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Dissection of Nodule Development by Supplementation of *Rhizobium leguminosarum* biovar *phaseoli* Purine Auxotrophs with 4-Aminoimidazole-5-Carboxamide Riboside[1]

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ABSTRACT

Purine auxotrophs of *Rhizobium leguminosarum* biovar *phaseoli* CFN42 elicit uninfected pseudonodules on bean (*Phaseolus vulgaris* L.). Addition of 4-aminoimidazole-5-carboxamide (AICA) riboside to the root medium during incubation of the plant with these mutants leads to enhanced nodule development, although nitrogenase activity is not detected. Nodules elicited in this manner had infection threads and anatomical features characteristic of normal nodules, such as peripheral vasculature rather than the central vasculature of the pseudonodules that were elicited without AICA riboside supplementation. Although 10^5 to 10^6 bacteria could be recovered from these nodules after full development, bacteria were not observed in the interior nodule cells. Instead, large cells with extensive internal membranes were present. Approximately 5% of the normal amount of leghemoglobin and 10% of the normal amount of uricase were detected in these nodules. To promote the development of true nodules rather than pseudonodules, AICA riboside was required no later than the second day through no more than the sixth day following inoculation. After this period, removal of AICA riboside from the root medium did not prevent the formation of true nodules. This observation suggests that there is a critical stage of infection, reached before nodule emergence, at which development becomes committed to forming a true nodule rather than a pseudonodule.

Purine auxotrophs of various species of *Rhizobium* are defective in symbiosis with their normal hosts (4, 6, 7, 10, 13, 14, 18). *Rhizobium meliloti* purine auxotrophs have been reported to induce ineffective nodules on alfalfa (6, 18). Thirty-one purine auxotrophs of *Rhizobium leguminosarum* bv *viceae* were described as noninfective (13), and one was reported to have a nonnodulating phenotype (14). A purine auxotroph of the broad host range *Rhizobium* strain NGR234 elicited root hair curling and nodule meristem initiation, but no infection threads formed on the tropical legume, siratro (4). On soybean, *Rhizobium fredii* purine auxotrophs induced pseudonodules that did not contain bacteria (7).

Purine auxotrophs of *R. leguminosarum* bv *phaseoli* elicit pseudonodules on bean (*Phaseolus vulgaris* L.) (10). These mutants cause root hair curling and nodule meristem initiation but do not elicit infection threads (22). Although supplementing the root medium with 0.1 mM purines or purine nucleosides has no effect on the nodulation phenotype, the addition of 0.1 mM AICA[2] riboside, the unphosphorylated derivative of the purine precursor AICAR, significantly enhances nodule development (10; J.D. Newman, unpublished observations). In the absence of AICA riboside, no bacteria can be isolated from the pseudonodules elicited by the mutants. Nodules elicited by the mutants in the presence of AICA riboside contain 10^5 to 10^6 bacteria per nodule, 1000-fold fewer than in nodules elicited by the wild type. These nodules are the same size as those elicited by the wild type but are unpigmented and lack nitrogenase activity. The enhancement of nodulation has been attributed to a restoration of the ability to infect (10). Recent experiments have shown that AICA riboside is unable to promote infection by a purine auxotroph that is also defective in the conversion of AICA riboside to AICAR (J.D. Newman, unpublished observations). This result suggests that AICA riboside does not act directly on the plant but rather must be taken up by the mutant bacteria and converted to AICAR to promote infection. The foregoing studies have led to the hypothesis that rhizobia must produce AICAR to initiate and/or sustain infection thread development, possibly using it in the production of a signal molecule (10).

Although there have been no detailed developmental studies of infection thread development in bean, such studies have been carried out in soybean (*Glycine max* L. Merr.), another plant that forms determinate nodules. These studies indicate that infection thread development is initiated within 24 h following inoculation (2, 20). By 10 d postinoculation, 2 d after soybean nodules begin to emerge, bacteria are observed in the process of being released from infection threads, and differentiation into the infected and uninfected cell types has begun (21). Although the general timing of infection thread development in bean can be inferred from these studies, the mechanism by which rhizobia induce infection thread development has not yet been elucidated.

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[2] Abbreviations: AICA, 4-aminoimidazole-5-carboxamide; AICAR, 4-aminoimidazole-5-carboxamide ribonucleotide; PRPP, 5-phosphoribosyl-1-pyrophosphate; Lb, leghemoglobin.

We report here a further characterization of bean nodulation by purine auxotrophs in the presence of AICA riboside. The question of how closely the development of these nodules resembles that of normal nodules was addressed by analysis of the protein composition and light microscopic observation at various stages of development. To gain a better understanding of the aspect of nodule development requiring AICA riboside, the period during nodule development at which AICA riboside is required by purine auxotrophs was determined. The time at which AICA riboside was first added and the duration of AICA riboside addition were varied. The resulting patterns of nodules and pseudonodules indicated that AICA riboside was required through the first 6 d following inoculation for nodule development to be enhanced. These studies also suggest that, if infection occurs, the plant commits to the development of a nodule-like structure rather than a pseudonodule approximately 6 d following inoculation.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

The bacterial strains used in this study were CE3, a streptomycin-resistant and symbiotically proficient derivative of wild isolate CFN42, and CE106, a Tn*5*-induced purine auxotroph derived from CE3 (11). *Rhizobium* strains were grown on rich medium or minimal medium at 30°C (22). Antibiotic concentrations were: streptomycin, 200 μg/mL; nalidixic acid, 20 μg/mL; and kanamycin, 30 μg/mL.

Plant Material

Bean (*Phaseolus vulgaris* cv Midnight) seeds were obtained from Johnny's Selected Seeds (Albion, ME). The seeds were surface sterilized, germinated for 2 d, transferred to plastic growth pouches (Northrup King) containing nitrogen-free plant nutrient solution (23), and inoculated as described previously (11). AICA riboside (Sigma) was added to both the plant medium and watering solutions at 0.1 mM.

Recovery of Nodule Bacteria

Nodules harvested 21 d after inoculation were surface sterilized, crushed, and assayed for viable bacteria as described previously (10).

Microscopy

Nodules were harvested and processed for microscopy as described by VandenBosch and Newcomb (21).

Protein Analysis

Nodules were harvested 22 d after planting. Plant proteins soluble at 13,000*g* were extracted from crushed nodules as described by VandenBosch *et al.* (22) and quantified by the bicinchoninic acid protein assay (Pierce). The extracts were separated by SDS-PAGE (8) and either stained with Coomassie blue or electroblotted onto nitrocellulose. After incubation of the blot with antiserum against soybean Lb (supplied by P. Ludden), bound antibodies were detected with goat alkaline phosphatase-conjugated anti-rabbit immunoglobulin-γ; (Sigma) followed by development with 5-bromo-4-chloro-3-indolyl phosphate (1). For quantitation of Lb and uricase, 2-μL aliquots of serially diluted extracts from nodules elicited by CE3 or CE106 in the presence of AICA riboside were spotted onto nitrocellulose, and the blots were developed by immunostaining with anti-Lb or antiserum against soybean uricase (supplied by D.P. Verma) as described above.

Delayed AICA Riboside Addition

Seedlings were inoculated with CE106 on the day of planting (day 0), and tracings of the root were drawn on the outside of the growth pouch. AICA riboside was added to the root medium to a final concentration of 0.1 mM after a delay of 0 to 9 d following inoculation. After the initial addition of AICA riboside to the root medium, the plants were watered with a solution of 0.1 mM AICA riboside when necessary. Upon completion of nodule development, the position of each infected nodule (large, white nodules rather than pseudonodules) was measured relative to regions of the root present at day 0. In control experiments for the effect of delayed infection on nodule distribution, inoculation with the wild type was delayed for various times after planting. Upon completion of nodule development, the position of each nodule was measured relative to root segments present at day 0.

AICA Riboside Removal Experiments

AICA riboside was added to the plant medium when the plants were inoculated with CE106 (day 0). On subsequent days, the AICA riboside-supplemented plant medium was poured from the appropriate pouches, and the inside of the pouches was then rinsed with sterile water followed by the addition of fresh unsupplemented root nutrient solution.

RESULTS

Infection Thread Formation in Emerging Nodules Elicited by Purine Auxotrophs in the Presence of AICA Riboside

Supplementation of the root medium with AICA riboside promoted infection by purine auxotroph CE106 such that the resulting nodules contained 10^5 to 10^6 bacteria, which is in agreement with earlier work (10). Without supplementation, less than 10 CE106 bacteria per pseudonodule could be recovered, compared with 10^8 to 10^9 bacteria per nodule from plants inoculated with wild-type strain CE3. To determine whether CE106 bacteria enter the nodules in a normal manner when supplemented with AICA riboside, emerging nodules (8 d postinoculation) were examined microscopically for the presence of infection threads. Emerging nodules elicited by CE106 in the presence of AICA riboside (Fig. 1, A and B) were indistinguishable from emerging nodules elicited by the wild-type strain CE3 (Fig. 1, C and D). In both cases, infection threads (arrows) containing bacteria were visible within root hairs associated with meristematic activity and within the interiors of the meristematic regions. As documented previously (22), emergent pseudonodules induced by unsupplemented CE106 also have extensive meristematic regions but not infection threads.

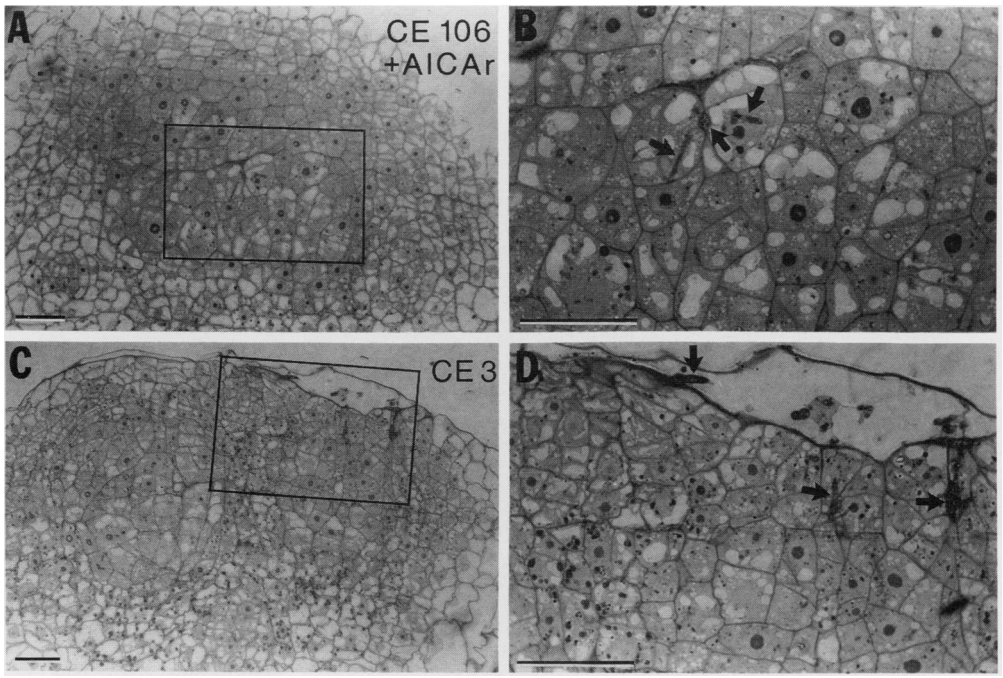

Figure 1. Infection thread formation. Emerging nodules elicited by CE106 in the presence of AICA riboside (A and B) or by CE3 (C and D) 8 d after inoculation. Areas outlined in A and C are shown at higher magnification in B and D, respectively. Arrows point to infection threads. Bars, 50 μm.

Morphology and Characteristics of Nodules Elicited by CE106 in the Presence and Absence of AICA Riboside

In the absence of AICA riboside, CE106 elicited pseudonodules that had a central vasculature (Fig. 2A, arrowhead; ref. 22) similar to that observed in lateral roots. Indeed, the pseudonodules frequently developed into lateral roots. As described previously (22), these pseudonodules were composed of highly vacuolate cells with prominent amyloplasts (Fig. 2B, double arrowheads).

Light microscopy of nodules elicited by CE106 in the presence of AICA riboside (Fig. 2C) revealed the peripheral vascular bundles (arrowheads) and cortical cell layers characteristic of normal nodule development as seen in nodules elicited by CE3 (Fig. 2E). Despite the ability to recover 10^5 to 10^6 bacteria from nodules elicited by CE106 in the presence of AICA riboside, infected cells were not evident. Instead, the central portion of the nodule contained vacuolate cells interspersed with cells having abundant cytoplasm containing numerous apparently empty vesicles (Fig. 2D, arrows), reminiscent of the vesicles observed in the meristematic regions of younger nodules (Fig. 1) and in nodule cells elicited by bacterial release mutants (Bar^-) (9, 15). Both cell types contained amyloplasts. Typical of determinate nodules, the central region of nodules elicited by the wild type (Fig. 2, E and F) were filled with large, darkly staining infected cells (asterisks) interspersed with smaller, uninfected, vacuolate interstitial cells having prominent amyloplasts. Although the nodule chosen for Figure 2C was smaller, the nodules elicited by CE106 in the presence of AICA riboside were of the same average size as those elicited by the wild type. The pseudonodules elicited by the mutant without supplementation were much smaller (10, 22).

Protein Analysis

Although the nodules elicited by CE106 in the presence of AICA riboside are morphologically similar to nodules elicited by wild-type bacteria, they are not pigmented and do not fix nitrogen (10). The composition of soluble plant proteins in these nodules was analyzed to determine whether any major nodule-specific plant proteins were present. The plant protein patterns of nodules elicited by CE3 and nodules elicited by CE106 in the presence and absence of AICA riboside were analyzed by SDS-PAGE and western blot to detect Lb (Fig. 3). The most prominent plant protein in extracts from nodules elicited by CE3 was Lb. A band that comigrated with Lb and reacted with antiserum against soybean Lb was also present in nodules elicited by CE106 in the presence of AICA riboside (Fig. 3, lanes 2). The absence of Lb in nodules elicited by CE106 in the absence of AICA riboside (Fig. 3, lanes 3) confirmed previously published results (3, 22). Dot immunoblots of nodule extracts analyzed with anti-Lb antiserum indicated that the nodules elicited by CE106 in the presence of AICA riboside contained approximately 5% of the amount of Lb polypeptide (per total nodule protein) found in wild-type nodules (data not shown).

In addition to Lb polypeptide, nodules elicited by CE106 in the presence of AICA riboside contained a characteristic nodule-specific protein of approximately 140 kD (Fig. 3A, band N). This protein was present in approximately the same abundance (per total nodule protein) as in nodules elicited by

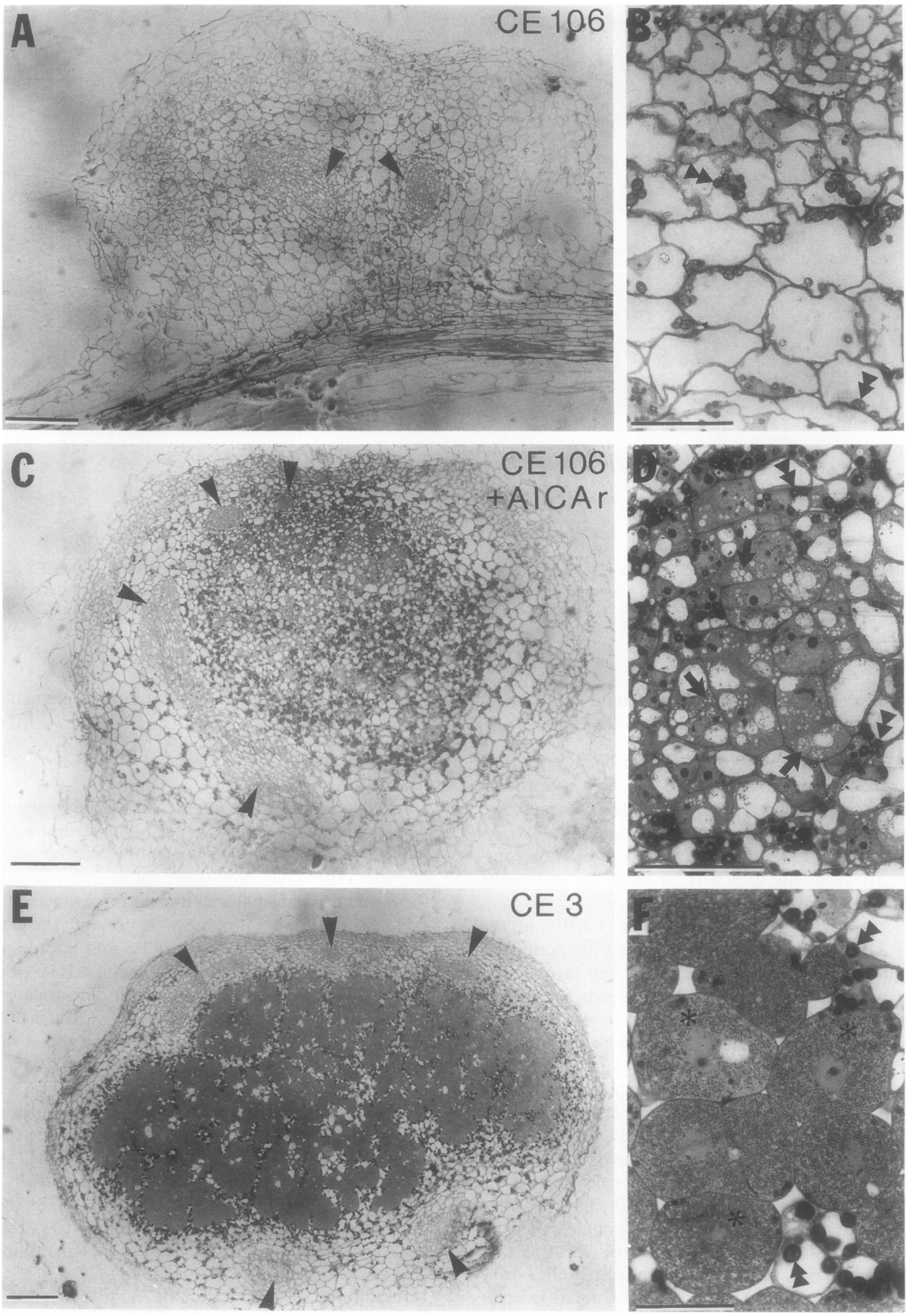

Figure 2. Morphology of mature nodules. Mature nodules (14 d postinoculation) elicited by CE106 in the absence (A and B) or presence (C and D) of AICA riboside or by CE3 (E and F). Arrowheads point to vascular bundles; double arrowheads point to amyloplasts; arrows point to vesicles; asterisks indicate infected cells. In A, C, and E, bars represent 200 μm; in B, D, and F, bars represent 50 μm.

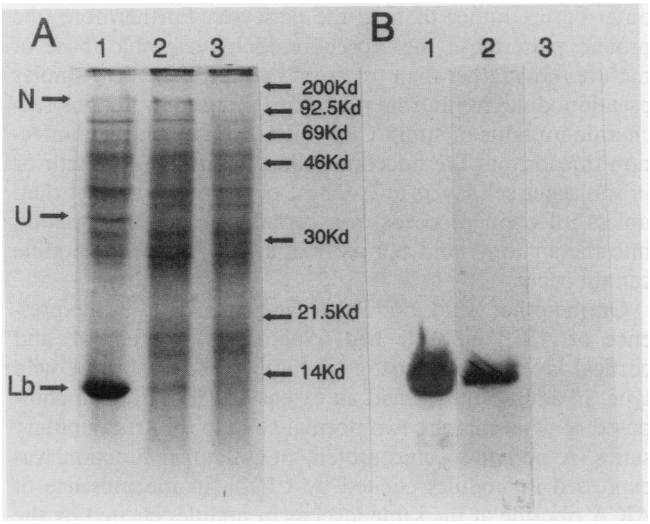

Figure 3. Analysis of soluble nodule plant proteins. The strain and conditions used to elicit the tissue from which the extracts were obtained are as follows: lanes 1, CE3; lanes 2, CE106 + AICA riboside; lanes 3, CE106 + H_2O. A, Coomassie-stained gel after SDS-PAGE; B western blot of a gel similar to that shown in A reacted with anti-soybean Lb. In A, 75 μg of total protein had been loaded into each lane. In B, lane 1 received 75 μg, whereas wells 2 and 3 received 150 μg of protein. Abbreviations: U, uricase, N, nodule-specific protein of unknown function.

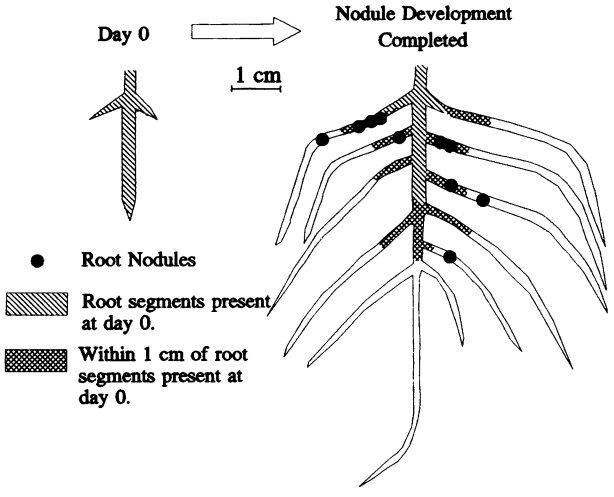

Figure 4. Representation of the distribution pattern of mature nodules after inoculation with wild-type bacteria. Day 0 is the day of inoculation. The nodulation pattern can be analyzed at any time after the nodules are well developed, because they emerge in a burst approximately 8 d after inoculation, and their numbers are stable thereafter (with a wild-type inoculum or a purine auxotroph supplemented with AICA riboside).

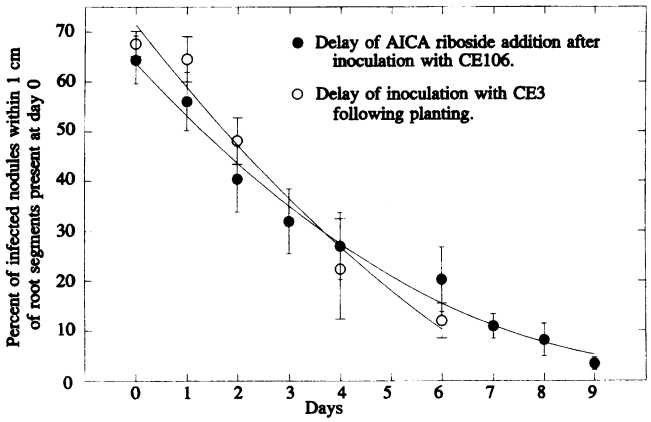

Figure 5. Effect of delayed AICA riboside addition on nodule distribution. For ●, the *x* axis indicates the delay in adding AICA riboside to plants that had been inoculated with the purine auxotroph, CE106, at day 0; for ○, the *x* axis represents the delay of inoculation with the wild type following planting. Each point represents the mean of the data collected from four to eight plants. The error bars associated with the points represent the SD.

the wild type. In the Coomassie-stained gel, uricase (Fig. 3A, band U) was not apparent in extracts of nodules elicited by CE106 in the presence of AICA riboside. However, it was detected by dot immunoblot with anti-soybean uricase antiserum and found to be present at 10% of the concentration found in normal nodules (data not shown).

Temporal Requirement for AICA Riboside: When Is AICA Riboside First Required?

Previous studies of nodule distribution in soybean (2) indicate that, after inoculation with the wild type, root nodules tend to form on regions of the root developing at the time of inoculation. A corollary of this observation is that a portion of the root is infectible only for a finite time during its development. Therefore, when inoculation is delayed, nodules appear on younger regions of the root. Under the conditions used in this study, bean nodules form primarily on lateral roots near the tap root rather than on the tap root itself; therefore, analysis of nodule distribution relative to the root tip as described by Calvert *et al.* (2) was not possible. To quantify nodule distribution such that changes could be demonstrated, the position of each nodule on a given plant was measured relative to regions of the root that were already present at the time of planting (day 0). This concept is diagramed in Figure 4. Root segments present at day 0 are indicated by the diagonal shading. Regions within 1 cm of these root segments are indicated by the cross-hatched shading. When seedlings were inoculated with the wild type at day 0, 60 to 70% of the nodules formed on the regions within 1 cm of root segments present at day 0. Although this is represented with only 10 nodules in Figure 4, the bean plants actually formed 50 to 150 nodules per plant.

Experiments with bean plants inoculated with CE3 (Fig. 5, ○) illustrated that, when inoculation was delayed, nodule distribution was altered such that there was a lower percentage

of nodules within 1 cm of the root segments present at day 0. This alteration in nodule distribution in response to delayed infection was used as an assay for the effect of delaying the addition of AICA riboside to plants inoculated with a purine auxotroph. The premise of this experiment is that, if AICA riboside is not required during an initial period, then delaying its addition for that length of time should have no effect on the distribution of infected nodules.

Seedlings were inoculated with CE106 on the day of planting (day 0). After a delay of 0 to 9 d, AICA riboside was added to the plant growth medium to a final concentration of 0.1 mM. Upon completion of nodule development, the position of each infected nodule was measured relative to root segments present at day 0 (Fig. 5, ●). Infected nodules were detected by the obvious difference in size and appearance.

Delaying AICA riboside addition by only 1 d caused a slight decrease in the percentage of infected nodules within 1 cm of root segments present at day 0, and infected nodule distribution was significantly altered when AICA riboside addition was delayed by 2 d. The trend toward fewer infected nodules near root segments present at day 0 continued such that, when AICA riboside was not added until day 9, only 3% of the infected nodules were within this region (Fig. 5).

When AICA riboside addition was delayed, numerous pseudonodules were observed along portions of the root nearest the segments present at day 0. In other words, nodule primordia initiated before AICA riboside addition became pseudonodules, even though AICA riboside was added before they emerged from the root.

How Long Is AICA Riboside Required?

Because AICA riboside seemed to be important within the first 2 d following inoculation, the next question we addressed was how long AICA riboside was required for nodulation to be enhanced. Plants were inoculated with the mutant on day 0 and, at the same time, AICA riboside was added. The growth pouches were then rinsed with sterile water on subsequent days to remove the AICA riboside and refilled with fresh unsupplemented plant medium. Thereafter, AICA riboside was absent from the plant medium. Control experiments indicated that this flushing procedure did not inhibit nodulation by the wild type, despite the probability of removing the bulk of the bacterial population that was not tightly bound.

When AICA riboside was removed before day 6, only pseudonodules were seen, except for three well-developed nodules on one of four plants in which AICA riboside was removed on day 5. AICA riboside removal 6 d after inoculation led to the enhancement of nodule development for almost half of the nodules initiated, whereas when the AICA riboside was removed on days 7 or 8, most of the nodules showed enhanced development. In these experiments, nodules first emerged from the root at approximately day 8.

DISCUSSION

Upon initiation of a meristem in differentiated regions of legume roots, two possible developmental pathways are the formation of either a lateral root or a root nodule. As described previously (22), purine auxotroph CE106 elicits pseudonodules that have a central vasculature similar to that observed in lateral roots, even though the meristem originates in the outer cortex rather than in the pericycle. Furthermore, the protein patterns in these pseudonodules resemble those of mature roots rather than true root nodules (22). Very similar pseudonodules result from meristems induced by lipopolysaccharide mutants of strain CE3. These mutants initiate infection threads, but the infections abort within the root hair or in subjacent cell layers (12). These observations suggest that, unless infection proceeds to a certain point, differentiation into the anatomy and cell types of a true *P. vulgaris* nodule cannot occur.

On the other hand, nodules elicited by CE106 in the presence of AICA riboside had a peripheral vasculature and cortical layers characteristic of nodules elicited by the wild type. In addition, they had an extensive central region composed of what may be two normal cell types in rudimentary states. A nodule-specific protein of unknown function was expressed in nodules elicited by CE106 in the presence of AICA riboside at the same level as in nodules elicited by the wild type. Lb and uricase were also present, although at much reduced levels compared with normal nodules. In summary, AICA riboside allows nodule morphogenesis to proceed at least to the point at which the plant forms a nodule structure that is clearly distinct from a root-like structure.

Despite the near-normal morphology of nodules elicited by CE106 in the presence of AICA riboside, no bacteroid-filled cells were observed, even though infection threads penetrated into the center of the meristematic region. It is possible that the bacteria were not released from the infection threads or that plant cells containing just a few released bacteria were present but due to their low number could not be unambiguously identified by light microscopy. Either scenario might account for the number of rhizobia that can be isolated from these nodules.

A possible explanation for the relatively normal abundance of the 140-kD protein is that it has a structural role that is required despite the lack of nodule function. Full expression of Lb and uricase, both involved in nodule function rather than structure, and other features of the complete differentiation of the nodule interior may be dependent on events beyond infection thread development, such as bacterial release and differentiation (17).

Delaying AICA riboside addition to mutant-inoculated plants had the same effect on nodule distribution as delaying inoculation with the wild type, *i.e.* as AICA riboside addition was delayed, infection was delayed, which shifted the location of infected nodules to younger segments of the root. Because the distribution of infected nodules was significantly altered when AICA riboside addition was delayed by 2 d, AICA riboside is apparently required within the first 2 d following inoculation. As infected nodules were shifted to younger segments by delaying AICA riboside addition, pseudonodules, rather than infected nodules, appeared in older portions of the root. This observation suggests that AICA riboside is unable to promote infection of nodules initiated before AICA riboside addition. More generally, these results also imply that, to be successful, the infection process must begin very soon after nodule initiation.

Experiments in which AICA riboside was removed at various times indicated that, for nodule development to be enhanced, AICA riboside must be present until day 6. AICA riboside may still be required for infection thread development beyond day 6; however, it seems that at this stage infection has proceeded to the point at which the plant commits to a nodule-specific developmental program rather than a developmental program that results in the formation of a structure similar to a lateral root. The removal of AICA riboside before this stage apparently blocks further infection thread development and consequently the nodule-specific developmental program. One caveat in assigning a time frame to this event is that it is not known when AICA riboside becomes internally depleted after it is removed exogenously.

The possibility that an event at day 6 or 7 triggers this nodule-specific commitment is intriguing because nodules do not emerge until 1 or 2 d later. Although possible, it seems unlikely that bacterial release from infection threads is the triggering event. Studies with soybean indicate that the first such bacterial release occurs 1 or 2 d after, not before, nodule emergence (21). Similarly, there was no indication of such release of wild-type bacteria in the emergent nodules of this study observed 8 d after inoculation (Fig. 1). Nodules at this stage are quite similar, whether induced by the wild type, CE106, or CE106 supplemented with AICA riboside (22; Fig. 1).

The main differences are that the meristematic regions of emerging nodules elicited by the wild type or by CE106 in the presence of AICA riboside are more compact and vigorous and contain infection threads that have penetrated to the center of this meristematic region. This latter process may be the key. Perhaps, bacteria within the infection thread continuously sustain plant cell division (*e.g.* by excreting the glycolipid produced by the *nod* gene products). Without this hypothetical reinforcement of meristem induction, the cells of the central nodule region simply might never become generated. The point that generation and differentiation of this central region do not depend on successful bacterial release has been illustrated previously with soybean nodulation by Bar^- mutant rhizobia (9). However, previous approaches could not suggest how early the commitment to this development occurs.

When AICA riboside is present only until day 5, the infection threads probably are becoming well developed. However, the plant does not commit to the nodule-specific developmental program unless infection continues through day 6. Thus, the use of AICA riboside to control infection thread development by purine auxotrophs permits the experimental separation of the processes of infection thread development and commitment to root nodule formation. Such a tool could be used to identify plant genes and regulatory elements specifically involved in this commitment to true nodule morphogenesis.

The temporal requirement for AICA riboside supplementation of the purine auxotroph supports the hypothesis that the symbiotic role of AICAR metabolism is to promote infection thread development. Relative to the original objective, this is the central conclusion of this study. Infection thread development is initiated within 24 h of inoculation in soybean (20). Because AICA riboside is required within 2 d and possibly sooner, and cannot confer infection upon nodules initiated before its addition, the AICA riboside metabolite may be needed as soon as infection starts. The requirement for AICA riboside supplementation continues until infection has progressed almost until bacteria are first released into nodule cells. The suggestion, then, is that AICA riboside is needed continuously throughout most of infection thread development.

Whether an AICA riboside derivative is needed for meristem induction or bacteroid differentiation/proliferation is more problematic. Clearly, the auxotrophs can induce meristematic activity in the absence of AICA riboside supplementation (22). On the other hand, without supplementation, pseudonodules are noticeably more sparse compared with the tight clustering of nodules obtained with supplementation. This difference could be due to a lower frequency of meristem induction or higher frequency of aborted meristematic activity. Exogenous supplementation does not provide bacteroid proliferation, but it may be impossible to deliver sufficient AICA riboside per bacterium at this point. However, increasing the AICA riboside concentration to 1.0 mM achieves no greater effect (J.D. Newman, unpublished data).

If AICA riboside acts as a precursor to a signal molecule, possible mechanisms by which it could promote development specifically of infection threads include (a) serving as a signal to the plant to stimulate production of infection thread wall components, (b) to target these components to the correct location, (c) as a signal for microtubule reorganization to define the orientation of newly deposited cell wall material, and (d) suppression of host defense responses against rhizobia. It has been suggested by others that in the broad host range *Rhizobium* strain NGR234, purine biosynthesis is involved in the repression of the plant's ability to induce a host defense response (4).

An interesting feature of AICA-based compounds is that they are often used as substrates by enzymes using adenine-based compounds. AICA can serve as a substrate for human adenine phosphoribosyltransferase (19), AICAR is synthesized by the same enzyme that synthesizes AMP (5), and AICAR triphosphate is synthesized from AICAR and PRPP by PRPP synthetase (16), which normally catalyzes the formation of PRPP from ribose-1-phosphate and ATP. This raises the possibility that a novel AICAR derivative produced by rhizobia could mimic or alter the levels of adenine-based cytokinins and thereby influence nodule development. Work is currently focused on determining the basis for the peculiar efficacy of AICA riboside, in preference to other purine sources, for restoring the infection of bean by the purine auxotrophs.

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