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Review article

# Peribacteroid Membrane Biogenesis in Mature Legume Root Nodules

ROBERT B. MELLOR and DIETRICH WERNER

*Department of Botany, Phillips University*
*D-3550 Marburg an der Lahn, Federal Republic of Germany*
*Tel. (06421) 282066; Telex 482372*

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**Abstract**

The origin and proliferation of the peribacteroid membrane in legume root nodules after cell colonization is reviewed. Evidence from electron microscopial and biochemical studies indicate that the theory on peribacteroid membrane biogenesis from the plasmamembrane is no longer tenable. A pluralistic alternative to this model is discussed.

Keywords: biogenesis, (*Brady)rhizobium*, compartmentation, fractionation, legumes, $N_2$-fixation, nodules, peribacteroid membrane, symbiosis.

Abbreviations: CDP: cytidine diphospho-; CURL: compartment of uncoupling of receptor and ligand; DMP: dolichol monophosphate; ER: endoplasmic reticulum; GDP: guanosine diphospho-; GERL: Golgi endoplasmic reticulum lysosome; Mito: mitochondria; PBM: peribacteroid membrane; TCA: Trichloroacetic acid; Tono: tonoplast

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## 1. Introduction

The infection of legumes by *Rhizobium* and *Bradyrhizobium* can be regarded as a beneficial plant disease (Vance, 1983). Recognition between symbiotic partners may involve lectins (Dazzo, 1981). Cell colonization is accomplished normally via an infection thread (Bauer, 1981). In, or during release from the infection thread, the bacteria differentiate into bacteroids (Sutton et al., 1981) whereupon all fix$^+$ and some fix$^-$ strains are surrounded by a host derived membrane, termed the peribacteroid membrane (Newcomb, 1981; Robertson et al., 1984). Due to the ease of cell fractionation of mature nodule tissue (see Mellor and Werner, 1986a), this system is attracting attention not only from workers interested in $N_2$-fixation and signal exchange between symbiotic partners, but also those interested in plant organelle development.

## 2. The Peribacteroid Membrane

The peribacteroid membrane was first observed by Bergersen and Briggs (1958) and consists of a conventional membrane bilayer 9–10 nm thick (Dart and Mercer, 1963). The membrane may play a part in protecting the bacteria from plant defence mechanisms (Vance, 1983), particularly since bacteroids which lie naked in the host cytoplasm elicit a host phytoalexin response (Werner et al., 1985). To date all effective (fix$^+$) symbioses have been found to have structurally intact peribacteroid membranes surrounding the prokaryote. It has been postulated that early in colonization the bacteria signal the host cell to induce peribacteroid membrane production, and this programme then runs independent of bacteroid proliferation (Regensburger et al., 1986). Plant genotypes which are not able to respond to bacterial messages are also known (Vance and Johnson, 1983). The prokaryotic genes responsible for the production of this undefined message are at present unknown. In ineffective associations this process may be modified, i.e. the fix$^-$ strain *Bradyrhizobium japonicum* RH 31 Marburg (Werner et al., 1984) induces only 2 of 4 membrane-building enzyme activities in soybean to the same extent as the nif$^+$ fix$^+$ wild type (Mellor and Werner, 1985; Mellor et al., 1986a). Furthermore, the peribacteroid membrane from nodules infected with strain RH 31 Marburg is different from that of nodules infected with the wild type 61-A-101 strain (Werner et al., 1986). Peribacteroid membrane envelopes may exist empty (Regensburger et al., 1986), or with disintegrated bacteroids inside (Basset et al., 1977) or appearing normally inhabited in a

host cell which also contains larger vacuoles (Werner et al., 1984), or may be absent, the bacteria lying naked in the cytoplasm (Werner et al., 1980). Peribacteroid membranes from ineffective symbioses have not yet been thoroughly enough studied to draw authoritative conclusions, however Werner et al. (1986) have shown both particle density and protein pattern to be different between peribacteroid membranes from effective ($fix^+$) and ineffective ($fix^-$) symbioses. Differences in the morphology of the host cell and peribacteroid membrane can also arise from bacterial factors unassociated with nod or fix genes (i.e. Arwas et al., 1985; Carter et al., 1978; Finan et al., 1983). Bacteroid and peribacteroid membrane replication are relatively independant of each other because when bacteroids replicate faster than peribacteroid membrane, it results in several bacteroids in one membrane envelope (see Verma and Long, 1983). Robertson and Lyttleton (1984) have suggested this is due to bacterial adhesion to the peribacteroid membrane, preventing peribacteroid membrane replication. Conversely fusion of peribacteroid envelopes may also occur (Newcomb and McIntyre, 1981).

## 3. Models of Peribacteroid Membrane Biogenesis

Bacteroids released into the host cell are surrounded by the infection thread membrane. This membrane is made from Golgi apparatus (Robertson et al., 1978a). A relationship(s) between the infection thread membrane and the plasmamembrane has not been definitely established, since the infection thread membrane has never been isolated. Thus although the two membranes may exist *in continuum* with each other, it is erroneous to think of this first invagination as actually being plasmamembrane. Not only do the two membranes fulfill different functions, but there is also a difficulty in accurately defining the plasmamembrane, due to it being spatially differentiated into patches (Galbraith and Northcote, 1977).

Peribacteroid membranes are host plant derived (Verma et al., 1978; Mellor et al., 1985). There are two basic models of peribacteroid membrane biogenesis (Figure 1). The model labelled "recycling" states that the peribacteroid membrane is derived in an as yet undefined manner, directly from the plasmamembrane (Bergersen and Briggs, 1958; Goodchild and Bergersen, 1966; Dixon, 1967; Newcomb, 1976; Verma et al., 1978; Blumwald et al., 1985). By contrast, the model labelled "direct differentiation" states that membrane material is provided directly through biosynthetic action of the endomembrane system and not the plasmamembrane. The first model relies on static comparisons between the peribacteroid membrane and the plasmamembrane.

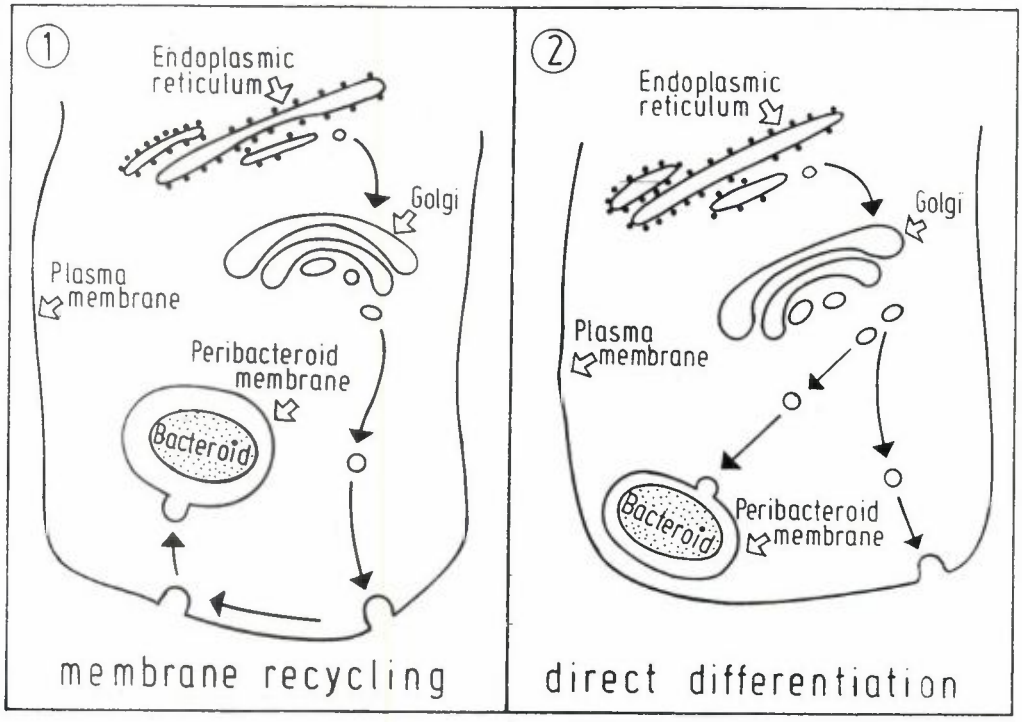


**Figure 1.** Models of peribacteroid membrane biogenesis. (1) "Membrane recycling": is similar to the scheme originally proposed by Bergersen and Briggs (1958). This model has similarities to that of plasmamembrane recycling proposed by Geuze et al. (1983) and called CURL. (2) "Direct differentiation": is basically that proposed by Robertson et al., (1978b). This scheme is similar to that proposed originally for animal cells by Novikoff et al. (1971), called GERL but, here the peribacteroid membrane replaces the lysosome membrane. Both schemes show the production of basically lytic vacuoles but GERL is modified from exocytitic models and stresses the relationship of the vacuole with the biosynthetic parts of the endomembrane system. CURL, on the other hand,is an endocytitic scheme and has as a central theme the controlling position of plasma-membrane in receptor-ligand interactions.

These include membrane thickness and staining properties (Dart and Mercer, 1963; Dixon , 1967; Newcomb, 1976), particle densities on membranes after freeze-fracturing (Tu, 1975; Robertson et al., 1978a) and enzymic and protein composition (Verma et al., 1978; Blumwald et al., 1985). The second model is based on observations of contact between peribacteroid membrane and plant endomembranes (Kijne, 1975; Robertson et al., 1978a; Kijne and Planqué, 1979), pulse-chase experiments on glycoconjugates (Mellor et al.,

1984a; see also Mellor and Werner, 1985) and phospholipids (Mellor et al., 1985). The differences between these two models are not as great as one might imagine. Since some plasmamembrane components are synthesized by endoplasmic reticulum and probably modified and sorted in the Golgi (Molnar, 1975), the only significant difference questioned is: are molecules specific for the peribacteroid membrane, or destined for sequestration within this compartment, ever exposed at the cell surface? This question will be considered in the following sections.

## 4. The Peribacteroid Space

Enzymatic activities in the peribacteroid space (the space between the bacteroid and the peribacteroid membrane) are not well characterized. Mellor et al. (1984b) have, however, reported the presence of hydrolytic activities there. Protease and some alpha-glucosidase were detected in the peribacteroid space, as was alpha-mannosidase (Mellor et al., 1984b). Fortin et al. (1985) have published SDS-PAGE profiles of peribacteroid space proteins where several polypeptides around the correct position for alpha-mannosidase (85 kD on gel filtration in 6 M Guanidine HCl, Bassarab, 1983) are to be seen. Alpha-mannosidase is translated on membrane-bound polyribosomes of the rough endoplasmic reticulum and sequestered in lytic compartments or cell walls according to its isoenzyme behaviour (Van de Wilden and Chrispeels, 1983). Alpha-mannosidase is often used as a marker for the vacuome or vacuole in various tissues (e.g. Leigh and Branton, 1976; Wiemken et al., 1979). The presence of alpha-mannosidase in various soybean nodule fractions has been well documented (Werner et al., 1983; Bassarab et al., 1984; Mellor et al., 1984a). Because alpha-mannosidase has not been definitely demonstrated in Golgi (Van der Wilden and Chrispeels, 1983; Mellor et al., 1984a), at present it is not possible to conclude either that it is, or is not, processed and/or segregated by the Golgi. How then does alpha-mannosidase get into the peribacteroid space? If the membrane recycling theory of peribacteroid membrane formation is correct, then alpha-mannosidase-carrying vesicles must fuse with the plasmamembrane, whereupon pits must be formed in the plasmamembrane and the alpha-mannosidase selectivity taken up again to be transported to the peribacteroid space. Currently we are not aware of evidence to support the concept of selective reuptake in plant cells. Alpha-mannosidase III (see Van der Wilden and Chrispeels, 1983) is known to be sequestered in the extracellular space, whereas isoenzymes I and II remain within the vacuome. Thus if isoenzymes I and/or

II are detected within the peribacteroid space this would support the idea that the peribacteroid space is in contact with the vacuome independently of the extracellular space. Kinnback et al. (1987) have reported that the peribacteroid space from effective nodules contains only the isoenzyme II of alpha-mannosidase. Thus, the vesicle lumen is delivered neither from the plasma membrane nor from diverted cell-wall building vesicles which have not reached the cell surface.

## 5. ATP-ase

Tu (1974), using histochemical methods, reported the presence of an adenyl cyclase activity in the peribacteroid membrane and the plasmamembrane of infected clover root nodule cells. Since this activity may not occur in legumes (Amrheim, 1977), Verma et al., (1978) have interpreted this to be an ATPase activity. An alternative hypothesis is that the staining was the result of protein kinase activity known to be associated with the peribacteroid membrane (Bassarab and Werner, 1986). Robertson et al. (1978b) reported ATPase activity to be associated with the peribacteroid membrane in lupine. Blumwald et al. (1985) characterized the soybean enzyme as a $Mg^{++}$-APTase, which was $K^+$ stimulated, $VO_3^-$-inhibited and had a pH-optimum at 6.3. The presence of a plasmamembrane-type, $H^+$-pumping ATPase was confirmed by Bassarab et al. (1986) (see also Mellor and Werner, 1986). Blumwald et al. (1985) however assayed their ATPase at 37°C, which makes it very difficult to detect ATPases other than the plasmamembrane type. The plasmamembrane ATPase is more highly stimulated by elevated temperature (Briskin and Poole, 1983) than the pH 8-optimum, $K^+$-poorly stimulated, $NO_3^-$-inhibited, $Mg^{++}$-ATPase found in Golgi and tonoplast membranes (O'Neill et al., 1983). Thus by using lower incubation temperatures, Bassarab et al. (1986) were able to demonstrate a minor Golgi-type ATPase (under 30% total activity) in addition to the major plasmamembrane-type ATPase in the peribacteroid membrane. This observation seriously questions the plasmamembrane-recycling model of peribacteroid membrane biogenesis, which requires all ATPase to be of the plasmamembrane type. The recycling must therefore be modified to allow a dual flow from the plasmamembrane and also Golgi into the peribacteroid membrane. The direct differentiation model is however unaffected by demonstration of two ATPases in the peribacteroid membrane. Indeed it is known that Golgi-derived vesicles share ATPase properties of both Golgi and plasmamembranes (Binari and Racusen, 1983).

## 6. Phospholipids

Robertson et al. (1978b) noted that the peribacteroid membrane of lupine contained large amounts of lipid in proportion to protein and speculated that this could reflect on carbon-nitrogen metabolism insofar as oxygen and ammonium both exhibit lipophilic properties. Verma et al. (1978) reported that soybean peribacteroid membranes, in common with endoplasmic reticulum and Golgi, could, in fixed sections, be solubilized using NP-40, implying that lipid is a major constituent of these membranes. Mellor et al. (1985) found phosphatidylcholine to be one of a variety of lipids present in the peribacteroid membrane. Phosphatidylcholine is made in plants via the CDP-choline pathway (see Mellor et al., 1985; Sauer and Robinson, 1985 and references therein). The first enzyme of this pathway, choline kinase, occurs in nodules where stable peribacteroid membrane is made, in two forms (Mellor et al., 1986a). Choline kinase I is constitutive and exists in roots, tissue culture cells and both $fix^+$ and $fix^-$ nodules with and without stable peribacteroid membrane. Whereas, choline kinase II is found only in nodules where a stable peribacteroid membrane exists, independent of the plant cultivar (Mellor et al., 1986a; Mellor et al., 1986b). Antigenicity of both choline kinase forms is poor (Mellor, unpublished data). Although choline kinase has earlier been described as a MW 35000 protein (Warfe and Harwood, 1979), recent evidence has established a molecular mass of about 60000 (Harwood, personal communication; see also Mellor et al., 1986a). Both forms of choline kinase are plant produced and are located in the plant cell cytoplasm.

The terminal enzyme of the CDP-choline pathway, choline phosphotransferase, is located in the ER (Mellor et al., 1985; Lord et al., 1972). Pulse chase experiments demonstrated that most of the phosphatidylcholine in infected nodule cells accumulates in the peribacteroid membrane. Other organelles, notably Golgi and mitochondria, also accumulated labelled phosphatidylcholine within three hours after chasing (Mellor et al., 1985). A more thorough examination of the published data revealed that the plasmamembrane region of the gradient also contained labelled phosphatidylcholine, although this could be accounted for as overlap from the Golgi region. In either way, chased or unchased, large quantities of labeled phosphatidylcholine did not appear in the plasmamembrane. Although the authors explain the labelling kinetics of the pulse-chase experiments in terms of a quick turnover of phosphatidylcholine in the Golgi *en route* to the peribacteroid membrane, the evidence presented cannot rule out a direct route from the endoplasmic retic-

ulum to the peribacteroid membrane and this deserves further investigation.

The subcellular distribution of the middle enzyme of the CDP-choline pathway, phosphorylcholine cytidyltransferase, has not been demonstrated in root nodules. It may be localized in either the endoplasmic reticulum (Moore, 1982) or Golgi (Sauer and Robinson, 1985). The distribution of phospholipid transfer proteins (Kader, 1977) is also unknown.

Robertson et al. (1978b) reported that the lipid content of lupine peribacteroid membrane increases with nodule age. Similarly the peak of choline kinase activity, which presumably reflects cellular demand for phosphatidylcholine, occurs relatively late in nodule development in soybean (Fig. 2). In nodules infected with bacterial strains making either deficient or unstable peribacteroid membranes, the stimulation of choline kinase activity was markedly lower (Fig. 2), presumably reflecting a lower demand for phosphatidylcholine.

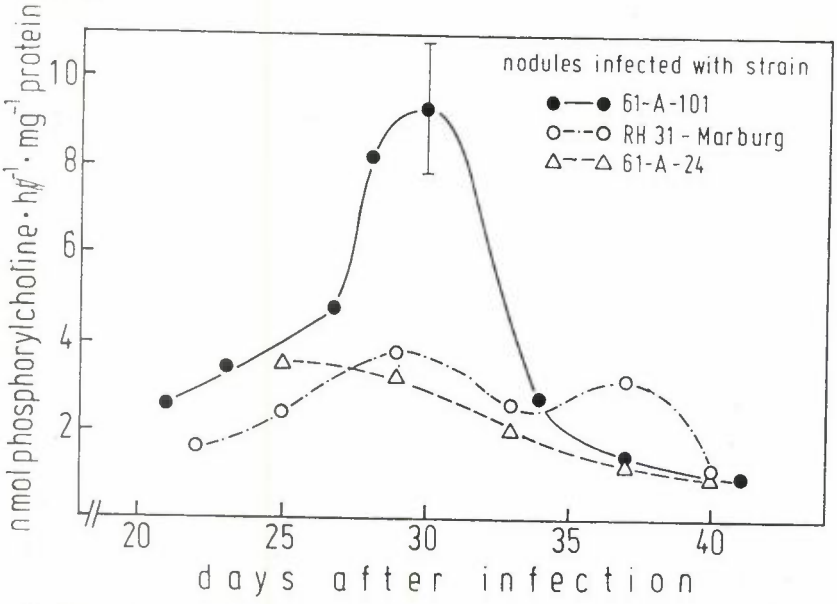


Figure 2. Choline kinase activity in soybean root nodules. Fluctuation in cytoplasmic choline kinase activity during the development of soybean nodules is dependent on the strain of *Bradyrhizobium japonicum* with which the nodules are infected. Closed circles: donate nodules infected with wild-type (nif$^+$, fix$^+$, builds stable PBM) 61-A-101. Open circles: nodules with mutant RH 31-Marburg (nod$^+$, fix$^-$, early fusion of PBM to give lytic compartments. Triangles: nodules with strain 61-A-24 (nod$^+$, fix$^-$, builds very short-lived PBM). For characterisation of strains see Mellor et al. (1986) and references therein. PBM = peribacteroid membrane.

## 7. Glycoconjugates

Protein N-glycosylation is achieved with help of lipid-linked intermediates (Behrens et al., 1973; Lennarz, 1975; Maelor-Davis and Delmer, 1982). Membrane protein may be glycosylated (Rothman and Lodish, 1977) and similar sugar transfer enzymes may be responsible for glycolipid glycosylation (Rauvala and Finne, 1979). Immediately after the infection of host cells with symbiotic (*Brady)rhizobium* spp. proliferation of endoplasmic reticulum is observed (Basset et al., 1977; Verma and Long, 1983). Such proliferation is reflected biochemically as an early stimulation of the endoplasmic reticulum marker enzyme activity GDP-DMP mannosyltransferase (Mellor et al., 1984a), the enzyme responsible for the first step in protein N-glycosylation (Mellor and Lord, 1979 and references therein). Mellor et al. (1984a) and Werner et al. (1983) reported that when ($^{14}$C) mannose is given to intact nodules, radioactive TCA-precipitates accumulate in, amongst other locations, the peribacteroid membrane. Using lectin probes Mellor and Werner (1985) found terminally glycosylated glycoconjugates containing the residue Gal $\beta$1-3 GalNac, in highest concentrations in the peribacteroid membrane. They also demonstrated that the enzyme responsible for assembling this residue on glycoproteins was localized in Golgi similar to that reported from animal systems (Morré et al., 1969). Some glycoproteins containing this residue have also been detected in the Golgi (Mellor and Werner, 1986b). Thus, it appears that the glycoconjugates found in the peribacteroid membrane have first been processed in the Golgi and those found in the Golgi have presumably been peribacteroid membrane proteins in transit. This hypothesis is supported by data (Werner et al., 1983; Mellor and Werner, 1985; Mellor et al., 1986a) which demonstrate glycosyltransferase and other membrane building enzyme activities reduced in symbioses containing reduced amounts of peribacteroid membranes (Table 1).

It is uncertain whether peribacteroid membranes contain unique sugar sequences. Brewin et al. (1985a) reported that monoclonal antibodies raised against a peribacteroid membrane glycoprotein reacted with plasmamembrane, peribacteroid membrane and Golgi membranes. Fortin et al. (1985) using polyvalent antibodies against peribacteroid membrane reported similar results. Since however in neither case was the antigen deglycosylated before use, and the antigenicity of plant glycoproteins lies largely in the sugar residues (Anderson et al., 1984), it may be implied that these three membrane systems share a common sugar conformation. Indeed Brewin et

Table 1. Glycosyltransferases in soybean root nodules. The subcellular locations of three glycosyltransferases in soybean nodules are given, along with the percent stimulation in nodules infected with the three *Bradyrhizobium japonicum* types indicated, at the time of peak enzyme activity (dpi = days post infection). The location and approx. amount of product was as determined by lectin binding (Mellor and Werner, 1985; Mellor and Werner, 1986b). Question marks indicate that not all subcellular fractions were tested.

| *Bradyrhizobium japonicum* strain | Location | % stimulation (over root tissue) | Peakday (d.p.i.) | Product location (lectin binding) |
|---|---|---|---|---|
| | | | MANNOSYLTRANSFERASE | |
| 61-A-101 | ER | 300 | 15 | tono (+++)<br>ER (+)<br>Golgi (++)<br>mito (++)<br>PBM (+++) |
| 61-A-24 | ER | 180 | 15 | tono (+)<br>ER (++)<br>mito (+) |
| RH 31 | ER | 280 | 15 | ? |
| | | | GALACTOSYLTRANSFERASE | |
| 61-A-101 | ER & Golgi | 780 | 16 | tono (+)<br>Golgi (++)<br>mito (+)<br>PBM (++) |
| 61-A-24 | ER & Golgi | 550 | 16 | tono (+)<br>Golgi (++)<br>mito (+) |
| RH 31 | ER & Golgi | 800 | 16 | ?<br>PBM (++) |
| | | | N-ACETYLGALACTOSAMINYLTRANSFERASE | |
| 61-A-101 | Golgi | 1600 | 17 | tono (+)<br>Golgi (+)<br>PBM (+++) |
| 61-A-24 | Golgi | 570 | 17 | Golgi (++)<br>? |
| RH 31 | Golgi | 800 | 17 | ?<br>PBM (+++) |

al. (1985a) reported the complete lack of antibody binding using deglycosylated membrane. This situation should be clarified using gold-lectin staining of Lowicryl-embedded thin sections (work in progress).

## 8. Labelling Studies

Histochemical studies using immunogold labeled antibodies have revealed that Golgi, plasmamembrane and peribacteroid membrane contain common sugar antigen(s) (Brewin et al., 1985a; Fortin et al., 1985) and that peribacteroid membrane contains a sugar antigen cross-reactive with bacterial lipopolysaccharide (Brewin et al., 1985b). Conclusive evidence that peribacteroid membranes actually contain patches of bacterial lipopolysaccharide is not available. Peribacteroid membrane contains at least six nodulespecific proteins, some of which may be distributed amongst both the periobacteroid membrane and the host endomembrane system (Fortin et al., 1985). One of the nodulins detected and declared to be specific for the peribacteroid membrane is nodulin-24 (Fortin et al., 1985; see also Kantinakis and Verma, 1985). This is rather surprising since nodulin-24 is translated as a MW 24,000 protein, processed by dog pancreatic microsomes to MW 20,000, then found on the peribacteroid membrane at a MW of 33,000 (Fortin et al., 1985; Katinakis and Verma, 1985). The ability of the protein to be processed and post-translationally modified fulfills the criteria for rough endoplasmic reticulum translated membrane glycoproteins (Katz et al., 1977; Bonatti et al., 1979). Unfortunately Fortin et al. (1985) did not test the endoplasmic reticulum or Golgi from infected nodule cells for reactivity with anti-nodulin-24. Thus the biosynthetic route of the final processed form of nodulin-24 is not known. After localizing nodulin-24 with immunogold methods, Fortin et al. (1985) concluded that nodulin-24 is located exclusively in the peribacteroid membrane since almost all gold granules accumulated there. It is unfortunate that the one electron-micrograph presented lacks the contrast to determine if the other gold grains really represent background staining or if they also lie over Golgi or other structures. If this is not the case, then we must assume that nodulin-24 mRNA may be translated on free polyribosomes and the protein later inserted into the peribacteroid membrane. Similar routes have been postulated for urate oxidase in mammalian microsomes (Goldman and Blobel, 1978; Kreibich et al., 1980; see also Perara et al., 1986), malate synthetase in plant glyoxysomes (Kindl, 1982; for review see Trelease, 1984) and the nodulin uricase II in the peroxisomes of uninfected nodule cells (Nguyen et al., 1985).

Labelling of whole protoplasts (Fowke et al., 1983; Tanchak et al., 1984) have shown that cationised ferritin is internalised into coated pits and coated vesicles in soybean tissue culture protoplasts within 30 sec. If peribacteroid membrane were to arise by membrane flow from the plasmamembrane, it would be expected that label given to infected nodule protoplasts would accumulate in the peribacteroid membrane or peribacteroid space. Ostrowski et al. (1986) labelled whole isolated infected protoplasts from soybean nodules with $^{125}I$ with and without colloidal gold for periods between fifteen and twenty-five min. No accumulation of radioactivity occured around the bacteroids. Double labelling of tissue culture protoplasts resulted in radioactivity being found in fractions possibly containing coated vesicles (Mersey et al., 1985, see also Depta and Robinson, 1986). Since this did not occur when infected nodule cell protoplasts were used, it may be that tissue culture cells reinternalise plasmamembrane, while infected nodule cells do so either much less, or much more slowly. In either event, only an undetectable amount of plasmamembrane reached the peribacteroid membrane. This could be further clarified by electron microscope studies using electron-dense markers similar to that of Tanchak et al. (1984) but using infected nodule protoplasts instead of tissue culture.

## 9. Vesicles

The major work on vesicle traffic in infected root nodule cells is that of Robertson and Lyttleton (1982; see also Robertson et al., 1985). These authors found both smooth and coated vesicles associated with the plasmamembrane as well as with the peribacteroid membrane and growing infection thread. Early evidence indicated a role for coated vesicles as well as for smooth vesicles in exocytosis or the provision of new plasmamembrane (Franke and Herth, 1974; Ryser, 1979). Since then a large body of data has accumulated supporting the inward flow from the plasmamembrane via coated vesicles (Dean, 1977; Pastan and Willingham, 1981; Pearse and Bretscher, 1981; Steinman et al., 1983; Gonatas et al., 1984; Joachim and Robinson, 1984; Hübner et al., 1985). In particular, Fowkes laboratory (Fowke et al., 1983; Tanchak et al., 1984) working with soybean protoplast have provided incontrovertable evidence that coated vesicles form from coated pits, enclosing endocytosed material, and flow back to the Golgi or multivesicular bodies, losing their coat *en route*. This scheme is consistent with work on animal systems (Herzog, 1980; Pastan and Willingham, 1981; see also Farquhar and Palade, 1981) in which coated vesicles lose or shorten

their coat underway to the Golgi, there fusing either with Golgi, forming coated ends to the dictyosome sacs, or with Golgi-derived smooth vesicles to form larger partially-coated vesicles. Herzog (1980) and Gonatas et al. (1984) further state that the vesicle contents direct Golgi to send the endocytosed material into lytic compartments or, if cell growth is required, to send more membrane *via* smooth vesicles to the cell surface. Should this occur, coated vesicles can be considered to act as a "primer" for smooth vesicles to deliver more membrane to the plasmamembrane. Thus, the observation that coated vesicles are more numerous at the growing point of the infection thread than elsewhere in the host cell (Robertson and Lyttleton, 1982) is consistent with their role in membrane internalization. The presence of coated vesicles at the peribacteroid membrane present a more complex problem. If the peribacteroid membrane contains the normally Golgi-located receptors for fusing with coated vesicles then these coated vesicles may be plasmamembrane-derived. It is, however, hard to imagine that coated vesicles could retain intact coats for the time required to travel to those peribacteroid membranes located in the center of the cell. No data is available about the distribution of coated vesicles per $\mu$m peribacteroid membrane from the center to the periphery of the cell. The electronmicrographs of coated vesicles attached to the peribacteroid membrane (Robertson and Lyttleton, 19822) also give no indication as to whether this structure was found in the center of the cell or at the periphery. Peribacteroid membranes share many more properties with plasmamembranes that with Golgi membranes (see Verma et al., 1978; Verma and Long, 1983; and references therein). Thus, it is more probable that coated vesicles budd off from peribacteroid membrane, as they do from plasmamembrane (Tanchak et al., 1984) rather than fuse with the peribacteroid membrane. This is an exciting theory since it allows for the possibility that molecules secreted from the bacteroid may be taken up by coated vesicles and transferred to the Golgi and then by, or through, transition elements (Morré et al., 1979; Finean et al., 1984; Morré et al., 1986) to the endoplasmic reticulum, thereupon influencing the whole biosynthetic apparatus of the eukaryotic cell. This theory still allows for the provision of the peribacteroid membrane via smooth vesicles from Golgi (Robertson et al., 1978a) and does not rule out that smooth (decoated coated) vesicles from the plasmamembrane fuse with the peribacteroid membrane. It is interesting to note that coated vesicles derived from the peribacteroid membrane are larger than those from the plasmamembrane (Robertson and Lyttleton, 1982). At least three classes of coated vesicles have been described in animal cells on the

basis of size (von Figuera et al., 1980; Pastan and Willingham, 1981; see also Pearse, 1980).

## 10. Conclusions

It appears unlikely that the peribacteroid membrane arises by recycling of membrane over the plasmamembrane roughly by the scheme given in Fig. 1 (see Dart and Mercer, 1963; Dixon, 1967; Tu, 1974, 1975; Newcomb, 1976). Evidence against this scheme includes the lack of internalized radioactivity when infected protoplasts are surface labelled with $^{125}$I (Ostrowski et al., 1986), and also the presence of alpha-mannosidase isoenzyme II in the peribacteroid space (Kinnback et al., 1987). Verma et al. (1978) and Verma and Long (1983) regard peribacteroid membranes as derived from modified plasmamembranes which, presumably after release from the Golgi, find their way to the peribacteroid membrane. This explanation is less tenable because of the presence of Golgi-type ATPase in the peribacteroid membrane (Bassarab et al., 1986). The direct differentiation model given in Fig. 1, similar to that favored by Robertson and co-workers (1978a), stresses the intimate connections between Golgi and the peribacteroid membrane, considering this to be the sole route whereby the peribacteroid membrane is provided (Brewin et al., 1985). Again this theory remains incomplete particularly when one considers the modifications needed in polypeptide profiles (Mellor and Werner, 1986a), for membrane thickness and staining (Dixon, 1967; Tu, 1974; Verma et al., 1978), and for freeze-fracture (Tu, 1975; Robertson et al., 1978a). Thus it may be erroneous to try to force the peribacteroid membrane into conceptual categories such as plasmamembrane-associated CURL (Geuze et al., 1983), or Golgi-associated GERL (Novikoff et al., 1971), but instead this compartment may be regarded as a separate organelle such as mitochondria or chloroplasts. How then is the peribacteroid membrane made? It may be overly dogmatic to consider exclusively any one source only. Indeed different components may travel to the periobacteroid membrane by quite different routes and the evidence given above points to the need for a pluralistic model, which is briefly outlined in Fig. 3.

The peribacteroid space contains acid hydrolase activities (Mellor et al., 1984b). These enzymes in *Phaseolus* and other plants are segregated by the endoplasmic reticulum (Chrispeels, 1980; Van der Wilden and Chrispeels, 1983; see also Jones, 1985). Membrane phospholipids may be differentially synthesized on specialized parts of the endoplasmic reticulum (see Jones, 1985). Since the data on phosphatidylcholine flow in nodules (Mellor et al.,

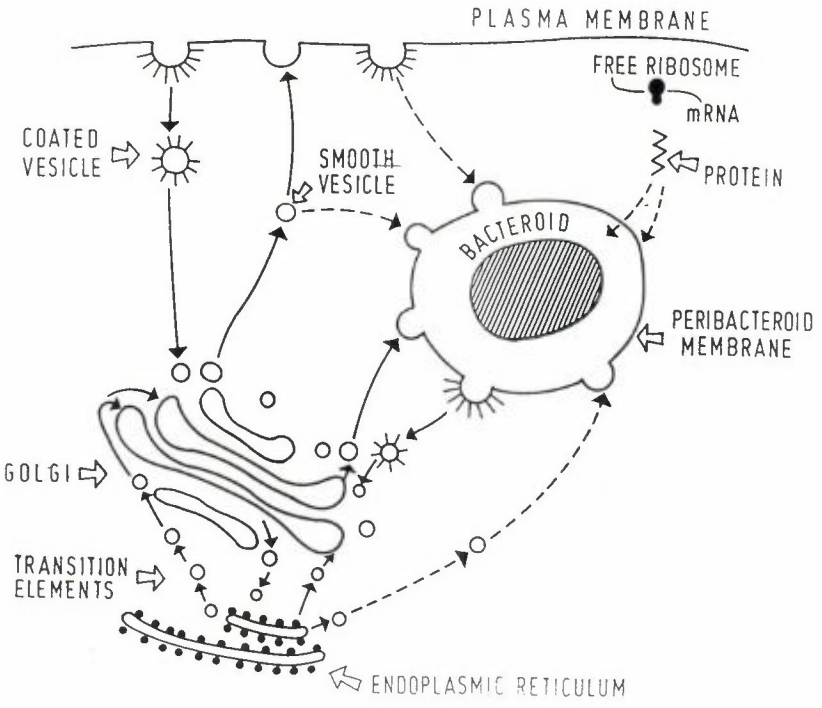


**Figure 3.** Proposed model of membrane traffic in mature nodule cells. Open arrows point to named structures. Closed arrows donate probable routes and directions of flow. Closed arrows with broken lines indicate pathways which cannot yet be ruled out. It is not yet possible to say that acid hydrolases do not flow directly from the ER to the PBM. This may also be the route for some lipid membrane components. The route to the PBM from plasmamembrane-bound coated vesicles or coated vesicles from the plasma-membrane seems unlikely due to the presence of alpha-mannosidase II in the peribacteroid space and the lack of radioactivity in the PBM from $^{125}I$ surface labelled protoplasts. Both membrane and lumen (peribacteroid space) proteins could be provided by free ribosomes. It appears that most PBM material, including glycoproteins, are provided by the Golgi, which in turn is supplied by the ER. Exciting is the prospect of a return flow *via* PBM-derived coated vesicles.

1985) is equivocal regarding flow by way of the Golgi, it is not yet possible to completely rule out that certain components by-pass the Golgi *en route* to the peribacteroid membrane. Kijne and Planqué (1979) published one electronmicrograph showing connections between endoplasmic reticulum and peribacteroid membrane. That a few peribacteroid membrane components could be provided by the endoplasmic reticulum is thus not impossible, a situation which could be similar to that postulated in animal cells for per-

oxisomes, according to the peroxisome reticulum hypothesis (Lazarow, 1980; Lazarow et al., 1980). Data have been furnished from a variety of systems that some organelle membrane and lumen components are translated on free polysomes and inserted into or through the membrane by an as yet unknown mechanism (Goldman and Blobel, 1978; Kreibich et al., 1980; Kindl, 1982; Trelease, 1984; Nguyen et al., 1985; Perara et al., 1986). This may possibly be true for nodulin-24 in the peribacteroid membrane (Fortin et al., 1985). Independently of whether or not this is so for nodulin-24, the sparse data presently available do not enable us yet to rule out that other, as yet unknown proteins, are provided for the peribacteroid membrane and space in this fashion.

Robertson's group (Robertson et al., 1978a; Robertson et al., 1978b; Robertson and Lyttleton, 1982; see also Robertson et al., 1985) have provided extensive electronmicroscope evidence that peribacteroid membranes are derived from the Golgi. Components thus delivered include glycoconjugates, which are core glycosylated in the endoplasmic reticulum (Mellor et al., 1984a) and N-acetylgalactosaminylated in the Golgi (Mellor and Werner, 1985; Mellor and Werner, 1986b) before being sequestered in the peribacteroid membrane (Mellor and Werner, 1985). That Golgi are intimately associated with the peribacteroid membrane is supported by the presence of a Golgi-type ATPase there (Bassarab et al., 1986).

We therefore conclude that the symbiotic bacteroid in its peribacteroid membrane is a distinct organelle, the outer membrane of which (the peribacteroid membrane) exhibits many similarities to the plasmamembrane, but also to Golgi and/or tonoplast membranes. The membrane components are primarily derived from Golgi and not plasmamembrane. The involvement to some extent of endoplasmic reticulum and free polyribosomes can not be ruled out.

Our further studies will concentrate on determining the history of the approximately 30 polypeptides present in the peribacteroid membrane (Werner et al., 1986).

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REFERENCES

Amrheim, N. 1977. The current status of cyclic AMPin higher plants. *Ann. Rev. Plant Physiol.* **28:** 123–132.

Anderson, M.A., Sandrin, M.S., and Clarke, A.E. 1984. A high proportion of hybridomas raised to a plant extract secrets antibody to arabinose or galactose. *Plant Physiol.* **75:** 1013–1016.

Arwas, R., McKay, I.A., Rowney, F.R.P., Dilworth, M.J., and Glenn, A.R. 1985. Properties of organic acid utilization mutants of *Rhizobium leguminosarum* strain 300. *J. Gen. Microbiol.* **131:** 2059–2066.

Bauer, W.D. 1981. Infection of legumes by rhizobia. *Ann. Rev. Plant Physiol.* **32:** 407–449.

Bassarab, S. 1983. Saccharidasen in der *Rhizobium-Glycine max* Symbiose. *Diplomarbeit.* University of Marburg.

Bassarab, S., Dittrich, W., Mellor, R.B., and Werner, D. 1984. Soybean root response to infection by *Rhizobium japonicum*: Saccharidases in root and nodule tissue. *Physiol. Plant Pathol.* **24:** 9–16.

Bassarab, S., Mellor, R.B., and Werner, D. 1986. Evidence for two types of $Mg^{++}$ ATPase in the peribacteroid membrane from *Glycine max* root nodules. *Endocyt. C. Res.* **3:** 189–196.

Bassarab, S. and Werner, D. 1986. Calcium dependent protein kinase in the peribacteroid membrane from nodules of *Glycine max. J. Plant Physiol.* (submitted).

Basset, B., Goodman, N., and Novacky, A. 1977. Ultrastructure of soybean nodules II: Deterioration of the symbiosis in ineffective nodules. *Can. J. Microbiol.* **23:** 873–883.

Behrens, N.H., Carminatti, H., Staneloni, R.J., Leloir, H., and Cantanella, A.I. 1973. Formation of lipid-bound oligosaccharides containing mannose. Their role in glycoprotein biosynthesis. *Proc. Natl. Acad. Sci. USA* **70:** 3390–3394.

Bergersen, F.J. and Briggs, M.J. 1958. Studies on the bacterial component of soybean root nodules: Cytology and organization in the host tissue. *J. Gen. Microbiol.* **19:** 482–490.

Binari, L.L.W. and Racusen, R.H. 1983. Membrane-associated ATP-ases in isolated secretory vesicles. *Plant Physiol.* **71:** 594–597.

Blumwald, E., Fortic, M.C., Rea, P.A., Verma, D.P.S., and Poole, R.J. 1985. Presence of host plasmamembrane type $H^+$-ATPase in the membrane envelope enclosing the bacteroids in soybean root nodules. *Plant Physiol.* **78:** 665–672.

Bonatti, S., Cancedda, R., and Blobel, G. 1979. Membrane biogenesis: *In vitro* cleavage, core glycosylation and integration into microsomal membranes of sudbis virus glycoproteins. *J. Cell Biol.* **80:** 219–224.

Brewin, N.J., Robertson, J.G., Wood, E.A., Wells, B., Larkins, A.P., Galfre, G., and Butcher, G.W. 1985a. Monoclonal antibodies to antigens in the peribacteroid membrane from *Rhizobium* induced root nodules of pea cross-react with plasma membranes and Golgi bodies. *EMBO J.* **4:** 605–611.

Brewin, N.J., Bradley, D.J., Wood, E.A., Wells, B., Larkins, A.P., Galfre, G., and Butcher, G.W. 1985b. Incorporation of lipopolysaccharide antigens from *Rhizobium* bacteroids into the peribacteroid membrane of pea root nodules. In: *Nitrogen Fixation Research Progress*, H.J. Evans, P.J. Bottomley and W.E. Newton, eds. Nijhoff, Dordrecht, p. 265.

Briskin, D.P. and Poole, R.J. 1983. Characterization of $K^+$-stimulated ATPase associated with the plasmamembrane of Red Beet. *Plant Physiol.* **71:** 350–355.

Carter, K.R., Jennings, N.T., Hanus, J., and Evans, H.J. 1978. Hydrogen evolution and uptake by nodules of soybeans inoculated with different strains of *Rhizobium japonicum*. *Can. J. Microbiol.* **24:** 307–311.

Chrispeels, M.J. 1980. The endoplasmic reticulum. In: *The Biochemistry of Plants* I. N.E. Tolbert, ed. Academic Press, New York, pp. 390–412.

Dart, P.J. and Mercer, F.V. 1963. Development of the bacteroid in the root nodule of the barrel medic (*Medicago tribuloides* Desr.) and subterranean clover (*Trifolium subterraneum* L.). *Arch. Mikrobiol.* **46:** 382–401.

Dazzo, F.B. 1981. Bacterial attachment as related to cell recognition in the *Rhizobium*-legume symbiosis. *J. Supramol. Struct. Cell Biochem.* **16:** 29–41.

Dean, R.T. 1977. Lysosomes and membrane recycling. *Biochem. J.* **168:** 603–605.

Depta, H. and Robinson, D.G. 1986. Isolation and enrichment of coated vesicles from suspension-cultured carrott cells. *Protoplasma* **130:** 162–170.

Dixon, R.O.D. 1967. The origin of the membrane envelope surrounding the bacteria and bacteroids and the presence of glycogen in clover root nodules. *Arch. Microbiol.* **56:** 156–166.

Farquhar, M.G. and Palade, G.E. 1981. The Golgi apparatus (complex)-(1954–1981)- from artifact to center stage. *J. Cell Biol.* **91:** 77–103.

Finan, T.M., Wood, J.M., and Jordan, D.C. 1983. Symbiotic properties of $C_4$-dicarboxylic acid transport mutants of *Rhizobium leguminosarum*. *J. Bact.* **154:** 1403–1413.

Finean, J.B., Coleman, R., and Michell, R.H. 1984. *Membranes and Their Cellular Functions.* 3rd ed. Blackwell, London.

Fortin, M.C., Zelechowska, M., and Verma, D.P.S. 1985. Specific targetting of membrane noduline to the bacteroid-enclosing compartment in soybean nodules. *EMBO J.* **4:** 3041–3046.

Fowke, L.C., Rennie, P.J., and Constabel, F. 1983. Organelles associated with the plasmamembrane of tobacco leaf protoplasts. *Plant Cell Reports* **2:** 292–295.

Franke, W.W. and Herth, W. 1974. Morphological evidence for *de dovo* formation of plasma membrane from coated vesicles in exponentially growing cultured plant cells. *Expl. Cell Res.* **89:** 447–451.

Galbraith, D.W. and Northcote, D.H. 1977. The isolation of plasma membrane from protoplast of soybean suspension cultures. *J. Cell Sci.* **24:** 295–310.

Geuze, H.J., Slot, J.W., Strous, G.J.A.M., Nodish, H.F., and Schwarz, A.L. 1983. Intracellular site of asialoglycoprotein receptor-ligand uncoupling: Double-label immunoelectronmicroscopy during receptor-mediated endocytosis. *Cell* **32:** 277–287.

Goldman, B.M. and Blobel, G. 1978. Biogenesis of peroxisomes: Intracellular site of synthesis of catalase and uricase. *Proc. Natl. Acad. Sci. USA* **75** : 5066–5070.

Gonatas, N.K., Stieber, A., Hickey, W.F., Herbert, S.H., and Gonatas, J.O. 1984. Endosomes and Golgi vesicles in absorptive and fluid phase endocytosis. *J. Cell Biol.* **99:** 1379–1390.

Goodchild, D.J. and Bergersen, F.J. 1966. Electron microscopy of the infection and subsequent development of soybean nodule cells. *J. Bact.* **92:** 204–213.

Herzog, V. 1980. Role of the Golgi complex in intracellular transport. In: *Biological Chemistry of Organelle Formation.* Th. Bücher, W. Sebald and H. Weiss, eds. Springer, Berlin, pp. 119–146.

Hübner, R., Depta, H., and Robinson, D.G. 1985. Endocytosis in maize root cap cells: Evidence obtained using heavy metal salt solutions. *Protoplasma* **129:** 214–222.

Joachim, S. and Robinson, D.G. 1984. Endocytosis of cationic ferritin by bean leaf protoplasts. *Eur. J. Cell Biol.* **34:** 212–216.

Jones, R.L. 1985. Endoplasmic reticulum. In: *Modern Methods of Plant Analysis.* H.F. Linskens and J.F. Jackson, eds. Springer, Berlin, pp. 304–324.

Kader, J.C. 1977. Exchange of phospholipids between membranes. In: *Dynamic Aspects of Cell Surface Organization.* G. Poste and G.L. Nicholson, eds. Elsevier, Amsterdam, pp. 127–204.

Katinakis, P. and Verma, D.P.S. 1985. Nodulin 24 gene of soybean codes for a peptide of the peribacteroid membrane and was generated by tandem duplication of a sequence resembling an insertion element. *Proc. Natl. Acad. Sci. USA* **82:** 4157–4161.

Katz, F., Rothman, J., Lingappa, V.J., Blobel, G., and Lodish, H. 1977. Membrane assembly *in vitro*: synthesis, glycosylation and asymmetric insertion of a transmembrane protein. *Proc. Natl. Acad. Sci. USA* **74:** 3275–3282.

Kreibich, G., Bar-Nun, S., Czako-Graham, M., Mok, W., Nack, E., Okada, Y., Rosenfield, M.G., and Sabatini, D.D. 1980. In: *Biological Chemistry of Organelle Formation.* Th. Bücher, W. Sebald, and H. Weiss, eds. Springer, Berlin, pp. 147–164.

Kijne, J.W. 1975. The fine structure of pea root nodules. 1. Vacuolar changes after endocytotic host cell infection by *Rhizobium leguminosarum. Physiol. Plant Pathol.* **5:** 75–79.

Kijne, J.W. and Planqué, K. 1979. Ultrastructural study of the endomembrane system in infected cells of pea and soybean root nodules. *Physiol. Plant Pathol.* **14:** 339–345.

Kindl., H. 1982. The biosynthesis of microbodies (peroxisomes, glyoxysomes). *Internat. Rev. Cytol.* **80:** 193–229.

Kinnback, A., Mellor, R.B., and Werner, D. 1987. Alpha-mannosidase II isoenzyme in the peribacteroid space of *Glycine max* root nodules. *J. Exp. Bot.* (in press).

Lazarow, P.B. 1980. Properties of the natural precursor of catalase: Implications for peroxisome biogenesis. *Ann. N.Y. Acad. Sci.* **343:** 293–303.

Lazarow, P.B., Shio, H., and Robbi, M. 1980. Biogenesis of peroxisomes and the peroxisome reticulum hypothesis. In: *Biological Chemistry of Organelle Formation.* Th. Bücher, W. Sebald, and H. Weiss, eds. Springer, Berlin, pp. 187–206.

Leigh, R.A. and Branton, D. 1976. Isolation of vacuoles from root storage tissue of *Beta vulgaris* L. *Plant Physiol.* **58:** 656–662.

Lennarz, W.J. 1975. Lipid linked sugars in glycoprotein synthesis. *Science* **188:** 986–991.

Lord, J.M., Kagawa, T., and Beevers, H.J. 1972. Intracellular distribution of enzymes of the cytidine diphosphate choline pathway in Castor Bean endosperm. *Proc. Natl. Acad. Sci. USA* **69:** 2429–2432.

Maelor-Davis, H. and Delmer, D.P. 1982. Protein glycosylation in higher plants: Recent developments. In: *Cellulose and Other Natural Polymer Systems.* R.M. Brown, ed. Plenum Press, New York, pp. 363–382.

Mellor, R.B. and Lord, J.M. 1979. Formation of lipid-linked mono- and oligo-saccharides from GDP-mannose by Castor Bean endosperm homogenates. *Planta* **146:** 91–99.

Mellor, R.B., Dittrich, W., and Werner, D. 1984a. Soybean root response to infection by *Rhizobium japonicum*: Mannoconjugate turnover in effective and ineffective nodules. *Physiol. Plant pathol.* **24:** 61–70.

Mellor, R.B., Mörschel, E., and Werner, D. 1984b. Legume root response to symbiotic infection: Enzymes of the peribacteroid space. *Z. Naturforsch.* **39c:** 123–125.

Mellor, R.B., Christensen, T.M.I.E., Bassarab, S., and Werner, D. 1985. Phospholipid transfer from ER to the peribacteroid membrane in soybean nodules. *Z. Naturforsch.* **40c:** 73–79.

Mellor, R.B. and Werner, D. 1985. Glycoconjugate interaction in soybean root nodules. *Lectins* **4:** 267–276.

Mellor, R.B. and Werner, D. 1986a. The fractionation of *Glycine max* root nodule cells: A methodological overview. *Endocyt. C. Res.* **3:** 317–336.

Mellor, R.B. and Werner, D. 1986b. Pea and peanut lectins as markers in subcellular fractionation: Differentiation between ER and Golgi membranes. *Lectins* **6** (in press).

Mellor, R.B., Christensen, T.M.I.E. and Werner, D. 1986a. Choline kinase II is present only in nodules that synthesize stable peribacteroid membranes. *Proc. Natl. Acad. Sci. USA* **83:** 659–663.

Mellor, R.B., Thierfelder, H., Pausch, G., and Werner, D. 1986b. The occurance of choline kinase II in the cytoplasm of soybean root nodules infected with various strains of *Bradyrhizobium japonicum*. *J. Plant Physiol.* (in press).

Mersey, B.G., Griffing, L.R., Rennie, P.J., and Fowke, L.C. 1985. The isolation of coated vesicles from protoplasts of soybean. *Planta* **163:** 317–327.

Molnar, J. 1975. Role of ER and Golgi apparatus in the biosynthesis of plasma glycoproteins. *Enzymes of Biological Membranes* **2:** 385–419.

Morré, D.J., Merlin, L.M., and Keenan, T.W. 1969. Localization of glycosyl transferase activities in a Golgi apparatus-rich fraction isolated from rat liver. *Biochem. Biophys. Res. Comm.* **37:** 813–819.

Morré, D.J., Kartenbeck, J., and Franke, W.W. 1979. Membrane flow and interconversions among endomembranes. *Biochim. Biophys. Acta* **559:** 71–152.

Morré, D.J., Paulik, M., and Nowack, D. 1986. Transition vesicle formation *in vitro*. *Protoplasma* **132:** 110–113.

Moore, T.S. 1982. Phospholipid biosynthesis. *Ann. Rev. Plant Physiol.* **33:** 235–259.

Newcomb, W. 1976. A correlated light and electron microscope study of symbiotic growth and differentiation in *Pisum sativum* root nodules. *Can. J. Bot.* **54:** 2163–2186.

Newcomb, W. 1981. Nodule morphogenesis and differentiation. *Internat. Rev. Cytol.* **313:** 247–298.

Newcomb, W. and McIntyre, L. 1981. Development of root nodules of mung bean (*Vigna radiata*): A reinvestigation of endocytosis. *Can. J. Bot.* **59:** 2478–2499.

Nguyen, T., Zelechowska, M., Foster, V., Bergmann, H., and Verma, D.P.S. 1985. Primary structure of the soybean nodulin -35 gene encoding uricase II localized in the peroxisomes of uninfected cells of nodules. *Proc. Natl. Acad. Sci. USA* **82:** 5040–5044.

Novikoff, P.M., Novikoff, A.B., Quintana, N., and Hauw, J.J. 1971. Golgi apparatus, GERL, and lysosomes of neurones in rat dorsal root ganglia, studied by thick section and thin section cytometry. *J. Cell Biol.* **50:** 859–886.

O'Neill, S.D., Bennet, A.B., and Spanswick, R.M. 1983. Characterization of a $NO_3^-$-sensitive $H^+$ ATPase from corn roots. *Plant Physiol.* **72:** 837–846.

Ostrowski, E., Mellor, R.B., and Werner, D. 1986. The use of colloid gold labelling in the detection of plasma membrane from symbiotic and non-symbiotic *Glycine max* root cells. *Physiol. Plant.* **66:** 270–276.

Pastan, I.H. and Willingham, M.C. 1981. Journey to the center of the cell: Role of the receptosome. *Science* **214:** 504–509.

Pearse, B.M.F. 1980. Coated vesicles. *TIBS* **5:** 131–134.

Pearse, B.M.F. and Bretscher, M.S. 1981. Membrane recycling by coated vesicles. *Ann. Rev. Biochem.* **50:** 85–101.

Perara, E., Rothman, R.E., and Lingappa, V.R. 1986. Uncoupling translocation from translation: Implications for transport of proteins across membranes. *Science* **232:** 348–352.

Rauvala, H. and Finne, J. 1979. Structural similarity of the terminal carbohydrate sequences of glycoproteins and glycolipids. *FEBS Lett.* **97:** 1–9.

Regensburger, B., Meyer, L., Filser, M., Weber, J., Studer,D., Lamb, J.W., Fischer, H.M., Hahn, M., an Hennecke, H. 1986. *Bradyrhizobium japonicum* mutants defective in root-nodule bacteroid development and nitrogen fixation. *Arch. Microbiol.* **144:** 355–366.

Ryser, U. 1979. Cotton fibre differentiation: Occurrence and distribution of coated and smooth vesicles during primary and secondary wall formation. *Protoplasma* **98:** 223–239.

Robertson, J.G., Lyttleton, P., Bullivant, S., and Grayston, G.F. 1978a. Membranes of lupin root nodules: 1.The role of Golgi bodies in the biogenesis of infection threads and peribacteroid membranes. *J. Cell Sci.* **30:** 129–149.

Robertson, J.G., Warburton, M.P., Lyttleton, P., Fordyce, A.M., and Bullivant, S. 1978b. Membranes in lupin root nodules: 2. Preparation and properties of peribacteroid membranes and bacteroid envelope inner membranes from developing lupin nodules. *J. Cell Sci.* **30:** 151–174.

Robertson, J.G. and Lyttleton, P. 1982. Coated and smooth vesicles in the biogenesis of cell walls, plasmamembranes, infection thread and peribacteroid membranes in root hairs and nodules of white clover. *J. Cell Sci.* **58:** 63–78.

Robertson, J.G. and Lyttleton, P. 1984. Division of peribacteroid membranes in root nodules of white clover. *J. Cell Sci.* **69:** 147–157.

Robertson, J.G., Lyttleton, P., and Tapper, B.A. 1984. The role of peribacteroid membrane in legume root nodules. In: *Advances in Nitrogen Fixation Research.* C. Veeger and W.E. Newton, eds. Nijhoff/Junk, Holland, pp. 475–481.

Robertson, J.G., Wells, B., Brewin, N.J., Wood, E., Knight, C.D., and Downie, J.A. 1985. The legume-*Rhizobium*-symbiosis: A cell surface interaction. *J. Cell Sci.* Suppl. **2**: 317–331.

Rothman, J.E. and Lodish, H.F. 1977. Synchronised transmembrane insertion and glycosylation of a nascent membrane protein. *Nature* **269:** 775–780.

Sauer, A. and Robinson, D.G. 1985. Subcellular localisation of enzymes involved in lecithin biosynthesis in maize roots. *J. Exp. Bot.* **36:** 1257–1266.

Steinman, R.M., Mellman, I.S., Muller, W.A., and Cohn, Z.A. 1983. Endocytosis and the recycling of the plasmamembrane. *J. Cell Biol.* **96:** 1–27.

Sutton, W.D., Pankhurst, C.E., and Craig, A.S. 1981. The *Rhizobium* bacteroid state. *Internat. Rev. Cytol.* S **13:** 149–177.

Tanchak, M.A., Griffing, L.R., Mersey, B.G., and Fowke, L.C. 1984. Endocytosis of cationized ferritin by coated vesicles of soybean protoplasts. *Planta* **162:** 481–486.

Trelease, R.N. 1984. Biogenesis of glyoxysomes. *Ann. Rev. Plant Physiol.* **35:** 321–347.

Tu, J.C. 1974. Relationship between the membrane envelope of rhizobial bacteroids and the plasmamembrane of the host cell as demonstrated by histochemical localization of adenyl cyclase. *J. Bact.* **119:** 986–991.

Tu, J.C. 1975. Structural similarity of the membrane envelopes of rhizobial bacteroids an the host plasmamembrane as revealed by freeze-fracturing. *J. Bact.* **133:** 691–694.

Vance, C.P. 1983. *Rhizobium* infection and nodulation: A beneficial plant disease? *Ann. Rev. Microbiol.* **37:** 399–424.

Vance, C.P. and Johnson, L.E.B. 1983. Plant determined ineffective nodules in alfalfa (*Medicago sativa*): Structural and biochemical comparisons. *Can. J. Bot.* **61:** 93–106.

Van der Wilden, W. and Chrispeels, M.J. 1983. Characterization of the isoenzymes of $\alpha$-mannosidase located in the cell wall, protein bodies and ER of *Phaseolus vulgaris* cotyledons. *Plant Physiol.* **71:** 82–87.

Verma, D.P.S. and Long, S. 1983. The molecular biology of *Rhizobium*-legume symbiosis. *Internat. Rev. Cytol.* **514:** 211–245.

Verma, D.P.S., Kazazian, V., Zogbi, V., and Bal, A.K. 1978. Isolation and characterization of the membrane envelope enclosing the bacteroids in soybean root nodules. *J. Cell Biol.* **78:** 919–936.

Von Figuera, K., Klein, U., and Hasilik, A. 1980. Origin and dynamics of lysosomes. In: *Biological Chemistry of Organelle Formation.* Th. Bücher, W. Sebald and H. Weiss, eds. Springer, Berlin, pp. 119–146.

Warfe, J. and Harwood, J.L. 1979. Purification of choline kinase from soybean. In: *Biochemistry and Physiology of Plant Lipids.* L.A. Appleqvist and C. Liljenberg, eds. Elsevier, Amsterdam, pp. 443–447.

Werner, D., Mörschel, E., Stripf, R., and Winchenbach, B. 1980. Development of nodules of *Glycine max* infected with an ineffective strain of *Rhizobium japonicum*. *Planta* **147:** 320–329.

Werner, D., Mellor, R.B., Bassarab, S., and Dittrich, W. 1983. *Glycine max* root response to symbiotic infection. *Endocytobiology* **2:** 585–586.

Werner, D., Mörschel, E., Mellor, R.B., and Bassarab, S. 1984. The host cell nucleus oriented lysis of bacteroid in an ineffective ($fix^-$) type *Glycine max* nodule. *Planta* **162:** 8–16.

Werner, D., Mellor, R.B., Hahn, M.G., and Grisebach, H. 1985. Soybean root response to infection: Glyceollin accumulation in an ineffective type of soybean nodule with an early loss of the periobacteroid membrane. *Z. Naturforsch.* **40c:** 171–181.

Werner, D., Mörschel, E., Garbers, C., Bassarab, S., Mellor, R.B. 1986. Particle density and protein composition of the peribacteroid membrane from soybean root nodules is affected by mutations in the microsymbiont *Bradyrhizobium japonicum*. *Planta* (submitted).

Wiemken, A., Schellenberg, M., and Urech, L. 1979. Vacuoles: The sole compartment of digestive enzymes in yeast cells (*Saccharomyces cereviseae*). *Arch. Microbiol.* **129**: 23–35.