Robards, E., and Robards, W. W. (1991) in *Endocytosis, Exocytosis and Vesicle Traffic in Plants* (Hawes, C. R., Coleman, J. O., and Evans, D. E., eds) pp. 199-232, Cambridge University Press, Cambridge, UK.

Kuroiwa, T. (1989) *Bot. Mag. Tokyo* **102**, 291-329.

Lanyon, V. S., Turner, J. C., and Mahlberg, P. G. (1981) *Bot. Gaz.* **142**, 316-319.

Lindstedt, I., and Liljenberg, C. (1990) *Physiol. Plant.* **80**, 1-4.

Mahlberg, P. G., and Kim, E. S. (1991) *Am. J. Bot.* **78**, 1113-1122.

Mahlberg, P. G., and Kim, E. S. (1992) *Am. J. Bot.* **79**, 166-173.

Mahlberg, P. G., Hammond, C. T., Turner, J. C., and Hemphill, J. K. (1984) in *Biology and Chemistry of Plant Trichomes* (Rodriguez, E., Healey, P. L., and Mehta, I., eds) pp. 23-51, Plenum Press, New York.

Malingr T., Hendriks, H., Batterman, S., Bos, R., and Visser, J. (1975) *Planta Med.* **28**, 56-61.

Murakami, S., Yamada, N., Nagano, M., and Osumi, M. (1985) *Protoplasma* **128**, 147-156.

Oliveira, M. M., and Pais, M. S. (1990) *J. Submicros. Cytol. Path.* **22**, 241-248.

Reynolds, E. S. (1963) *J. Cell Biol.* **17**, 208-212.

Robinson, D. G. (1985) *Plant Membranes, Endo- and Plasma Membranes of Plant Cells*, Wiley & Sons, New York.

Schnepf, E. (1969) *Protoplasma* **67**, 195-203.

Spurr, A. (1969) *J. Ultrastr. Res.* **26**, 31-43.

Turner, J. C., Hemphill, J. K., and Mahlberg, P. G. (1978) *Am. J. Bot.* **65**, 1103-1106.

Tzen, J. T. C., Lie, G. C., and Huang, A. H. C. (1992) *J. Biol. Chem.* **267**, 15626-15634.

Wellburn, A. R. (1984) in *Chloroplast Biogenesis* (Baker, N., and Barber, J., eds) pp. 255-303, Elsevier, Amsterdam.

Werker, E., and Fahn, A. (1981) *Bot. Gaz.* **142**, 461-476.