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VisN and VisR Are Global Regulators of Chemotaxis, Flagellar, and Motility Genes in *Sinorhizobium (Rhizobium) meliloti*†

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The known 41 flagellar, chemotaxis, and motility genes of *Sinorhizobium (Rhizobium) meliloti* contained in the "flagellar regulon" are organized as seven operons and six transcription units that map to a contiguous 45-kb chromosomal region. By probing gene expression on Western blots and with *lacZ* fusions, we have identified two master regulatory genes, *visN* and *visR*, contained in one operon. The gene products probably form a heterodimer, VisNR, acting as a global transcription activator of other flagellar genes. The related 27-kDa VisN and VisR proteins are LuxR-type proteins with typical ligand- and DNA-binding domains. The *vis* operon itself is constitutively transcribed; however, to activate flagellar genes, VisNR seemingly requires the binding of a yet-unknown effector. Gene expression in tester strains with known deficiencies revealed a hierarchy of three classes of flagellar genes: class I comprises *visN* and *visR*; class II, controlled by VisNR, comprises flagellar assembly (class IIA) and motor (class IIB) genes; and class III comprises flagellin and chemotaxis genes that require functional class I and class IIA genes for expression. In contrast to their enterobacterial counterparts, *mot* genes belong to class II without exerting control over class III genes. While the general hierarchy of gene expression resembles the enterobacterial scheme, the assignment of *mot* genes to class IIB and the global control by a LuxR-type VisNR activator are new features distinguishing the *S. meliloti* flagellar gene system.

Bacterial motility and chemotaxis are essential qualities for optimum adaptation to different environments. Flagellar synthesis and motility are maximal under nutritional stress and require 2 to 3% of the energy of a cell. Therefore, the expression of some 50 genes involved in this process is strictly regulated by a hierarchy of controls (19). The current paradigm of flagellar gene regulation was derived from studies of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (13, 15, 18, 20, 29). The *E. coli* chemotaxis, flagellar, and motility genes map in four separate clusters often referred to as the flagellar regulon. The genes of this regulon are organized in three classes that are expressed in hierarchial order, although their map positions do not necessarily reflect the assignments to these classes. Class I is represented by a master operon that encodes the transcription activators, FlhC and FlhD, which in turn regulate the expression of class II (18). This includes genes that determine the flagellar basal body and the flagellin-specific export apparatus and *fliA*, which encodes a σ^{28} (σF) transcription factor for class III.

Sinorhizobium (Rhizobium) meliloti, a member of the alpha subgroup of proteobacteria, exhibits significant deviations from the enterobacterial (gamma subgroup) paradigm of chemotaxis in its flagellar structure and mode of rotation (23). The complex, rigid flagellar filaments of *S. meliloti* consist of four closely related flagellin subunits, FlaA, FlaB, FlaC, and FlaD, encoded by four linked but independently transcribed genes (24, 33). The right-handed flagellar helices rotate exclusively in the clockwise mode, and swimming cells respond to tactic stimuli by modulating their flagellar rotary speed (31, 32). Two novel motor proteins, MotC and MotD, are essential players in the control of flagellar rotary speed (23). The organization of the *S. meliloti* chemotaxis (*che*), flagellar (*fla*, *flg*, *flh*, and *fli*), and motility (*mot*) genes is distinctly different from that in enterobacteria, since all known 41 genes are clustered in one contiguous 45-kb chromosomal region (33). Among these are 10 genes of a *che* operon, four *mot* genes (23), 15 *flg*, *flh*, and *fli* genes encoding components of the basal body and the flagellar export apparatus, 4 flagellin (*fla*) genes, and 5 genes of hitherto unknown function. Notably, MotA is encoded in the same operon as FliM, FliN, and FliG, and a new gene, *orf38*, necessary for flagellum formation, is part of the *motB-motC-motD* operon (33). In keeping with established nomenclature (20), we refer to the entirety of these genes as the flagellar regulon.

We have identified in *S. meliloti* two related members of the LuxR family, VisN and VisR (for "vital for swimming"), formerly named Orf12 and Orf13, respectively (33), that function as global activators of the flagellar regulon. Transcriptional activators of the LuxR family have been found in various bacterial species, where they regulate such different processes as conjugation, cell division, and antibiotic production (6, 34). Typical members of this family are TraR, which regulates plant-pathogenic genes of *Agrobacterium tumefaciens* (5, 22), and RhiR and RaiR, which regulate the nodulation genes of *Rhizobium leguminosarum* and *Rhizobium etli* (3, 8, 26). LuxR is best known for its role in quorum sensing, i.e., the ability to monitor population density by sensing the external concentration of autoinducer molecules, notably homoserine lactones. All these transcription factors have similar structures with a ligand-binding domain and DNA-binding domains of the helix-turn-helix type, which place them in the LuxR-FixJ-UhpA-NarL superfamily of transcription factors (6, 9). The LuxR-like regulators, VisN and VisR, of *S. meliloti* described here act as master controls of a gene cascade that encodes flagellar, motor, and chemotaxis proteins.

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Markers	Source or reference
E. coli		
S17-1	*recA endA thi hsdR* RP4-2 Tc::Mu::Tn*7* Tp^r Sm^r	30
S. meliloti		
RU10/406	Wild-type strain	7, 14
RU11/001	Sm^r; spontaneous streptomycin-resistant derivative of RU10/406 (wild type)	25
RU11/011[a]	Δ(*flaA-flaC*)::Sm^r Nm^r; nonmotile	25
RU11/300A	Sm^r Tc^r; RU11/001(pRU1770)	This study
RU11/300B	Sm^r Tc^r; RU11/317(pRU1770)	This study
RU11/300C	Sm^r Tc^r; RU11/318(pRU1770)	This study
RU11/317	Sm^r Δ*visR*	This study
RU11/318	Sm^r Δ*visN*	This study
RU11/391	Sm^r Nm^r Gm^r; RU11/318(pRU1755)	This study
RU11/397	Sm^r Nm^r Gm^r; RU11/317(pRU1757)	This study
RU11/513	Sm^r Δ*motBC*	23
RU11/800	Sm^r Δ*fliM*	This study
RU11/801	Sm^r Δ*orf38*	This study
RU11/802	Sm^r Δ*motA*	This study
Plasmids		
pML122	Km^r Gm^r	16
pPHU234[b]	Tc^r; promoterless *lacZ*	11
pRU1755	Km^r Gm^r; recombinant of pML122 and 969-bp *Hin*dIII-*Xba*I fragment containing *visN*	This study
pRU1756	Km^r Gm^r; recombinant of pML122 and 1.7-kb *Xba*I-*Xho*I fragment containing *visN* and *visR*	This study
pRU1757	Km^r Gm^r; recombinant of pML122 and 930-bp *Sac*I-*Xho*I fragment containing *visR*	This study
pRU1770	Tc^r; *visN-lacZ* fusion	This study
pRU2250	Tc^r; *tlpA-lacZ* fusion	This study
pRU2269	Tc^r; *motA-lacZ* fusion	This study
pRU2274	Tc^r; *flaA-lacZ* fusion	This study
pRU2278	Tc^r; *orf38-lacZ* fusion	This study

[a] This strain was erroneously defined as Δ(*flaA-flaB*) (25).
[b] pPHU235 and pPHU236 are pPHU234 derivatives with different translational phasing of *lacZ* (11).

MATERIALS AND METHODS

Bacterial strains and plasmids. Derivates of *E. coli* K-12 and *S. meliloti* MVII-1 (12) and the plasmids used in this study are listed in Table 1.

Media and growth conditions. *E. coli* strains were grown in Luria broth (19) at 37°C. *S. meliloti* strains were grown in TYC at 30°C (23). Motile cells prepared for immunoblots and β-galactosidase assays (see below) were grown overnight in TYC, diluted in 15 ml of RB (7) to an optical density at 600 nm (OD_{600}) = 0.05, layered on Bromfield plates (31), and grown on a slowly rotating platform at 30°C for 17 h to an OD_{600} of ca. 0.1 to 0.5. Swarm plates containing Bromfield medium and 0.3% Bacto Agar were inoculated with 3-μl droplets of the test culture and incubated at 30°C for 2 days. Antibiotics were used at the following final concentrations: for *E. coli*, kanamycin, 50 mg/liter; gentamicin, 10 mg/liter; and tetracycline, 10 mg/liter; for *S. meliloti*, neomycin, 100 mg/liter; streptomycin, 600 mg/liter; gentamicin, 20 mg/liter; and tetracycline, 10 mg/liter.

DNA methods. *S. meliloti* chromosomal DNA was isolated and purified as previously described (31). Plasmid DNA was purified with Qiagen spin columns or Qiagen 100 tips, as described by the manufacturer (Qiagen, Hilden, Germany). DNA fragments or PCR products were purified from agarose gels by use of the QiaEX DNA purification kit (Qiagen). PCR amplification of chromosomal DNA (33) and Southern analyses followed previously published protocols (23, 33). DNA was sequenced by the method of Sanger et al. (27) with a Quick Denature Sequenase kit (Amersham Buchler, Braunschweig, Germany) or with an ABI 310 automatic sequencer (Applied Biosystems, Weiterstadt, Germany). Sequences were aligned and compared by using GCG sequence analysis software (4); similarities were calculated by the method of Henikoff and Henikoff (9).

Gene replacement and complementation. Deletions were generated in vitro by using two PCR steps as described by Higuchi (10). PCR products containing the desired deletions were cloned into the mobilizable suicide vector pK18 *mob sacB* (28) and used to transform *E. coli* S17-1. Clones were sequenced to ascertain the accuracy of PCR-generated portions. Filter crosses of *E. coli* S17-1 and *S. meliloti* and sequential selections on neomycin and on 10% sucrose were performed as previously described (31). Complementation with pML122-based recombinant genes (Table 1) followed established protocols (16).

Immunoblots. Aliquots (1 ml) of cell culture of wild-type *S. meliloti* RU11/001 and various deletion strains at an OD_{600} of 0.3 were harvested by centrifugation at 20,000 × *g* for 6 min, resuspended in 20 μl of sodium dodecyl sulfate sample buffer, and heated to 100°C for 8 min. Samples were stored at −20°C. Fla, FliM, and MotC proteins were separated electrophoretically in a 10 to 15% linear acrylamide gradient and CheY1 was separated in a 12.5 to 20% linear gradient by the method of Laemmli (17). Electrophoretic transfer of proteins from gels to 0.45-μm-pore-size nitrocellulose (Hybond ECL; Amersham) was performed in a tank blot device (Biological Laboratories, Harvard University) for 1.5 h at 500 mA. The nitrocellulose blots were blocked overnight at room temperature in 80 mM Na_2HPO_4–20 mM NaH_2PO_4–100 mM NaCl–0.1% (vol/vol) Tween 20 (pH 7.5) (Bio-Rad)–5% instant nonfat dry milk on a shaking platform. The blots were probed with anti-FliM-MalE, anti-Fla, and anti-CheY1 polyclonal antibodies at a 1:1,000 dilution for 2 h. Anti-MotC polyclonal antibody was purified, as described by Platzer et al. (23), at a 1:250 dilution for 3 h. The blots were washed three times (10 min each), incubated with donkey anti-rabbit horseradish peroxidase-linked whole immunoglobulin antibody (Amersham) diluted 1:2,500, and washed four times (10 min each). Detection of bands by enhanced chemiluminescence (Amersham) was performed as specified by the manufacturer.

Construction of *lacZ* fusions and β-galactosidase assay. The broad-host range vector pPHU234 and two derivates, pPHU235 and pPHU236 (Table 1), served as vehicles for translational fusions to a promoterless *lacZ* (11). Five *lacZ* fusions were constructed to test gene expression in *S. meliloti*: (i) pRU1770, a recombinant of a 217-bp *Xba*I-*Hin*dIII *visN* (9-bp) fragment and pPHU234; (ii) pPHU2250, a recombinant of a 1,969-bp *Eco*RI-*Pst*I *tlpA* (165-bp) fragment and pPHU235; (iii) pRU2269, a recombinant of a 786-bp *Eco*RI-*Hin*dIII *motA* (15-bp) fragment and pPHU235; (iv) pRU2274, a recombinant of a 550-bp *Eco*RI-*Pst*I *flaA* (62-bp) fragment and pPHU236; and (v) pRU2278, a recombinant of a 561-bp *orf38* (23-bp) fragment and pPHU 234 (lengths of coding sequences are given in parentheses). The resulting *lacZ* fusion plasmids were used to transform *E. coli* S17-1 and then conjugally transferred to wild-type and mutant *S. meliloti* strains by a streptomycin-tetracycline double selection, as described by Labes et al. (16). Cultures of *S. meliloti* cells containing *lacZ* fusions were sampled, diluted in Z-buffer to an OD_{600} of 0.2, permeabilized with 1 drop of toluene, and assayed for β-galactosidase activity by the method of Miller (21).

RESULTS

VisN and VisR are LuxR-type regulatory proteins. The *visN* and *visR* genes form an operon located centrally in the *S. meliloti* flagellar regulon between *fliF* and two operons (start-

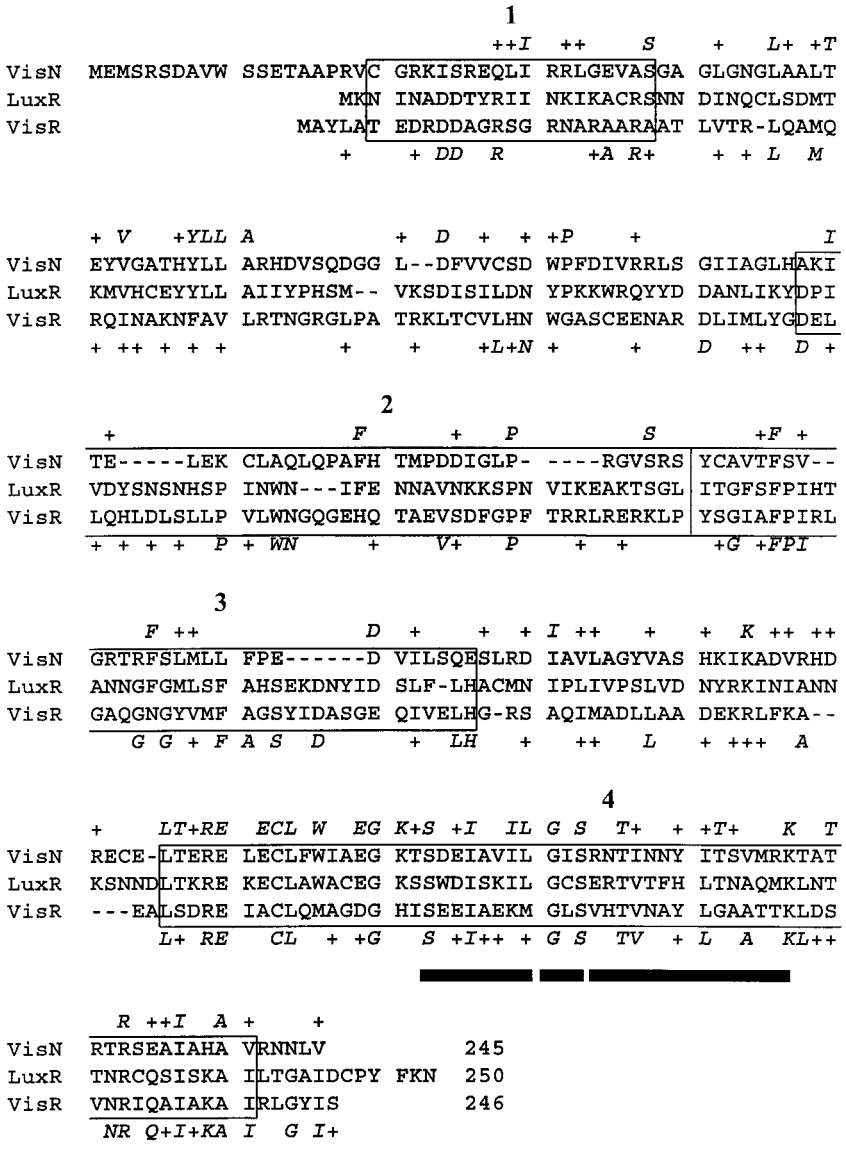

FIG. 1. Alignment of the LuxR (6) and VisN and VisR (33) polypeptide sequences. Comparisons of VisN and VisR each to LuxR with regard to identical (one-letter amino acid symbols) or similar residues (+; assigned according to the system of Henikoff and Henikoff [9]) are depicted above (VisN and LuxR) and below (VisR and LuxR) the sequences, respectively. LuxR-type functional domains are numbered as follows: 1, autoregulation; 2, ligand binding; 3, oligomerization; 4, transcription activation. The three components of the conserved helix-turn-helix DNA-binding motif (1, 9) are marked by black bars.

ing with *flhB* and *motA*, respectively) with opposite transcription polarity relative to all other genes (33). The derived polypeptide sequences of the structurally related 27-kDa VisN and VisR proteins revealed distinct similarities to the transcription activator LuxR (Fig. 1). LuxR-like factors typically consist of a receptor module with ligand-binding and oligomerization domains (located in the N-terminal half) and an activator module with the DNA-binding site featuring a helix-turn-helix motif and the transcription activation domain (located in the C-terminal half) (5). The entire LuxR with its autoinducer binding, oligomerization, and DNA-binding domains exhibits some 40% similarity (9) to the VisN and VisR polypeptides. The similarity to LuxR is more prominent among the DNA-binding domains with 39% identical and 60% similar residues for VisN and 42% identical and 60% similar residues for VisR, respectively. Both VisN and VisR contain the characteristic helix-turn-helix motifs, which have 36% identity and 60% similarity to each other. With respect to LuxR, the lowest sequence conservation prevails in the ligand-binding domains of VisN and VisR, suggesting a new specificity for a yet-unknown ligand.

visN and *visR* knockout mutants were used to study the presumptive regulatory role of these genes. In-frame deletions of *visN* and *visR* constructed to avoid polar effects were used for allelic exchange of the wild-type genes by homologous recombination (28, 31). The resulting mutants, RU11/318 (Δ*visN*) and RU11/317 (Δ*visR*), were nonmotile under phase-contrast microscopy and on swarm plates (Fig. 2, spot 2). Electron microscopy revealed that both mutants were nonflagellate, pointing to impaired flagellar synthesis. Complementation by separate expression of the homologous wild-type alleles introduced on plasmids pRU1755 (*visN*) and pRU1757 (*visR*) completely restored swimming and swarming proficiency (Fig. 2, spot 3). The data clearly suggest that the gene products

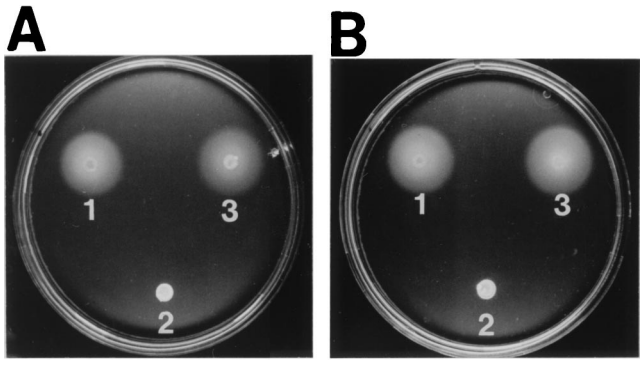

FIG. 2. Effects on swarming of in-frame deletions of *visR* (A) and *visN* (B) and complementation by the wild-type alleles. (A) Swarm 1, RU11/001 (wild type); swarm 2, RU11/317 (Δ*visR*); swarm 3, RU11/397 (Δ*visR*/*visR*). (B) Swarm 1 RU11/001 (wild type); swarm 2, RU11/318 (Δ*visN*); swarm 3 RU11/391 (Δ*visN*/*visN*). Strains to be tested were transferred by micropipette (3 μl) onto Bromfield swarm plates and incubated at 30°C for 2 days. The diameter of a swarm ring reflects the motile proficiency of a given strain.

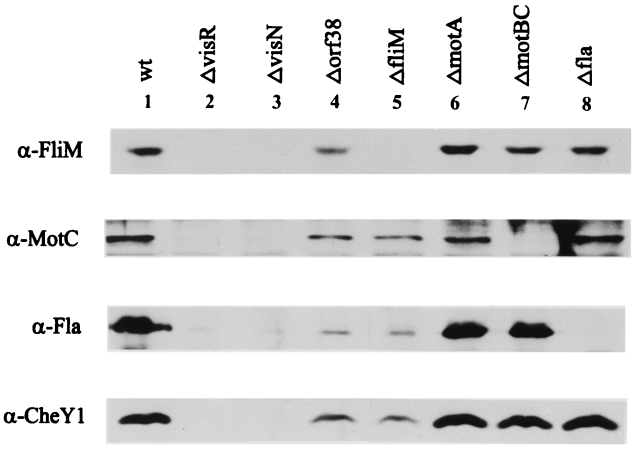

FIG. 3. Western blot analysis of gene expression in wild-type and mutant *S. meliloti* by using polyclonal anti-FliM (α-FliM), anti-MotC (α-MotC), anti-flagellin (α-Fla), and anti-CheY1 (α-CheY1) antibodies. Equal amounts of total cell protein from strains 1 (RU11/001 [wild type]), 2 (RU11/317 [Δ*visR*]), 3 (RU11/318 [Δ*visN*]), 4 (RU11/801 [Δ*orf38*]), 5 (RU11/800 [Δ*fliM*]), 6 (RU11/802 [Δ*motA*]), 7 (RU11/513 [Δ*motBC*]), and 8 (RU11/011 [Δ*flaA–flaC*]) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, blotted on nitrocellulose, and detected with specific antibody as described in Materials and Methods. Note the lack of a signal where the indicator gene was deleted.

of *visN* and *visR* are acting in *trans* and are both required for flagellar synthesis.

VisN and VisR are master controls of the flagellar regulon. Features of the Δ*visN* and Δ*visR* deletion mutants, notably the total absence of flagella, led us to ask whether VisN and VisR are global regulators of the entire cluster of chemotaxis, flagellar, and motility genes (flagellar regulon). We therefore tested the expression of representative basal-body, motor, flagellin, and chemotaxis genes by Western analysis (Fig. 3) and by *lacZ* fusions (Table 2) in wild-type *S. meliloti* and in defined tester mutant background. Polyclonal antibodies against recombinant FliM, MotC, CheY1, and purified flagellar filaments (Fla) were used to probe gene expression of the four "indicator" genes, i.e., *fliM*, encoding a constituent of the basal flagellar body; *motC*, encoding a flagellar motor protein; *flaA* to *flaC*, encoding the flagellin subunits; and *cheY1*, encoding a chemotaxis response regulator (23, 24, 31, 32, 33). The results, shown in Fig. 3 (lanes 2 and 3), confirm the presumed role of VisN and VisR as global regulators, since none of the three genes representing major operons and the *fla* genes were expressed, neither in the Δ*visN* (RU11/318) nor in the Δ*visR* (RU11/317) mutant background. In a control experiment with wild-type *S. meliloti*, each indicator gene produced a strong signal (lane 1). Since *visN* and *visR* mutants each failed to express the *fli*, *mot*, *fla*, and *che* genes, we postulate that VisN and VisR may form a functional heterodimer, VisNR, that acts as transcription activator on target promoters. This is an intriguing new function for a LuxR-like regulatory protein.

Immunoblots of the four indicator genes have been extended to extracts of six additional strains bearing in-frame deletions in *orf38*, *fliM*, *motA*, *motBC*, and *flaA* to *flaC* (Fig. 3, lanes 4 to 8). By including these knockout mutants, a hierarchial order of gene expression within the flagellar regulon was derived. The results in Fig. 3 reveal differential expression of the four indicator genes depending on the mutant allele tested. The data facilitate a subdivision of the flagellar regulon into three classes of expression, with *visNR* as the master operon (class I); *orf38*, *fliM*, *motA*, and *motBC* as class II; and the *che* and *fla* genes as class III.

This scheme has been refined and confirmed by probing transcription from the promoters that correspond to the four indicator genes plus the *vis* operon itself. Table 2 lists the β-galactosidase activities of different *lacZ* fusions measured in seven tester strains and expressed in proportion to activities determined in wild-type *S. meliloti* RU11/001 (not shown). Ideally, the ratio of mutant to wild-type activity is 1 if the mutant allele exerts no control over the promoter tested and is

TABLE 2. Expression of five promoters fused to *lacZ* in seven different mutant tester strains

Plasmid (*lacZ* fusion)	Relative promoter activity in following mutant background[a]:						
	RU11/317 (Δ*visR*) (class I)	RU11/318 (Δ*visN*) (class I)	RU11/801 (Δ*orf38*) (class IIA)	RU11/800 (Δ*fliM*) (class IIA)	RU11/802 (Δ*motA*) (class IIB)	RU11/513 (Δ*motBC*) (class IIB)	RU11/011 (Δ*flaA-C*) (class III)
pRU1770 (*vis* operon)[b]	0.79	1.24	0.92	1.05	1.16	1.03	1.12
pRU2269 (*mofli* operon)[b]	0.01	0.03	1.42	1.43	1.31	1.02	1.53
pRU2278 (*mot* operon)[b]	0	0.04	1.23	1.31	0.65	1.26	1.20
pRU2274 (*flaA*)	0.04	0.02	0.34	0.22	0.97	0.96	1.52
pRU2250 (*che* operon)[b]	0	0	0.07	0.05	0.60	0.68	1.05

[a] Relative activities are expressed as the ratio of β-galactosidase activities in a given mutant background to the wild-type background (not shown). Transcription from five promoters (left column) was determined via plasmid-borne *lacZ* fusions (see Materials and Methods). Mean values of four Miller assays (21) were averaged, with standard deviations ranging between 0.01 and 0.15.

[b] Operons controlled by the specified promoters contain the following genes (33): *vis*, *visN-R*; *mofli*, *motA fliMNG*; *mot*, *orf38 motBCD*; *che*, *tlpA orf2 cheY1AWRB Y2D orf10*.

TABLE 3. In vivo *vis* promoter activity in exponentially growing and starved wild-type and mutant strains of *S. meliloti*

Strain[a]	β-Galactosidase activity[b] (Miller units) in:	
	Exponentially growing cells[c]	Cells after starvation[d]
RU11/300A (wild type)	99 ± 8	132 ± 10
RU11/300B (Δ*visR*)	98 ± 7	127 ± 9
RU11/300C (Δ*visN*)	111 ± 8	149 ± 12

[a] Each strain contains the reporter plasmid pRU1770 (Table 1).
[b] Values of β-galactosidase activity (21) with standard deviations were averaged from five independent experiments.
[c] About 70% motile cells grown to an OD_{600} of 0.15.
[d] Nonmotile cells starved in RB (7) at 30°C for 48 h.

close to 0 if the native allele is needed for gene expression. Accordingly, *visN* and *visR* (class I) are required for transcription of basal-body (*fli*), motor (*mot*), flagellin (*flaA*), and chemotaxis (*che*) genes but not for *vis* transcription itself (except for moderate repression exerted by VisN on *vis* promoter activity, as seen in strain RU11/317). *fliM* and *orf38* (class IIA) exert control over flagellin (*flaA*) and chemotaxis (*che*) gene transcription but not over basal-body and motor genes. The *mot* genes (class IIB) have no control over the other classes, except for a slight stimulation of chemotaxis gene expression, which was, however, not seen on immunoblots (Fig. 3, lanes 6 and 7). The *flaA* gene (class III) has no control over other indicator genes. Increased expression levels listed under Δ*flaA* (Table 2, right-hand column) may reflect the titration of sigma factor that is normally engaged by strong *fla* promoters (24). We conclude that the *vis* operon lies at the top of the hierarchy as the sole class I operon, with both its gene products being absolutely required for the expression of the other genes in the flagellar regulon, i.e., those belonging to class II and class III. Class II genes have been subdivided into classes IIA (*orf38* and *fliM*) and IIB (*motA* and *motBC*) depending on whether they control class III genes (*fla* and *che*) or not. Expression of the latter depends on class I and class IIA but not class IIB genes (Fig. 3 and Table 2).

Genetic control of the *vis* operon. The combination of a *lacZ* fusion (pRU1770) and in-frame deletions of *visN* and *visR* used to probe transcription control of the *vis* genes (Table 2) has been extended to testing their expression at two extremes of bacterial growth. Exponentially growing motile cells and starved nonmotile cells (Table 3) with and without VisN or VisR were compared for expression of the *vis* operon. High β-galactosidase activities observed under these two conditions indicate constitutive expression (again, with some 20% repression by VisN alone [Table 2]) of *visN* and *visR* throughout all stages of growth with a 25% up-regulation in response to starvation (low energy). On the other hand, immunoblots and observations of swimming cells (data not shown) tell us that flagellar and motility genes are preferentially expressed in a "window" between early and mid-exponential growth but not in stationary or low-energy phases. Therefore, an additional regulatory element that controls the onset and close of VisNR activity (controlling motility) during growth must exist. We propose that it is the binding of a hitherto unknown effector to VisN, VisR, or both that triggers the transcription of class II genes.

DISCUSSION

The stepwise assembly of enterobacterial flagella is reflected by a regulatory cascade of three classes of genes securing the sequential biosynthesis of flagellar and motor components as needed (20). Similarly, the present study of gene expression within the *S. meliloti* flagellar regulon yielded three major classes of operons and genes that are expressed in hierarchical order. However, the molecular nature of two LuxR-type global activators and the different class assignment of *mot* genes are distinguishing new features of the *S. meliloti* system. The interdependence of gene expression deduced from Western blots and reporter gene assays (Fig. 3 and Table 2) is diagrammed in Fig. 4. Class I, represented by the *vis* operon, encodes two LuxR-type master regulatory proteins, VisN and VisR, both required for the expression of all flagellar, motility, and chemotaxis genes tested. The *vis* operon itself is constitutively expressed, with possible modulation by VisN and low metabolic energy; it does not require other gene products for transcription. However, quasiconstitutive transcription of *vis* (Table 3), on one hand, and the fact that motility is limited mostly to exponential growth, on the other hand, are contradictory unless one assumes posttranslational activation of VisNR by the binding of an effector (see below). Class II includes genes encoding basal-body components and motor proteins. Their expression requires the function of *visN* and *visR* but of no other genes of the flagellar regulon. Class III comprises *cheY1*, representing the chemotaxis operon (33), and *flaA*, a principal flagellin gene. Their expression requires class I and class IIA genes. The *mot* genes are also contained in class II operons, but they do not control chemotaxis and flagellation and have thus been assigned to class IIB.

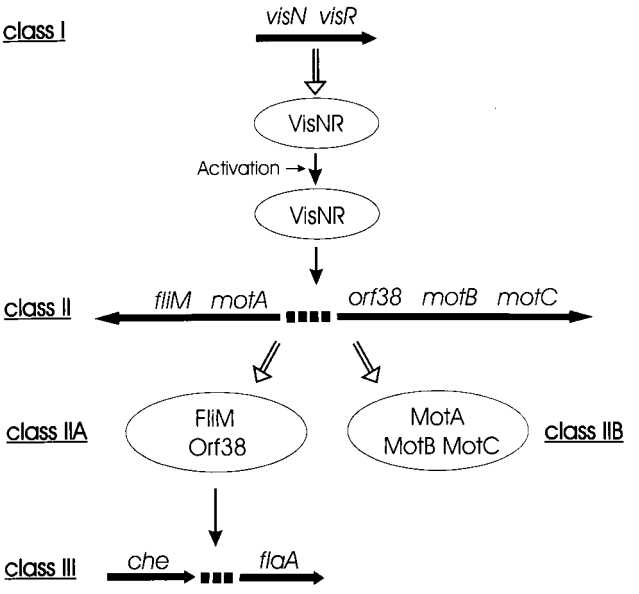

FIG. 4. Regulation scheme of the *S. meliloti* flagellar, motility, and chemotaxis gene system falling into a hierarchy of three expression classes. The transcription polarities of operons and *flaA* are indicated by horizontal arrows drawn below the gene symbols (33). Translation to gene products (ellipsoids) is indicated by open white arrows, and positive regulatory controls are indicated by solid arrows. The postulated heterodimeric structure of VisNR, posttranslational activation by an unknown effector, and subclassification of basal-body (IIA) and motor (IIB) genes are included.

Although a similar hierarchy of gene expression exists within the enterobacterial flagellar regulon (20), there are new features in the *S. meliloti* regulatory cascade. The global regulators, VisN and VisR, are of the LuxR type without structural resemblance to the enterobacterial master controls, FlhC and FlhD (18). Since both VisN and VisR are needed for gene

activation, it is plausible to assume that they function as a heterodimer, VisNR. Multimerization is similarly reported of LuxR, the transcription activator of *Vibrio fischeri* luminescence (2). Like LuxR (6), VisNR supposedly requires the binding of a yet unknown effector for function. Unlike in quorum sensing (6), such ligand molecules are not present among those excreted into the medium by motile or densely grown bacteria (34), since *S. meliloti* cells showed no response when concentrated culture medium from motile or from densely grown cells was added (data not shown). We are currently testing endogenous metabolites and chemotactic attractants as candidate effectors of VisNR. Another complication is introduced by dissimilar polypeptide structures of the ligand-binding sites of VisN and VisR (Fig. 1) that may reflect different ligand-binding specificities. Given the heterodimeric molecular structure of functional VisNR, two different receptor domains conceivably require two different ligands for activation. Obviously, the correct mixture of two ligand molecules may not be readily available in the cell. Alternatively, the heterodimeric receptor domains may cooperate in binding a single effector molecule at their common interface.

The Mot proteins (notably MotA and MotB) of *S. meliloti* are encoded by different class II operons (33), unlike in *E. coli* and *S. enterica* serovar Typhimurium, where they map in one operon assigned to class III (20). The *S. meliloti motA* gene (class IIB) is part of an operon that also contains the C-ring component genes, *fliMNG* (class IIA). The *motBCD* genes (class IIB), on the other hand, belong to a separate operon together with *orf38* (class IIA), which may encode a structural component of the basal body. Does this genetic (and regulatory) separation of the *motA* and *motBCD* genes and their linkage to basal-body genes reflect known differences in the *S. meliloti* mode of flagellar rotation requiring a more complex motor structure (23)? It needs to be elucidated whether the basal-body (rotor) and the four motor (stator) genes require coassembly as a way of securing their correct positioning relative to each other inside and outside the cytoplasmic membrane.

While VisN and VisR directly control class II genes, their control over class III genes (*flaA* and the *che* operon) is most likely to be an indirect one. The dependence of class III gene expression on the completion of basal-body structure and flagellar export (class IIA genes) implies a similar mode of control to that which operates in enterobacteria (20). In *E. coli* and *S. enterica* serovar Typhimurium, σ^{28}-mediated transcription of class III genes is inhibited by an anti-sigma factor, FlgM, which is expelled into the medium upon completion of the flagellar export apparatus, thus releasing σ^{28} for class III gene transcription. We expect that ongoing efforts toward completing the genetic map of the *S. meliloti* flagellar regulon (33) will, among other genes, also reveal class II genes that control class III transcription.

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