COATED AND SMOOTH VESICLES IN THE
BIOGENESIS OF CELL WALLS, PLASMA
MEMBRANES, INFECTION THREADS AND
PERIBACTEROID MEMBRANES IN ROOT
HAIRS AND NODULES OF WHITE CLOVER

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SUMMARY

Involvement of coated and smooth vesicles in the infection by rhizobia of root hairs and
nodule cells of white clover was examined using electron microscopy of thin sections. Coated
vesicles, about 100 nm in diameter including coat, were observed free in the plant cell cytoplasm
and associated with root hair plasma membranes, infection thread membranes and peribac-
teroid membranes. The number of coated vesicles seen associated with segments of the plasma
membrane at the tip of the root hair was about 20-fold greater than the number associated
with the plasma membrane towards the base of the root hair. Similarly, the number seen
associated with segments of the infection thread membrane near the tip of the thread was about
20-fold greater than the number associated with the membrane towards the base of the thread.
The number seen associated with segments of the peribacteroid membranes in recently infected
nodule cells was comparable to the number associated with the plasma membrane towards the
base of the root hair or with the infection thread membrane towards the base of the thread.

Coated vesicles were observed in the vicinity of and associated with both proximal and distal
faces of the Golgi bodies; they often possessed, while still attached to the Golgi cisternae,
shorter coats than those observed on coated vesicles free in the cytoplasm. Coated vesicles
contained electron-dense material that did not stain positively with Thieiry stain for poly-
saccharides, and which appeared to have been released into the region of the cell wall or peri-
bacteroid space as a result of fusion of the vesicles with plasma membranes or peribacteroid
membranes.

Smooth vesicles were also observed in the vicinity of and associated with Golgi bodies, and
in association with root hair plasma membranes, infection thread membranes and peribac-
teroid membranes. The smooth vesicles commonly contained material of varying electron
density, which sometimes occurred in the form of droplets. Similar droplets seen in the region
of the cell wall and in the peribacteroid space appeared to have resulted from the fusion of
smooth vesicles with these membranes.

It is proposed that both smooth and coated vesicles derived from Golgi bodies are involved
in the biogenesis of cell walls, plasma membranes, infection threads and peribacteroid mem-
branes in root hairs and nodules of white clover.
INTRODUCTION

Infection of legumes by *Rhizobium* usually occurs near the tips of growing root hairs and involves the invagination of the plant cell wall and plasma membrane to form an infection thread (Robertson & Farnden, 1980). The thread grows through the root cortical cells to the developing nodule meristem where the rhizobia are released from the tip into the cytoplasm of the meristematic cells by endocytosis. During endocytosis each rhizobial cell is surrounded by the infection thread membrane, which then becomes the peribacteroid membrane (Robertson, Lyttleton, Bullivant & Grayston, 1978). The rhizobia and peribacteroid membranes continue to multiply and divide in the nodule cell cytoplasm until there is no space left for further division.

The mechanisms by which the rhizobia trigger the formation of the infection thread and the infection of the meristematic cells are not yet understood (Robertson, Lyttleton & Pankhurst, 1981). Nor is it known to what degree plasma membranes of root hairs, infection thread membranes and peribacteroid membranes differ from each other, though one difference based on evidence from freeze-fracture studies of plasma membranes and peribacteroid membranes has been reported (Robertson et al. 1978). However, it appears likely that the synthesis of both infection threads and peribacteroid membranes involves functional modification by the rhizobia of the plant endomembrane system for plasma membrane and cell wall biogenesis (Robertson et al. 1978; Robertson & Farnden, 1980).

Biogenesis of plasma membranes of plant cells appears to involve fusion with the plasma membrane of both smooth and coated vesicles originating from Golgi bodies (Newcomb, 1980; Ryser, 1979; Whaley & Dauwalder, 1979; Mollenhauer & Morré, 1980). Previously we reported evidence to suggest that Golgi bodies produced smooth vesicles of varying diameter, which fused with the peribacteroid membrane in lupin nodules (Robertson et al. 1978). Here we present evidence that indicates that coated vesicles and smooth vesicles are involved in the biogenesis of the infection thread and peribacteroid membranes, as well as the cell walls and plasma membranes, in root hairs and nodules of white clover. A preliminary report of this work has been presented (Robertson & Lyttleton, 1982).

MATERIALS AND METHODS

Preparation of root and nodule tissue

Seedlings of white clover (*Trifolium repens* L. cv. Grasslands Huia) were grown in microscope-slide chambers using a modified version of the Fåhraeus method (Fåhraeus, 1957; Nutman, 1959; Sahlman & Fåhraeus, 1963). To each microscope slide (26 mm x 75 mm x 0.9 mm) were glued eight glass spacers (5 mm x 5 mm x 0.9 mm) in such a way that two coverslips of different sizes (22 mm x 22 mm; 22 mm x 40 mm) could be supported to form a chamber for root growth (Fig. 1). For studies of roots using the light microscope alone, both coverslips were glued to the spacers. Where root tissue was required for processing for electron microscopy, only the smaller coverslip was glued to the spacers, the larger being placed temporarily in position for examination of the root hairs in the light microscope. The slide chambers were sterilized in Petri dishes using dry heat and all subsequent steps were carried out aseptically.
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Fig. 1. A microscope-slide chamber containing a nodulated white clover seedling grown for three weeks after inoculation with *R. trifolii* NZP561. Actual size.

Plant nutrient medium was prepared by diluting stock solutions and contained, per litre: 75 mg KCl, 246 mg MgSO₄·7H₂O, 102 mg KH₂PO₄, 43 mg Na₂HPO₄, 5 mg FeCl₃·6H₂O, 2·9 mg H₃BO₃, 1·8 mg MnCl₂·4H₂O, 0·4 mg ZnSO₄·H₂O, 0·08 mg CuSO₄·5H₂O, 0·1 mg CoSO₄·7H₂O, 0·05 mg Na₂MoO₄·2H₂O. After autoclaving and cooling, sterilized CaCl₂ solution was added to give a final concentration of 147 mg CaCl₂ per litre. This nutrient medium was 20 mosM (pH 6·5) and contained only a very slight sediment. To prepare nutrient agar medium for the slide chambers, 0·35 % agar (Davis Gelatine (N.Z.) Ltd) was included in the mixture prior to autoclaving. The CaCl₂ solution was then added on cooling to 35 °C at which temperature 0·4 ml of the liquid nutrient agar medium was pipetted underneath the smaller coverslip of the slide chamber.

Seeds of white clover were surface-sterilized for 6 min in concentrated sulphuric acid, washed five times with sterile distilled water over a period of 45 min and incubated for 24 h on inverted, tap-water agar plates at 26 °C in the dark. Seedlings, approximately 5 mm in length, were transferred with a small spatula to the still liquid agar in the slide chambers. After the agar had set the slides were transferred to sterile test tubes (30 mm x 150 mm) containing approximately 25 ml of plant nutrient medium, sufficient to cover the lower edge of the smaller coverslip. The tubes were plugged with cotton wool and placed in a controlled environment room with a 12-h day length, a day/night temperature and relative humidity regime of 25/21 °C and 86/92 % RH and a light intensity of 150 Wm⁻¹ over a spectrum range of 390 to 780 nm.

Samples of root tissue were taken for electron microscopy at intervals following emergence of the root tip below the edge of the smaller coverslip. Emergence usually occurred 3–4 days after insertion of the seedling radicle into the slide chamber. Some of the slide chambers containing seedlings with emerging root tips (1–2 mm) were replaced in sterile Petri dishes. The root tips were inoculated with a suspension of *Rhizobium trifolii* NZP561 that had been cultured to
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mid-log phase in yeast extract medium (Keele, Hamilton & Elkan, 1969), harvested at 16,000 rpm for 10 min and washed three times by resuspending in the plant nutrient medium. Following inoculation, the larger coverslips were put in position to prevent drying of the root tips. After 1 h the coverslips were removed, excess inoculum was washed off with sterile nutrient medium and the slides replaced in the test tubes and returned to the growth room. The slide chambers were removed from the tubes at intervals over a period of 24-48 h and examined in the light microscope under phase-contrast to monitor root hair development and infection prior to taking samples for electron microscopy. At this stage aseptic conditions were maintained as nearly as possible.

Samples of root tips for electron microscopy were also taken from uninoculated seedlings grown from seeds incubated for 36 h at 26 °C in the dark on filter paper soaked with sterilized tap-water.

Root nodules were obtained from white clover plants grown for 35 days in pumice culture (Boland, Fordyce & Greenwood, 1978) following inoculation of seedlings with R. trifolii NZP566.

Figs. 2-25. Abbreviations used: b, bacteroid; cv, coated vesicle; cw, cell wall; d, droplet; g, Golgi body; it, infection thread; itm, infection thread membrane; itw, infection thread wall; ma, matrix material; n, nucleus; pbm, peribacteroid membrane; pbs, peribacteroid space; pl, plasma membrane; r, rhizobium; sv, smooth vesicle.

Figs. 2, 3, 4, 7, 10. Thin sections of young root hairs from white clover seedlings grown in slide chambers. Stained with uranium and lead.

Figs. 5, 6, 8, 9. Thin sections of young root hairs from white clover seedlings grown on filter paper. Stained with uranium and lead.

Figs. 2-5. Thin sections of the tip regions of root hairs.

Fig. 2. Coated vesicles are present free in the cytoplasm and associated (arrows) with the plasma membrane. A large invagination of the plasma membrane contains fibrous material and droplets. Smooth vesicles present in the cytoplasm are associated with the plasma membrane (double arrowheads) with the apparent release of contents, occasionally in the form of a droplet (large arrowhead). × 34,000.

Fig. 3. Coated vesicle near the plasma membrane at high magnification. × 126,000.

Fig. 4. Association of many coated vesicles (arrows) with the plasma membrane when in close apposition to the cell wall, but not with the large vesicles or invaginations of the plasma membrane. × 21,000.

Fig. 5. Coated and smooth vesicles in the region of a Golgi body sectioned near the outer edge of the cisternae. Some smooth vesicles contain distinct droplets (double arrowhead) or electron-dense material (arrowhead) (enlarged in Fig. 8). Droplets of varying electron density lie in the region of the cell wall. Association of a coated vesicle with the neck region of a large invagination of the plasma membrane (arrow) is evident. × 29,000.

Fig. 6. Coated vesicle associated with the plasma membrane, with the contents in the cell wall region (arrow). × 61,000.

Fig. 7. Droplet or vesicle within a vesicle in the cytoplasm of a root hair. × 105,000.

Fig. 8. Area outlined in Fig. 4 at higher magnification, showing a smooth vesicle with an internal droplet (double arrowhead) and another vesicle containing electron-dense material (arrowhead). × 73,000.

Fig. 9. Smooth vesicle associated with the plasma membrane with contents present in the form of a droplet or vesicle in the cell wall region (arrowhead). × 21,000.

Fig. 10. Coated vesicles in the region of a Golgi body near the base of a root hair and adjacent to a cortical cell. Some of the coated vesicles are clearly attached to smooth vesicles (arrowheads) and polygonal units of the coat are visible (double arrowhead). Only one association (arrow) of a coated vesicle with the plasma membrane is evident. × 46,000.
Electron microscopy

Roots of inoculated and uninoculated seedlings grown in the slide chambers were processed approximately 42 h following emergence of the tip below the edge of the smaller coverslip. The roots were flooded with 3% glutaraldehyde in 0.025 M Na, K phosphate buffer (pH 6.8). This and all subsequent steps were carried out at 18–22 °C. The roots were cut sequentially into 1-2 mm pieces, evacuated in fresh fixative for 10 min, transferred to fresh fixative for 1 h, washed in phosphate buffer three times, fixed in 2% OsO₄ in phosphate buffer for 1 h, washed in 1% NaCl three times, immersed in 0.5% magnesium uranyl acetate in 1% NaCl for 1 h, dehydrated in acetone, and infiltrated and embedded in Epoxy resin (Spurr, 1969) in rubber moulds (3 mm x 3 mm x 10 mm) in which a 1.5 mm layer of resin had been previously polymerized. The embedded specimens were examined under bright field to locate infection threads in the root hairs.

Root tips from seedlings grown on filter paper were immersed in 3% glutaraldehyde in 0.05 M piperazine-N,N-bis-2-ethane sulfonic acid (PIPES) buffer (pH 6.8) (Salama & Brandão, 1973) cut into 1-2 mm pieces, evacuated in fresh fixative for 15 min, transferred to fresh fixative for 1.5 h, then washed in 0.05 M PIPES buffer three times and in 0.025 M Na, K phosphate buffer three times. Fixation in OsO₄ and subsequent steps were carried out as before.

Root nodules were sliced longitudinally into a modified Karnovsky (1965) fixative containing 3% glutaraldehyde, 2% formaldehyde, 0.1 M Na, K phosphate buffer (pH 7.2), evacuated in fresh fixative for 5 min, transferred to fresh fixative for 1 h at 4 °C and 1 h at 22 °C, washed in phosphate buffer three times, fixed in 1% OsO₄ in phosphate buffer, washed in 1% NaCl three times, immersed in 0.5% magnesium uranyl acetate in 1% NaCl for 1 h, dehydrated in ethanol and propylene oxide and embedded in Epon 812. Thin sections cut with a diamond knife were mounted on unsupported copper grids and stained at 18–22 °C with saturated uranyl acetate in 50% ethanol for 5 min, followed by lead citrate (Venable & Coggeshall, 1965) for 3 min. Sections mounted on unsupported gold grids were stained for polysaccharides (Thiery, 1967; Robertson, Lyttleton, Williamson & Batt, 1975) by floating grids on 1% periodic acid for 30 min, 0.2% thiocarbohydrazide in 20% acetic acid for 22 h, and 1% silver proteinate for 30 min, with appropriate washing steps between each reagent. Sections were examined with a Philips EM 200 or 201c microscope at 60 kV.

Numbers of coated vesicles associated with membranes were determined by counting the number of obvious associations in micrographs over a range of lengths from 2.5 to 30 μm of a particular membrane. Associations were expressed as coated vesicles/μm membrane. The word association is used in the sense that coated or smooth vesicles were seen at stages of either fusion or budding (Ryser, 1979).

RESULTS

Coated and smooth vesicles in the biogenesis of root hairs

Electron micrographs of thin sections of very young root hairs of white clover showed the presence of a large number of coated vesicles in the cytoplasm (Figs. 2-5). The range in diameters, including coats, for 31 coated vesicles was from 72 to 103 nm. The range in diameters of the vesicles of 14 of the most clearly defined of these coated vesicles was from 26 to 51 nm with a range in the length of the coats from 21 to 31 nm. Many of these coated vesicles were observed free in the cytoplasm in the vicinity of Golgi bodies, associated with smooth vesicles in the vicinity of Golgi bodies (Figs. 5, 10) or associated with the Golgi cisternae themselves. Coated vesicles were also observed associated with the plasma membrane (Figs. 2, 4). The electron-dense contents of the coated vesicles (Fig. 3) were occasionally observed in the form of
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droplets in the region of the cell wall (Fig. 6). Segments of plasma membrane showing relatively high numbers of associated coated vesicles (1.7 coated vesicles/\mu m plasma membrane) were noted near the tip of the root hair (Fig. 4). However, behind the tip (Fig. 10) the number of associations was much lower (0.1 coated vesicles/\mu m plasma membrane). Coated vesicles were rarely observed associated with the large invaginations of the plasma membrane, which were commonly seen at the tip of the root hair (Figs. 2, 4) and where observed, the associations occurred in the neck region of the invaginations (Fig. 5).

The micrographs also showed the presence of smooth vesicles of variable diameter (100–300 nm) containing material which varied in electron density between vesicles (Figs. 2, 4, 5, 7, 10). Many of these smooth vesicles were observed either attached to or in the vicinity of Golgi bodies (Figs. 4, 5, 10). Some very large smooth vesicles (Fig. 4), which appeared to have been formed by coalescence of smaller smooth vesicles (Fig. 2), opened out onto the plant cell wall to form invaginations of the plasma membrane (Figs. 2, 4). These invaginations apparently flattened out (Fig. 5) as the root hair grew, since they were rarely observed behind the tip. They often contained loose fibrous material and round electron-dense bodies or droplets of varying sizes (Figs. 2, 5). These droplets were similar in appearance to those observed in smooth vesicles in the cytoplasm. The contents of these vesicles sometimes possessed a distinct boundary, giving the impression of a droplet or vesicle within a vesicle (Figs. 5, 7, 8). Smooth vesicles containing droplets were also observed in association with the large invaginations and with the plasma membrane when in apposition to the root hair cell wall (Figs. 2, 9). Droplets in the cell wall region adjacent to the plasma membrane were more distinct than those remote from the plasma membrane (Figs. 2, 4, 5).

Coated and smooth vesicles in the biogenesis of infection threads

The process of root hair infection in white clover involves the initial curling of the root hairs around rhizobia attached to the exterior surface of the root hair wall, followed by the invagination of the cell wall and plasma membrane to form an infection thread, which grows down through the root hair cytoplasm (Robertson & Farnden, 1980; Callaham & Torrey, 1981). Electron micrographs of sections of root hairs, near the region at which the infection thread originated, showed rhizobia enclosed in a layer of matrix material, infection thread wall and an infection thread membrane (Fig. 11). Coated vesicles were present in the cytoplasm surrounding the infection thread (Fig. 11) and occasionally observed associated with the infection thread membrane (0.1 coated vesicles/\mu m infection thread membrane). Cross-sections near the tip of the infection thread, still within the root hair, showed matrix material but very little cell wall material (Fig. 13). In contrast with the base of the infection thread, associations of coated vesicles with segments of membrane near the tip (Figs. 12, 13) were much more frequent (2.3 coated vesicles/\mu m infection thread membrane).

Smooth vesicles also occurred in the cytoplasm around the infection threads (Figs. 11, 13) and often appeared in association with the infection thread membrane (Figs. 12, 13).
Coated and smooth vesicles in the biogenesis of peribacteroid membranes

Following entry of the infection thread into the region of the nodule meristem in the root cortex, the rhizobia in the tip of the thread move into the plant cytoplasm by endocytosis. Electron micrographs of this process commonly showed coated vesicles free in the cytoplasm (Figs. 14–16) and also associated with infection thread membranes and peribacteroid membranes (Figs. 14, 17). The number associated with the peribacteroid membranes varied at this early stage of cell infection but was never greater than 0.5 coated vesicle/µm peribacteroid membrane (Figs. 14, 25). The range in diameters, including coat, of 41 coated vesicles was 73 to 115 nm. The range in diameters of the vesicles of 19 of the most clearly defined coated vesicles was 31 to 63 nm with a range in coat lengths from 21 to 28 nm (Figs. 15, 16). Occasionally coated vesicles of even larger diameter, about 140 nm including coat, were observed free in the cytoplasm and associated with the peribacteroid membrane (Fig. 17).

Coated vesicles associated with peribacteroid membranes were frequently observed to contain electron-dense material (Fig. 18). Occasionally this material was not evident (Fig. 19) and sometimes the amount of material was large in relation to the apparent volume of the associated coated vesicle (Fig. 20). In newly infected nodule cells coated vesicles were observed associated with plasma membranes as well as with peribacteroid membranes in close proximity (Fig. 25).

Coated vesicles were commonly observed in the vicinity of and associated with Golgi bodies adjacent to the tips of infection threads (Fig. 21) and throughout the cytoplasm of infected cells (Figs. 15, 22, 23, 25). They appeared as coated evaginations of cisternae on both proximal and distal faces of the Golgi bodies (Fig. 22). In some cases these coated evaginations occurred on large smooth vesicles in the vicinity of, or attached to, the Golgi cisternae (Fig. 23). The coats of the evaginations were in the range from 7 to 14 nm, which was noticeably shorter than the length of the coat of vesicles free in the cytoplasm (Figs. 15, 22, 23).

When thin sections were stained for polysaccharides using the Thiéry (1967) method, the membranes of the coated vesicles and also the peribacteroid membranes stained positively, while the coats and contents of the coated vesicles did not stain (Fig. 24).

Smooth vesicles of varying size, which frequently, but not always, contained electron-dense material, occurred in the cytoplasm of infected cells in the vicinity of peribacteroid membranes (Figs. 14, 25) and also in the vicinity of Golgi bodies (Fig. 21).

Figs. 11–13. Cross-sections of infection threads in root hairs of white clover seedlings grown in slide chambers. Stained with uranium and lead.
Fig. 11. Infection thread close to the origin of the thread on the root hair cell wall. The rhizobium is surrounded by matrix material and the infection thread wall. Coated and smooth vesicles are present near the infection thread membrane. ×25,000.
Fig. 12. Coated vesicle (arrow) and smooth vesicle (double arrowhead) associated with the infection thread membrane near the tip of the thread. ×83,000.
Fig. 13. Coated vesicles (arrows) and smooth vesicles (double arrowheads) associated with the infection thread membrane near the tip of the thread. ×26,000.
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The membranes and, in some cases, the contents of these smooth vesicles stained positively for polysaccharides. In some vesicles the stained contents resembled a loose network, in others a droplet (Fig. 24). Droplets were also observed in the peribacteroid space and sometimes in invaginations of the peribacteroid membranes (Figs. 14, 25).

DISCUSSION

This study provides evidence that both coated and smooth vesicles are involved in the biogenesis of infection threads and peribacteroid membranes, as well as cell walls and plasma membranes in white clover. The coated vesicles, of diameter 73–115 nm including the coat, appear similar to coated vesicles reported in other vascular plants (Newcomb, 1980). It is not clear whether the somewhat larger coated vesicles observed in association with the peribacteroid membranes (Fig. 17) represent a different population of vesicles from those observed in root hairs (Fig. 3).

Coated vesicles were most commonly observed in the vicinity of Golgi bodies in root hairs and nodule cells, and near the plasma membrane of the root hair tip and infection thread tip of white clover. This distribution of coated vesicles between possible sites of origin and destination is similar to that observed in growing root hairs of radish (Bonnett & Newcomb, 1966) and in phloem cells of *Cucurbita* (Cronshaw & Esau, 1968). In the present study coated vesicles were associated with cisternae on both proximal and distal faces of the Golgi bodies. Ryser (1979) reported that coated vesicles seemed to bud from the most distal cisternae of the Golgi stacks in cotton fibres.

Although not all coated vesicles associated with Golgi bodies are necessarily involved in a transport system to a target membrane, some being possibly associated with an internal transfer system (Rothman, 1981), a direction of flow of vesicles from the Golgi to the root hair plasma membrane, to the tip of the infection thread or to the peribacteroid membrane, is suggested by the following evidence. Firstly, it seems likely that the Golgi bodies are sites of origin of coated vesicles, since coated evagina-

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Figs. 14–21. Thin sections of the early infection zone of white clover nodules from 35-day plants grown in pumice culture. Stained with uranium and lead.

Fig. 14. Rhizobia at the point of release by endocytosis from the infection thread. Coated vesicles are present in the cytoplasm and associated with the peribacteroid membrane (arrows). Droplets, some of which appear to have originated from associated smooth vesicles (arrowheads), are evident in the peribacteroid space. ×39,000.

Fig. 15. Coated vesicle in the vicinity of the plasma membrane and a Golgi body in an infected nodule cell. The length of the coat of the vesicle is about 24 nm in comparison with coats of about 10 nm on the evaginations (arrowheads) of the Golgi body. ×81,000.

Fig. 16. Coated vesicle close to the peribacteroid membrane. ×81,000.

Fig. 17. Large coated vesicle associated with the peribacteroid membrane. ×81,000.

Figs. 18–20. Coated vesicles (arrows) associated with peribacteroid membranes showing differences in the size of the coated invaginations and in the electron density of the contents. ×81,000.

Fig. 21. Golgi bodies with associated coated and smooth vesicles near the tip of an infection thread. A smooth vesicle contains a droplet (arrow). ×45,000.
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tions of the cisternae possessed shorter coats than those occurring on vesicles free in the cytoplasm or associated with other membranes. This is consistent with the suggestion of van der Valk & Fowke (1981) that maturation of coated vesicles was completed after the vesicles were pinched off from the Golgi cisternae. Secondly, the coated vesicles contained dense material, which was often observed in what appeared to be a stage of release from these vesicles after fusion with the target membrane (Figs. 6, 12, 18). A similar observation in bindweed was interpreted as indicating fusion of coated vesicles with the plasma membrane (Bonnett, 1969; Newcomb, 1980). We obtained no evidence to support the view that coated vesicles move in the reverse direction, though the possibility cannot be ruled out at this stage.

The involvement of Golgi-derived smooth vesicles in the biogenesis of root hair plasma membranes and cell walls, infection threads and peribacteroid membranes in white clover was indicated by the following observations: smooth vesicles, still attached to, or in the vicinity of, Golgi bodies, often contained material of varying electron density (Figs. 22, 23), which sometimes resembled droplets (Figs. 21, 25); smooth vesicles containing similar material occurred in the cytoplasm (Figs. 7, 8, 24, 25); invaginations of plasma membranes and peribacteroid membranes containing electron-dense material, sometimes in the form of droplets, were frequently observed (Figs. 24, 25) and it seems likely that these invaginations represent sites of fusion of smooth vesicles with concomitant release of contents. This concept of a flow of smooth vesicles from the Golgi bodies to the plasma membranes has been proposed in studies of a variety of plant systems, including root hairs (Sievers, 1964; Bonnett & Newcomb, 1966; Evert & Eichhorn, 1974; Ryser, 1979; Mollenhauer & Morré, 1966, 1980). It has been suggested that movement of smooth vesicles containing fibrous material away from the tip of the infection thread occurs in nodules of soybean (Bassett, Goodman

Figs. 22–25. Thin sections of the early infection zone of white clover nodules from 35-day plants grown in pumice culture.

Fig. 22. Coated vesicles associated with both faces of a Golgi body in an infected cell. A coated vesicle with a short coat is associated with a Golgi cisterna (arrow). Smooth vesicles in the vicinity of the Golgi contain electron-dense material. Stained with uranium and lead. × 44000.

Fig. 23. Golgi body in an infected cell. A smooth vesicle associated with the cisterna shows an evagination with a short coat (arrowhead). A similar smooth vesicle contains a droplet (arrow). Stained with uranium and lead. × 43000.

Fig. 24. Thin section stained with silver by the Thiéry (1967) method for polysaccharides showing a coated vesicle and smooth vesicles close to or associated with the peribacteroid membrane (double arrowheads). The membranes and contents of the smooth vesicles are stained with silver as is the membrane of the coated vesicle. The coat and the contents of the coated vesicle are unstained. One smooth vesicle contains material resembling a droplet (arrow), while in others the contents form a loose network. × 45000.

Fig. 25. Bacteroid and Golgi body in close proximity to the plasma membrane. Coated vesicles are observed associated with the Golgi, free in the cytoplasm and associated with the peribacteroid membrane and the plasma membrane (arrows). Note the presence in the peribacteroid space of a droplet apparently originating from a smooth vesicle associated with the peribacteroid membrane (arrowhead). Stained with uranium and lead. × 48000.
& Novacky, 1977) and pea (Kijne & Planqué, 1979), as part of a system of degradation of thread wall material. Although such a mechanism may occur, a system of net synthesis of infection threads and peribacteroid membranes must also exist and the present results, together with those obtained in lupin (Robertson et al. 1978), strongly suggest that this is by way of fusion of smooth and coated vesicles derived from Golgi bodies.

Although the role of coated vesicles in plants has yet to be defined, they are clearly involved in cell wall and plasma membrane biogenesis (Franke & Herth, 1974; Ryser, 1979; Newcomb, 1980). Bonnett & Newcomb (1966) did not record a higher frequency of fusion of coated vesicles with the plasma membrane at the tip of the root hair in radish compared with other regions of the hair. However, in later studies Newcomb (1980) reported the presence of patches of polygonal coat material close to the plasma membrane, suggesting a high rate of fusion of coated vesicles with this membrane in rapidly growing cells. In white clover the number of coated vesicles associated with segments of the plasma membrane near the tip of the root hair and with the membrane at the tip of the infection thread was 20-fold higher than at other regions of the root hair and on the peribacteroid membranes, which have no adjoining cell walls. Although the interpretation of these results is complicated by a lack of knowledge of the relative rates of synthesis of the different membranes, they support the idea that coated vesicles in white clover play a role in the synthesis of primary cell wall material, as well as membrane synthesis, in agreement with Ryser (1979) working with cotton fibres. Coated vesicles may also play a role in the synthesis of the matrix material in the infection thread (Fig. 13). However, the origin and nature of this material is uncertain (W. Newcomb, 1981). The fact that the contents of the coated vesicles did not stain for polysaccharides (Fig. 24) and appeared to disperse following release into the cell wall space, is consistent with the contents being proteinaceous. Callaham & Torrey (1981) have considered the possible role of hydrolases in the infection of legumes by rhizobia. Coated vesicles could play a role in the transport of these and other enzymes to the region of the cell wall (Bonnett & Newcomb, 1966; Bonnett, 1969) and also to the infection thread and peribacteroid space.

The concept that coated vesicles fuse with the various membranes in roots and nodules of white clover (Fig. 25) raises the question of how these membranes, and the coated vesicles themselves, differ from each other and whether there is a system of recognition or direction that ensures that they arrive at the correct destination. One possibility might be that the coated vesicles are directed to a particular site by a cytoskeletal system (Hyams & Stebbings, 1979; Seagull & Heath, 1980). Coated vesicles near the tip of root hairs appeared to have fused with the plasma membrane when in close apposition to cell wall material, but not with the large smooth vesicles or invaginations (Figs. 2, 4). Apparently these large vesicles did not possess, at least for a short time after fusion with the plasma membrane, the system of recognition or direction to allow fusion of coated vesicles.

In conclusion, a definition of the role played by coated and smooth vesicles in the system of cell wall and membrane biogenesis is essential to our understanding of the processes of infection and nodule development in the legume–Rhizobium symbiosis.
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