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Arabinogalactan-Rich Glycoproteins Are Localized on the Cell Surface and in Intravacuolar Multivesicular Bodies[1,2]

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ABSTRACT

We investigated the subcellular distribution of antigenic sites immunoreactive to the monoclonal antibody 16.4B4 (PM Norman, VPM Wingate, MS Fitter, CJ Lamb [1986] Planta 167: 452–459) in tobacco (*Nicotiana tabacum*) leaf cells. This antibody is directed against a glycan epitope in a family of plasma membrane arabinogalactan proteins of 135 to 180 kilodaltons, elaborated from a polypeptide of relative molecular mass 50 kilodaltons (PM Norman, P Kjellbom, DJ Bradley, MG Hahn, CJ Lamb [1990] Planta 181: 365–373). We demonstrated by immunogold electron microscopy that the epitope reactive with monoclonal antibody 16.4B4 is localized on the cell surface in the leaf parenchyma cell periplast. The 16.4B4 antigen is also localized in multivesicular invaginations of the plasma membrane also known as plasmalemmasomes, implying a biochemical and, hence, functional interrelationship between these structures. Monoclonal antibody 16.4B4 also labels intracellular multivesicular bodies that appear to represent internalized plasmalemmasomes. Antibody reactivity was also observed in partially degraded multivesicular bodies sequestered within the central vacuole. We propose that the subcellular distribution of the epitope reactive with monoclonal antibody 16.4B4 defines a plasmalemmasome (or multivesicular body-mediated) pathway for the internalization of the periplasmic matrix for vacuolar mediated disposal. The multivesicular bodies appear to be equivalent to the well-characterized endosomes and multivesicular bodies of animal cells involved in the internalization and lysosome-mediated degradation of extracellular materials.

Elaborations of the plasma membrane have often been observed in EM studies of plant cells (for examples, see refs. 19 and 28). Evaginations of the plasma membrane, termed plasma tubules, are particularly abundant in transfer cells and other cell types exhibiting high rates of solute transfer. Concave-shaped invaginations of the plasma membrane containing numerous vesicles, termed plasmalemmasomes, are found in diverse cell and tissue types (for examples, see ref. 19). The physiological function of such plasma membrane elaborations has remained elusive, although roles in cell wall biogenesis (19), endocytosis (28), and transport (22) have all been proposed.

The determination of the function of plant cell plasma membrane elaborations has been hindered by the lack of known specific macromolecular components. A number of monoclonal antibodies to epitopes present on the plant cell surface have been generated, starting from crude microsomal membrane preparations or intact protoplasts as immunogens (8, 24). Mab[3] 16.4B4, which was generated by immunization with microsomes from suspension-cultured cells of *Nicotiana glutinosa* (24), was shown to react with an epitope located on the external face of the plasma membrane by immunofluorescence visualization of antibody binding to the surface of isolated protoplasts (24). This was further confirmed by (a) specific agglutination of protoplasts, (b) ELISA of antibody binding to the surface of intact protoplasts, and (c) topologically specific labeling of the surface of intact protoplasts followed by analysis of membrane partitioning on sucrose density gradients (8, 23, 24). The epitope reactive with Mab 16.4B4 resides on glycan moieties that define a family of plasma membrane arabinogalactan-rich glycoproteins elaborated from a polypeptide with relative molecular mass of 50 kD, rich in alanine, glycine, serine, and threonine (23).

In the present paper, the cellular distribution of the epitope reactive with Mab 16.4B4 is examined at the ultrastructural level by immunogold EM. We show that this epitope is widely distributed along the external face of the plasma membrane. We also observed dense immunogold labeling of multivesicular plasma membrane invaginations (plasmalemmasomes), as well as similar structures sequestered within the cytoplasm and the central vacuole, suggesting an ontogenic relationship between these structures. The existence and possible functional significance of a pathway for internalization of periplasmic material mediated by plasma tubules and plasmalemmasomes is discussed.

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[2] This paper is dedicated to the memory of ASPP member Dr. Leland (Lee) M. Shannon (1927–1991), Professor of Biochemistry and Dean of the Graduate Division at the University of California, Riverside. Lee was widely known for his laboratory's research on the legume seed lectins. Lee will be greatly missed by his friends and colleagues.

[3] Abbreviations: Mab, monoclonal antibody; TBST, 50 mM Tris-HCl (pH 7.4), 0.15 M NaCl, and 0.5% Tween-20.

MATERIALS AND METHODS

Axenic explants of tobacco (*Nicotiana tabacum* L.) and tobacco callus cultures were maintained as previously described (6). The monoclonal antibodies used were designated 16.4B4 against a tobacco membrane epitope, 27.1E8 which is directed against the core polypeptide of the 16.4B4 glycan epitope, and 40.2.B2 which is a control Mab directed against a cell wall epitope of the phytopathogenic bacterium *Pseudomonas syringae* pv *glycinea* (23, 24, 36). Leaves were cut into approximately 2-mm^2 pieces and fixed in 4% (v/v) EM grade formaldehyde, 2% (v/v) glutaraldehyde (Polysciences,[4] Warrington, PA), and 0.1 M potassium phosphate (pH 7.4) for 1 h at room temperature and then transferred to 7°C for 18 h. The samples were then postfixed in aqueous 1% (w/v) OsO_4 (Polysciences) for 2 h at room temperature. The samples were dehydrated in a graded ethanol series and embedded in the hard grade of LR White resin (Polysciences). Silver-colored sections were obtained and mounted on 300-mesh nickel grids.

The grids were labeled by a 1:20 dilution of the hybridoma culture supernatants containing monoclonal antibodies 16.4B4 and 40.2B2 in TBST for 1 h at room temperature. The grids were washed with TBST and then indirectly labeled with 5 or 15 nm colloidal gold coupled to goat anti-mouse IgG/IgM (Janssen Life Sciences, Piscataway, NJ) for 15 min at room temperature. The grids were washed with TBST, then distilled water, and stained with 5%, w/v, aqueous uranyl acetate (Polysciences) for 30 min and 33 mg/ml alkaline lead citrate (Polysciences) for 10 min. The grids were examined and photographed with Hitachi (Tokyo, Japan) H300 and H500 electron microscopes.

RESULTS

Mab 16.4B4 Labels the Periplasm of Tobacco Callus Cells

Previous fluorescent microscopy studies of tobacco suspension cell protoplasts indicated that the epitope reactive with MAb 16.4B4 was localized on the exterior face of the plasma membrane. We have further investigated the subcellular distribution of this epitope in undifferentiated callus cells with EM immunogold assays. The periplasmic space was specifically labeled by gold particles with the label associated with the outer surface of the plasma membrane and the inner surface of the cell wall (Fig. 1). It is important to note that the spatial error associated with the immunogold assay of 25 nm is larger than the bilayer thickness of the plasma membrane. Therefore, although the label appears to be associated with the outer surface of the plasma membrane, this is within the spatial error of the assay. The label associated with the inner surface of the cell wall is outside of the spatial error associated with the plasma membrane. Therefore, the aggregate labeling of the plasma membrane and inner surface verifies that the epitope reactive with Mab 16.4B4 is associated with the periplasmic space of the callus cells. Additional gold label is associated with fibrous and vesicular inclusions in the periplasmic space. The plasma membrane of the callus cells exhibits the corrugated appearance typical of chemically fixed plant cells. However, the very close juxtaposition of the plasma membrane and inner surface of the cell wall indicates that chemical fixation did not induce extensive plasmolysis (Figs. 1 and 2). Elaborated domains of the plasma membrane were observed. These structures that invaginate into the cytoplasm enclose a space containing numerous vesicles and fibrous inclusions and have been termed plasmalemmasomes or multivesicular bodies by other investigators. By analogy with more extensively characterized structures of animal cells, we have used the term multivesicular bodies in this paper. The vesicular and fibrous contents of the multivesicular bodies are densely labeled by gold particles. In contrast, the contiguous plasma membrane enclosing the vesicular contents was observed to be almost completely devoid of gold particle label (Fig. 2).

Structurally similar multivesicular bodies were observed in the vacuole of the callus cells. However, the intravacuolar multivesicular bodies were enclosed by a double-unit membrane (Fig. 3), compared to the single membrane that encloses the plasma membrane elaborations (Fig. 2). Mab 16.4B4 densely labels the vesicular contents but not the double-unit membrane of the intravacuolar multivesicular bodies (Fig. 3). We infer that these intravacuolar multivesicular bodies are sequestered within the vacuole with the inner membrane derived from the plasma membrane and the outer membrane derived from the tonoplast. Very similar intravacuolar structures including the double-unit membrane result from autophagy of cellular constituents by vacuoles (for examples, see ref. 13). Control-labeling experiments using Mab 40.2B2 which is directed against a bacterial cell wall glycan (36) exhibited only sparse nonspecific labeling of the cell surface (Fig. 4).

Arabinogalactan-Rich Glycoproteins Are Localized on the Cell Surface and Multivesicular Bodies of Leaf Cells

We examined whether the arabinogalactan-rich glycoprotein reactive with Mab 16.4B4 was expressed in differentiated cells, which would facilitate experiments on spatial and temporal regulation. We determined that tobacco leaves contained the epitope reactive with Mab 16.4B4. Tobacco leaf tissues embedded in the acrylic LR White resin were observed to have structural preservation comparable to that which typically results from epoxy resin protocols. However, the hydrophilic properties of acrylic resins yields superior labeling with immunoglobulins. One notable difference compared to epoxy resin-embedded leaf tissues is the high electron density of the chloroplast stroma in LR White-embedded specimens (reviewed in ref. 12). All of the micrographs shown in this paper are densely stained with uranyl acetate and lead citrate to provide sufficient contrast of the immunolabeled structures. The overstaining with electron-dense stains was found to be necessary to visualize the structural details of the immunogold-labeled subcellular constituents. The consequences

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

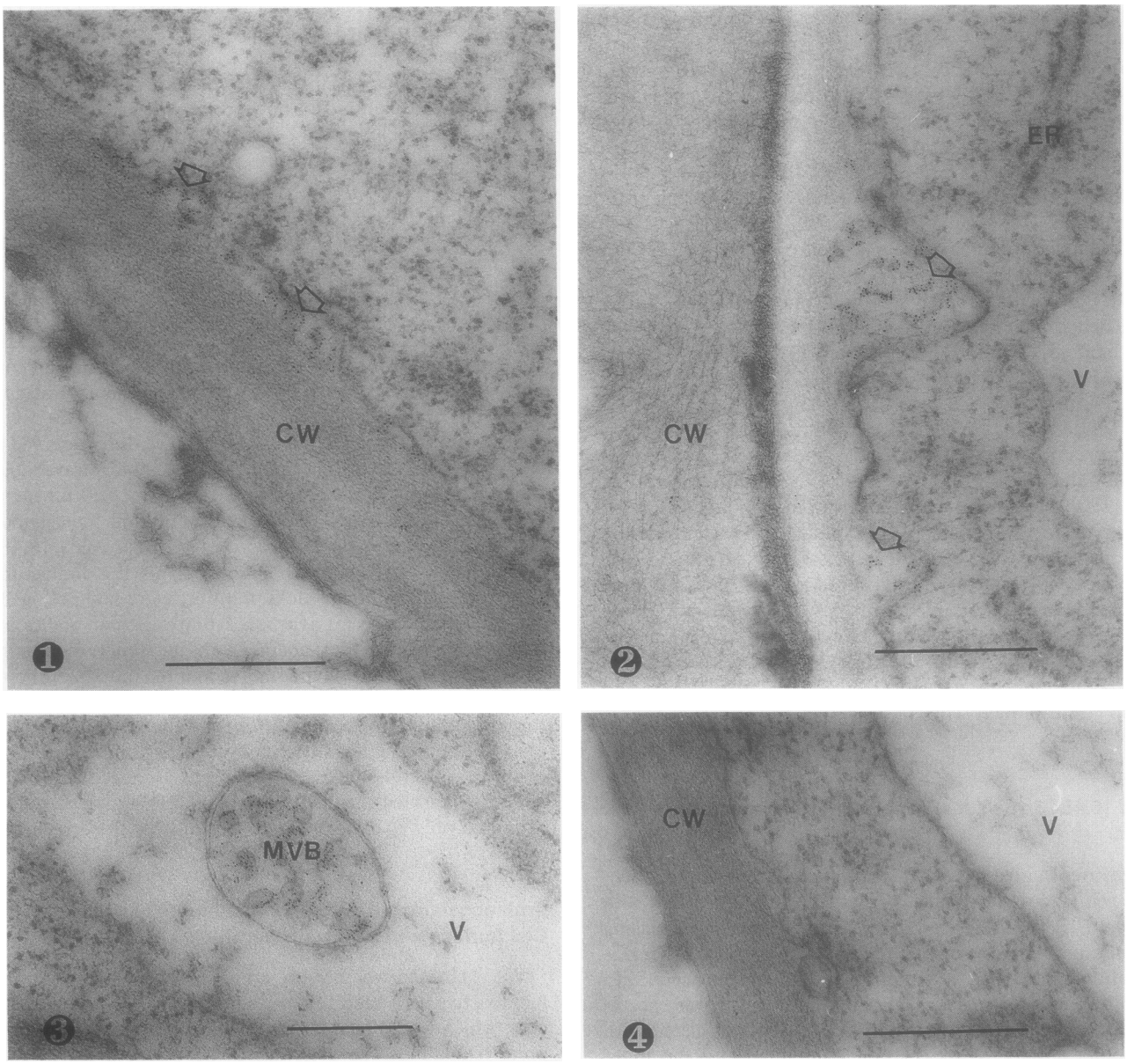


Figure 1. Immunogold labeling of the epitope reactive with Mab 16.4B4 on the plasma membrane of a tobacco callus cell (arrowheads). The gold particles specifically label vesicular and fibrous inclusions in the periplasmic space between the cell wall (CW) and plasma membrane. Only a few apparently nonspecific gold particles are localized on the adjacent cytoplasm and cell wall. ×48,000; bar, 0.5 μm.

Figure 2. The immunogold labeling of invaginations of the plasma membrane, termed plasmalemmasomes or multivesicular bodies, is shown. Note that vesicular contents contained within the plasma membrane invagination are densely labeled. In contrast, very little gold label is observed to be associated with the plasma membrane enclosing the vesicles. Adjacent segments of the endoplasmic reticulum (ER), cell wall (CW), and vacuole (V) are labeled by only a few nonspecific gold particles. ×48,000; bar, 0.5 μm.

Figure 3. A multivesicular body (MVB) apparently sequestered within the vacuole (V) of a tobacco callus cell is shown. Like the plasma membrane invaginations, the vesicular contents of this multivesicular body is densely labeled. Note that the intravacuolar multivesicular body is enclosed by two-unit membranes in contrast to the plasma membrane invaginations which are enclosed by only a single-unit membrane. ×36,000; bar, 0.5 μm.

Figure 4. A portion of a callus cell from a control-labeling experiment using Mab 40.2B2. Note the absence of gold particles on the periplasmic space and a vesicular inclusion (arrowhead). The cell wall (CW) and vacuole (V) are also devoid of gold particle label. ×48,000; bar, 0.5 μm.

of the intense staining is that some organelles, most notably the chloroplasts, are excessively stained in these micrographs.

Indirect immunogold labeling of tobacco leaf tissue confirmed that Mab 16.4B4 was directed at an epitope present at the plasma membrane of differentiated parenchyma cells. However, this ultrastructural analysis revealed that, although this epitope is widely distributed along the external face of the plasma membrane or in the periplasmic space (Fig. 5), antibody reactivity is particularly strong in vesicles that protrude into the periplasmic space. The occurrence and labeling of vesicular structures was particularly marked at the site of triple-cell junctions (Fig. 5) in comparison to adjacent double-cell junctions. Inspection of the periplasm at the triple-cell junction at high magnification indicates that in these regions the majority of the gold particles resulting from immunolabeling are associated with periplasmic inclusions (Fig. 5, solid arrowheads).

Apparent Endocytosis of Multivesicular Bodies Containing Arabinogalactan-Rich Glycoproteins

Abundant immunogold-labeled multivesicular bodies were observed to be associated with the parenchyma cell plasma membrane. Additional multivesicular bodies containing numerous labeled vesicles were often observed in the cytoplasm at sites adjacent to the triple-cell junction of the leaf cell parenchyma (Fig. 5). Figures 6 through 11 are a series of electron micrographs that reconstruct a presumptive pathway for internalization of the leaf cell multivesicular bodies. Figure 6 shows a multivesicular body in which the space containing the vesicular contents is contiguous with the periplasmic space. Mab 16.4B4 labels the vesicular inclusions of the multivesicular body. The periplasmic space adjacent to the multivesicular body is also labeled with gold particles (Figs. 5, 6, 8, and 10). The gold particles on the periplasmic space appear to be associated with the exterior surface of the plasma membrane, the adjacent inner surface of the cell wall, and periplasmic material.

Figure 7 shows another immunolabeled multivesicular body which appears to be almost entirely enclosed by the cytoplasm and the apposed vacuole. This is interpreted as an intermediate structure in the internalization of the multivesicular body. Immunological control specimens labeled with Mab 40.2B2 exhibited a complete absence of gold particles on the multivescular body and periplasmic space (Fig. 14). Figure 8 shows an immunolabeled multivesicular body which appears to be separated from the plasma membrane while invaginating into the vacuole. The origin of the double-unit membrane of the intravacuolar multivesicular body can be seen in Figures 6 through 9. The outer membrane of the multivesicular body is contiguous with the tonoplast (Figs. 6 and 8). Figure 9 shows a multivesicular body that has apparently been sequestered within the vacuole. This multivesicular body does not exhibit any apparent structural disruption. Figure 10 shows another immunogold-labeled multivesicular body sequestered in the vacuole. This multivesicular body exhibits partial disruption of the peripheral membrane and the internal vesicular contents. Apparent leakage of the epitope reactive with Mab 16.4B4 antigen into the vacuolar sap is indicated by the gold particles associated with a juxtaposed vacuole membrane (solid arrowheads). Other multivesicular bodies located in the interior of the vacuole exhibit extensive disruption of the internal vesicles, consistent with the presumptive degradative action of vacuolar hydrolases (Fig. 11). The static micrographs presented in Figures 6 to 11 in an

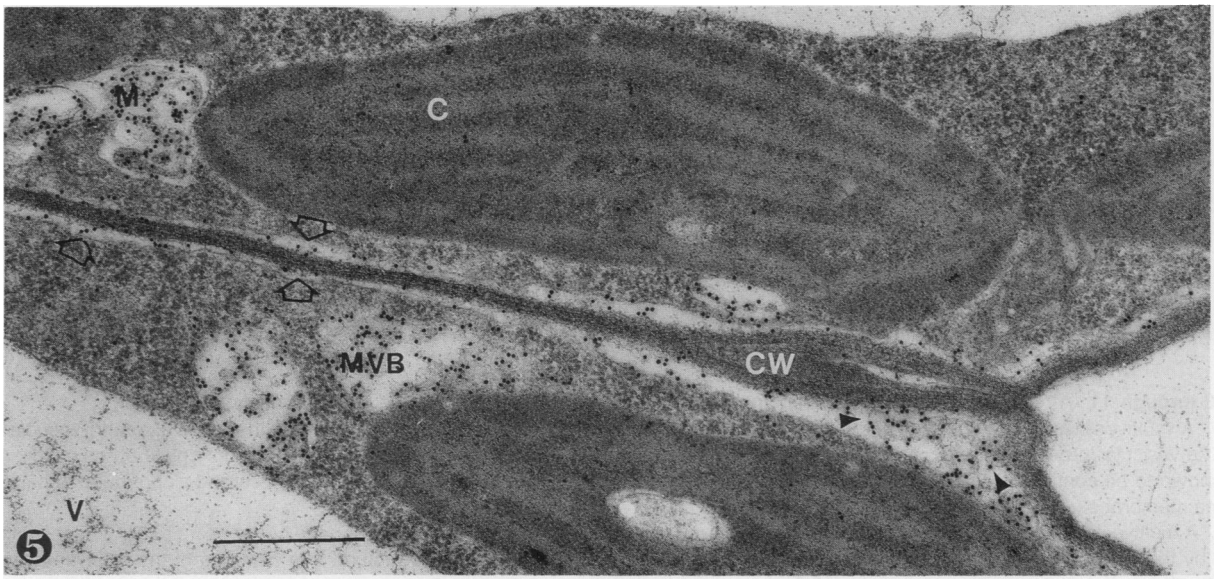
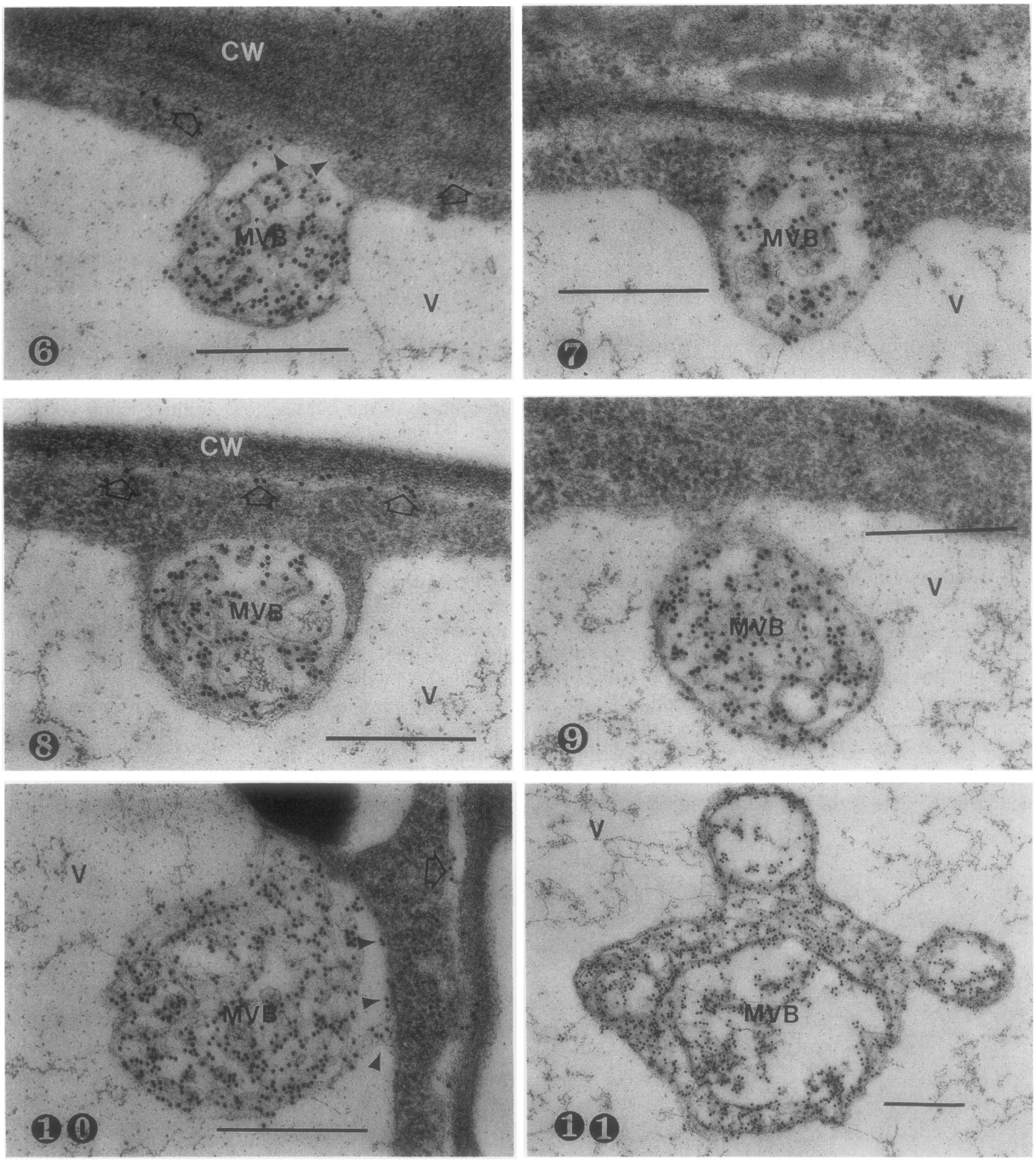


Figure 5. A low magnification micrograph of two tobacco leaf cells at a triple cell junction is shown. Immunogold labeling with Mab 16.4B4 is specifically localized on the periplasmic space of the cells in the triple-cell junction. The label is associated with small vesicular structures marked with solid arrowheads. The vesicular/tubular contents in the multivesicular bodies or plasmalemmasomes (MVB) are also densely labeled. Association of the 16.4B4 antigen with the external face of the plasma membrane is indicated by open arrows. The chloroplasts (C), mitochondria (M), vacuolar contents (V) are not labeled other than by sparse nonspecific gold particles. CW, Cell wall. ×30,000; bar, 0.5 μm.



Figures 6 through 11. A reconstruction of an apparent internalization of multivesicular bodies for vacuolar mediated disposal.

Figure 6. A multivesicular body or plasmalemmasome (MVB) invaginating from the periplasmic space into the cytoplasm and vacuole (V) is shown. The vesicular/tubular contents of the multivesicular body are specifically labeled by Mab 16.4B4. The interior space of the multivesicular body is shown to be contiguous with the periplasmic space (solid arrowheads). Additional gold particles are localized along the external face of the plasma membrane (open arrows). CW, Cell wall. ×47,000; bar, 0.5 μm.

Figure 7. A multivesicular body (MVB) is shown which is apparently closely associated with the cell surface but appears to be almost completely enclosed by the cytoplasm. This is interpreted as an early stage in the internalization of the structure. The contents of the multivesicular body are specifically labeled. V, Vacuole. ×47,000; bar, 0.5 μm.

Figure 8. An immunogold-labeled multivesicular body (MVB) apparently sequestered in the cytoplasm is shown. Additional gold particle labeling is also shown along the external face of the plasma membrane. V, Vacuole; CW, cell wall. Additional gold particles are localized along the external face of the plasma membrane (open arrows). ×47,000; bar, 0.5 μm.

Figure 9. An immunogold-labeled multivesicular body (MVB) apparently sequestered within the vacuole (V) is shown. This multivesicular body does not exhibit any morphological degradation that is a possible consequence of vacuolar sequestration. Gold particle labeling is also localized on constituents of the periplasmic space. ×47,000; bar, 0.5 μm.

apparent ontogenic series are interpreted as representative of stages of a dynamic pathway that mediates the internalization of an apoplastic glycoprotein for vacuolar disposal. Several intravacuolar multivesicular bodies in different apparent stages of degradation are shown in Figure 12. Although the intravacuolar multivesicular bodies are densely labeled with gold particles, these structures are extensively disrupted, consistent with the action of vacuole hydrolases. Further evidence that the immunogold-labeled multivesicular bodies are sequestered in the vacuole is apparent by the observation that these structures are colocalized with intravacuolar protein aggregations as shown in Figure 13. Interestingly, the intravacuolar protein aggregation is not labeled, demonstrating that Mab 16.4B4 reactivity is restricted to those intravacuolar structures that originate from the putative endocytotic pathway. Control labeling experiments with Mab 40.2B2 resulted in specimens in which the plasmalemmasomes and intravacuolar multivesicular bodies were completely devoid of gold label (Fig. 14).

Our attempts to use Mab 27.1E8 to label the core polypeptide of the arabinogalactan-rich glycoprotein were unsuccessful. The core protein is extensively glycosylated (23) which renders the polypeptide epitope reactive with Mab 27.1E8 inaccessible.

DISCUSSION

The observations presented here demonstrate that the glycan epitope reactive with Mab 16.4B4 is highly abundant at the surface of the plasma membrane and in multivesicular bodies often referred to as plasmalemmasomes. Moreover, our data establish that multivesicular vacuolar inclusions in the central vacuole carry the same glycan epitope. The core polypeptide that carries the Mab 16.4B4 epitope is a 50-kD hydrophilic protein especially rich in neutral amino acids which is peripherally associated with the plasma membrane (23). The EM localization of the Mab 16.4B4 epitope on the cell surface, as well as prior fluorescent microscopy observations of tobacco suspension cell protoplasts (23), supports the biochemical characterization of these arabinogalactan-rich glycoproteins as a constituent of the cell surface. Sedgley and Clarke (30) localized arabinogalactan-rich glycoproteins in style cells on the cell surface and in cytoplasmic multivesicular bodies. However, they did not observe significant arabinogalactan-rich glycoprotein concentrations within vacuoles. The size heterogeneity of this family of proteins previously observed in SDS/PAGE immunoblots (23) may reflect processing events associated with the distribution of the protein between the cell surface, plasmalemmasomes/multivesicular bodies, and vacuolar inclusions. Recent results of Stacey *et al.* (31) and Knox *et al.* (18) have shown that members of an arabinogalactan-rich glycoprotein family (26) are regulated in a spatial and temporal pattern in meristematic cells undergoing differentiation. Their observations indicate that individual members of the arabinogalactan-rich glycoprotein family accumulate as cells differentiate. Furthermore, their observations imply that preexisting arabinogalactan-rich glycoproteins must also be removed during differentiation. Whether the loss of a particular epitope is the consequence of the modification or turnover of that specific protein has not yet been determined.

The plasmalemmasomes or multivesicular bodies and plasma tubules have often been observed in cells specialized for high solute fluxes (2, 3, 5, 9, 10, 11, 22, 28) that would require an increase in the available surface area of the plasma membrane. Some investigators have questioned whether plasma membrane elaborations constitute genuine subcellular structures. This question has been partially resolved by Chaffey and Harris (2) who used cryofixation and freeze substitution to demonstrate that plasma tubules were present in cells prepared without the use of chemical fixation, indicating that these structures are not a fixation artifact. Griffing and coworkers (personal communication) observed multivesicular bodies in cryofixed soybean suspension cells, demonstrating that these are genuine structures. The results presented in this paper provide further evidence that multivesicular bodies in plant cells are not artifacts. Even if multivesicular bodies did result from the action of fixatives, it is difficult to envision any mechanism that would form cell surface and intravacuolar inclusions containing the same discretely localized glycan. Fixation artifacts are generally considered to induce a more random and extensively distributed epitope that would be detectable in immunocytochemical assays as gold particles associated with organelles that do not have an ontogenic relationship. Our observations that periplasmic vesicles and multivesicular bodies contain a specific, characteristic epitope provide additional evidence that these structures are not artifacts but are genuine subcellular features.

Our data indicate that Mab 16.4B4 labels the arabinogalactan-rich glycoproteins of intracellular structures, which we have interpreted to be characteristic components of an internalization pathway. We interpret our observations as indicating an ontogenic relationship between these structures such that the multivesicular bodies are internalized for vacuolar mediated destruction. Robards and Kidwai (28) and Marchant and Robards (19) first proposed an ontogenic model in which plasmalemmasomes are internalized for vacuole-mediated disposal. This hypothesis is strongly supported by our immunocytochemical observations of the intracellular distribution of the epitope reactive with Mab 16.4B4. However, endocytosis and consequent vacuolar mediated disposal of extracellular material is still controversial among plant cell biologists. Because our data is derived solely from static electron micrographs, it is difficult to ascertain direction of

Figure 10. A partially disrupted multivesicular body (MVB) sequestered within the central vacuole (V) is shown. Note that the immunogold particles are specifically localized on the apparently partially disrupted and degraded vesicular/tubular inclusions of the multivesicular bodies. The disruption of the vacuole-sequestered plasmalemmasome is interpreted as the consequence of the action of vacuolar hydrolases. Other gold particles are localized on material outside of the plasmalemmasome adjacent to the vacuole membrane (arrowheads). This is interpreted as resulting from the leakage of material from the disrupted plasmalemmasome. ×47,000; bar, 0.5 μm.

Figure 11. A disrupted immunogold-labeled multivesicular body (MVB) located in the interior of a leaf cell vacuole (V) is shown. Note that the contents of the plasmalemmasome are disorganized, which is typical of organelles sequestered in the vacuole for autophagic digestion. ×24,000; bar, 0.5 μm.

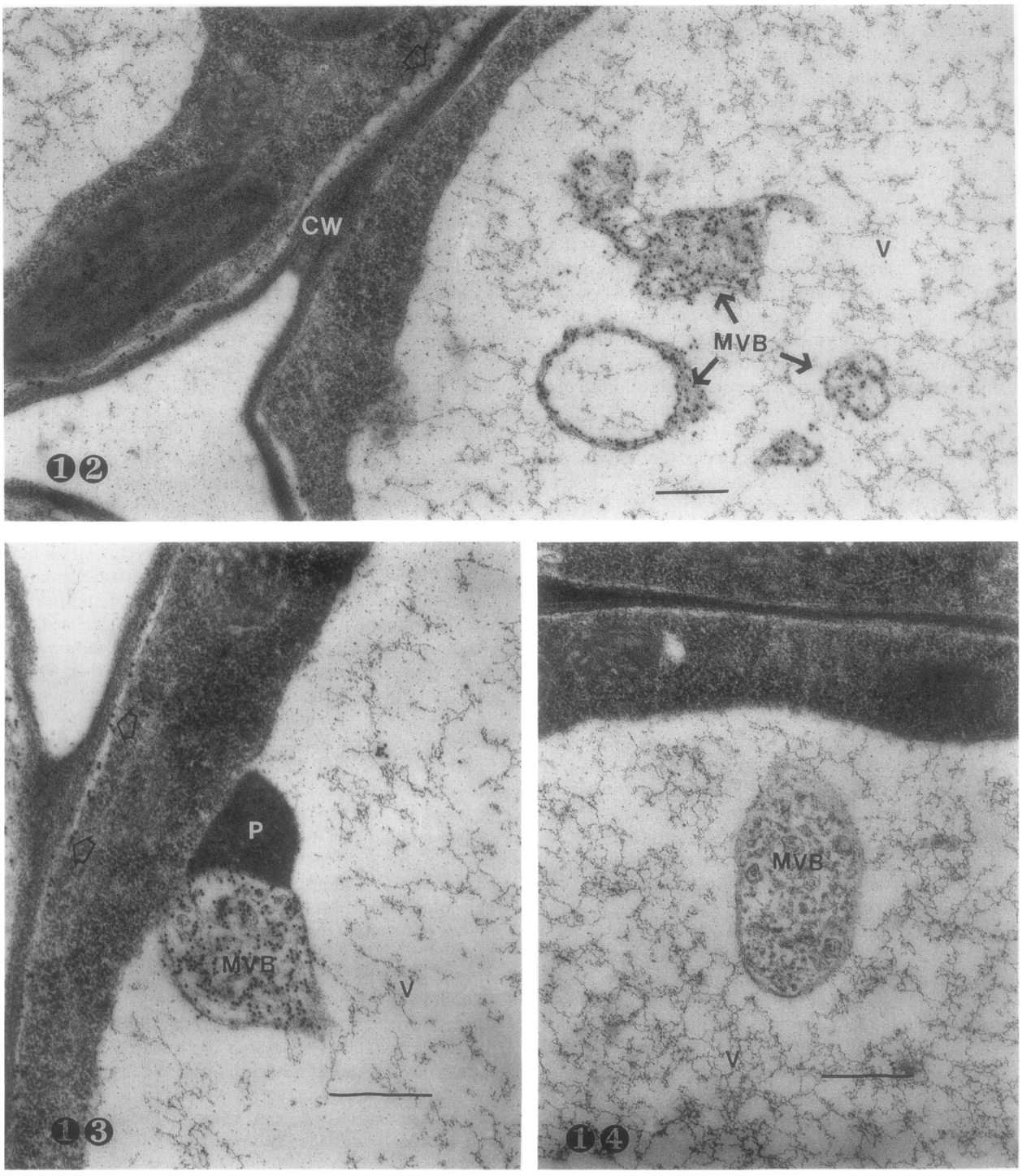


Figure 12. A leaf cell vacuole (V) in which is sequestered four extensively disrupted multivesicular bodies (MVB, arrows) is shown. Note that immunogold label is associated only with disrupted multivesicular bodies. CW, Cell wall. ×24,000; bar, 0.5 μm.

Figure 13. A multivesicular body (MVB) sequestered within the vacuole (V) that is adjacent to an intravacuolar protein aggregation is shown. This demonstrates that the labeled multivesicular body is colocalized with vacuolar proteins in the vacuolar sap. This provides direct evidence that multivesicular bodies are sequestered within the vacuole for disposal. The protein aggregation (P) is not labeled by the Mab 16.4B4. Additional gold label is shown on the periplasmic space (arrows). ×32,000; bar, 0.5 μm.

Figure 14. A multivesicular body (MVB) sequestered within the vacuole (V) is shown. This section was labeled with Mab 40.2B2 to provide an immunological control. Note the complete absence of gold particle label on the vesicular/tubular contents of the multivesicular body and the periplasmic space. This demonstrates the specificity of Mab 16.4B4 in the specifically labeled sections. ×30,000; bar, 0.5 μm.

flow in such an ontogenic reconstruction. Although we have interpreted these observations to represent a multivesicular body-mediated endocytosis pathway, an alternative interpretation of our results is that intravacuolar structures containing arabinogalactan-rich glycoproteins are expelled from the cell. The extensive *O*-linked glycosylation of the arabinogalactan-rich glycoproteins is a biochemical characteristic of cell surface and cell wall proteins, whereas vacuolar proteins have been shown to contain only *N*-linked glycan side chains. This pattern of glycosylation suggests that the arabinogalactan-rich glycoproteins are likely to be primary constituents of the cell surface and become associated with the vacuole only by a secondary process(es).

Plant cell walls are thought to exclude particles larger than 4 nm (1) which should limit the physiological necessity for endocytotic processes during normal growth and development. However, observations of the uptake of cationic ferritin (33, 34), lectin-gold conjugates (14), lucifer yellow dye (25), and heavy metal salts (17) into plant cells have clearly demonstrated endocytosis by the coated vesicle pathway to the partially coated reticulum associated with the Golgi apparatus. Other observations have shown that incubation of plant cells with heavy metal salts results in the sequestering of the metals in the periplasmic space (29, 35) and in small cellular vacuoles (17). In a process closely analogous to endocytosis in animal cells (for review, see ref. 32), the protoplasts of soybean suspension culture cell protoplasts have been shown to take up surface-bound cationic ferritin into multivesicular bodies (27, 33). Griffing and Fowke (7) showed that cytoplasmic multivesicular bodies contain peroxidase activity, an enzyme characteristic of extracellular spaces. Furthermore, the cationic ferritin-labeled multivesicular bodies were shown to be deposited in protoplast vacuoles, which was interpreted to result from the fusion of the multivesicular bodies with the tonoplast (27, 33).

The functional role of the vacuole in autophagic degradation of cellular constituents in plant cells is well documented. Autophagy has been shown to occur during growth and development as well as during senescence (for review, see ref. 20). Recent observations of Melroy and Herman (21) showed that a specific seed tonoplast protein, α-tonoplast intrinsic protein, is internalized for turnover during seedling growth. Furthermore, Hofte *et al.* (15) showed that α-TIP expressed in transgenic tobacco leaves is apparently internalized into the vacuole on multivesicular bodies, which appear to be identical with the intravacuolar multivesicular bodies described in this paper. Although there is only scant physiological information concerning internalization in plants, such an internalization pathway may potentially have important functions during attempted infection, *e.g.* in the internalization of viruses, transduction of elicitor signals, and the lysis of microbial pathogens. Recent observations by Horn *et al.* (16) have shown that fluorescent derivatives of elicitors are internalized by suspension cells and accumulated in the vacuole. Observations by Drews and coworkers (4) suggest that multivesicular bodies or plasmalemmasomes may proliferate as a consequence of infection of soybean leaves by *Pseudomonas*. The identification of the antigen reactive with Mab 16.4B4 as a component of the cell surface and multivesicular bodies will provide a specific marker to study the regulation and development of plasma membrane specializations, particularly during cellular growth and differentiation, as well as in response to stresses such as viral and microbial infection.

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