Isolation of the *Rhizobium leguminosarum* NodF Nodulation Protein: NodF carries a 4'-Phosphopantetheine Prosthetic Group

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Rhizobium species produce a protein product of the *nodF* gene that has a limited but recognizable homology to the well-characterized acyl carrier protein (ACP) of *Escherichia coli*. NodF functions together with NodE in generating a host-specific response to the plant host in the interchange of signals leading to the effective nodulation of roots (H. P. Spaink, J. Weinman, M. A. Djordjevic, C. A. Wijffelman, R. J. H. Okker, and B. J. J. Lugtenberg, EMBO J. 8:2811-2818, 1989; B. Scheres, C. van de Wiel, A. Zalusky, B. Horvath, H. Spaink, H. van Eck, F. Zwartkruis, A.-M. Wolters, T. Gloudemans, A. van Kammen, and T. Bisseling, Cell 60:281-294, 1990). The *nodFE* region of *Rhizobium leguminosarum* has been cloned into a multicopy plasmid and has been shown in *R. leguminosarum* to code for a flavonoid-inducible protein that is effectively labeled by radioactive β -alanine added to the growth medium. After purification, the labeled protein migrates as a single band with an apparent molecular weight of 5,000 during sodium dodecyl sulfate-polyacrylamide gel electrophoresis, more rapidly than *E. coli* ACP. In contrast, in native gels the protein is resolved into two bands, both identified as NodF by analysis of the amino terminus and both migrating more slowly than *E. coli* ACP. Pulse-chase experiments with labeled β -alanine suggested that the slower-moving band may be the precursor of the faster band. The NodF protein carries a 4'-phosphopantetheine as a prosthetic group. A NodF fusion protein under the control of the *lac* promoter is expressed in *E. coli* and is labeled with β -alanine, indicating that it is recognized by the ACP synthase of *E. coli*. The ACP phosphodiesterase of *E. coli*, which catalyzes the release of phosphopantetheine from *E. coli* ACP, does not remove phosphopantetheine from NodF.

Bacteria of the genus *Rhizobium* are able to interact symbiotically with specific plant hosts, leading to the formation of nitrogen-fixing root nodules. In the exchange of signals prior to nodulation, flavonoids secreted by the plant cause the induction of rhizobial nodulation (*nod*) genes. The *nodABCIIJ* genes are termed common *nod* genes because they are functionally interchangeable among different *Rhizobium* species. In contrast, genes determining the host range of nodulation are termed host specific. Among them are genes of the *nodFEL* operon (7, 22, 37) (Fig. 1). NodF functions together with NodE in the production of a host-specific rhizobial signal (31).

Some indication of the function of NodFEL proteins can be derived from studying their homology, deduced from the gene sequence, to known proteins. The NodL protein appears to be homologous to the acetyl transferases LacA and CysE (9, 44), and NodE shares homology with a group of β -ketoacyl synthases, such as FabB (the condensing enzyme of fatty acid biosynthesis of *Escherichia coli*), or those presumed to be involved in the synthesis of β -ketide antibiotics in *Streptomyces* species (2, 34). The NodF sequence was found to resemble that of the acyl carrier protein (ACP) of *E. coli* in the region near the serine to which the phosphopantetheine is attached, although amino acid identity to the ACP was very low in the rest of the molecule (see Fig. 6A). Because the NodF protein is inducible and because mutants lacking NodF are viable, it is clear that it cannot be required for the synthesis of essential cell lipids. A consti-

tutive ACP, which presumably does function in the synthesis of essential membrane lipids, has been recently isolated from *R. meliloti* and studied in this laboratory (27).

The periplasmic cyclic β -1,2-glucans of *Rhizobium* species are also involved in the cell signalling leading to nodulation (13) in a way that is so far not understood. These glucans are very similar to the periplasmic membrane-derived oligosaccharides of *E. coli* (26). The ACP of *E. coli* is an essential component of a UDP-glucose-requiring transglucosylase system that catalyzes the synthesis of the β -1,2 backbone of membrane-derived oligosaccharides (39). This novel function does not require the phosphopantetheine prosthetic group of ACP (38). These findings raise the possibility that the function of the NodF protein could be related to the synthesis or modification of a cell surface carbohydrate, which might possibly not require a phosphopantetheine prosthetic group.

An understanding of the molecular basis of the function of the NodF protein in the nodulation process clearly requires its isolation and characterization, which we now report.

MATERIALS AND METHODS

Materials. 3-[(3-Cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (CHAPS) and phenylmethylsulfonyl fluoride were obtained from Sigma Chemical Co.; the polypeptide molecular weight calibration kit, Mono Q, and Superose 12 were obtained from Pharmacia; leupeptin and pepstatin were obtained from Boehringer Mannheim; Centricon TM3 was obtained from Amicon; silica Gel H (Precotes) was obtained from Applied Science Laboratories; and DEAE-cellulose (DE-52) was obtained from Whatman. Reagents for electrophoresis and protein standards were obtained from

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FIG. 1. Construction of plasmids. In this map of the *nod* region of Sym plasmid pRL1J1, the sizes and positions of *nod* genes according to published data (7, 27) are indicated by solid arrows. The directions and start sites of transcription (7) are indicated by thin arrows. Strongly conserved sequences (*nod* boxes), which precede every inducible *nod* operon, are indicated by solid triangles. The restriction fragments (hatched boxes) were cloned in the vectors pIC20H (24) and pMP190 (35). The N-terminal part of the NodF fusion protein, as predicted from the nucleotide sequence, is indicated by the one-letter amino acid code. Abbreviations: Bg, BgIII; Ba, BamHI; Bc, BclI; Ba/Bc, BamHI-BclI fusion; Ec/Bg, EcoRI-BgIII fusion; p.LAC, promoter of *lacZ*; Cm^R, chloramphenicol resistance; Sm^R, streptomycin resistance; Ap^R, ampicillin resistance.

Bio-Rad Laboratories and β -[3-³H(N)]alanine (specific activity, 120 Ci/mmol) was obtained from New England Nuclear. *E. coli* constitutive ACP labeled with tritium in the β -alanine moiety of the phosphopantetheine residue, ACP phosphodiesterase from *E. coli*, and unlabeled 4'-phosphopantetheine were the kind gifts of T. Fischl of this laboratory, who prepared them as described previously (11).

Bacterial strains and plasmids. The construction of plasmids is shown in Fig. 1. The broad-host-range plasmid pMP1255 was mobilized from *E. coli* KMBL 1164 to *R. leguminosarum* by using pRK2013 as a helper plasmid (8) as previously described (35). *E. coli* SJ16 (*pand*) (16), a β -alanine auxotroph, was transformed with pMP1715 and pMP248, an IncQ plasmid containing the *lacI* gene of *E. coli*.

Growth of cultures. Cultures of *R. leguminosarum* RBL5560(pMP1255) were grown on medium B (42) containing 0.5 mg of streptomycin per ml at 30°C on a gyratory shaker. To some cultures, flavonoid inducer (0.4 μ g of naringenin per ml) or β -[3-³H]alanine (10⁷ cpm/ml; 120 Ci/mmol) was added at a density of 5×10^7 cells per ml. Cells were usually harvested after three generations of further growth.

PAGE. Polyacrylamide gel electrophoresis (PAGE) in 15% polyacrylamide gels containing 0.1% (wt/vol) sodium dodecyl sulfate (SDS) was carried out by the method of Laemmli (19). Alternatively, when a high resolution of proteins with apparent molecular weights of 1,000 to 20,000 was required, electrophoresis in the presence of SDS was performed as described by Schaeffer and von Jagow (30), with the separating and stacking gels containing 16.5 and 4% acrylamide, respectively. Electrophoresis in nondenaturing gels was performed as described by Jackowski and Rock (17).

RESULTS

Labeling of a flavonoid-inducible protein with β -alanine.

β -Alanine is a precursor of 4'-phosphopantetheine, the prosthetic group of ACP (5). If NodF carries a 4'-phosphopantetheine, it should become labeled with radioactive β -alanine. In order to obtain a high level of expression, the *nodFE* region of *R. leguminosarum* was cloned into a multicopy plasmid (pMP1255) (Fig. 1). In *R. leguminosarum* RBL5560 harboring pMP1255, a flavonoid-inducible protein band was effectively labeled with radioactive β -alanine (Fig. 2). In addition, other noninducible radioactive bands were detected. Fractionation of the cells showed that the inducible labeled protein is found in the cytoplasmic fraction of *R. leguminosarum* RBL5560(pMP1255) (data not shown). Its mobility in the SDS gel electrophoresis system of Schaeffer and von Jagow was that of a protein of apparent M_r of 5,000, which is considerably faster than that of the *E. coli* ACP.

Purification of flavonoid-inducible, β -alanine-labeled protein. Cells of *R. leguminosarum* RBL5560(pMP1255) were grown in 3 liters of culture medium as described in Materials and Methods. At a density of 5×10^7 cells per ml, naringenin was added to a final concentration of 1.5 μ M, and after about 24 h of further growth, cells were harvested at a density of 5×10^8 cells per ml and stored in a freezer at -20°C. The frozen cells (31 g) were suspended in 50 ml of buffer A (50 mM Tris-HCl [pH 6.8], 0.1 M KCl, 20 mM dithiothreitol, 1 μ M pepstatin, 1 μ M leupeptin, 0.2 mM phenylmethylsulfonyl fluoride) and passed twice through a French pressure cell at 20,000 lb/in². The suspension was centrifuged at $110,000 \times g$ for 60 min to obtain the cell extract. At this point, a similar extract, obtained from cells of *R. leguminosarum* RBL5560 (pMP1255) grown in the presence of β -[3-³H]alanine, was

FIG. 2. Detection of NodF by autoradiography after SDS-PAGE. The high-speed supernatant fraction of extracts of cells grown in medium containing labeled β -alanine was analyzed by SDS-PAGE in 15% gels by the method of Laemmli (19). Lanes: 1, *R. leguminosarum* RBL5560(pMP1255) without inducer; 2, *R. leguminosarum* RBL5560(pMP1255) with naringenin; 3, labeled ACP from *E. coli* (*E. coli* SJ16 harboring pMP1715 and pMP248 was grown at 37°C in minimal medium E [43] containing carbenicillin [0.1 mg/ml], chloramphenicol [20 μ g/ml], methionine [0.01%], thiamine [0.001%], and β -alanine [0.25 μ M; 1.3×10^7 cpm/ml]); 4, same as lane 3 with glucose (0.2%) also added; 5, same as lane 3 with lactose (0.2%) also added.

added. The cell extract was applied to a 150-ml column of DEAE 52-cellulose, which had been equilibrated with buffer B (10 mM bis-Tris-HCl [pH 6.0], 0.1 M NaCl, 1 mM CHAPS). The column was washed with 300 ml of buffer B. Elution was performed with a linear gradient from 0.1 to 1 M NaCl in buffer B in a total volume of 600 ml. Fractions (4.8 ml) were collected and assayed for radioactivity. Radioactive fractions were diluted with an equal volume of water, and a portion, usually about 18 mg of protein, was applied to a 1 ml Mono Q column which had been equilibrated with buffer B. The column was eluted with a linear gradient from 0.1 to 0.6 M NaCl in buffer B in a total volume of 22.5 ml. Fractions (0.75 ml) were collected, analyzed on polyacrylamide gels, and assayed for radioactivity. Fractions of the flavonoid-inducible, radioactive peak which eluted between 0.21 and 0.3 M NaCl were concentrated by rechromatography on Mono Q, and the radioactivity was eluted with 0.6 M NaCl. Concentrated radioactive fractions so obtained were applied to a Superose 12 column equilibrated with buffer B. The column was eluted with buffer B at the rate of 0.5 ml/min. Fractions (0.5 ml) were collected, and those containing radioactivity were pooled and concentrated in a Centricon TM3 device. A portion of the concentrated radio-labeled fractions was subjected to preparative electrophoresis under nondenaturing conditions essentially as described by Jackowski and Rock (17). A thin vertical strip between R_f values 0.57 and 0.74 was cut out from the gel. The flavonoid-inducible radioactivity was then recovered from the corresponding region of the preparative gel with an electroelution device (Elutrap; Schleicher & Schuell).

A summary of the purification of the flavonoid-inducible, β -alanine-labeled protein is shown in Table 1. Analysis of the purified protein by SDS-PAGE (Schaeffer and von Jagow

TABLE 1. Purification of NodF

Step	Protein (mg)	Radioactivity (cpm)	Sp act (cpm/mg)
Extract	221	2.6×10^6	11,765
DEAE-cellulose	37	1.6×10^6	43,240
Mono Q	5.8	2.6×10^5	44,828
Superose 12	1.25	2.2×10^5	176,000
Preparative PAGE	0.32	1.54×10^5	481,250

[30]) (Fig. 3) revealed a single protein band stained with Brilliant Blue G, which moves at the same position as the flavonoid-inducible, β -alanine-labeled radioactivity corresponding to an apparent M_r of 5,000, much faster than expected from the predicted molecular weight for NodF of 9,946.

Identification of flavonoid-inducible protein as NodF gene product. Although only a single band was detected by electrophoresis in SDS gels (Fig. 3), electrophoresis of the purified flavonoid-inducible, β -alanine-labeled protein in a native polyacrylamide gel revealed two protein bands (Fig. 4). After separation of the two bands, they were transferred to a polyvinylidene difluoride membrane in 10 mM CAPS [3-(cyclohexylamino)-1-propanesulfonic acid] buffer (pH 11) containing 10% (vol/vol) methanol, as described by Matsudaira (25), and subjected to automated Edman degradation sequence analysis in the Microchemical Facility of the Harvard Biological Laboratories. The sequences of the first 10 residues from the amino terminus are A, D, Q, L, T, L, E, I, I, and S. Both proteins (NodF_s and NodF_f) possess the same N terminus, exactly that expected for the NodF protein. We therefore conclude that the two bands represent distinct forms of NodF.

Slower-moving NodF (NodF_s) may be a precursor of faster-moving NodF (NodF_f). *R. leguminosarum* RBL5560 (pMP1255) was pulsed with radioactive β -alanine. After removal of the label, a flavonoid inducer was added and the

FIG. 3. SDS-PAGE of the purified NodF protein from *R. leguminosarum* RBL5560(pMP1255). Proteins were analyzed by a procedure of Schaeffer and von Jagow (30), as described in Materials and Methods. Lanes: 1, ACP from *E. coli*; 2, NodF protein from *R. leguminosarum* RBL5560(pMP1255); 3, peptide molecular weight markers (17,201, 14,632, 8,235, 6,383, 2,556, and 1,695); 4, low-molecular-weight protein markers (97,400, 66,200, 42,699, 31,000, 21,500, and 14,400).

FIG. 4. Purification of NodF protein. Native PAGE was performed with cell extract from *R. leguminosarum* RBL5560 (pMP1255) grown without inducer (lane 1) or in the presence of naringenin (lane 2). Also shown are pooled fractions from induced cells after DE-52 (lane 3), pooled fractions after Mono Q (lane 4), pooled fractions after Superose 12 (lane 5), NodF protein after preparative electrophoresis (lane 6), constitutive ACP from *E. coli* (lane 7), and constitutive ACP from *R. meliloti* (lane 8).

appearance of the different NodF forms over time was determined (Fig. 5). The NodF_s band was quickly labeled, and during growth on unlabeled medium, radioactivity was lost from this band after several generations of chase (data not shown). In contrast, the NodF_f band acquired label more slowly, and during the chase in unlabeled medium, it increased in radioactivity. These data suggest that the slower-

FIG. 5. Pulse-chase with labeled β -alanine. A culture of *R. leguminosarum* RBL5560(pMP1255) (5 ml) was grown as described in Materials and Methods for three generations in the presence of [β - 3 H]alanine without inducer to label the cellular pool of phosphopantetheine precursor. The cells were washed twice and resuspended in 50 ml of unlabeled medium containing naringenin. Growth was continued, and samples were taken after various times during the chase. The soluble cell extracts were analyzed by autoradiography after separation by native gel electrophoresis. Lanes: 1, 0 h (start of chase); 2, 8 h (chase generation 1); 3, 16 h (generation 2); 4, 24 h (generation 3).

moving NodF may be a precursor of the faster-moving NodF. We investigated the possibility that the slow-moving precursor NodF might be a free, unacylated ACP, whereas the fast-moving form might carry an acyl residue because this would account for its faster migration in native gels (28). Neither treatment with hydroxylamine at neutral pH by the method of Rosenfeld et al. (29) nor treatment with dithiothreitol at pH 10 by the method of Lakin-Thomas and Brody (20), conditions expected to remove an acyl thioester, was able to convert the fast-moving NodF into the slow-moving form (data not shown), making it unlikely that the difference in migration results from an acyl residue on NodF_s. In the pulse-chase experiment shown in Fig. 5, the intensity of an additional band which moves slightly slower than the NodF_s band seems to increase during the chase period. This band, however, was also found in *nodF* mutant strains and is therefore not NodF related. Further, it is not induced by flavonoids; its appearance seems to be correlated with cultures grown to high cell densities.

Identification of a prosthetic group of NodF protein as 4'-phosphopantetheine. Constitutive ACP from *E. coli* (20,000 cpm), which is known to carry 4'-phosphopantetheine as a prosthetic group, and NodF protein labeled with β -alanine (11,000 cpm) were treated with 50 mM NaOH at 70°C for 1 h (pH 12 in the reaction mixture), as described by Majerus et al. (23). In both cases, a labeled low-molecular-weight compound was quantitatively released which, after neutralization, was separated from the apo protein in a Centricon TM3 ultrafiltration device. Unlabeled 4'-phosphopantetheine was added as carrier to the labeled products. The labeled products, both from the constitutive ACP from *E. coli* and the β -alanine-labeled NodF protein, were indistinguishable from that of the 4'-phosphopantetheine carrier during chromatography on DEAE-cellulose and on thin-layer silica Gel H plates developed in *n*-butanol-acetic acid-water (5:2:4, vol/vol), as described by Jackowski and Rock (16). After treatment with alkaline phosphatase from *E. coli* (23), the labeled compounds obtained from the constitutive ACP from *E. coli* or the β -alanine-labeled NodF protein behaved like authentic pantetheine when analyzed by thin-layer chromatography on silica Gel H layers with ethanol-28% ammonium hydroxide (4:1, vol/vol) (16).

Expression of NodF in *E. coli*. We cloned the *nodFE* region also into plasmid pMP1715 (Fig. 1), resulting in an in-frame fusion of the *lacZ* 5' terminus with the *nodF* gene, as confirmed by nucleotide sequencing. The predicted molecular weight of the fusion protein is 11,515. After induction with IPTG (isopropyl- β -D-thiogalactopyranoside), *E. coli* strains harboring pMP1715 produced a high level of NodE protein, as confirmed by testing with antibodies (36) and also a protein with mobility in SDS gels corresponding to a protein with an M_r of 8,000 (data not shown). In *E. coli* SJ16, a β -alanine auxotroph containing pMP1715, the IPTG-inducible protein with an apparent M_r of 8,000 was labeled after growth on [β - 3 H]alanine (Fig. 2). This result indicates that ACP synthase of *E. coli* recognizes the apo LacZ'-NodF protein as a substrate. *E. coli* ACP phosphodiesterase, however, an enzyme known to hydrolyze 4'-phosphopantetheine from the constitutive ACP in *E. coli*, did not release any phosphopantetheine from labeled NodF, not even at enzyme concentrations 1,000-fold higher than that needed to obtain a total release of 4'-phosphopantetheine from the constitutive ACP in *E. coli* (data not shown).

FIG. 6. Homologs to ACP. (A) Alignment of amino acid sequences around the predicted active serine (*), the 4'-phosphopantetheine binding site. Amino acid sequence lengths are shown. Also shown are identities to *E. coli* ACP and to *R. leguminosarum* NodF, a and b, respectively. Data were obtained from the following references for the sequences listed at the left in descending order: 41, 3, 15, 18, 6, 33, 32, 34, 2, and 12. (B) Evolutionary tree of ACPs. The cladogram was obtained by progressive sequence alignment by the method of Feng and Doolittle (10).

DISCUSSION

The NodF protein is involved in the synthesis of a signalling compound that determines the specificity of *Rhizobium* species for particular plant hosts (31). That such rhizobial signal substances may be carbohydrates is suggested by two general lines of evidence. (i) Other signalling substances are thought to be recognized by lectins of the host plant, proteins with affinity for specific sugar residues. (ii) Recently, an oligosaccharide containing sulfated and acylated glucosamine residues has been reported to be a host-specific signal compound produced by *R. meliloti* (21).

The NodF protein is homologous to the ACP of *E. coli* in the region of serine-36 of the *E. coli* protein, site of substitution with phosphopantetheine (Fig. 6A). The two proteins, however, share only 19 amino acid identities in a total of 77 *E. coli* ACP residues and 93 NodF residues (Fig. 6A). The overall similarity of the two proteins therefore is rather low, and because little is known about the structural requirements for substitution with phosphopantetheine, it certainly could not have been concluded from comparison of the two structures that NodF necessarily carries a phosphopantetheine prosthetic group. Support for the notion that substitution with phosphopantetheine might in fact not be needed is provided by the report that in *Azorhizobium caulinodans* an ACP-like protein does not contain a serine residue corresponding to serine-36 of *E. coli* ACP (12). Further, it is known that the function of *E. coli* ACP in the synthesis of periplasmic carbohydrates, the membrane-derived oligosac-

charides, does not require the phosphopantetheine prosthetic group (38). The results of the present study therefore provide needed, direct evidence that the NodF protein is in fact substituted with phosphopantetheine. Considering that, in spite of the low overall homology between classical ACPs and NodF proteins, both groups possess a 4'-phosphopantetheine, one is tempted to suggest that open reading frames, thought to be involved in β -ketide biosynthesis in *Streptomyces* species (2, 34) and which are more similar to NodF proteins than to classical ACPs (Fig. 6B), also carry a 4'-phosphopantetheine.

We detected two distinct forms of NodF, differing in their rates of migration during electrophoresis in native polyacrylamide gels. Pulse-chase experiments with labeled β -alanine were consistent with the conclusion that the slower-moving form is the precursor of the more rapidly moving form. Studies with *E. coli* ACP have revealed that fatty acyl-S-ACP migrates more rapidly than nonacylated ACP (29). However, the faster-moving form of NodF could not be converted to the slower-moving form by treatment with hydroxylamine or dithiothreitol under conditions known to cause deacylation. Thus, the situation somewhat resembles the finding by Jackowski and Rock (18) of a distinct, more rapidly migrating form of ACP in a *fabF* mutant of *E. coli*. The faster rate of migration could not be attributed to acylation nor to any change in the primary structure and remains without explanation.

When the *nodF* gene was fused to the 5' region of the *lacZ* gene, under the control of the *lac* promoter, the resultant fusion protein was expressed in *E. coli* after induction with isopropyl thiogalactoside. Further, it was labeled with β -alanine added to the growth medium, indicating a rather low degree of specificity for the enzymic attachment of phosphopantetheine. This was rather surprising, because studies in vitro with ACP synthase, an enzyme catalyzing the transfer of phosphopantetheine from coenzyme A to ACP, had indicated that certain residues close to the amino terminus are of crucial importance for recognition by this enzyme (14). The amino terminus of the fusion protein is very different from that of *E. coli* ACP. There are at least three possibilities. (i) The specificity of ACP synthase is different in the living cell from that seen in vitro. (ii) Although the N termini of ACP and LacZ'-NodF are different, they might be recognized as similar tertiary structures by a single ACP synthase, leading to a substitution with phosphopantetheine. (iii) There is some alternative enzymic route for the transfer of phosphopantetheine to ACPs. The constitutive ACP of *R. meliloti* is very similar to *E. coli* ACP and quite different from the rhizobial NodF proteins, raising the question of whether a distinct enzyme is needed for transfer of phosphopantetheine to NodF or whether a single enzyme which recognizes only the region near the serine to be substituted functions in both cases.

ACP phosphodiesterase, an enzyme that cleaves the phosphopantetheine residue from *E. coli* ACP, does not remove phosphopantetheine from NodF labeled with β -alanine. The phosphodiesterase thus appears to be more highly specific than the enzyme(s) that transfers phosphopantetheine residues to species of ACP. The function of ACP phosphodiesterase, which has recently been purified and shown to be a protein of an apparent M_r of 25,000, remains obscure, but it is believed to participate in the turnover of *E. coli* ACP in living cells (11). Our results show that not every phosphopantetheine attached to proteins is turned over by ACP phosphodiesterase.

Although ACPs are relatively small (M_r s, ca. 10,000), they appear to be quite versatile. Thus far, only one ACP has been found in *E. coli*. It is an essential component of the fatty acid synthase system of this organism, to which nascent fatty acids are attached during the process of elongation (40), and it appears to also function in the transfer of the completed long-chain fatty acid residues to phospholipid precursors (4) as well as in the transfer of highly specific residues to the lipid A component of lipopolysaccharides (1). In a quite different metabolic role, it is needed for the enzymic synthesis of membrane-derived oligosaccharides (39).

In contrast, *Rhizobium* species produce not only a constitutive ACP, presumably functioning in the biosynthesis of essential cell lipids, but they also produce the inducible NodF protein for the specialized functions (still unidentified) needed for cell signalling to nodulation. Open reading frames identified in *Streptomyces* species indicate the presence of a specialized ACP functioning in the synthesis of polyketide antibiotics in that organism. The occurrence of these specialized ACPs may perhaps be part of a mechanism by which the synthesis of unusual lipids needed for special purposes can be carried out in a separate pathway without interfering with the synthesis of essential cellular lipids. Remarkably, the specialized ACPs, *Rhizobium* NodF and *Streptomyces* ORF3, involved in β -ketide synthesis, are more similar to each other than either group is to classical ACPs (Fig. 6B). This finding suggests that a common ancestor of these

specialized ACPs originated by gene duplication from the classical ACP and diverged only later on.

ACKNOWLEDGMENTS

This research was supported by grants GM19822 and GM22057 from the National Institute of General Medical Sciences. Otto Geiger is a Feodor Lynen Fellow of the Alexander von Humboldt Foundation, Federal Republic of Germany. Herman P. Spink was supported by a NATO grant from the Netherlands Organization for Scientific Research (NWO).

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