dons, however, is not affected by chloramphenicol while in rice and maize seedlings it is induced by the antibiotic. Dixit et al. reported the inhibition of induction of nitrate reductase by chloramphenicol in maize leaves. Such conflicting results exist in the literature. The present study therefore suggests the need for investigating and understanding the effect of chloramphenicol directly or indirectly on eukaryotic protein synthesis inhibition.

**Table 3**

**Effect of chloramphenicol on cytosolic isocitrate dehydrogenase malate dehydrogenase and isocitrate lyase activities from Aspergillus niger**

<table>
<thead>
<tr>
<th>Conditions of the growth</th>
<th>Isocitrate dehydrogenase*</th>
<th>Malate dehydrogenase*</th>
<th>Isocitrate lyase*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.9</td>
<td>556.2</td>
<td>34.8</td>
</tr>
<tr>
<td>With chloramphenicol (2 mg/ml)</td>
<td>6.6</td>
<td>328.4</td>
<td>18.4</td>
</tr>
<tr>
<td>With chloramphenicol (4 mg/ml)</td>
<td>5.6</td>
<td>206.7</td>
<td>18.5</td>
</tr>
</tbody>
</table>

* (units/mg protein)
13 December 1982


**REGULATION OF NITROGENASE EXPRESSION IN RHIZOBIUM**

**S. S. MOHAPATRA AND P. M. GRESSHOFF**

Botany Department, Australian National University, Canberra, ACT, Australia.

**INTRODUCTION**

The catalytic reduction of atmospheric dinitrogen to ammonia by nitrogenase and its subsidiary enzyme systems (nitrogen fixation), is of immense agricultural importance. Research on biological nitrogen fixation was pioneered by intensive studies of legume-Rhizobium symbioses, since legumes contribute more to the nitrogen economy of the world than any other system. Reductionist efforts to characterise these symbioses have been made with excised root cultures, detached leaves and pods, nodules, nodule protoplasts, and isolated bacteroids. Although potentially valuable, the application of these isolated experimental systems is limited because of the enormous complexities involved in the symbiosis. Development of an effective symbiosis comprises a multi-step cascade of events, namely root colonisation, root hair adhesion, infection, nodule initiation and development, bacteroid development and ultimately nitrogen fixation. This requires a tightly co-ordinated reciprocal communication between plant and bacterium, as shown in figure 1. The range of non-fixing Rhizobium mutants isolated
Figure 1: A scheme for function of Legume-
Rhizobium symbiotic genes. The success of symbiotic process requires the co-ordinated read-off of genetic signals between plant and the bacterium, both being subjected to control by environment. Plants cells undergo differentiation (Sym. A, B etc) for cortical via meristematic to bacteroid filled cells. Meanwhile the vegetative bacterial cells undergo a morphogenesis (Sym. 1, 2 etc) and develop into nitrogen fixing bacteroids.

indicates that each step of the symbiosis is governed by at least one prokaryotic gene. Further complications at different biochemical and physiological levels arise in the study of the nodule, an organ sui generis often plagued by changes in macro and microenvironmental conditions. Thus, isolated plant organs have a restricted value for the study of the central features of the symbiosis such as the expression of nitrogenase.

The last decade has seen considerable progress towards an understanding of the more subtle aspects of the nitrogenase regulation. Attempts have been made to mimic the plant-Rhizobium symbiosis in explanta co-cultures of plant cells and Rhizobium. Additionally, under appropriate conditions nitrogenase activity (the final operative phenotype in the root nodule symbiosis previously thought to be expressed only in bacteroids) has been demonstrated in axenic cultures of Rhizobium. Although it is not known yet, whether or not such symbiotic activity by Rhizobium occurs in the rhizosphere, this discovery was significant as it permitted the development of the genetics of Rhizobium nitrogenase and its regulation. This article discusses these developments.

IN VITRO SYMBIOSIS

Recent advances in cell and tissue culture opened up new avenues for studying symbiotic nitrogen fixation at the cellular level. Co-cultures of plant callus or cell suspension with rhizobia were initially used as model systems. Progress in this area has been recently reviewed and is summarised in table 1.

(a) Infection and nodulation:

Original interest in in vitro co-culture was directed towards its potential as a system to study the infection process. Although many workers demonstrated Rhizobium infection of plant cell cultures to be similar to the in planta situation (table 1), others have referred to the phenomena as a superficial, morphological artifact rather than a genetically-controlled developmental process. Furthermore, the behaviour of cultured plant cells does not always correspond to the genetic constitution of the plant from which they were derived. Thus, the conclusions drawn from infection studies made in vitro were anomalous. Ability of Rhizobium strains to interact with legume stems and non-legume derived callus culture conclusively demonstrated that genetic barriers to in planta nitrogen fixation (infection, nodule initiation, etc.) either were not operable or were bypassed in vitro. The recent discovery of promiscuous Rhizobium strains (belonging to the cowpea miscellany) capable of nodulating legume stems and various non-leguminous tree species (belonging to the Parasponia genus) shows a relaxed stringency of plant—Rhizobium association similar to in vitro studies. These findings have thus helped to dispel the existing dogma that a Rhizobium strain is capable of nitrogen fixation only when associated with the root of particular legumes defined by its cross inoculation specificity.

(b) Nitrogen fixation in vitro

The capability of Rhizobium strain 32H1 to derepress nitrogenase activity (as measured by acetylene reduction or ¹⁵N₂ incorporation) in the presence of both legume and non-legumes callus and later in the absence of any plant cells elicited two important features: (a) the genetic information for nitrogenase is encoded in the bacterium and (b) that the diffusible but yet nutritionally substitutable substances produced by the plant cells were probably responsible for stimulating Rhizobium nitrogenase activity. Interestingly some of the nutritional and environmental factors controlling success of the in planta symbiosis also regulate expression of nitrogenase in vitro. For example, nitrate, ammonia and glutamine as well as oxygen have repressing effects. Succinate, a carbon metabolite passing from the plant cytoplasm to the bacteroid, was a key compound to supporting high levels of in vitro nitrogenase activity. Further investigations using a trans-filter apparatus (i.e. Rhizobium-plant cell suspension co-cultures) extended these initial observations made on separated agar cultures.

Nitrogenase activity was derepressed and/or stimulated in normally non-derepressable or moderately
<table>
<thead>
<tr>
<th>Associations involved</th>
<th>Aspects of symbiosis studied</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Rhizobium-legume associations</td>
<td>(a) Cellular differentiation</td>
<td>13, 14, 15, 16, 17</td>
</tr>
<tr>
<td>(1) <em>R. japonicum-Glycine max</em></td>
<td>(b) Intracellular symbiosis</td>
<td>18, 19, 20</td>
</tr>
<tr>
<td></td>
<td>(c) Nutritional and cultural parameters:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hormonal</td>
<td>13, 14, 15, 16, 17, 18, 22</td>
</tr>
<tr>
<td></td>
<td>inorganic combined N2</td>
<td>15, 16, 17, 18, 23, 24, 25, 26</td>
</tr>
<tr>
<td></td>
<td>Carbohydrates</td>
<td>17, 27</td>
</tr>
<tr>
<td></td>
<td>(d) Host symbiont genetic effect</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>(e) Stimulatory/inhibitory effect of diffusible factors</td>
<td>17, 25, 28, 29, 44, 45, 46</td>
</tr>
<tr>
<td></td>
<td>(f) Non-specificity</td>
<td>29, 30</td>
</tr>
<tr>
<td>(2) <em>R. vigna-Arachis hypogaea</em> (cowpea miscellany)</td>
<td>Intracellular symbiosis</td>
<td>31, 32</td>
</tr>
<tr>
<td></td>
<td>— <em>Stylosanthes gracilis</em></td>
<td>non-specificity (stem callus used)</td>
</tr>
<tr>
<td></td>
<td>— <em>Glycine max</em></td>
<td>non-specificity</td>
</tr>
<tr>
<td></td>
<td>— <em>Pisum sativum</em></td>
<td>(differential cross inoculation group combinations)</td>
</tr>
<tr>
<td></td>
<td>— <em>Trifolium Spp</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>— <em>Vicia hajastana</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>— Cowpea</td>
<td>Stimulatory effects of diffusible factors</td>
</tr>
<tr>
<td>(3) <em>R. trifolii</em> - <em>Trifolium</em></td>
<td>Organogenesis and cellular differentiation</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>— <em>T. repens</em></td>
<td></td>
</tr>
<tr>
<td>(4) <em>R. lupini</em> - <em>Lupinus polyphyllus</em></td>
<td>Stimulatory effects of diffusible factors</td>
<td>36</td>
</tr>
<tr>
<td>2. Rhizobium (strain 32H1) - Non-legume associations</td>
<td>Stimulatory factors</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>— <em>Brassica napus</em></td>
<td>deviation of</td>
</tr>
<tr>
<td></td>
<td>— <em>Bromus inermis</em></td>
<td><em>Rhizobium</em> - legume</td>
</tr>
<tr>
<td></td>
<td>— <em>Triticum monococcum</em></td>
<td>symbiosis <em>in vitro</em> and stimulatory effects of diffusible factors</td>
</tr>
<tr>
<td></td>
<td>— <em>Nicotiana tabacum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>— <em>Nemesia strummuusa</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>— <em>Portulaca grandiflora</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>— <em>Petunia hybrida</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>— <em>Daucus carota</em></td>
<td></td>
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<tr>
<td></td>
<td>— <em>Triticum aestivum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>— <em>Sorghum nigricans</em></td>
<td></td>
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</tbody>
</table>

Derepressed *Rhizobium* strains during co-culture with plant cells. This effect was also obtained when vegetative *Rhizobium* cells were exposed to co-culture supernatant. It was postulated that this phenomenon involved an exchange of molecular signals between plant and bacterial cells. Recently legume cell cultures of soybean, pea, and white clover were shown to respond to certain bacterial substances by synthesizing dialysable plant factors (also called nitrogenase factors) which were claimed to accumulate in the conditioned plant cell medium (PCM)\textsuperscript{36,38}, subsequently causing derepression of nitrogenase.

Fractionation of plant cell conditioned medium indicated that only certain fractions were able to stimulate expression of nitrogenase activity in pure cultures of *Rhizobium*. PCM fractionation led to the
postulate by Reporter that copper-containing metallothioneins and peptidoglycans were involved in the in vitro interaction. PCM was reported to increase oxidative phosphorylation. In parallel, the derepression of nitrogenase was apparently associated with the loss of exopolysaccharide (Reporter, personal communication). However, there are many gaps, inconsistencies and experimental weaknesses that cloud the reliability and interpretation of the above mentioned studies and the numerous related investigations by other workers. It remains obscure in the absence of reproducible results, whether PCM components represent complex molecular signals. Whether such "signals" exist, or if they are a collection of "single" organic molecules involved in normal cellular metabolism still remains unclear, although the in vitro culture on completely defined media of some strains (e.g. CB756, ANU289 or 32H1) indicated that at least for some strains there was no need for signal complexity.

**NITROGENASE IN FREE-LIVING RHIZOBIUM**

Although attempts to show asymbiotic nitrogen fixation by rhizobia were first documented in 1945, the conclusive evidence for such activity was not obtained until 30 years later. The reports on in vitro symbiosis between plant cells and rhizobia (Table 1) provided the primary impetus to efforts and subsequently discovery of nitrogen fixation in free-living rhizobia. The demonstration of nitrogen fixation by 15N2 incorporation and nitrogenase activity (acetylene reduction) inhibition by specific inhibitors has confirmed the early observations. Since then either agar, stationary/shaken liquid or chemostat culture of *Rhizobium* in defined media has been used to produce nitrogenase activity. To date more than 50 strains (Table 2) show the nitrogenase positive phenotype under in vitro conditions.

However, most of these strains belong to slow growing *Rhizobium* species such as *R. japonicum* and 'cowpea strains'. Several strains belonging to these species cannot be derepressed for nitrogenase under similar conditions as used for derepressable strains. Since the strains differ with regard to the requirements for expression of nitrogenase activity, it may be that yet optimal conditions necessary for derepression of nitrogenase in these strains have not been found. Alternately, derepression of nitrogenase in the laboratory cultures may be under genetic control. The results of some relatively recent genetic exchange experiments and DNA:DNA hybridization studies indicated that strains labelled 'cowpea rhizobia' and *R. japonicum* may represent at least two and three different sub-species, respectively. Since many strains belonging to one sub-species of *R. japonicum* were nitrogenase positive in vitro it was thought that nitrogenase derepression in culture may be confined to one sub-species. The organisation of nitrogen fixation genes was reported to be very different in *R. japonicum* strains USDA110 and 61A76, belonging to the same sub-species (on the basis of DNA homology classification), of which only strain 61A76 is capable of nitrogenase expression in vitro. Thus it appears that the genetic basis for in vitro nitrogenase expression may be strain specific. The precise molecular architecture for such differences between strains remains to be worked out.

In contrast, as shown in Table 2 there are only a few unsubstantiated reports in the literature of nitrogenase activity with fast growing *Rhizobium* species. Demonstration of nitrogenase activity in *R. trifolii* strain T1, the spectinomycin resistant derivative T1 spec or by exposure to plant-cell conditioned medium was not repeatable in our laboratory and in that of others (Beringer pers. comm.). Furthermore, other reports of nitrogenase activity in fast growing *Rhizobium* strains are either characterised by a lack of repeatability or proper contamination tests. It is thus our opinion that as yet a repeatable derepression of nitrogenase in fast growing *Rhizobium* strains such as *R. meliloti*, *R. trifolii* and *R. leguminosarum* under defined or in associated in vitro culture is not demonstrable.

<table>
<thead>
<tr>
<th><strong>Rhizobium species</strong></th>
<th><strong>Nitrogenase positive (agar liquid)</strong></th>
<th><strong>Nitrogenase negative (agar liquid)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>'Cowpea' strains</td>
<td>12 4 9 —</td>
<td></td>
</tr>
<tr>
<td><em>R. japonicum</em></td>
<td>8 26 5 13</td>
<td></td>
</tr>
<tr>
<td><em>R. lupini</em></td>
<td>— — 4 —</td>
<td></td>
</tr>
<tr>
<td><em>R. meliloti</em></td>
<td>— 2(?) 4 2</td>
<td></td>
</tr>
<tr>
<td><em>R. trifolii</em></td>
<td>— 2(?) 16</td>
<td></td>
</tr>
<tr>
<td><em>R. leguminosarum</em></td>
<td>1(?) — 6 —</td>
<td></td>
</tr>
<tr>
<td>Paraporia</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td>1 1 14(?) —</td>
<td></td>
</tr>
</tbody>
</table>

—, not known
(?) not confirmed results
Several factors influence derepression of nitrogenase in \textit{Rhizobium in vitro}. Some recent reviews\textsuperscript{56, 52, 63} have discussed these factors. Here, we discuss some new developments which have not been reviewed previously.

(a) Regulation by Oxygen

Nitrogenase activity on agar cultures was measured under atmospheric oxygen tension (0.2 atm), which was later found to be the optimal concentration\textsuperscript{52}. Attempts\textsuperscript{52} to derepress nitrogenase in liquid culture under air were unsuccessful as a consistently low O\textsubscript{2} tension was obligatory for derepression. Oxygen concentrations in the range of 0.06 to 0.36% were effective\textsuperscript{56, 64}. Studies in chemostat cultures of strain 32H1 indicated that cultures growing with about 1 micro-mole dissolved oxygen gave high specific rates. The optimal level of oxygen in the gasphase differed with carbon and nitrogen sources used in the medium\textsuperscript{65, 66}. Different strains showed variable O\textsubscript{2} optima, all being within a small range generally classified as microaerobic\textsuperscript{65}.

It was not known then, whether the lack of activity under aerobic conditions was due to O\textsubscript{2} repression of nitrogenase synthesis, O\textsubscript{2} inactivation of nitrogenase or auto-oxidation of reduced electron donors. Measurement of antigenically cross-reacting material\textsuperscript{66} in aerated continuous cultures of strain 32H1 showed oxygen repression of nitrogenase synthesis. Exposure to moderate oxygen concentrations (20–30 μM dissolved oxygen tension) of the culture with established nitrogenase activity also resulted in inactivation of nitrogenase\textsuperscript{65}. Similar effects of O\textsubscript{2} on nitrogenase synthesis were observed by pulse labelling derepressed cells with a \textsuperscript{14}C labelled amino acid in free-living \textit{R. japonicum}\textsuperscript{67}. In \textit{Klebsiella pneumoniae} oxygen is thought to interact with the \textit{nif/LA} regulatory protein complex which in turn controls transcription of the \textit{nif} HDK operon responsible for the synthesis of the Fe and Mo/Fe component protein of nitrogenase. Whether O\textsubscript{2} regulates nitrogenase synthesis in \textit{Rhizobium} in a manner similar to that observed in \textit{Klebsiella} remains to be answered.

(b) Regulation by Carbon sources

\textit{Rhizobium} strains differ in their preference for a carbon source for growth as well as derepression of nitrogenase. Initially a combination of two carbon sources (a pentose sugar, e.g. arabinose and a TCA cycle intermediate, e.g. succinate) was advocated\textsuperscript{50, 54} to derepress nitrogenase activity in strain 32H1. From our work with \textit{Parasponia-Rhizobium} strain ANU289, it appears that of the two, succinate seems to play a dominating role in derepression. Comparable results were also obtained\textsuperscript{58} using a single carbon source (gluconate or succinate) in strain 32H1 and several \textit{R. japonicum} strains. However, it must be noted that the later reports utilised the liquid derepression rather than agar culture system as well as different oxygen levels.

The exact role of a carbon source in derepression of nitrogenase is yet not understood. Using an experimental approach that allows the quantitative determination of the \textit{de-novo} biosynthesis of the constituent polypeptides of nitrogenase, the amount of synthesis was shown to be dependent upon the carbon source used in free living \textit{R. japonicum}\textsuperscript{67}. Cells grown on mannitol or glycerol produced only trace amounts of the nitrogenase polypeptides whereas gluconate supported maximal synthesis and whole cell nitrogenase activity.

Since catalytic activity of nitrogenase depends on the supply of reducing equivalents and energy, the carbon source may exert its effect by modulating general metabolism of the cell\textsuperscript{68}. A negative correlation between exopolysaccharide production and nitrogenase activity was found in liquid cultures in a survey of 20 \textit{Rhizobium} strains\textsuperscript{66}. It was envisaged that nitrogenase and exopolysaccharide (EPS) synthesis compete for energy (in energy limiting microaerobic conditions). Thus in strains, which produce large amounts of EPS, most available energy was utilized in the production of EPS rather than synthesis and maintenance of nitrogenase. EPS synthesis and nitrogenase depression in \textit{Parasponia-Rhizobium} strain ANU289 (non-mucoid on mannitol containing medium) and its isogenic mucoid derivative strain ANU289\textsuperscript{66, 67} supported the above hypothesis\textsuperscript{68}. Further studies with inhibitors specific for exopolysaccharides synthesis or further isolation of specific mutants such as strain ANU288 and ANU289 lacking any one of the enzymes necessary for exopolysaccharide synthesis may be helpful in understanding the correlation between EPS synthesis and nitrogenase activity.

(c) Regulation by ammonium

\textit{Rhizobium} strains with the exception of \textit{Sesbania} (Dreyfus, pers. comm.) strains in contrast to other free-living nitrogen fixing organisms exhibit the nitrogen fixing phenotype only in a developmental state which is different from their normal vegetative growth state. Attempts to grow \textit{Rhizobium} on its own fixed nitrogen have been difficult because derepression of nitrogenase occurred only in the presence of a utilizable nitrogen source, such as glutamate, glutamine, ammonium chloride, potassium nitrate, aspartate,
aspargine and casaminoacids. The type and amount of the particular nitrogen sources varies between strains.

For example, inhibition of nitrogenase activity by 10 mM ammonium was observed in agar culture at 20% oxygen in the gas phase. In contrast, similar concentration of ammonium had no inhibitory effect on nitrogenase in strains 32H1 and 31-1b-83 in dilute shaken culture under low oxygen tension. Thus a close interaction between oxygen and ammonium concentrations and culture method was thought to be involved. Furthermore, studies with R. japonicum strain 31-1b-83 revealed that the degree of ammonium inhibition was pH dependent and the maximum inhibition was found at the optimum pH for nitrogenase activity. Variable effects of ammonium depending upon the carbon source used in the medium observed in our laboratory indicated that the inhibition may be mediated by a possible metabolic effect. Thus the oxygen and carbon effects can be explained by differential growth and thus depletion. As ammonium effects on nitrogenase activity are strain specific and affected by carbon sources, oxygen levels, culture regime and the presence of other nitrogenous compounds, it is at present difficult to develop a generalised mechanism of ammonium regulation in Rhizobium. In the absence of evidence for lack of polypeptide synthesis, it is not known whether inhibition is due to repression of nitrogenase synthesis or simply a modulation of activity. More recently, some new findings on ammonia assimilation in Rhizobium have been reported. Glutamine (Gln) auxotrophs of Rhizobium strain 32H1 failed to derepress synthesis of nitrogenase both in culture and in planta. The nitrogen fixation (nif) defects in these strains were shown to be the direct result of glutamine auxotrophy as reversion to prototrophy simultaneously recovered nitrogenase derepression ability.

The current model which states that unadenylated GS mediates derepression of nitrogenase is consistent if not an extrapolation by hyperbole, with the regulation of GS by the classical adenylation cascade established by Stadtman and his colleagues for E. coli, and more recently in Klebsiella. Regulation of other nif operons in K. pneumoniae by the nif LA operon which is subjected to control by products of gln operons is shown in figure 2. Whether the regulatory patterns as shown for Klebsiella also hold true for Rhizobium in general is still unknown. Perhaps arguing against a general concept is the fact that (a) Rhizobium has two GS (GS1 + GSII) enzymes, (b) the sequence homology of the regulatory region of K. pneumoniae nif/HDK is minimal, compared to that of R. meliloti and R. trifolii and (c) in some Rhizobium strains (notably Parasponia-Rhizobium strain ANU289) the nifH (Fe-protein) and nifD (component of the Mo-Fe protein) are not in the same transcriptional unit as they are either in K. pneumoniae or R. trifolii and R. meliloti. (Scott and Shine, Personal Communication).

Before one builds models for Rhizobium nitrogenase regulation based on Klebsiella data, it is essential that more fundamental Rhizobium work is carried out.

CONCLUDING REMARKS

In essence, the so-called reductionist approach using in vitro co-cultures of plant callus-cell suspension with rhizobia has not provided such a simplified experimental system compared to the intact nodule as was formerly anticipated. The approaches outlined however permitted an elaboration of factors controlling nitrogenase activity in vitro and ultimately led to the discovery that the genes for nitrogenase are encoded by the Rhizobium genome. Additionally, the derepression of nitrogenase activity in free-living Rhizobium has undoubtedly initiated investigations aimed towards the understanding of several aspects of regulation of nitrogenase and related assimilatory enzymes.

Understanding the expression the Rhizobium nitrogenase, has recently acquired a commercial character stimulated by the need to increase protein

Figure 2: The current model of nif regulation in Klebsiella pneumoniae. General nitrogen control, genes such as gln G (ntr C), gln F (ntr A) and gln L (ntr B) are involved in regulation of nif. gln F product activates gln G, which in turn activates transcription from nif LA promoter. gln L product either by itself or in combination of gln G repress nif LA transcription. In nif cluster, nif L and nif A gene act respectively to repress (solid line) or activate (dotted line) all other nif operons.
production and to reduce the use of expensive inorganic fertilizers on a world-wide basis. The advent of new recombinant DNA techniques has advanced our knowledge of Rhizobium participation in symbiosis. For example some of the genes responsible for both nodulation and nitrogen fixation processes have been identified and cloned \( ^{25-74} \). The physical mapping of nif genes in Rhizobium is currently undertaken in several laboratories. Whether our increased understanding of the molecular biology, genetics and biochemistry of nitrogenase and symbiotic nitrogen fixation actually will result in increased crop production or just constitute a further in-depth analysis of a complex developmental process is beyond the scope of this paper.

12 October 1982