CMLS, Cell. Mol. Life Sci. 58 (2001) 61–71
1420-682X/01/010061-11 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Ammonia and amino acid transport across symbiotic membranes in nitrogen-fixing legume nodules

D. A. Day[a,*], P. S. Poole[b], S. D. Tyerman[c] and L. Rosendahl[d]

[a]Biochemistry Department, University of Western Australia, Crawley, WA 6009 (Australia),
Fax + 61 8 93801148, e-mail: dday@cyllene.uwa.edu.au
[b]School of Animal and Microbial Sciences, University of Reading, Reading, RG6 6AJ (UK)
[c]School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide 5001 (Australia)
[d]Plant Biology and Biogeochemistry Department, Risø National Laboratory, DK-4000 Roskilde (Denmark)

Received 5 June 2000; received after revision 13 July 2000; accepted 14 July 2000

Abstract. Biological nitrogen fixation involves the reduction of atmospheric N_2 to ammonia by the bacterial enzyme nitrogenase. In legume-rhizobium symbioses, the nitrogenase-producing bacteria (bacteroids) are contained in the infected cells of root nodules within which they are enclosed by a plant membrane to form a structure known as the symbiosome. The plant provides reduced carbon to the bacteroids in exchange for fixed nitrogen, which is exported to the rest of the plant. This exchange is controlled by plant-synthesised transport proteins on the symbiosome membranes. This review summarises our current understanding of these transport processes, focusing on ammonia and amino acid transport.

Key words. Legumes; rhizobia; symbiosomes; peribacteroid membrane; nitrogenase; ammonium; amino acids.

Nitrogen is an essential nutrient for plants and is often a limiting factor in crop growth. Consequently, large quantities of fertiliser are often applied to crops. This is an energy-consuming and expensive procedure. Some plants, many of them legumes, form symbioses with soil bacteria (rhizobia) that are able to biologically convert atmospheric N_2 to NH_3 for use in the plant, a process termed symbiotic N_2 fixation. These plants are able to grow in the absence of added nitrogen fertiliser with obvious advantages for sustainable agriculture.

Biological N_2 fixation involves the conversion of atmospheric N_2 to NH_3, a reaction catalysed by the enzyme nitrogenase that is found only in certain prokaryotes, including members of the Rhizobiaceae that form symbioses with legumes.

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \quad (1)$$

Symbiotic N_2 fixation in legumes takes place in nodules on the roots of the host plant (fig. 1). The infected cells of these organs house the nitrogenase-producing forms of the rhizobia, called bacteroids. Within the specialised, low-oxygen environment of nodule cells, the bacteroids fix atmospheric N_2 into NH_3 that serves as a source of nitrogen to the host in return for reduced carbon, probably in the form of dicarboxylic acids such as malate [1, 2].

Bacteroids inside the infected root cells are surrounded by a symbiosome or peribacteroid membrane (PBM) of plant origin that effectively segregates the bacteroids from the plant cytoplasm and determines the type and quantity of compounds that pass between the symbionts. Rhizobia are totally dependent, therefore, upon

* Corresponding author.

their plant hosts for nutrients when living within the nodule. The PBM, by controlling the flux of metabolites between the two symbionts, is obviously important in the regulation of N_2 fixation and the maintenance of the symbiosis. Studies with isolated symbiosomes [organelle-like structures consisting of bacteroids, the peribacteroid space (PBS) and the PBM; fig. 2] have shown the PBM to have selective permeability to metabolites [1, 3]. For example, the PBM is poorly permeable to sugars but contains a transporter specific for dicarboxylic acids, as does the bacteroid membrane [1]. The PBM also possesses an H^+-ATPase that pumps H^+ into the symbiosome to establish electrical and pH gradients across the PBM. In addition, the bacteroid respiratory electron transport chain pumps H^+ out of the bacteroid and into the PBS. The end result is acidification of the PBS (although the precise pH will be determined by the relative activities of the proton pumps and counter ion movements). Thus in vivo, the PBM and bacteroid inner membrane delineate three compartments of contrasting pH: a slightly alkaline plant cytosol, an acidic PBS and an alkaline bacteroid interior. Furthermore, the PBM and bacteroid inner membrane have opposite electrical polarities. These relationships will determine in part the nature of transport systems involved in the exchange of metabolites between plant and bacteroid.

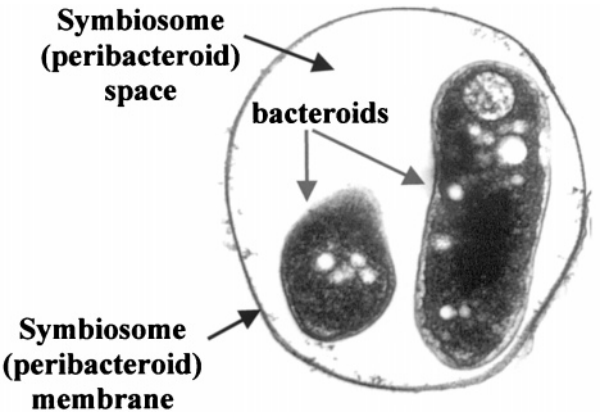

Figure 2. Structure of a symbiosome. The plant-derived peribacteroid membrane (PBM) surrounds one or more bacteroids to form an organelle-like structure which effectively segregates the bacteroid from the plant host cytoplasm.

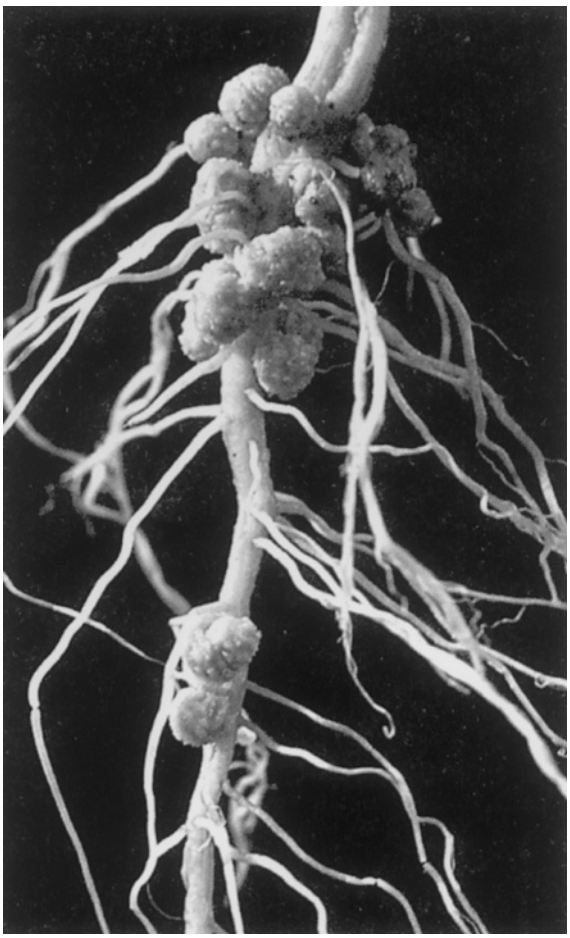

Figure 1. Nodules on a root of soybean inoculated with *B. japonicum*.

Although the principal metabolic exchange between the symbiotic partners is reduced carbon to the bacteroid for fixed nitrogen to the plant, other important nutrient exchange also occurs [1]. In addition to the PBM, symbiotically important transport proteins are also expressed on bacteroid membranes, including the well-studied dicarboxylate transporter (Dct) and various amino acid transporters [1, 4]. This review will focus on transport of fixed nitrogen across the PBM and bacteroid membranes. Ammonia/ammonium has been considered the most likely form of fixed nitrogen delivered to the plant, but the possible involvement of amino acids has been suggested based on the findings that pea symbiosomes supplied in vitro with radiolabelled dicarboxylic acids excrete radiolabelled amino acids, primarily alanine and asparate [5, 6]; and that soybean bacteroids incubated in $^{15}N_2$ excrete ^{15}N-alanine [7].

Ammonia/ammonium production and transport in bacteroids and symbiosomes

Ammonia transport and assimilation in rhizobia

Free-living rhizobia assimilate NH_3 largely via the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway [8, 9]. Under conditions of both low oxygen

and nitrogen supply, rhizobia express, in addition to nitrogenase, GS and GOGAT, a high-affinity NH_4^+ transporter to reabsorb any NH_3 that might leak from the cells [10] (fig. 3). N_2 fixation by symbiotic bacteroids, on the other hand, is regulated by oxygen concentration rather than nitrogen [11, 12]. Since bacteroids are not nitrogen starved in nodules, the NH_3 assimilation pathway is not derepressed and activities of the high-affinity NH_4^+ transporter, GS and GOGAT are all low relative to nitrogenase activity [13–16]. Mutations in the *ntr* genes that are required for induction of these enzymes have little or no effect on the symbiotic performance of rhizobia [17–19].

Free-living rhizobia, like other bacteria, transport NH_3 in two ways (fig. 3). First, when concentrations of NH_3 in the surrounding medium are low, NH_4^+ transporters allow rapid accumulation of NH_4^+ in the cell [20]. These transporters (Amts) have been characterised in a number of rhizobia grown in culture [10, 14–16, 21] and appear to be under the control of the Ntr system [22]. The second mechanism involves NH_3 entering the cells by diffusion. At high concentrations of NH_3, the Amt system is repressed and diffusion of NH_3 provides the cell with sufficient nitrogen for growth [15].

Bacteroids isolated from N_2-fixing nodules do not express the Amt system, and NH_3 transport occurs by simple diffusion of NH_3 out of the cell [14, 15, 23]. Since NH_3 assimilation is also repressed in bacteroids (see above), most of the NH_3 that is produced by bacteroid N_2 fixation is likely to be lost from the bacteroid by diffusion down a concentration gradient [24]. In the absence of an NH_4^+ transporter, the exported NH_3 cannot be effectively recovered. Repression of the Amt system in N_2-fixing bacteroids, therefore, avoids a futile, energy-consuming cycle [20]. Consistent with this, forced expression of the Amt protein in bacteroids disrupts the symbiosis [25].

While assimilatory enzymes in the bacteroid are repressed, those in the plant fraction are elevated [2, 8]. Consequently, there is a very strong 'sink' driving NH_3 efflux from the bacteroid. The bulk of NH_3 that leaves the bacteroid is likely protonated in the PBS, forming NH_4^+. Using the Henderson-Hasselbach equation, the concentration of NH_4^+ can be calculated to be 178 times greater than that of NH_3 at pH 7. In vivo, the acidity of the PBS will enhance the formation of NH_4^+. Protonation of NH_3 ensures that there is a concentration gradient for it to move into the so-called acid-trap [26]. However, a transporter is required on the PBM for the rapid movement of NH_4^+ across the membrane (fig. 4).

An ammonium channel on the PBM

Initial studies, using the NH_4^+ analogue methylammonium, failed to find any evidence for such a carrier [27],

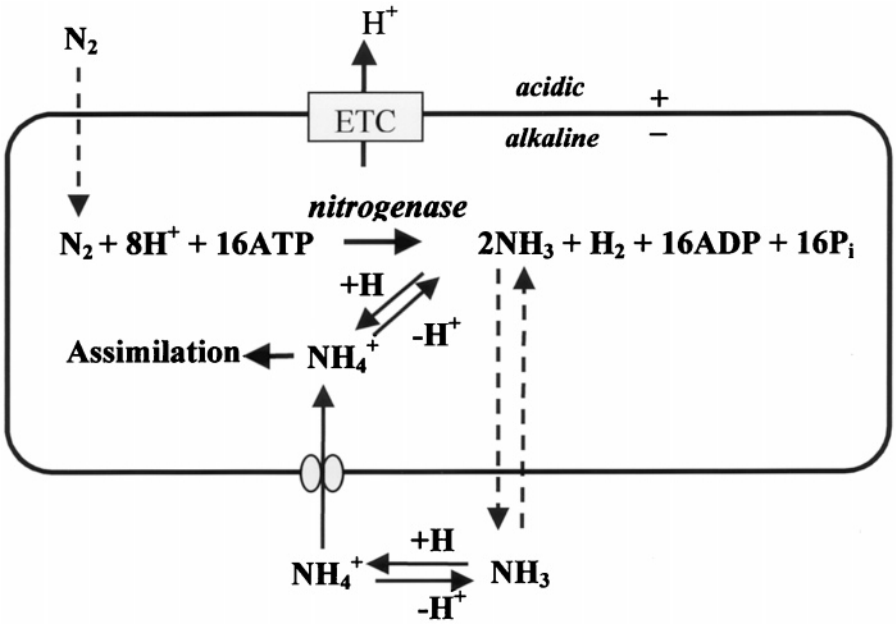

Figure 3. In free-living N_2-fixing bacteria, the ammonium transporter (Amt) ensures recycling of ammonia lost from the cell and scavenging of exogenous ammonium.

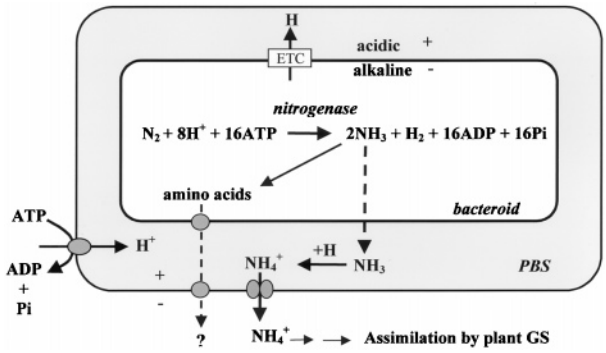

Figure 4. Ammonium transport in symbiosomes. Ammonia produced in the bacteroid diffuses into the peribacteroid space (PBS) where it is protonated (trapped) in the acidic environment. Suppression of rhizobial Amt prevents cycling of ammonium back into the bacteroid. Movement of ammonium ions into the plant cytoplasm requires the operation of a channel. Ammonia assimilation into amino acids also occurs and these may also be provided to the plant.

but this technique has limitations in intact symbiosomes when measuring efflux rather than uptake. Subsequently, the movement of NH_4^+ in isolated symbiosomes was reinvestigated using the patch-clamp technique and a novel voltage-gated monovalent cation channel capable of transporting NH_4^+ across the PBM from soybeans was identified [28]. A very similar channel has been observed in *Lotus japonicus* PBM using patch-clamp [D. Roberts and S. D. Tyerman, unpublished results], and in pea nodules, using ^{14}C-methylammonium and energised bacteroid-side-out PBM vesicles [29]. This channel opens to allow NH_4^+ efflux from the symbiosome when a positive (inside PBS) membrane potential is generated across the PBM, as will occur in vivo by the action of the H^+-ATPase on the PBM (see below).

In PBM patches, NH_4^+ currents were rectified so that the movement of NH_4^+ was unidirectional—effectively out of the PBS [29]. Subsequent experiments indicated that this rectification depends on the concentration of calcium and magnesium on either face of the PBM, with both cations inhibiting ion flow [30]. Since magnesium concentrations in the plant cytosol are expected to be in the millimolar range, the channel will probably be outwardly rectified in vivo, preventing backflow of NH_4^+ into the symbiosome (i.e. ions will move out of the PBS). The role of calcium in regulating the channel is less clear: calcium concentrations in the plant cytosol are usually very low, but we have found substantial quantities of calcium in the PBS of soybean nodules. We assume that little of this is free, since NH_4^+ currents were detected in patch-clamp experiments of intact symbiosomes [28]. The PBM channel is selective for small monovalent cations, with K^+ and NH_4^+ being the most active, but does not transport divalent cations, larger cations such as choline or anions [28–30]. At low, physiological concentrations (10–20 mM), the channel shows a preference for NH_4^+ over K^+. Most plant K^+ channels are permeable to NH_4^+ but to a lesser extent than K^+ [30]. Thus the channel identified in the PBM very likely functions as an NH_4^+ channel in vivo.

Channel-mediated ammonia flux across the PBM

The channel described above has properties that one would expect of a transporter delivering NH_4^+ to the plant, and the magnitude of the NH_4^+ currents across the patch, extrapolated to the whole symbiosome, is adequate to account for estimates of N_2 fixation rates in symbiosomes in vivo [29]. However, identification of the channel does not rule out the possibility that some NH_3 diffuses across the PBM. In vivo, this will depend on the pH of the PBS [27, 31].

Recently, Tyerman and Niemietz [32] measured the NH_3 permeability of PBM vesicles and showed that it can be significant (8×10^{-5} m/s). They measured NH_3 influx into PBM vesicles that were predominantly PBS side facing outwards, thus corresponding to the normal direction of flux from the symbiosome to the plant cytoplasm. A substantial portion of this flux could be inhibited by Hg, as was the water permeability of the vesicles. Hg inhibition of water transport indicates aquaporin-mediated transport and the results with NH_3 suggest that it can also move through such a channel. Whether this channel is the well-known PBM aquaporin Nodulin 26 [33] has yet to be determined, but treatment of PBM vesicles with adenosine triphosphate (ATP) inhibits NH_3 transport and stimulates water movement [32]. Nodulin 26 is known to be phosphorylated by ATP and is permeable to other small uncharged molecules [34]. Solute movement and water movement through Nodulin 26 possibly occur via different pathways that are regulated differently by ATP-induced conformational changes. The closure of the channel to NH_3 when cytosolic ATP concentrations are high may be important to prevent NH_3 cycling in and out of the symbiosome, since the PBM will be polarised and channel-mediated NH_4^+ efflux will be favoured. Under these conditions, the PBS will be acidified by the H^+-ATPase, decreasing the symbiosome NH_3 concentration to the point where there may be a gradient for NH_3 influx to the PBS from the cytoplasm. These conditions also enhance malate uptake by symbiosomes [35] and will help to co-ordinate efflux NH_4^+ and uptake of malate across the PBM. However, if plant cytosol ATP concentrations are limited, as may occur when nodule oxygen supply is restricted by environmental

stress, then the pH of the PBS may rise and NH_3 could efflux. Figure 5 summarises these ideas.

Recent measurements of neutral solute transport across PBM vesicles indicate that there is asymmetric transport across the membrane and probably through Nodulin 26 [32]. Such rectification is common for ion channels and may also be possible for some aquaporins [36]. Water and glycerol fluxes into PBM vesicles equivalent to efflux from the PBS to the cytoplasm in vivo were significantly larger than for the other direction. For glycerol, this difference was enhanced by Hg treatment. For alanine, the asymmetry was the most spectacular with the (equivalent) PBS-to-cytoplasm flux, being similar to that for glycerol, but 100 times higher than the flux in the other direction and strongly inhibited by Hg [S. D. Tyerman and C. M. Niemietz, unpublished data] (fig. 6). As will be discussed below, alanine is a possible alternative product of N_2 fixation in the bacteroid, under some conditions.

Proteins involved in nodule ammonium transport

An attempt has been made to isolate a cDNA encoding the PBM NH_4^+ channel using functional complementation of yeast [37]. Several different transformations of a yeast mutant unable to grow on low NH_4^+, with a cDNA library from soybean nodules, yielded a single cDNA that was termed *GmSAT1* (*Glycine max* symbiotic ammonium transport). Transcripts of the *GmSAT1* gene were readily detected in N_2-fixing nodules but not in other tissues of soybean, indicating that

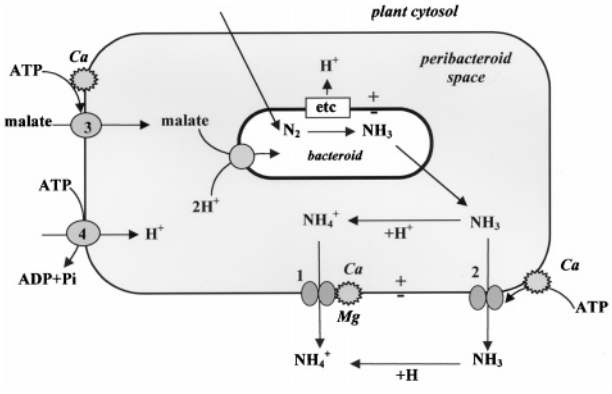

Figure 5. Channel-mediated NH_3 and NH_4^+ transport out of the symbiosome. NH_4^+ can exit via a monovalent cation channel (1) which is regulated by Ca and Mg on either side of the PBM. NH_3 can move though an aquaporin channel (2) which is regulated by a Ca-sensitive protein kinase. This kinase also regulates malate transport (3) into the symbiosome. Operation of the ATPase (4) on the PBM also influences transport by changing the energisation of the PBM and the pH of the PBS.

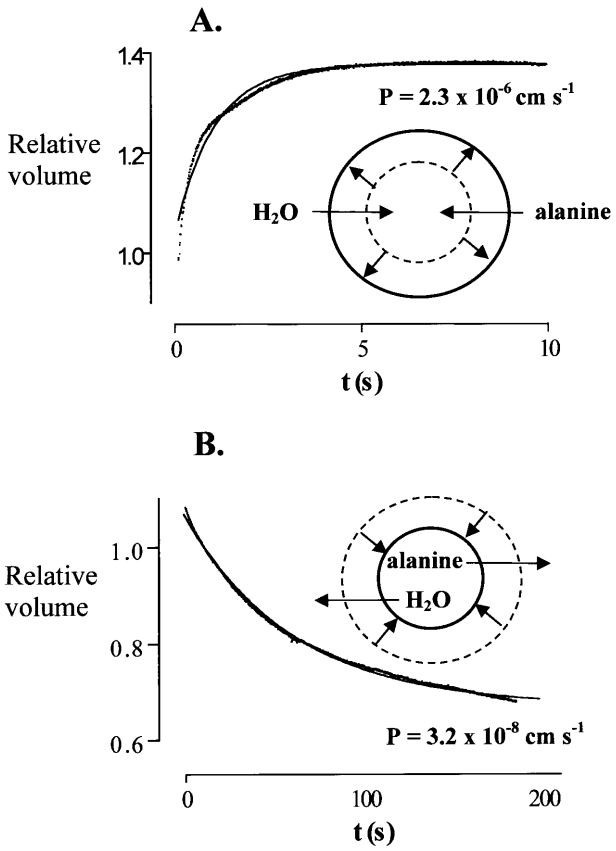

Figure 6. Demonstration of asymmetry in transport of alanine across the PBM [S. D. Tyerman and Niemietz, unpublished results]. PBM vesicle volume was measured using light scattering in a stopped-flow spectrofluorimeter [32]. (*A*) When vesicles loaded with sucrose are suddenly exposed to an external concentration of alanine but with equal total osmolarity, alanine influxes and the vesicles swell osmotically. (*B*) When vesicles loaded with alanine are suddenly exposed to a lower external concentration of alanine but with equal total osmolarity, alanine efflux occurs and the vesicles shrink osmotically (note the difference in time scale). The permeability can be calculated from the swelling/shrinking kinetics. The vesicles were 70–80% oriented such that the cytoplasmic face was to the inside. Thus influx in the figure corresponds to alanine movement to the cytoplasm from the PBS in vivo.

GmSAT1 is a nodulin. GmSAT1-transformed yeast displayed biochemical and electrophysiological characteristics similar to the soybean PBM monovalent-cation channel described above. Longer-term studies with NH_4^+ demonstrated that yeast transformed with *GmSAT1* transport NH_4^+ as well as methylammonium. An antibody raised against GmSAT1 expressed in *Escherichia coli* reacted with a 39-kDa protein in the PBM but not in the nodule soluble fraction, indicating that the protein is membrane bound [38]. However, the deduced amino acid sequence of GmSAT1 shows no extended homology to transport proteins in the data

banks, and hydropathy analysis indicates that GmSAT1 has a single putative membrane-spanning region. Further experiments with different yeast mutants have indicated that GmSAT1 is not itself a channel but that it interacts with one of the NH_4^+ transporters of yeast to promote its activity and change its properties [39; B. N. Kaiser and D. A. Day, unpublished results]. Whether this occurs at the level of mRNA or protein is not known, but it should be noted that GmSAT1 contains a sequence homologous to the DNA-binding domain of basic helix-loop-helix (bHLH) transcription factors. If GmSAT1 acts as a transcription factor in nodules, it is likely to be a receptor/sensor protein, since it is clearly located on the PBM [37]. In this context, it is worth noting that the secondary structure of GmSAT1 is similar to that of the membrane-bound Notch receptors of animal and insect cells.

Two cDNA clones that encode proteins with strong homology to high-affinity NH_4^+ transporters of other species (Amts) have recently been isolated from soybean nodules [38]. However, the location of these proteins within nodule cells has yet to be determined.

Amino acid production and transport in bacteroids and symbiosomes

Labelling experiments with bacteroids and symbiosomes

Early studies of $^{15}N_2$ fixation by detached nodules of soybean and serradella indicated that $^{15}NH_3$ was the earliest detectable product [40]. Later studies with anaerobically prepared nodule breis and washed bacteroids showed that $^{15}NH_3$ accounted for most of the total ^{15}N fixed from $^{15}N_2$ [40–42]. In addition, bacteroids appeared to have low activities of the appropriate enzymes required for assimilation of NH_4^+, while the plant cytosol has high activities of GS and GOGAT (see above). Consequently, it was accepted that fixed N_2 is secreted from bacteroids exclusively as NH_4^+ that is then assimilated into amino acids in the plant cytosol. More recent results suggest that amino acids must also be considered.

Exposure of *Bradyrhizobium japonicum* bacteroids to ^{14}C-succinate, or whole pea and soybean nodules to $^{14}CO_2$, resulted in substantial synthesis of ^{14}C-glutamate, ^{14}C-aspartate and ^{14}C-alanine [43–46]. The labelling of the glutamate pool in soybeans turned over slowly, suggesting a low rate of metabolism [44]. The high rate of glutamate synthesis has been attributed to inhibition of the 2-ketoglutarate complex of the tricarboxylic acid (TCA) cycle under microaerobic conditions due to a lowered $NADH/NAD^+$ ratio [46]. Consistent with this, mutation of the 2-ketoglutarate complex results in the synthesis and secretion of large quantities of glutamate by *Rhizobium leguminosarum* b.v. *viciae* [47]. The labelling of alanine by pea bacteroids was particularly interesting since although it was the most highly labelled amino acid in the first 3 min in isolated bacteroids, it was overtaken by glutamate after 6 min, suggesting that the alanine pool turned over rapidly [45]. Feeding ^{14}C-labelled malate, glutamate or 2-oxoglutarate, individually to N_2-fixing pea bacteroids did not result in the efflux of specific compounds [5]. However, feeding aspartate to the pea bacteroids resulted in some efflux of glutamate that was increased tenfold when 2-oxoglutarate was also added. Under these conditions, the bacteroids also excreted malate and alanine. Likewise, supplementing malate with glutamate resulted in efflux of alanine, 2-oxoglutarate and aspartate. Bacteroids of *R. lupini* also secrete alanine and aspartate when fed malate and allowed to reduce N_2 [48]. This exchange of intracellular amino acids from bacteroids is likely to occur via the general amino acid permease [49, 50]. Results such as these led to the suggestion that a malate/aspartate shuttle operates across the symbiotic interface [e.g. 51]. However, isolated symbiosomes take up amino acids poorly [22, 52] and the results of the labelling studies with intact N_2-fixing symbiosomes (see above) are not compatible with the operation of coordinated shuttles [6, 46]. Consistent with the lack of formal shuttle mechanisms, mutation of *aap*, which prevents growth on glutamate as a carbon source in free-living *R. leguminosarum*, did not affect symbiotic N_2 fixation [47]. Nonetheless, the results described above do indicate that if amino acids are synthesised in significant quantities, then they are likely to move out of the bacteroids.

When isolated pea and soybean symbiosomes were fed ^{14}C-malate under microaerobic conditions, synthesis and secretion of primarily alanine but also aspartate and glutamate were very rapid, with up to 12% of the label recovered in the reaction mixture as amino acids after 30-min incubation [6]. This indicates rapid synthesis of amino acids and secretion across both the bacteroid and PBM.

Amino acids versus ammonia as products of N_2 fixation

Although labelling data suggested that bacteroids synthesise substantial pools of amino acids under microaerobic conditions, comparative rates of NH_4^+ and amino acid synthesis were not measured until very recently and NH_3 was considered to be the only nitrogen product secreted to the plant. However, using $^{15}N_2$, isolated, sucrose-gradient-purified soybean bacteroids were shown to synthesise and secrete alanine under conditions of oxygen deprivation in a closed system [7]. This agrees with the endogenous labelling studies in soybean and pea bacteroids [5, 6, 44, 48]. Remarkably though, alanine was the sole nitrogen secretion product of these

bacteroids. The failure to detect alanine in previous studies was suggested to have been due to the use of crude differential centrifugation to purify bacteroids resulting in contamination with plant alanine deaminase activity [7], but this has subsequently been discounted [53]. A further unusual feature of this work was the low enrichment of the NH_3 pool with ^{15}N, even though NH_3 is undoubtedly the first product of nitrogenase action. Although endogenous NH_3 was present [7], which may have diluted any $^{15}NH_3$, even if alanine is the sole secreted nitrogen product, NH_3 would be expected to be highly labelled with ^{15}N when bacteroids are incubated in 99 atom percent excess $^{15}N_2$, since NH_3 supplies the nitrogen of alanine. The only way to explain this difference is to propose that N_2 reduction by nitrogenase and NH_3 assimilation into alanine must be so tightly coupled that the NH_3 formed by nitrogenase is never released into the bulk NH_3 pool in the cell.

In an attempt to address some of these contradictions, pea bacteroids were isolated anaerobically and purified on Percoll gradients that produce clean bacteroid preparations without any associated alanine deaminase activity, and the products of N_2 fixation were measured [9]. NH_4^+ was the sole secretion product when the bacteroids were kept at low–moderate density in a closed assay system [9]. As the bacteroid density was increased, alanine synthesis began at high rates. The switching on of alanine synthesis at high bacteroid densities was caused by accumulation of NH_4^+ (formed from N_2 fixation) in the fixed volume of the assay. To confirm this, exogenous NH_4^+ was titrated into low-density bacteroid preparations and alanine synthesis began immediately at high rates. Using $^{15}N_2$, both the NH_3 and alanine pools became highly labelled, consistent with NH_3 being the direct precursor of alanine. The whole cell K_m for NH_4^+ secretion was 3.1 mM. This may explain why amino acid production has not been measured in soybean bacteroids in flow cells [42, 53]: under these conditions, the NH_3 is continuously removed in the flowing medium, thereby preventing its accumulation. The measured steady-state concentration of NH_4^+ in soybean bacteroids is 12 mM, suggesting that the requirement for several millimolar NH_4^+ for alanine synthesis is still physiologically relevant [54]. However, subsequent experiments with Percoll-purified bacteroids from soybean in a closed system have shown that although some alanine is formed under some conditions, ammonia is the major product when nitrogenase activity is optimised [Y. Li, D. A .Day and F. J. Bergersen, unpublished results].

Pathway of alanine synthesis in bacteroids

Alanine dehydrogenase (AldA) has been shown to be the pathway for alanine synthesis in pea bacteroids [9]. It was identified by its ability to suppress an alanine degradation (*dadR)* mutant that does not grow on alanine as the sole source of carbon. AldA is present in the wild type and in the *dadR* mutant but is unable to compensate for the *dad* mutation unless it is present in multiple copies on a plasmid. Thus, AldA is not the primary pathway for alanine catabolism in *R. leguminosarum*, but, rather, alanine synthesis is its main function

Peas inoculated with an *aldA* deletion strain were able to fix N_2 (and by inference to produce NH_3) at the same rate as the wild type. Bacteroids from these plants also secreted NH_3 at the same rate as wild-type bacteroids, but lacked any significant alanine secretion [9]. This led to lower total rates of nitrogen secretion (NH_3 plus alanine) by bacteroids of the *aldA* mutant compared to the wild type. Consistent with this, 6-week-old plants inoculated with the *aldA* mutant showed a small but significant (20%) decrease in dry weight. This is consistent with pea bacteroids secreting both alanine and NH_3 [9], but is not absolute proof that alanine reaches the plant. The small drop in plant biomass could be explained by a knock-on effect on dicarboxylate or general metabolism.

AldA has been detected at moderate–high activity in soybean, alfalfa, pea and lupin bacteroids [9, 56–59]. In soybean bacteroids, AldA activity increased approximately threefold from day 19 to day 35 after infection and activities ranged from 0.3–1 μmol min^{-1} (mg protein)$^{-1}$ [58, 59]. These activities are much higher than in pea bacteroids [0.08 μmol min^{-1} (mg protein)$^{-1}$] suggesting that alanine production in soybean bacteroids may be significant at lower ammonium concentrations than is the case in pea bacteroids The apparent K_ms of the soybean enzyme for NADH, NH_4^+ and pyruvate are 86 μM, 8.9 mM and 0.49 mM, respectively. In *R. leguminosarum*, the K_m for NH_4^+ is 5.1 [9]. Obviously, the affinity for NH_4^+ in rhizobia is much higher than in other bacteria where K_ms in the range of 20–300 mM are common, suggesting that the enzyme has an aminating role in bacteroids [58]. In lupin and soybean bacteroids, AldA is a tetramer with subunit molecular weights of 41,000 and 43,000 and holoenzyme molecular weights of 180,000 and 190,000, respectively [57, 58].

Amino acid transport in rhizobia

Transport studies with isolated bacteroids confirm that they are able to accumulate certain amino acids quite rapidly [60–62]. A general amino acid permease (Aap) that transports a wide range of L-amino acids with very high affinity (K_m for glutamate 81 nM) has been described in free-living *R. leguminosarum* [50, 63, 64]. This system has been cloned and shown to be an ATP-bind-

ing cassette (ABC) transporter consisting of four genes: *aapJQMP*. AapJ is a periplasmic binding protein, AapQM are integral membrane proteins and AapP is the ABC subunit characteristic of this class of transporter [49, 63, 64]. The Aap is the principal transporter in *R. leguminosarum* for a wide range of amino acids including glutamate, aspartate, proline and histidine and is also a major transporter of aliphatic amino acids. The uniquely broad specificity for amino acids of the Aap (for a transporter of the ABC class) means that heterologous amino acids can exchange for one another. Thus intracellular alanine will leave the cell in exchange for a wide range of amino acids including acidic, basic and aliphatic amino acids. This has led to the suggestion that this transporter is either bidirectional or regulates another efflux channel. Consistent with a bidirectional role, Aap is responsible for 76% of glutamate export in cells where glutamate export was induced by growth on glucose and aspartate [49]. The proposal that the Aap itself may transport amino acids out of the cell is controversial since it is widely accepted that periplasmic-binding-protein-dependent ABC systems promote unidirectional amino acid uptake [65, 66]. Regardless of whether the Aap enables amino acid efflux itself or regulates another system, it is a prime candidate for regulating amino acid export by *R. leguminosarum*. Another key feature of the Aap is that under conditions where amino acid export is most active, uptake by the Aap is inhibited even though transcription of the *aap* operon is unaffected [63, 64, 67]. This suggests that Aap may be post-translationally modified to enable high rates of export when uptake is inhibited. Inhibition of uptake occurs when intracellular amino acids are at high levels, conditions that may occur inside bacteroids [7, 9, 44, 49].

Knock-out mutants of the Aap still show some glutamate uptake and considerable uptake of aliphatic amino acids, such as alanine and leucine. A second ABC transport system with high homology to the leucine, isoleucine, valine (liv) transporter of *E. coli* and the equivalent branched-chain (bra) amino transporter in *Pseudomonas* has also been mutated and cloned in *R. leguminosarum*. Designated Bra, it is composed of *braDEFGC*, with BraDE as the integral membrane components, BraFG as the ABC subunits and BraC as a periplasmic binding protein [A. M. Hosie, personal communication]. While this is clearly a transporter of aliphatic amino acids, it also shows some uptake of other amino acids such as glutamate. Double mutants in *aap* and *bra* are devoid of almost all detectable high-affinity amino acid uptake, including that of glutamate, alanine, leucine and histidine. This is very unusual and suggests that almost all high-affinity amino acid uptake in *R. leguminosarum* is regulated by just two ABC transporters. Some amino acids, such as alanine, are also taken up via low-affinity catabolic uptake systems (K_m for alanine 819 μM). This system has recently been cloned and sequenced and appears to be a C_3-monocarboxylate carrier with high affinity for lactate [K_m 4 μM; A. M. Hosie, personal communication]. Doubtless there are other specific catabolic systems induced by growth on some amino acids as sole carbon sources. However, under most growth conditions, the Aap and Bra appear to dominate high-affinity amino acid uptake and both are capable of exchanging amino acids. This is potentially of great importance to amino acid export by bacteroids. While specific export systems for amino acids such as alanine may be induced in bacteroids, Bra and Aap may well dominate export.

In *R. meliloti*, glutamate and aspartate share a common low-affinity transporter that has a K_m for aspartate of 1.5 mM, although there was evidence for a higher-affinity carrier as well [55]. Southern blots using AapJ from *R. leguminosarum* revealed strong hybridisation to *R. meliloti*, suggesting the Aap may be the high-affinity amino acid permease [64]. Interestingly, aspartate can be transported by the Dct system in *R. meliloti* and *R. leguminosarum* [67, 68]. However, while the K_m for succinate by the Dct in *R. leguminosarum* is 5 μM, the K_i for inhibition by aspartate is 5 mM [67]. This indicates that the Dct is unlikely to act as a significant transport system for aspartate in the presence of even moderate concentrations of succinate, malate or fumarate. Likewise, malate and succinate completely blocked uptake of aspartate by competitive inhibition in alfalfa bacteroids at concentrations that occur in nodules [69]. The Dct system is necessary for growth of *R. meliloti* on aspartate as a sole carbon source [68]. Phenylalanine, methionine, leucine, proline and glycine are also taken up by isolated bacteroids, albeit at much slower rates than glutamate, but transport mechanisms have not been investigated [22].

B. japonicum bacteroids isolated from soybean nodules take up glutamate, aspartate and alanine in an energy-dependent manner [22, 30, 52]. Aspartate uptake displayed an apparent K_m of 27.5 μM and a V_{max} of 5.14 nmol min^{-1} mg^{-1} and was competitively inhibited by glutamate with an apparent K_i of 9.7 μM, indicating that these amino acids share a common carrier (presumably the Aap discussed above). Bacteroids loaded with ^{14}C-aspartate exchanged label for external cold aspartate and glutamate. Alanine uptake into bacteroids also occurs via a high-affinity carrier with a K_m of 12.2 μM and V_{max} of 3.5 nmol min^{-1} mg^{-1}.

Amino acid transport across the PBM

Several models that integrate carbon and nitrogen exchange via shuttles and cycles involving uptake and efflux of amino acids across the PBM have been pro-

posed [51, 70, 71]. Transport studies with isolated symbiosomes from a number of different legumes have failed to identify the amino acid transporters on the PBM that are needed to co-ordinate the proposed schemes [1], but efflux of amino acids from the bacteroid does not necessarily require co-ordinated exchange with other metabolites. For example, both aspartate and alanine efflux from isolated soybean and pea bacteroids and symbiosomes has been detected when incubated with malate and other organic acids [7, 6, 9, 72]. When free bacteroids are loaded with C-aspartate or glutamate, the amino acid can exchange for external unlabelled aspartate, glutamate and malate [52]. Likewise, the efflux of ^{14}C-amino acids from N_2-fixing pea symbiosomes that are supplied in vitro with ^{14}C-malate as the sole carbon source was increased threefold when unlabelled glutamate was supplied as an additional carbon substrate [6]. Presumably, the amino acids synthesised in the bacteroid exchange for malate or glutamate in the PBS. The exchanged amino acid may then diffuse or be transported from the symbiosome down a concentration gradient (that will be large when isolated symbiosomes are suspended in a relatively large volume of reaction buffer).

Radioactive loading experiments with isolated symbiosomes failed to detect rapid uptake or exchange of aspartate or alanine across the PBM [52]. However, in these experiments, loading significant quantities of radioactively labelled amino acid into the symbiosome was difficult (because uptake was so limited) and the PBM was energised with ATP. Many amino acid transporters operate as proton symporters [73] and the pH gradient across the PBM (acid inside) is in the wrong direction to allow uptake. Proton symport transport out of the symbiosome, on the other hand, may occur when the PBM is energised and amino acid concentrations in the PBS are adequate. Indeed, Rubeck et al. [74] have demonstrated operation of a proton-aspartate symporter in energised bacteroid-side-out pea PBM vesicles with an apparent K_m for aspartate of 0.2 mM. The substrate specificity of the transporter has yet to be determined and, therefore, if it also operates with alanine is not known. However, diffusion of alanine out of the symbiosome can occur via the Nodulin 26 aquaporin channel (see above). Further work is needed before firm conclusions can be drawn about amino acid transport out of symbiosomes in vivo, but current results indicate that it can occur and in a controlled fashion.

Most of these studies have been performed with symbiosomes from mature nodules. In developing nodules, before the bacteroids begin to fix atmospheric nitrogen, nitrogen is likely transported from the plant to the differentiating and dividing rhizobia. The permeability of the PBM to amino acids, therefore, possibly changes during development.

Conclusions

Our current understanding of nitrogen metabolism and transport in legume symbiosomes is summarised in figure 7. The presence of transporters for both NH_4^+/NH_3 and amino acids in the PBM suggests that fixed nitrogen may efflux from the symbiosome in either form. Although the relative significance of the different forms in efflux of fixed nitrogen from the symbiosomes in planta cannot be deduced from our present knowledge, the results with the *aldA*$^-$ mutant pea bacteroids indicate that the symbiosis can function effectively with ammonia as the sole transported species but that alanine production may enhance it. We now need additional studies on efflux mechanisms for amino acids across the PBM, ideally measured under N_2-fixing conditions. However, even with detailed knowledge on the kinetics and other characteristics of the PBM transporters, their in planta significance can only be estimated, since information on the microenvironment of the different compartments of the symbiosomes is lacking. Accurate estimation of the translocation and assimilation of fixed nitrogen in nodules requires non-invasive and non-destructive measurements of the fate of the fixed nitrogen. In vivo ^{15}N-nuclear magnetic resonance (NMR) spectroscopy may provide this possibility since this method ideally can provide real-time

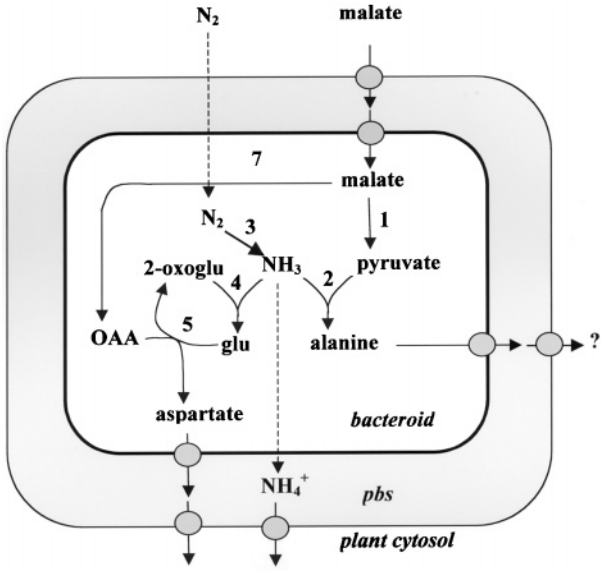

Figure 7. Summary of nitrogen fixation and transfer in the symbiosome. 1, malic enzyme; 2, alanine dehydrogenase; 3, nitrogenase; 4, glutamate dehydrogenase; 5, aspartate amino transferase; 7, malate dehydrogense. Transport mechanisms for malate, ammonium and aspartate have been identified on the PBM of either soybean or pea; transport of alanine across the PBM may occur via an aquaporin channel. Dotted lines indicate passive diffusion.

information on identity, levels and the environment of intracellular free nitrogen metabolites over a time course. The use of this technique with pea nodules containing *aldA*$^-$ bacteroids should help to provide more definitive answers concerning the importance of alanine in N_2 fixation.

Acknowledgments. We thank Dr Fraser Bergersen for critical reading of the manuscript and Dr Brent Kaiser and Mr Youzhong Li for making unpublished results available.

1 Udvardi M. K. and Day D. A. (1997) Metabolite transport across symbiotic membranes of legume nodules. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48:** 493–523
2 Poole P. S. and Allaway D. (2000) Carbon and nitrogen metabolism in *Rhizobium*. Adv. Microb. Physiol. **43:** 117–163
3 Whitehead L. F. and Day D. A. (1997) The peribacteroid membrane. Physiol. Plant **100:** 30–44
4 Rosendahl L., Dilworth M. J. and Glenn A. R. (1991) Organic and inorganic inputs into legume root nodule nitrogen fixation. In: Biology and Biochemistry of Nitrogen Fixation, pp. 259–292, Dilworth M. J. and Glenn A. R. (eds), Elsevier, Amsterdam
5 Appels M. A. and Haaker H. (1991) Glutamate oxaloacetate transaminase in pea root nodules – participation in a malate/aspartate shuttle between plant and bacteroid. Plant Physiol. **95:** 740–747
6 Rosendahl L., Dilworth M. J. and Glenn A. R. (1992) Exchange of metabolites across the peribacteroid membrane in pea root nodules. J. Plant Physiol. **139:** 635–638
7 Waters J. K., Hughes B. L., Purcell L. C., Gerhardt K. O., Mawhinney T. P. and Emerich D. W. (1998) Alanine not ammonia, is excreted from N_2-fixing soybean nodule bacteroids. Proc. Nat. Acad. Sci. USA **95:** 12038–12042
8 Atkins C. A. (1991) Ammonia assimilation and export of nitrogen from the legume nodule. In: Biology and Biochemistry of Nitrogen Fixation, pp. 293–319, Dilworth M. J. and Glenn A. R. (eds), Elsevier, Amsterdam
9 Allaway D., Lodwig E., Crompton L. A., Wood M., Parsons T. R., Wheeler T. et al. (2000) Identification of alanine dehydrogenase and its role in mixed secretion of ammonium and alanine by pea bacteroids. Mol. Microbiol. **36:** 508–515
10 Gober J. W. and Kashket E. R. (1983) Methylammonium uptake by *Rhizobium* sp. strain 32H1. J. Bacteriol. **153:** 1196–1201
11 Fischer H. M. (1994) Genetic regulation of nitrogen fixation in rhizobia. Microbiol. Rev. **58:** 352–386
12 Batut J. and Boistard P. (1994) Oxygen control in *Rhizobium*. Antonie Van Leeuwenhoek **66:** 129–150
13 Brown C. M. and Dilworth M. J. (1975) Ammonia assimilation by rhizobium cultures and bacteroids. J. Gen. Microbiol. **86:** 39–48
14 Glenn A. R. and Dilworth M. J. (1984) Methylamine and ammonium transport systems in *Rhizobium leguminosarum* MNF3841. J. Gen. Microbiol. **103:** 1961–1968
15 Howitt S. M., Udvardi M.K., Day D. A. and Gresshoff P. M. (1986) Ammonia transport in free-living and symbiotic *Rhizobium* sp. ANU289. J. Gen. Microbiol. **132:** 257–261
16 O'Hara G. W., Riley I. T., Glenn A. R. and Dilworth M. J. (1985) The ammonium permease of *Rhizobium leguminosarum* MNF3841. J. Gen. Microbiol. **131:** 757–764
17 Pawlowski K., Ratet P., Schell J. and Bruijn F. J. de (1987) Cloning and characterization of *nifA* and *ntrC* genes of the stem nodulating bacterium ORS571, the nitrogen fixing symbiont of *Sesbania rostrata*: regulation of nitrogen fixation (*nif*) genes in the free living versus symbiotic state. Mol. Gen. Genet. **206:** 207–219
18 Szeto W. W., Nixon B. T., Ronson C. W. and Ausubel F. M. (1987) Identification and characterization of the *Rhizobium meliloti ntrC* gene: *R. meliloti* has separate regulatory pathways for activation of nitrogen fixation genes in free-living and symbiotic cells. J. Bacteriol. **169:** 1423–1432
19 Udvardi M. K., Lister D. L. and Day D. A. (1992) Isolation and characterization of a ntrC mutant of *Bradyrhizobium* (*Parasponia*) sp. ANU289. J. Gen. Microbiol. **138:** 1019–1025
20 Kleiner D. (1985) Bacterial ammonium transport. FEMS Microbiol. Rev. **32:** 87–100
21 Pargent W. and Kleiner D. (1985) Characteristics and regulation of ammonium (methylammonium) transport in *Rhizobium meliloti*. FEMS Microbiol. Lett. **30:** 257–259
22 Udvardi M. K., Salom C. L. and Day D. A. (1988) Glutamate transport across the bacteroid but not the peribacteroid membrane of soybean nodules. Mol. Plant Microbe Interact. **1:** 250–254
23 Jin H. N., Glenn A. R. and Dilworth M. J. (1988) How does L-glutamate transport relate to selection of mixed nitrogen sources in *R. leguminosarum*? Arch. Microbiol. **153:** 448–454
24 Bergersen F. J. and Turner G. L. (1967) Nitrogen fixation by the bacteroid fraction of breis of soybean root nodules. Biochim. Biophys. Acta **141:** 507–515
25 Tate R., Riccio A., Merrick M. and Patriaca E. J. (1998) The *Rhizobium etli amtB* gene coding for an NH_4^+ transporter is down-regulated early during bacteroid differentiation. Mol. Plant Microbe Interact. **11:** 188–198
26 Brewin N. J. (1991) Development of the legume root nodule. Annu. Rev. Cell Biol. **7:** 191–226
27 Udvardi M. K. and Day D. A. (1990) Ammonia (^{14}C-methylamine) transport across the bacteroid and peribacteroid membranes of soybean root nodules. Plant Physiol. **94:** 71–76
28 Tyerman S. D., Whitehead L. F. and Day D. A. (1995) A channel-like transporter for NH_4^+ on the symbiotic interface of N_2-fixing plants. Nature **378:** 629–632
29 Mouritzen P. and Rosendahl L. (1997) Identification of a transport mechanism for ammonium in the symbiosome membrane of pea root nodules. Plant Physiol. **115:** 519–526
30 Whitehead L. F., Day D. A. and Tyerman S. D. (1998) Divalent cation gating of an ammonium permeable channel in the symbiotic membrane from soybean nodules. Plant J. **16:** 313–324
31 Szafran M. M. and Haaker H. (1995) Properties of the peribacteroid membrane ATPase of pea root nodules and its effect on the nitrogenase activity. Plant Physiol. **108:** 1227–1232
32 Tyerman S. D. and Niemietz C. M. (in press). Solute and water permeation across the symbiosome membrane of legumes: asymmetry of water and solute permeation through Nod26. Proc. MIP2000, Gotenburg, Sweden
33 Rivers R. L., Dean R. M., Chandy G., Hall J. E., Roberts D. M. and Zeidel M. L. (1997) Functional analysis of nodulin 26, an aquaporin in soybean root nodule symbiosomes. J. Biol. Chem. **272:** 16256–16261
34 Dean R. M., Rivers R. L., Zeidel M. L. and Roberts D. M. (1999) Purification and functional reconsititution of soybean nodulin 26: an aquaporin with water and glycerol transport properties. Biochemistry **38:** 347–353
35 Ou Yang L. J., Whelan J., Weaver C. D., Roberts D. M. and Day D. A. (1991) Protein phosphorylation stimulates the rate of malate uptake across the peribacteroid membrane of soybean nodules. FEBS Lett. **293:** 188–190
36 Tyerman S. D., Bohnert H. J., Maurel C., Steudle E. and Smith J. A. C. (1999) Plant aquaporins: their molecular biology, biophysics and significance for plant water relations. J. Exp. Bot. **50:** 1055–1071
37 Kaiser B. N., Finnegan P. M., Tyerman S. D., Whitehead L. F., Bergersen F. J., Day D. A. et al. (1998) Characterisation of an ammonium transport protein from the peribacteroid membrane of soybean nodules. Science **281:** 1202–1206
38 Kaiser B. N. (1999) Ammonium Transport Across Symbiotic Membranes from Soybean Nodules. PhD thesis, Australian National University, Canberra

39 Marini A. M., Springael J. Y., Frommer W. B. and Andre M. (2000) Cross-talk between ammonium transporters in yeast and interference by the soybean SAT1 protein. Mol. Microbiol. **35:** 378–385

40 Bergersen F. J. (1965) Ammonia – an early stable product of nitrogen fixation by soybean root nodules. Austr. J. Biol. Sci. **18:** 1–9

41 Kennedy I. R. (1966) Primary products of symbiotic nitrogen fixation. II. Pulse labelling of *Serradella* nodules with $^{15}N_2$. Biochem. Biophys. Acta **120:** 295–303

42 Bergersen F. J. and Turner G. L. (1990) Bacteroids from soybean root nodules: respiration and N_2 fixation in flow-chamber reactions with oxyleghaemoglobin. Proc. Soc. Lond. B **238:** 295–320

43 Rosendahl L., Vance C. P. and Pedersen W. B. (1990) Products of dark CO_2 fixation in pea root nodules support bacteroid metabolism. Plant Physiol. **93:** 12–19

44 Salminen S. O. and Streeter J. G. (1987) Involvement of glutamate in the respiratory metabolism of *Bradyrhizobium japonicum* bacteroids. J. Bacteriol. **169:** 495–499

45 Salminen S. O. and Streeter J. G. (1992) Labeling of carbon pools in *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* BV *viciae* bacteroids following incubation of intact nodules with Co-14(2). Plant Physiol. **100:** 597–604

46 Salminen S. O. and Streeter J. G. (1990) Improved analysis of metabolite uptake by *Bradyrhizobium japonicum* bacteroids. Can. J. Microbiol. **37:** 238–243

47 Walshaw D. L., Wilkinson A., Mundy M., Smith M. and Poole P. S. (1997) Regulation of the TCA cycle and the general amino acid permease by overflow metabolism in *Rhizobium leguminosarum*. Microbiology **143:** 2209–2221

48 Kretovich V. L., Karyakina T. I., Sidelnikova L. I., Shaposhnikov G. L. and Kaloshina G. S. (1986) Nitrogen fixation and biosynthesis of aspartic acid and alanine by bacteroids of *Rhizobium lupini* on various carbon sources. Dokl. Akad. Nauk. SSSR **291:** 1008–1011

49 Walshaw D. L. and Poole P. S. (1996) The general L-amino acid permease of *Rhizobium leguminosarum* is an ABC uptake system that influences efflux of solutes. Mol. Microbiol. **21:** 1239–1252

50 Poole P. S., Franklin M., Glenn A. R. and Dilworth M. J. (1985) The transport of L-glutamate by *Rhizobium leguminosarum* involves a common amino acid carrier. J. Gen. Microbiol. **131:** 1441–1448

51 Kahn M. L., Kraus J. and Sommerville J. E. (1985) A model of nutrient exchange in the *Rhizobium*-legume symbiosis. In: Nitrogen Fixation Research Progress, pp. 193–199, Evans H. J., Bottomley P. J. and Newton W. E. (eds), Nijhoff, Dordecht

52 Whitehead L. F., Young S. and Day D.A. (1998) Aspartate and alanine movement across symbiotic membranes of soybean nodules. Soil Biol. Biochem. **30:** 1583–1589

53 Li Y., Green L. S., Day D. A. and Bergersen F. J. (2000) Ammonia and alanine efflux from nitrogen-fixing soybean bacteroids. In: Nitrogen Fixation: from Molecules to Crop Productivity, p. 391, Pedrosa F. O., Hungria M., Yates M. G. and Newton W. E. (eds), Kluwer, Dordecht.

54 Streeter J. G. (1989) Estimation of ammonia concentration in the cytosol of soybean nodules. Plant Physiol. **90:** 779–782

55 Miller R. W., McRae D. G. and Joy K. (1991) Glutamate and gamma-aminobutyrate metabolism in isolated *Rhizobium meliloti* bacteroids. Mol. Plant Microbe. Interact. **4:** 37–45

56 Dunn S. D. and Klucas R. V. (1973) Studies on possible routes of ammonium assimilation in soybean root nodule bacteroids. Can. J. Microbiol. **19:** 1493–1499

57 Kazakova O. V., Tsuprun V. L., Ivanushkin A. G., Kaftanova A. S., Pushkin A. V. and Kretovich V. L. (1988) Quaternary structure and kinetic characteristics of alanine dehydrogenase from *Rhizobium lupini* bacteroids. Dokl. Akad. Nauk. SSSR **300:** 479–482

58 Smith M. T. and Emerich D. W. (1993) Alanine dehydrogenase from soybean nodule bacteroids: purification and properties. Arch. Biochem. Biophys. **304:** 379–385

59 Stripf R. and Werner D. (1978) Differentiation of *Rhizobium japonicum*. II. Enzymatic activities in bacteroids and plant cytoplasm during the development of nodules of *Glycine max*. Z. Naturforsch **33:** 373–381

60 Dilworth, M. J. and Glenn, A. (1981) Control of carbon substrate utilization by rhizobia. In: Current Perspectives in Nitrogen Fixation, pp 244–251, Gibson A. H. and Newton W. E. (eds), Australian Academy of Science, Canberra

61 Jin H. N., Glenn A. R. and Dilworth M. J. (1990) How does L-glutamate transport relate to selection of mixed nitrogen sources in *Rhizobium leguminosarum* biovar *trifolii* MNF1000 and cowpea *Rhizobium* MNF2030? Arch. Microbiol. **153:** 448–454

62 Day D. A. and Udvardi M. K. (1992) Metabolite exchange across symbiosome membranes. Symbiosis **14:** 175–189

63 Walshaw D. L., Lowthorpe S., East A. and Poole P. S. (1997) Distribution of a sub-class of bacterial ABC polar amino acid transporter and identification of an N-terminal region involved in solute specificity. FEBS Lett. **414:** 397–401

64 Walshaw D. L., Reid C. J. and Poole P. S. (1997) The general amino acid permease of *Rhizobium leguminosarum* strain 3841 is negatively regulated by the Ntr system. FEMS Microbiol. Lett. **152:** 57–64

65 Higgins C. F. (1992) ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. **8:** 67–113

66 Holland I. B. and Blight M. A. (1999) ABC-ATPases, adaptable energy generators fuelling transmembrane movement of a variety of molecules in organisms from bacteria to humans. J. Mol. Biol. **293:** 381–399

67 Reid C. J., Walshaw D. L. and Poole P. S. (1996) Aspartate transport by the Dct system in *Rhizobium leguminosarum* negatively affects nitrogen-regulated operons. Microbiology **142:** 2603–2612

68 Watson R. J., Rastogi V. K. and Chan Y.-K. (1993) Aspartate transport in *Rhizobium meliloti*. J. Gen. Microbiol. **139:** 1315–1323

69 McRae D. G., Miller R. W., Berndt W. B. and Joy K. (1989) Transport of C4-dicarboxylates and amino acids by *Rhizobium meliloti* bacteroids. Mol. Plant Microbe Interact. **2:** 273–278

70 Kim Y. S. and Chae H. Z. (1990) A model of nitrogen flow by malonamate in *Rhizobium japonicum*-soybean symbiosis. Biochem. Biophys. Res. Commun. **169:** 692–699

71 Kohl D. H., Schubert K. R., Carter M. B., Hagedorn C. H. and Shearer G. (1988) Proline metabolism in N_2-fixing root nodules: energy transfer and regulation of purine synthesis. Proc. Natl. Acad. Sci. USA **85:** 2036–2040

72 Kouchi H., Fukai K. and Kihara A. (1991) Metabolism of glutamate and aspartate in bacteroids isolated from soybean root nodules. J. Gen. Microbiol. **137:** 2901–2910

73 Rentsch D., Boorer K. J. and Frommer W. B. (1998) Structure and function of plasma membrane amino acid, oligopeptide and sucrose transporters from higher plants. J. Membrane Biol. **162:** 177–190

74 Rubeck A., Mouritzen P. and Rosendahl L. (1999) Characterisation of aspartate transport across the symbiosome membrane in pea root nodules. J. Plant Physiol. **155:** 576–583