MICROBIOLOGICAL REVIEWS, Sept. 1994, p. 352–386
0146-0749/94/$04.00+0

Vol. 58, No. 3

Genetic Regulation of Nitrogen Fixation in Rhizobia

HANS-MARTIN FISCHER*

Mikrobiologisches Institut, Eidgenössische Technische Hochschule, ETH-Zentrum, CH-8092 Zürich, Switzerland

INTRODUCTION 352
ORGANIZATION AND FUNCTION OF NITROGEN FIXATION GENES IN *R. MELILOTI*, *B. JAPONICUM*, AND *A. CAULINODANS* 353
Genomic Organization of *nif* and *fix* Gene Clusters 353
***nif* Genes** 354
***fix* Genes** 355
fixABCX 355
fixNOQP 356
fixGHIS 356
fixR 356
REGULATION OF *nif* AND *fix* GENE EXPRESSION 356
Regulatory Cascades in *R. meliloti*, *B. japonicum*, and *A. caulinodans* 356
FixLJ 357
Identification of *fixLJ* genes 357
Structure and function of FixL and FixJ 358
(i) FixL 358
(ii) FixJ 358
Regulation by FixLJ 359
FixK 360
Identification of *fixK*-like genes 360
Structure and function of FixK-like proteins 360
Regulation by FixK 363
RpoN (σ^{54}) 364
Structure and function of RpoN 364
RpoN-dependent promoters 364
RpoN of rhizobia 365
NifA 366
Identification of *nifA* genes 366
Structure and function of NifA 366
Control of NifA synthesis 369
(i) *R. meliloti* 370
(ii) *B. japonicum* 370
(iii) *A. caulinodans* 371
Control of NifA activity 371
NtrBC and NtrYX 372
REGULATION BY FixLJ-FixK AND NifA IS NOT RESTRICTED TO NITROGEN FIXATION GENES 373
FixLJ-FixK-Regulated Functions 373
NifA-Regulated Functions 373
Nodule development and bacteroid persistence 373
R. meliloti nfe 374
R. meliloti mos 374
R. leguminosarum* bv. phaseoli *melA 374
***B. japonicum groESL*$_3$** 374
B. japonicum ndp 374
B. japonicum glnII 375
CONCLUSIONS AND PERSPECTIVES 375
ACKNOWLEDGMENTS 375
REFERENCES 376

INTRODUCTION

The ability to convert atmospheric dinitrogen ammonia is strictly limited to prokaryotes. However, within this group of organisms, nitrogen fixation is observed in a large number of species belonging to both prokaryotic kingdoms, *Archaebacte-*

* Mailing address: Mikrobiologisches Institut, Eidgenössische Technische Hochschule, ETH-Zentrum, Schmelzbergstrasse 7, CH-8092 Zürich, Switzerland. Phone: 41-1-632-4419. Fax: 41-1-632-1148. Electronic mail address: hfischer@micro.biol.ethz.ch.

ria and *Eubacteria* (418). As well as free-living diazotrophs (e.g., *Klebsiella*, *Azotobacter*, and *Rhodobacter* spp.), many bacteria living in close association (e.g., *Azospirillum* spp. [120]) or in an intimate intracellular symbiosis with their host plant(s) can fix dinitrogen. The latter group of symbiotic diazotrophs includes species of the three rhizobial genera *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium* (hereafter collectively referred to as the rhizobia). Together with two additional genera, *Agrobacterium* and *Phyllobacterium*, they constitute the family *Rhizobiaceae* of the α subdivision of the proteobacteria, (119, 418).

The free-living diazotroph *Klebsiella pneumoniae* has been regarded as a paradigm for the analysis of biological nitrogen fixation. Genetic, biochemical, and physiological studies done on the *K. pneumoniae* nitrogen fixation system have revealed a number of basic concepts that are common to many diazotrophs (for reviews, see references 60, 85, 126, 169, and 257). Moreover, these studies also contributed significantly to a better understanding of bacterial gene expression in general. Nitrogen fixation has also been studied extensively in various other diazotrophs such as *Azotobacter* spp. (40, 201), photosynthetic bacteria (213, 314), cyanobacteria (56, 157, 158), *Azospirillum* spp. (120), rhizobia (27, 87, 96, 102, 163), and methanogenic bacteria (235).

The interaction between the two partners performing symbiotic nitrogen fixation is initiated by a molecular dialog which has been studied extensively over the last years (for reviews, see references 99, 133, 142, 212, and 340). Flavonoids or isoflavonoids secreted by the host plants induce the expression of a number of nodulation (*nod*) genes in the cognate rhizobial bacteria. The products of *nod* genes are enzymes involved in the biosynthesis of species-specific, substituted lipooligosaccharides, called Nod factors (98, 315). These signal compounds, which are released by induced rhizobial cells, elicit the curling of plant root hairs and division of meristematic cells, eventually leading to the formation of root nodules. Rhizobial cells, which are attracted to host plants by chemotaxis, attach to root hairs and start to infect plant tissue inside a host-derived infection thread that progressively penetrates into the root cortex (46, 170, 202, 351). Subsequently, the bacterial cells are released into plant cells, where they further divide and differentiate physiologically and sometimes also morphologically into so-called bacteroids that will eventually reduce atmospheric nitrogen to ammonia. The fixed nitrogen is used by the plant as the nitrogen source, and in turn, photosynthates and amino acids are provided to the bacteroids as carbon, energy, and nitrogen sources (413).

Rhizobial genes required for symbiotic nitrogen fixation include those involved in Nod factor synthesis, nodule development, synthesis of the nitrogen-fixing apparatus, and bacteroid metabolism. On the other hand, a number of plant genes (nodulin genes) whose expression is specifically induced in root tissue as a consequence of the interaction with rhizobia are also known (for reviews, see references 329 and 395). Hence, the formation of effective (i.e., nitrogen-fixing) root nodules requires a coordinated temporal and spatial expression of both plant and bacterial genes (96, 135, 170). During the early stages of symbiosis, this is brought about by the exchange of highly specific chemical signals as described above. At a later stage, expression of certain bacterial genes is coordinated, together with nodule morphogenesis, via the decreasing oxygen concentrations to which infecting bacteria are exposed (393, 417). The combined effects of specialized plant cells acting as an oxygen diffusion barrier and an abundant nodulin, leghemoglobin, which reversibly binds oxygen, result in a very low concentration of free oxygen (3 to 30 nM) within infected nodule tissue (see references 86, 165, and 179 and references therein). This is a factor of about 10^4 to 10^5 times lower than the ~250 μM dissolved-oxygen concentration present in standard aerobic cultures. In response to this dramatic physiological switch, rhizobia initiate the expression of both nitrogen fixation genes and genes whose products relate to the altered environmental conditions, e.g., genes encoding a high-affinity terminal oxidase (165).

This review is focused on the genetics and functions of regulatory elements involved in the control of nitrogen fixation in three representative rhizobial species, *Rhizobium meliloti*, *Bradyrhizobium japonicum*, and *Azorhizobium caulinodans*. They were chosen for this comparison because they possess distinct regulatory circuitries and because comparable data are available. Relevant information on other rhizobial species (e.g., *Rhizobium leguminosarum* bv. viciae, *R. leguminosarum* bv. trifolii, and *R. leguminosarum* bv. phaseoli) will also be mentioned where appropriate. Typical host plants for *R. meliloti* and *B. japonicum* are the agronomically important plants alfalfa (*Medicago sativa*) and soybean (*Glycine max*), respectively. The symbiosis between *A. caulinodans* and its typical host, the tropical legume *Sesbania rostrata*, is unique in the sense that nodules are elicited not only on the roots but also on stems (for a review, see reference 87). Furthermore, *A. caulinodans*, unlike *R. meliloti* and *B. japonicum*, is able to grow in pure culture with molecular dinitrogen as the sole nitrogen source (109). Thus, *A. caulinodans* shares features of both free-living and symbiotic diazotrophs.

The first part of this review will present an overview on the organization of nitrogen fixation genes in *R. meliloti*, *B. japonicum*, and *A. caulinodans*. In the second part the regulatory genes and their products, functions, and interactions in species-specific regulatory networks will be described. Finally, the third section will briefly review cumulative evidence indicating that the regulatory function of these networks extends beyond nitrogen fixation genes.

ORGANIZATION AND FUNCTION OF NITROGEN FIXATION GENES IN *R. MELILOTI*, *B. JAPONICUM*, AND *A. CAULINODANS*

Genomic Organization of *nif* and *fix* Gene Clusters

Symbiotic nitrogen fixation genes in the broadest sense can be divided into *nod*, *nif*, and *fix* genes. The *nod* gene products are required for the early steps in nodule formation and will not considered here (for reviews, see references 142 and 212). Rhizobial *nif* genes are structurally homologous to the 20 *K. pneumoniae nif* genes (19), and it is inferred that a conserved *nif* gene plays a similar role in rhizobia as in *K. pneumoniae*. At least nine different rhizobial *nif* genes have been identified so far in *R. meliloti*, *B. japonicum*, and *A. caulinodans* (Table 1). The term "*fix* gene" is used for genes that are essential for nitrogen fixation but do not have a homologous counterpart in *K. pneumoniae*. This general definition has three consequences. First, *fix* genes represent a very heterogeneous class including, e.g., genes involved in development and metabolism of bacteroids. Second, genes originally termed *fix* genes may have to be renamed later when the functions of the corresponding gene products have become known. Third, genes that were identified originally in the context of symbiotic nitrogen fixation may also play a role in other processes not related to nitrogen fixation or may even be present in nondiazotrophs, making the term "*fix* gene" rather inappropriate. Conversely, it is evident that gene products involved in central cellular functions may at the same time play an essential role in

TABLE 1. *nif* and *fix* genes identified in *R. meliloti*, *B. japonicum*, or *A. caulinodans* and their known or proposed functions[a]

Gene	Product and/or (proposed) function	Reference(s)[b]		
		Rm	Bj	Ac
nif genes[c]				
nifH	Fe protein of nitrogenase	381	137	285
nifD	α subunit of MoFe protein of nitrogenase	75, 326	196	94, 108, 284
nifK	β subunit of MoFe protein of nitrogenase	75, 326	379	94, 108, 284
nifE	Involved in FeMo cofactor biosynthesis	249	6	285
nifN	Involved in FeMo cofactor biosynthesis	4, 5	6	
nifB	Involved in FeMo cofactor biosynthesis	57	136, 287	108
nifS	Cysteine desulfurase (421); activation of sulfur for metallocluster synthesis?		116	
nifW	Unknown function; required for full activity of FeMo protein			18, 200
nifX	Unknown function		163	
nifA	Positive regulator of *nif*, *fix*, and additional genes	58, 403	377	278, 304
fix genes				
fixABCX	Unknown function; required for nitrogenase activity; FixX shows similarity to ferredoxins	115	153, 154	18
fixNOQP	Microaerobically induced, membrane-bound cytochrome oxidase	42	299	242
fixGHIS	Redox process-coupled cation pump?	194	299	244
fixLJ	Oxygen-responsive two-component regulatory system involved in positive control of *fixK* (Rm, Bj, Ac) and *nifA* (Rm)	82	13	198
fixK/*fixK*$_2$	Positive regulator of *fixNOQP* (Rm, Bj, Ac), *nifA* (Ac), *rpoN*$_1$, and "nitrate respiration" (Bj); negative regulator of *nifA* and *fixK* (Rm)	28	14	199
Rm *fixK'*	Reiterated, functional copy of *fixK*	28		
Bj *fixK*$_1$	*fixK* homolog of unknown function; not essential for nitrogen fixation		15	
fixR	Unknown function; not essential for nitrogen fixation		377	
nfrA	Regulation of *nifA*			197

[a] Abbreviations: Rm, *R. meliloti*; Bj, *B. japonicum*; Ac, *A. caulinodans*.
[b] Wherever possible, citations refer to publications that include sequence data of the relevant rhizobial gene; see the text for additional references.
[c] See reference 85 for a detailed list of all 20 *K. pneumoniae nif* genes.

symbiosis, although the corresponding genes have not been named *fix* genes. Examples of such genes which are not further discussed in this article include the *dct* genes involved in dicarboxylic acid transport (96) or the glycine-biosynthetic gene *glyA* of *B. japonicum* (322).

The *nif* and *fix* genes of *R. meliloti*, *B. japonicum*, and *A. caulinodans* are organized in distinct clusters whose structure and genomic location are species specific (Fig. 1). Generally, linkage between nitrogen fixation genes in rhizobia is not as tight as in *K. pneumoniae*, in which 20 adjacent *nif* genes are organized in eight operons within ca. 24 kb of DNA (19).

R. meliloti carries two extremely large plasmids (megaplasmids) of about 1,400 kb (pSym-a or megaplasmid 1) and 1,700 kb (pSym-b or megaplasmid 2) (25, 59, 173, 181, 321, 352). Both cluster I (*nifHDKE*, *nifN*, *fixABCX nifA nifB frdX*) and cluster II (*fixLJ*, *fixK*, *fixNOQP*, *fixGHIS*) (Fig. 1A) are located on megaplasmid 1 (31, 83). The cluster II genes map at about 220 kb downstream of the *nifHDKE* operon and are transcribed in opposite orientation to it. Interestingly, a functional duplication of the region spanning *fixK* and *fixNOQP* is present at ca. 40 kb upstream of *nifHDKE* (308). A cluster of *nod* genes including the common *nod* genes (*nodABC*) is located in the 30-kb region between *nifE* and *nifN* (238). Additional genes required for an effective symbiosis are located on megaplasmid 2 (see references 173, 181, 230, 309, 310, and 402 and references therein) and on the chromosome (114, 173).

B. japonicum and apparently also *A. caulinodans* do not have plasmids. Hence, the *nif* and *fix* (and *nod*) genes are located on the chromosome in these organisms, and they are organized as depicted in Fig. 1B and C. In *B. japonicum* three clusters of *nif* and *fix* genes have been characterized by now and, in addition, a *fixK*-like gene (*fixK*$_1$) was identified at a fourth locus. Recently, a correlated physical and genetic map of the circular *B. japonicum* chromosome was established and its size was estimated to be ca. 8,700 kb (219). Cluster II of *B. japonicum*, which includes the *fixRnifA* operon, *fixA*, and at least 11 *nod* genes (not shown in Fig. 1B), is located at a distance of ca. 240 kb away from cluster I, which contains the nitrogenase structural genes. Cluster III, harboring *fixLJ*, *fixK*$_2$, and the *fixNOQP* operon, maps at ca. 770 kb away from cluster II. Hence, in *B. japonicum* all known *nif* and *fix* genes required for symbiotic nitrogen fixation and the common *nod* genes are located within about 1,000 kb on the chromosome. Moreover, additional gene regions that are involved in nodulation (*nodVW* [143]) or whose expression is induced under symbiotic conditions (*rpoN*$_1$ [217], *groESL*$_3$ [128], and *ndp* [406]) are present close to or within this segment of the *B. japonicum* chromosome (219). One might speculate that the symbiotic region of *B. japonicum* was located originally on a plasmid similar to the Sym plasmids of *R. meliloti* and became then part of the chromosome by integration at one stage during evolution. Alternatively, the symbiotic plasmids of *R. meliloti* (and other rhizobia) may have evolved by excision of a chromosomal region. In *A. caulinodans* four clusters of *nif* or *fix* genes and two additional loci carrying single nitrogen fixation genes (*nifB*, *nfrA*) have been characterized; however, their relative positions on the chromosome are not known (Fig. 1C) (94, 108, 198–200, 296).

The structural *nif* and *fix* genes known to date in *R. meliloti*, *B. japonicum*, and *A. caulinodans* and their known or proposed functions will be briefly described next, whereas the regulatory *nif* and *fix* genes will be discussed in greater detail further below.

nif Genes

The *nifD* and *nifK* genes specify the α and β subunits, respectively, of the $\alpha_2\beta_2$ FeMo protein (component I or

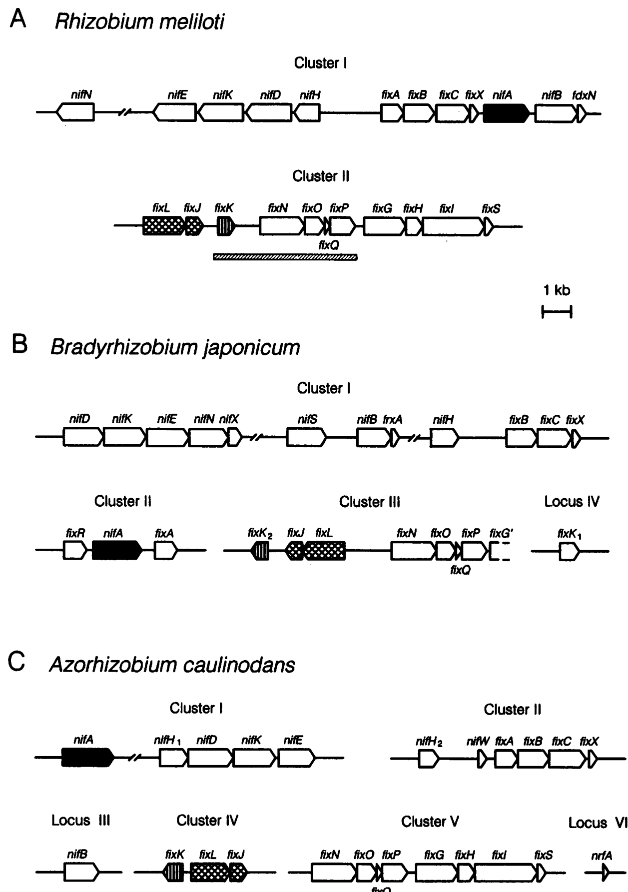


FIG. 1. Organization of *nif* and *fix* gene clusters in *R. meliloti* (A), *B. japonicum* (B), and *A. caulinodans* (C). Several known ORFs associated with some of the *nif* or *fix* genes are not shown here. In *R. meliloti* both clusters of *nif* and *fix* genes are located on the megaplasmid pSym-a, whereas in *B. japonicum* and *A. caulinodans* all of the depicted genes are present on the chromosome. Homologous regulatory genes are marked by identical patterns. The hatched bar below cluster II of *R. meliloti* refers to a region which is duplicated on the megaplasmid. For details and references, see the text and Table 1.

dinitrogenase; $M_r \approx 220,000$). The homodimeric Fe protein (component II or dinitrogenase reductase; $M_r \approx 60,000$) is encoded by *nifH*. In *R. meliloti* the *nifHDK* genes are organized in an operon along with *nifE*, whereas *nifHDK* and *nifE* in *A. caulinodans* form two separate transcriptional units (94, 249). In *B. japonicum* the *nifH* gene is located 17 kb downstream of the *nifDKENX* operon (6, 131, 163). In *A. caulinodans* a second *nifH* gene (*nifH*$_2$) that differs in only six nucleotides from *nifH*$_1$ was found in cluster II upstream of the *fixABCX* genes (285). This is reminiscent of *R. leguminosarum* bv. phaseoli, which possesses three identical, functional copies of *nifH* (303). No *nifN*-like gene has been described in *A. caulinodans* so far. On the basis of complementation data, the *nifB* gene of *R. meliloti* was suggested to form part of a *nifA-nifB-fdxN* transcriptional unit. However, no relevant transcript data are available, and DNA sequence data imply that the *nifB* gene is transcribed at least partly from its own −24/−12 promoter (57, 209). The same promoter type was also found upstream of the *B. japonicum nifB* gene, which is cotranscribed with the *frxA* gene (117, 287). The transcriptional organization of the *A. caulinodans nifB* gene is not known.

Synthesis of the FeMo cofactor of component I requires the products of the *nifE*, *nifN*, and *nifB* genes; however, the exact biochemical functions of the respective proteins are not known (see reference 84 and references therein). Interestingly, the amino acid sequences of the NifE and NifN proteins show a significant similarity to those of NifD and NifK, respectively. It was suggested that the *nifEN* genes originated from duplication of the *nifDK* genes and that the NifEN complex may provide a scaffold for FeMo cofactor biosynthesis (47).

Recently, NifS of *Azotobacter vinelandii* was purified and shown to be a pyridoxal phosphate-containing dimeric desulfurase ($M_r \approx 87,500$) that catalyzes the formation of L-alanine and elemental sulfur by using L-cysteine as a substrate (421). This finding and the fact that NifS is required for full activity of both components of nitrogenase suggested that the released sulfur is used for the synthesis of nitrogenase metalloclusters.

The function of the *nifW* gene (originally termed *nifO* [200]) located upstream of the *fixA* gene in *A. caulinodans* is not clear. Mutations in the homologous genes of *K. pneumoniae* (19, 37, 294) and *Azotobacter vinelandii* (186, 187) lead to a reduced activity of the FeMo protein, whereas *A. caulinodans nifW* mutants show a complete lack of nitrogenase activity (18, 200).

Downstream of the *nifB* genes of both *R. meliloti* and *B. japonicum* are the genes *fdxN* and *frxA*, respectively, encoding ferredoxin-like electron transfer proteins. In contrast to *frxA* of *B. japonicum*, the *R. meliloti fdxN* gene is absolutely essential for nitrogen fixation (117, 209, 250). Another ferredoxin-like protein is encoded by *fixX*; this gene is located downstream of *fixC* in all three rhizobial species discussed here.

fix Genes

fixABCX. The *fixABCX* genes were originally identified in *R. meliloti* (75, 115, 301, 326) and later also in *B. japonicum* (136, 153), *A. caulinodans* (18, 200), *R. leguminosarum* bv. viciae (149), *R. leguminosarum* bv. trifolii (182), and *R. leguminosarum* bv. phaseoli (264). They are organized in a single operon in all species except *B. japonicum*, in which *fixA* and *fixBCX* form distinct transcriptional units present in clusters II and I, respectively. The *B. japonicum fixBCX* operon includes a proximal open reading frame (ORF35), which is not essential for nitrogen fixation activity but whose translation significantly stabilizes *fixBCX* mRNA (154).

Mutations in any one of the *fixABCX* genes of *R. meliloti*, *B. japonicum*, and *A. caulinodans* completely abolish nitrogen fixation. It has been proposed that the *fixABCX* gene products might be involved in electron transport to nitrogenase (115, 153). Support for this hypothesis came from the finding that FixX contains five conserved cysteines of which three are arranged in a Cys-X-X-Cys-X-X-Cys cluster typical of ferredoxins (49). However, results from in vitro nitrogenase assays with crude extracts prepared from wild-type, *fixC*, and *fixB A. caulinodans* cells indicate that at least the FixB and FixC proteins are probably not involved in the direct electron transfer to nitrogenase (200). The FixB proteins of *R. meliloti* and *A. caulinodans* show significant similarity to the α subunits of rat and human electron transfer flavoproteins (18), and FixC of *A. caulinodans* displays some local similarity to human electron transfer flavoprotein-ubiquinone oxidoreductase (141). Thus, it remains to be resolved whether the *fixABCX* genes in rhizobia are involved in a redox process that is indirectly related to nitrogen fixation. Interestingly, a cluster of four ORFs homologous to the *fixABCX* genes has been identified recently in *Escherichia coli* by systematic sequencing. However, no functional data on these putative genes are available yet (419).

fixNOQP. The *fixNOQP* genes were first described in *R. meliloti* as a duplicated *fix* region that is linked to the regulatory genes *fixLJ* and *fixK* and whose expression is induced under symbiotic conditions (31, 83, 308). Homologous genes were then identified in *B. japonicum* (299), *A. caulinodans* (242, 243), and *R. leguminosarum* bv. viciae (180a). They are probably organized in an operon in all three species. *R. meliloti* mutant strains deleted for both *fixNOQP* regions and *B. japonicum fixNOQP* mutants are defective in symbiotic nitrogen fixation, whereas a corresponding mutant of *A. caulinodans* retained 50% of wild-type nitrogenase activity under both symbiotic and free-living conditions (242, 243, 299, 308). In addition, *B. japonicum* mutants are affected in bacteroid development and exhibit a decreased whole-cell oxidase activity when grown microaerobically or anaerobically. The predicted amino acid sequences of the FixNOQP proteins imply that they constitute a membrane-bound, cytochrome *c*-containing heme/copper cytochrome oxidase (193, 243, 299). It is postulated that this oxidase complex is required to support bacteroid respiration under conditions of low oxygen present in root nodules (165, 299).

fixGHIS. Four tightly linked genes, named *fixGHIS*, have been identified by mutational analysis and subsequent DNA sequence determination downstream of the *fixNOQP* operon in cluster II of *R. meliloti* (194). On the basis of hybridization experiments, homologous genes seem to exist in various members of the genus *Bradyrhizobium*, in *A. caulinodans*, and in *R. leguminosarum* bv. viciae and bv. phaseoli. In fact, partial DNA sequence analysis confirmed the presence of *fixG*, *fixI*, and probably *fixH* downstream of *fixP* in *A. caulinodans*, and there is at least a *fixG*-like gene present at an analogous position in *B. japonicum* (244, 299). *R. meliloti* mutant strains carrying transposon insertions in *fixG*, *fixH*, or *fixI* are unable to fix nitrogen. On the other hand, Tn*5* insertions in *fixG* or *fixI* of *A. caulinodans* result in only a reduced symbiotic nitrogen fixation activity and affect nitrogenase activity only marginally under free-living conditions. On the basis of the deduced amino acid sequences, all four *fixGHIS* gene products are predicted to be transmembrane proteins. FixG is likely to be involved in a redox process, because it contains two cysteine clusters typical of iron-sulfur centers present in bacterial ferredoxins. FixI is homologous to the catalytic subunit of bacterial and eukaryotic ATPases involved in cation pumping. It is speculated that FixI is a symbiosis-specific cation pump whose function is coupled to a redox reaction catalyzed by the FixG subunit (194). Clearly, further biochemical analysis is required to define the function of the *fixGHIS* gene products in rhizobial nitrogen fixation.

fixR. In *B. japonicum* a gene termed *fixR* is located upstream of the regulatory *nifA* gene, and the two genes form an operon (377). No *fixR*-like gene has been described so far in other rhizobia, but interspecies hybridization experiments indicate the existence of homologous DNA regions in other slow-growing rhizobia and in the nonsymbiotic bacterium *Rhodopseudomonas palustris* (375). The predicted FixR gene product of ca. 30 kDa in molecular mass is not essential for nitrogen fixation by *B. japonicum*, as shown by nonpolar mutations in *fixR* (377). It shows significant similarity to the NodG protein of *R. meliloti* and also to human and rat steroid dehydrogenases (24), which implies that FixR is involved in an oxidation-reduction process. The fact that FixR is encoded in an operon along with *nifA* raises the possibility that FixR is involved in the redox-dependent activation and inactivation of the NifA protein. However, no experimental data are available yet to support this hypothesis.

REGULATION OF *nif* AND *fix* GENE EXPRESSION

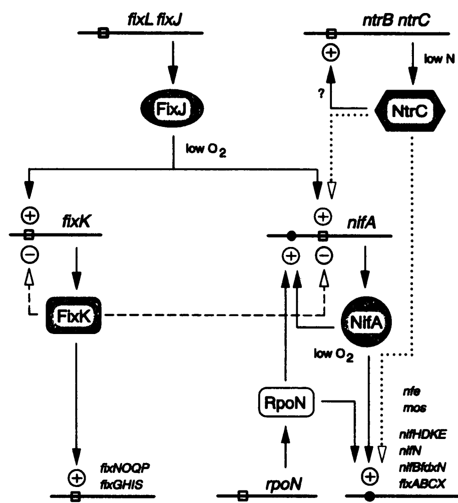
Regulatory Cascades in *R. meliloti*, *B. japonicum*, and *A. caulinodans*

In view of the high energy demands of nitrogen fixation (up to 40 mol of ATP may be required in vivo for the reduction of 1 mol of dinitrogen to ammonia [169]) and the marked oxygen sensitivity of nitrogenase, it makes perfect sense that all diazotrophs studied so far have evolved sophisticated regulatory mechanisms that guarantee a tight control of nitrogenase synthesis. The expression of nitrogen fixation genes is controlled by cascades of hierarchically organized regulatory genes. Their concerted action enables the bacteria to sense optimal environmental conditions required for nitrogen fixation and to transmit this information to the level of gene expression. Similar principles are known from the regulation of bacterial developmental processes such as sporulation in *Bacillus subtilis* (122) or cell type differentiation in *Caulobacter crescentus* (279). Some free-living diazotrophs such as *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, *Azospirillum lipoferum*, or *Azospirillum brasilense* are able to down-regulate the activity of nitrogenase at the posttranslational level in response to the presence of combined nitrogen (for a review, see reference 239). This control is based on the reversible ADP-ribosylation of nitrogenase reductase. A similar "switch off-on" mechanism also operates in *A. caulinodans* (221) but is not further discussed here.

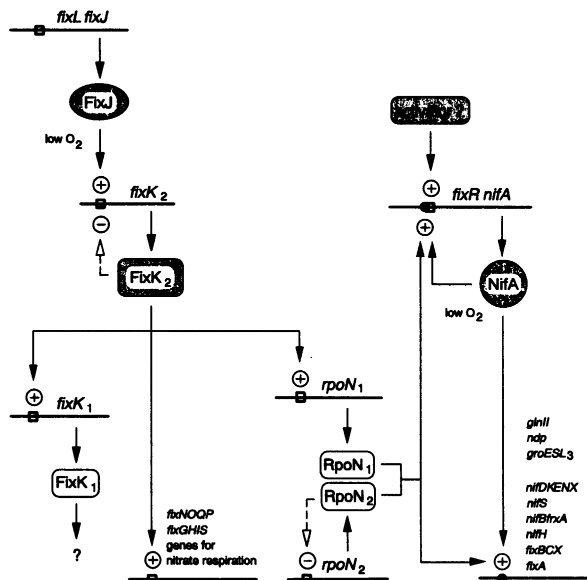
Environmental nitrogen and oxygen conditions are critical signals for the regulation of *nif* gene expression in *K. pneumoniae*. This dual physiological control is mediated via the general nitrogen regulatory system (*ntr*) and the *nif*-specific regulatory genes, *nifLA* (257). By contrast, expression of nitrogen fixation genes in symbiotic diazotrophs is regulated predominantly in response to the cellular oxygen conditions. The lack of a strict nitrogen control mechanism probably relates to the physiological conditions under which symbiotic nitrogen fixation takes place. Bacteroids are already provided with combined nitrogen by their host plant while fixing nitrogen. Thus, it seems reasonable to uncouple symbiotic nitrogen fixation from nitrogen control. Nevertheless, elements of an *ntr*-like system are also found in rhizobia; however, their regulatory role in nitrogen fixation is different from that in *K. pneumoniae*. *A. caulinodans* is exceptional among rhizobia in being able to fix nitrogen both symbiotically and in pure culture. Accordingly, nitrogen control does play an important role for free-living nitrogen fixation specifically in this bacterium, and components of the *ntr* system are also partly relevant for symbiosis.

Figures 2A to C depict current models of regulatory circuitries involved in the regulation of nitrogen fixation of *R. meliloti*, *B. japonicum*, and *A. caulinodans*, respectively. The individual components will be discussed in detail in the following sections. It is obvious that all three organisms use largely identical regulatory elements (FixL, FixJ, FixK, NifA, RpoN); however, these are integrated into different, species-specific networks. Two regulatory cascades which are present in all three species control different groups of target genes. The cascade including NifA controls expression of the nitrogenase structural genes and genes encoding accessory functions by means of their RpoN-dependent −24/−12 promoters. Thus, this type of regulation is analogous to the situation in *K. pneumoniae*. On the other hand, the FixLJ and FixK proteins of *R. meliloti*, *B. japonicum*, and *A. caulinodans* constitute a distinct regulatory cascade which may be specific for symbiotic diazotrophs since no functionally homologous proteins have been found in free-living diazotrophs. A common function of this cascade

A *Rhizobium meliloti*



B *Bradyrhizobium japonicum*



C *Azorhizobium caulinodans*

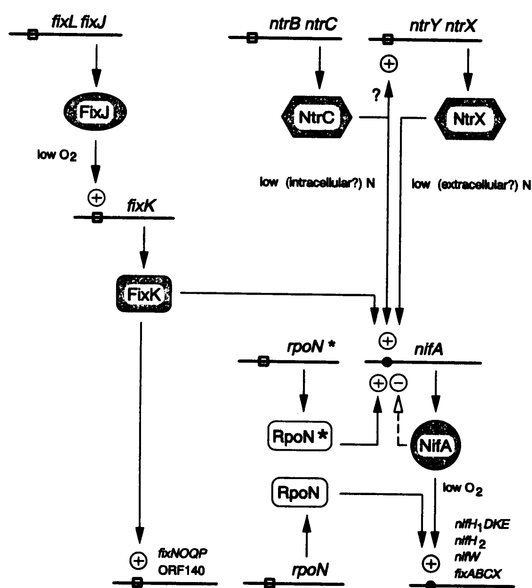


FIG. 2. Comparative models of *nif* and *fix* gene regulation in *R. meliloti* (A), *B. japonicum* (B), and *A. caulinodans* (C). Homologous regulatory proteins are symbolized identically in panels A, B, and C. Solid arrows and open arrowheads with dashed lines indicate positive and negative regulation, respectively. Dotted lines with open arrowheads denote NtrC-mediated activation of the *R. meliloti nifHDKE*, *nifBfdxN*, and *fixABCX* promoters which is observed in free-living, aerobically growing cells under nitrogen-limiting conditions but which is of no relevance for symbiotic nitrogen fixation. Solid circles indicate σ^{54}-dependent $-24/-12$-type promoters, whereas open boxes indicate other promoters. Key references describing substantial components of the models presented include 28, 39, 82, 103, 319, 373, 396, and 403 for *R. meliloti*; 13–15, 127, 217, 376, and 377 for *B. japonicum*; and 198, 199, 278, 295, 296, 304, and 362 for *A. caulinodans*. For additional references and further details, see the text.

concerns the regulation of the *fixNOQP* genes, whereas it differs substantially with respect to its involvement in *nifA* regulation. In *R. meliloti* and *A. caulinodans*, FixJ and FixK, respectively, act as direct positive regulators of *nifA*. By contrast, *nifA* expression in *B. japonicum* is largely independent of the FixLJ-FixK cascade and there exists only a loose linkage between the two mentioned cascades via the FixLJ-$FixK_2$-dependent regulation of one of two *rpoN* genes. Similarly, the significance of the nitrogen response regulator NtrC with regard to the regulation of nitrogen fixation varies among *R. meliloti*, *B. japonicum*, and *A. caulinodans*. No such role has been attributed to NtrC of *B. japonicum*, whereas NtrC of *R. meliloti* and *A. caulinodans* contributes to the regulation of *nifA*, albeit at different levels of biological significance.

FixLJ

Identification of *fixLJ* genes. The *fixLJ* genes of *R. meliloti* were originally identified genetically by their requirement for the transcriptional activation of the *fixNOQP* and *fixGHIS* operons as well as for the *nifA* gene under low-oxygen conditions (82, 83, 308). Subsequently, homologous genes were cloned from *B. japonicum* (13) and *A. caulinodans* (198), and recent reports indicate that similar genes are also present in *R. leguminosarum* bv. viciae (180a) and in the genus *Frankia*, the nitrogen-fixing symbiont of actinorhizal plants (277). Recently, a two-component regulatory pair, RegBA, and an activator protein, PrrA, have been shown to be involved in oxygen regulation of photosynthesis genes of *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*, respectively (32, 121, 297, 346). However, neither RegA nor PrrA belongs to the FixJ subclass of regulators (see below), and RegA is not involved in oxygen regulation of the *Rhodobacter capsulatus nifHDK* operon.

The rhizobial *fixLJ* genes encode proteins of ca. 55 kDa (FixL) and 22 kDa (FixJ), and corresponding proteins of the three rhizobial species are about 50% identical. DNA sequence analysis of the *fixLJ* genes of *R. meliloti*, *B. japonicum*, and *A. caulinodans* strongly implies that these genes are organized in an operon in all three species; however, no

transcript-mapping data are available. Similarly, only limited information is available on a possible regulation of the *fixLJ* genes. The *fixLJ* operon in *B. japonicum* was found to be expressed both under aerobic and anaerobic conditions, albeit at slightly different levels (13). Null mutations in *fixL* or *fixJ* of *R. meliloti* and *A. caulinodans* result in the formation of symbiotically inefficient (Fix^-) nodules, and nitrogenase activity in free-living *A. caulinodans* mutant cells is drastically diminished (31, 82, 198, 396). Similarly, *fixLJ* mutants of *B. japonicum* show a loss of about 90% wild-type symbiotic nitrogen fixation activity and are unable to grow anaerobically with nitrate as the terminal electron acceptor, suggesting a regulatory role of FixLJ also in nitrate respiration (13).

Structure and function of FixL and FixJ. The FixL and FixJ proteins are members of the ubiquitous two-component regulatory systems that enable bacteria to respond to environmental or cytoplasmic signals with specific cellular activities (8, 82, 320). Typically, signal sensing and transduction include autophosphorylation at a conserved histidine residue in the C-terminal domain of the sensor protein and transfer of the phosphate to an aspartate residue in the N-terminal region of the cognate response regulator protein (for reviews, see references 45, 289, 290, 363, and 364). On the basis of sequence similarities, many of the response regulators and cognate sensors can be classified into either the NtrC-, OmpR- or FixJ-type subfamily (8, 364). Other members of the FixJ subfamily include the NodV-NodW (143), NarX-NarL (361), UhpB-UhpA (414), RcsC-RcsB (368), DegS-DegU (166, 220), ComP-ComA (407, 408), and BvgS-BvgA (17) regulatory pairs.

The FixL and FixJ proteins of *R. meliloti* have both been purified after overproduction in *E. coli* (139). Purification and subsequent characterization of FixL were facilitated by the use of a truncated, soluble form of FixL (FixL*) lacking 126 N-terminal amino acids. The apparent molecular masses of the native FixL* and FixJ proteins are 93 and 50 kDa, respectively, which is in good agreement with the predicted values for dimeric forms of FixL* (86 kDa) and FixJ (46 kDa) (139).

(i) FixL. The FixL protein of *R. meliloti* (505 amino acids) is a membrane-bound, oxygen-regulated hemoprotein kinase/phosphatase which can be dissected into three functional domains (97, 139, 140, 140a, 236, 266). The N-terminal domain, of ca. 120 amino acids, contains four membrane-spanning segments as suggested by hydropathy analysis and the use of *fixL-phoA* fusions (236). This portion of the protein is dispensable for both in vivo and in vitro FixL activity (97, 236). The FixL protein of *A. caulinodans* also shows a hydrophobic N terminus, but no hydrophobic segments are present in the corresponding region of *B. japonicum* FixL (13, 198).

The central domain of *R. meliloti* FixL, from amino acids 127 to 260, contains one heme moiety per monomer and binds oxygen (140a, 266). Two of three histidine residues present in this domain (His-138 and His-194) are conserved in FixL of *B. japonicum* and *A. caulinodans*, suggesting a possible role in heme binding. However, replacement of His-138 by glutamine did not alter the spectral properties of FixL*, which makes it unlikely that His-138 is directly involved in heme ligation (265). Interestingly, a region exhibiting homology to the central domain of FixL was recently identified in the N terminus of the *Azotobacter vinelandii* NifL protein which is involved in oxygen regulation of *nif* gene expression (41). However, no evidence is available that NifL is a hemoprotein, too.

The C-terminal domain of the FixL proteins (amino acids 260 to 505 in *R. meliloti* FixL) corresponds to the most highly conserved domain of the sensor proteins of two-component systems. A truncated derivative of the *R. meliloti* FixL protein

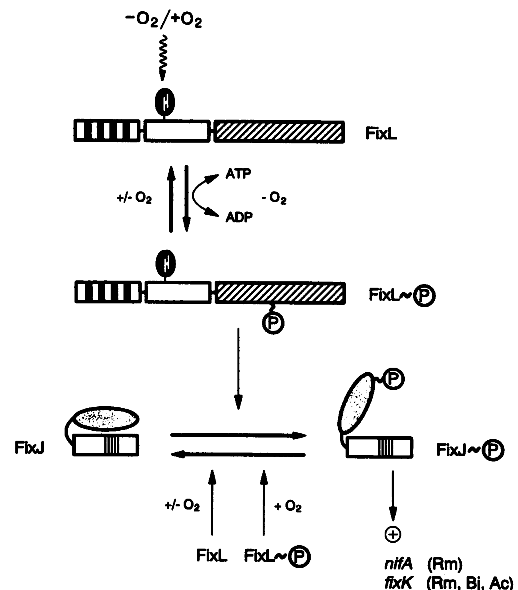


FIG. 3. Model of FixLJ-mediated oxygen control of *nifA* expression in *R. meliloti* (Rm) and expression of *fixK* in *R. meliloti*, *B. japonicum* (Bj), and *A. caulinodans* (Ac). For details and references, see the text. Four membrane-spanning segments in the N-terminal domain of the sensor kinase protein FixL are indicated by vertical black bars. Oxygen sensing or the sensing of microaerobiosis requires the heme moiety (H) attached to the central, cytoplasmic domain of FixL. The C-terminal domain (hatched) corresponds to the most highly conserved domain of sensor proteins of two-component regulatory systems and includes the autophosphorylation site of FixL. The nonphosphorylated N-terminal domain (shaded) of the FixJ protein masks the transcriptional activation function of the C-terminal domain (open box) which contains a putative helix-turn-helix DNA-binding motif (vertical lines inside box). Phosphorylation of the N-terminal domain by FixL-phosphate induces a conformational change in the protein, thereby releasing the inhibitory effect of the N-terminal domain. Control by oxygen occurs at two levels. Low-oxygen conditions stimulate the autophosphorylation activity of FixL and repress the phosphatase activity of FixL-phosphate (but not that of FixL), which together results in increased levels of phosphorylated FixJ under these conditions. Conversely, FixJ-phosphate accumulation is efficiently prevented under high-oxygen conditions by the decreased autophosphorylation of FixL and the stimulated phosphatase activity of FixL-phosphate. Note that the phosphoryl transfer from FixL-phosphate to FixJ is not affected by the oxygen conditions.

corresponding to the C-terminal domain exhibits in vitro autophosphorylation and FixJ kinase activity, although these activities are lower than those observed with FixL* (266). Replacement of the conserved His-285 by glutamine abolishes both activities, supporting the idea that His-285 is the site of autophosphorylation (265). FixL* autophosphorylation (but not the reverse reaction or the phosphotransfer reaction to FixJ) is specifically stimulated by low oxygen tension, and this regulation requires the heme-binding domain. FixL* also possesses phosphatase activity. In contrast to nonphosphorylated FixL*, this activity is suppressed under anaerobic conditions when FixL* is phosphorylated (Fig. 3) (140, 237, 266).

(ii) FixJ. The FixJ proteins of *R. meliloti*, *B. japonicum*, and *A. caulinodans* are very similar in size (204, 205, and 211 amino acids, respectively) and consist of two functional domains of comparable homology (ca. 50% identity). The N-terminal regulatory domain contains four conserved amino acid residues (Asp-10, Asp-11, Asp-54, and Lys-104 in *R. meliloti* FixJ)

which are characteristic for two-component response regulators (290). An aspartic acid residue corresponding to Asp-54 in FixJ has been shown to be the phosphorylation site in the CheY, VirG, and NtrC proteins (190, 330, 331). Phosphorylation of FixJ at Asp-54 has not yet been demonstrated directly; however, two FixJ mutant proteins containing amino acid substitutions at positions thought to be located near Asp-54 in the FixJ tertiary structure (Glu-12 to Gly, Thr-82 to Ile) are severely affected in their ability to be phosphorylated by FixL* (410). Unexpectedly, replacement of Asp-54 by Asn resulted in a FixJ mutant protein whose transcriptional activity was only marginally affected both in vivo and in vitro (311). Moreover, its activity was strongly stimulated via phosphorylation by a soluble form of FixL independently of the oxygen conditions, suggesting that this particular FixJ mutant protein can be phosphorylated at an alternative residue. However, it is not known yet whether this type of phosphorylation also occurs in the wild-type FixJ protein and, if so, whether it is of biological relevance to *R. meliloti.*

Recently, four mutant *R. meliloti* FixJ proteins which showed increased activation of the *R. meliloti nifA* promoter in *E. coli* were described (409). All of them had amino acid exchanges in a region of the N-terminal domain which is likely to be involved in phosphorylation. Further biochemical analysis revealed that one of the mutant proteins (Gly-83 to Glu), although less efficiently phosphorylated than wild-type FixJ, is more resistant to dephosphorylation by FixL*. This could mean that this particular amino acid position plays a role in an effective interaction between FixL and FixJ.

The C-terminal domain of FixJ shares homology with a number of transcriptional activators and also with the C-terminal part of σ factors which interact with the -35 region of bacterial promoters (195). On the basis of the latter observation, one might speculate about similar activation mechanisms of FixJ-like activators and σ factors. The region with most pronounced homology includes a putative helix-turn-helix motif likely to be involved in sequence-specific promoter recognition. The functional role of this motif is supported by the effect of site-directed mutations introduced into the second (recognition) helix which drastically reduce the in vivo activity of the resulting *R. meliloti* FixJ mutant proteins (195). On the basis of results obtained from in vivo activity tests with individually expressed domains of *R. meliloti* FixJ, Kahn and Ditta (195) proposed a model for the regulation of FixJ activity (Fig. 3). In the nonphosphorylated state, the N-terminal domain of FixJ masks the transcription activation function of the C-terminal domain. Phosphorylation of the N-terminal portion by FixL induces a conformational change, thereby relieving the inhibitory effect of the N-terminal domain. Upon dephosphorylation, the transcription activation function of FixJ is inhibited again. This model was further corroborated by recent in vitro transcription experiments with the purified C-terminal activator domain of FixJ (81a).

Figure 3 summarizes the model of the signal transduction mechanism via the FixLJ cascade. The cellular oxygen conditions are sensed by the heme moiety bound to the central, cytoplasmic FixL domain and, in turn, modulate inversely the kinase and phosphatase activities of the C-terminal domain. Low-oxygen conditions favor the kinase activity, resulting in an increased amount of phosphorylated FixJ protein, which then activates transcription of target genes. Conversely, when the oxygen tension increases, the level of phosphorylated FixJ protein is diminished by the phosphatase activity of FixL phosphate which is stimulated under these conditions.

Regulation by FixLJ. All three species, *R. meliloti*, *B. japonicum*, and *A. caulinodans*, require the *fixLJ* genes for microaerobic induction of the *fixK* gene (*fixK*$_2$ in *B. japonicum*) under microaerobic conditions (Fig. 2A to C, respectively) (14, 82, 198). Unlike in *B. japonicum* and *A. caulinodans*, FixJ directly controls *nifA* expression in *R. meliloti* (82, 283, 396).

The regulatory function of the *R. meliloti fixLJ* gene products has also been studied in a heterologous *E. coli* background and in vitro. In the absence of FixL, overexpressed FixJ protein can activate in *E. coli* expression of both an *R. meliloti fixK'-'lacZ* and a *nifA'-'lacZ* fusion regardless of the aeration status of the culture, and transcription of the latter fusion was shown to start at the same position as in the homologous *R. meliloti* background (167). When the *fixL* and *fixJ* genes are expressed simultaneously at low levels in *E. coli*, activation of both target genes, *nifA* and *fixK*, is observed only under microaerobic conditions (95). Thus, the FixL protein is responsible for oxygen control of FixJ-mediated activation of *nifA* and *fixK* in *E. coli* also. At high levels of FixJ, constitutively synthesized FixL protein has no effect on *fixK* expression whereas that of *nifA* is activated under microaerobic conditions and repressed in aerated cultures (95). This finding suggests functional differences in the interaction of FixJ with the promoters of *nifA* and *fixK*; it is further supported by the fact that mutations in *fixJ* can have different effects on the activation of these promoters by the corresponding mutant proteins (195, 410). It remains to be elucidated whether differential regulation of the *fixK* and *nifA* genes by the FixLJ regulatory proteins is also observed in the homologous *R. meliloti* background and, if so, whether this has any biological significance with respect to an efficient symbiosis.

Originally, in vitro FixJ activity was demonstrated in a coupled transcription-translation system through activation of a *fixK'-'lacZ* fusion by using extracts prepared from *E. coli* cells that overproduced the *R. meliloti* FixJ protein (30). Subsequently, purified FixJ protein was used to activate in vitro transcription from the *fixK* and *nifA* promoters in conjunction with *E. coli* σ^{70} RNA polymerase or *R. meliloti* RNA polymerase (3, 312). In addition, it was demonstrated that phosphorylation of FixJ by FixL, which preferentially occurs under low-oxygen conditions, greatly enhances in vitro transcriptional activity of FixJ. Thus, by using the FixL and FixJ proteins, it has been possible for the first time to transduce in vitro an environmental signal (low oxygen conditions) to the level of gene expression.

The rhizobial genes known to be activated by FixJ include *nifA*, *fixK*, and *fixK'* of *R. meliloti* (*fixK'* refers to the reiterated *fixK* gene in the duplicated *fixK-fixNOQP* region [Fig. 1A]); *fixK*$_2$ of *B. japonicum*; and *fixK* of *A. caulinodans* (14, 28, 199, 396). However, no direct interaction of FixJ with the promoter region of any of these genes has been demonstrated as yet. Mutational analyses aimed at the identification of *cis*-acting regulatory elements in FixJ-dependent promoters allowed the definition of minimal upstream DNA requirements, but the nature of the postulated FixJ-binding site and the core promoter still remains to be elucidated. Sequence comparison reveals the presence of a common C(C/G)NAAT(T/A)T_{-33} element in the promoter regions of all five target genes (Fig. 4). A 10-bp insertion into this motif of the *R. meliloti nifA* promoter abolishes induction by microaerobiosis (2). It is not known, however, whether this motif is part of a FixJ recognition site or whether it makes contact with the RNA polymerase (or both). An additional, conserved element (TAAG) is present around position -64 in the promoter regions of all four *fixK* genes but not in that of *R. meliloti nifA*. Deletion of part of this motif drastically reduces microaerobic induction of *R. meliloti fixK*, whereas deletion of the corresponding (nonconserved) region in the *R. meliloti nifA* promoter has no effect

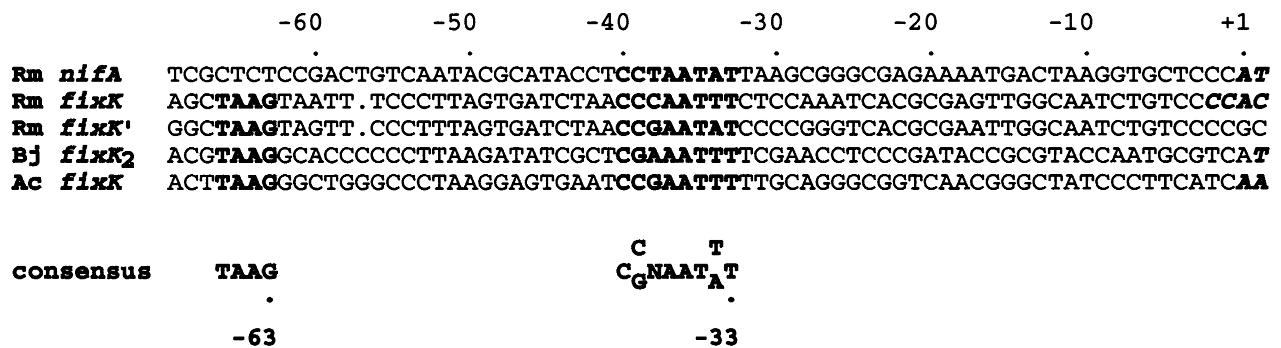


FIG. 4. Promoter regions of FixJ-dependent genes of *R. meliloti* (Rm), *B. japonicum* (Bj), and *A. caulinodans* (Ac). *fixK'* refers to the second *fixK* gene of *R. meliloti*, which is located in the duplicated *fixK-fixNOQP* region ca. 40 kb upstream of *nifHDKE* (Fig. 1A). Where known, the transcriptional start sites are emphasized in italic boldface characters. The numbering is indicated as defined for *R. meliloti nifA* in reference 2. To optimally align the −63 consensus motif, a 1-nucleotide gap has been introduced into the *R. meliloti fixK* and *fixK'* sequences as originally proposed by Waelkens et al. (397). Conserved DNA sequence motifs potentially involved in contacting RNA polymerase and/or FixJ binding are highlighted (boldface letters), and proposed consensus motifs are indicated. For details, see the text. References: *R. meliloti nifA*, 2, 396; *fixK*, 28; *fixK'*, 397; *B. japonicum fixK*$_2$, 14; *A. caulinodans fixK*, 199.

(199, 397). It is speculated that this could account for the differential activation pattern of *nifA* and *fixK* by overproduced wild-type or mutant FixJ mutant proteins (95, 195, 397, 410).

FixK

Identification of *fixK*-like genes. Expression studies with an *R. meliloti fixN'-'lacZ* fusion in *E. coli* had originally led to the finding that the *R. meliloti fixK* gene was required for activation of the *fixN* promoter (28). In *R. meliloti* the *fixK* gene is located between the *fixLJ* and the *fixNOQP* operons and is part of the 5-kb DNA region that is reiterated on megaplasmid 1 (cf. Fig. 1A). Similarly, a *fixK*-like gene (*fixK*$_2$) was recently identified downstream of the *fixLJ* operon in *B. japonicum* (Fig. 1B) (14). Another *fixK*-like gene had been described previously in *B. japonicum*; however, no functional role could be assigned to it (15). Hereafter, this gene will be referred to as the *B. japonicum fixK*$_1$ gene in order to distinguish it from the FixJ-controlled *fixK*$_2$ gene. In *A. caulinodans*, a *fixK*-like gene is present upstream of the *fixLJ* genes and orientated opposite to them (Fig. 1C) (199). There may also exist another *fixK*-like gene in *A. caulinodans*, as inferred from the description of a gene that is homologous to *E. coli fnr* but whose mutation results in a different phenotype from that of a mutation in the *fixLJ*-linked *fixK* gene (89). Finally, in *R. leguminosarum* bv. viciae a *fixK*-like gene (*orf240*, *fnrN*) was identified on the basis of its ability to promote microaerobic activation of a heterologous *R. meliloti fixN'-'lacZ* fusion and to partially complement the symbiotic defects of an *R. meliloti fixJ* mutant (73).

Null mutations in the *fixK* genes of *R. meliloti* (*fixK fixK'* double mutants) and *A. caulinodans*, as well as in *fixK*$_2$ of *B. japonicum*, abolish nitrogen fixation by these organisms. In addition, *B. japonicum fixK*$_2$ mutants are defective in nitrate respiration, as was described previously for *B. japonicum fixLJ* mutant strains (13, 14). This differs from *R. meliloti*, in which the activity of the dissimilatory ("respiratory") nitrate reductase is apparently not affected by a mutation in *fixK* (28). No data are available on nitrate respiration in *A. caulinodans*. *B. japonicum fixK*$_1$ mutants and *R. leguminosarum* bv. viciae *fnrN* mutants are still able to fix nitrogen, but nitrogenase activity and nodule number are diminished to some extent in the latter case. No other mutant phenotypes have been reported for these strains.

In addition to being positively regulated, the *R. meliloti fixK* genes and the *B. japonicum fixK*$_2$ gene are subject to negative autoregulation, but it is not known whether this control is direct or indirect (Fig. 2) (14, 28).

Structure and function of FixK-like proteins. The FixK proteins, which are similar in size (211 to 248 amino acids) (Table 2), belong to a family of prokaryotic transcriptional regulators, named the Crp-Fnr family for its best-characterized members. The *E. coli* cyclic AMP (cAMP) receptor protein (Crp) or catabolite gene activator protein (Cap) primarily regulates transcription of genes involved in catabolic functions (for reviews, see references 44 and 211). DNA-binding and transcriptional activation by Crp are regulated allosterically through the binding of cAMP. The Fnr protein of *E. coli* is a transcriptional regulator which controls the expression of a variety of genes involved mainly in anaerobic respiratory processes (for reviews, see references 358 and 388). It is postulated that the switch from inactive Fnr under aerobic conditions to active Fnr under anaerobic conditions is accompanied by its conversion from the monomeric to dimeric form (225). The molecular mechanisms underlying redox sensing are not well understood (see below). The regulatory functions of Crp and Fnr include both positive and negative control. Crp and Fnr share at least two common structural elements that seem to be conserved in all members of the Crp-Fnr family (71, 350). First, five glycine residues in the N-terminal half of the proteins (corresponding to positions 33 and 62, 45 and 74, 56 and 85, 67 and 96, and 71 and 100 of Crp and Fnr, respectively) are almost always invariant. In Crp they were shown to separate consecutive β-strands which form a β-roll structure involved in Crp dimerization and nucleotide binding (404). Second, a helix-turn-helix motif characteristic of many DNA-binding proteins is present in the C-terminal part of all Crp-Fnr-homologous proteins (corresponding to positions 169 to 192 and 196 to 219 of Crp and Fnr, respectively [Fig. 5]). Determination of the X-ray crystal structure of the Crp-DNA complex ultimately confirmed the direct involvement of this motif in DNA binding (345).

The members of the Crp-Fnr protein family can be divided into three classes based on their overall amino acid sequence similarity and functional differences (Table 2). Class I includes Fnr of *E. coli* and 11 additional proteins whose similarity to Fnr varies from 24 to 74% identical amino acids. Common to all

TABLE 2. Crp-/Fnr-homologous regulatory proteins

Class[a]	Protein(s)	Species	No. of aa[b]	Function(s) or gene(s) regulated	Reference(s)
IA	Fnr	*E. coli*	250	Anaerobic respiration	349, 385
IA	EtrA	*Shewanella putrefaciens*	250	Anaerobic respiration	327
IA	HlyX	*Actinobacillus pleuropneumoniae*	240	Hemolysin synthesis?	240
IA	Anr	*Pseudomonas aeruginosa*	244	Nitrate respiration, arginine deiminase, HCN production	338, 423
IA	FnrA	*Pseudomonas stutzeri*	244	Arginine deiminase	81
IA	Btr	*Bordetella pertussis*	241	Unknown	26
IB	$FixK_1$	*B. japonicum*	237	Unknown	15
IB	AadR	*Rhodopseudomonas palustris*	239	Anaerobic growth on 4-hydroxybenzoate	101
IB	FnrN	*R. leguminosarum* bv. viciae	240	Nitrogen fixation (marginally)	73
IC	$FixK_2$	*B. japonicum*	235	Nitrate respiration, expression of *fixNOQP*, *fixK*$_1$, *rpoN*$_1$, and *fixK*$_2$	14
IC	FixK	*A. caulinodans*	248	*nifA* expression	199
IC	FixK	*R. meliloti*	211	Expression of *fixNOQP*, *fixGHIS*, *nifA*, and *fixK*	28
II	Crp	*E. coli*	210	Catabolic gene activation	7, 76
II	Crp	*Salmonella typhimurium*	210	Catabolic gene activation	343
II	Crp	*Shigella flexneri*	210	Catabolic gene activation	77
II	Crp	*Klebsiella aerogenes*	210	Catabolic gene activation	288
II	Crp	*Haemophilus influenzae*	224	Competence development	69
II	Clp	*Xanthomonas campestris* pv. campestris	230	Phytopathogenicity	91
III	NtcA	*Synechococcus* sp. strain PCC 7942	222	Nitrogen assimilation	394
III	NtcA	*Synechocystis* sp. strain PCC 6803	225	Unknown	EMBL accession no. X71607
III	NtcA, BifA	*Anabaena* sp. strain PCC 7120	223	Nitrogen assimilation and unknown other functions	405 (EMBL accession no. X71608)
III	CysR	*Synechococcus* sp. strain PCC 7942	206	Sulfur assimilation	224

[a] For classification criteria, see the text.
[b] Total number of amino acids (aa) as predicted from the DNA sequence of respective genes.

functionally defined proteins of this class is their involvement in oxygen control of various cellular processes.

Class II comprises Crp of *E. coli* and highly conserved Crp-like proteins from numerous other bacteria. Interestingly, the functions of the Crp-like proteins of *Haemophilus influenzae* and *Xanthomonas campestris* pv. campestris are related to the development of competence and the regulation of phytopathogenicity, respectively, rather than to the regulation of catabolic genes. The amino acid sequences of the Crp proteins from *E. coli*, *Salmonella typhimurium*, *Shigella flexneri*, and *Klebsiella aerogenes* are extremely well conserved (at most one amino acid replacement), whereas Crp of *Haemophilus influenzae* and Clp of *Xanthomonas campestris* pv. campestris show a lower degree of homology to *E. coli* Crp (76 and 44% identical amino acids, respectively).

Class III proteins includes a group of cyanobacterial proteins that are involved in the control of nitrogen or sulfur metabolism. Possibly NtcA of *Anabaena* sp. strain PCC 7210 has additional functions, because it was originally identified as BifA, a protein that binds to the upstream region of the *xisA* gene which encodes a site-specific recombinase synthesized during heterocyst development (70). The similarity between the NtcA proteins is very pronounced (77 to 88% identity), whereas CysR of *Synechococcus* sp. strain PCC 7942 is less similar to NtcA (~32% identity).

The proteins of class I can be further divided into subclasses IA, IB, and IC. Fnr and all other class IA proteins show a strictly conserved Cys-X_2-Cys-X_5-Cys motif in their N termini and an additional conserved cysteine residue in the central part of the polypeptide (the cysteine residues correspond to positions 20, 23, 29, and 122 of Fnr). These four cysteine residues are essential for Fnr activity, and it is speculated that they contribute to formation of an iron-binding domain that may be involved in redox sensing (147, 254, 347, 357).

In the proteins of class IB, which includes $FixK_1$ of *B. japonicum* and FnrN of *R. leguminosarum* bv. viciae, the four cysteine residues are also conserved; however, their spacing in the N-terminal motif is slightly variable (Cys-X_{2-3}-Cys-X_7-Cys). It seems likely that this motif plays a similar role in redox sensing as does the corresponding motif in Fnr.

The proteins of class IC are characterized by the lack of both the N-terminal and central cysteine residues. Although it remains to be demonstrated explicitly, one can speculate that the activity of class IC regulators is not redox controlled, in contrast to that of class IA and IB proteins. Notably, the overall similarities of proteins within subclasses IA, IB, and IC are higher (≥33, ≥35, and ≥40% identical amino acids, respectively) than between the proteins of those subclasses.

The structural similarity of *B. japonicum* $FixK_1$ and *R. leguminosarum* bv. viciae FnrN with Fnr of *E. coli* is also

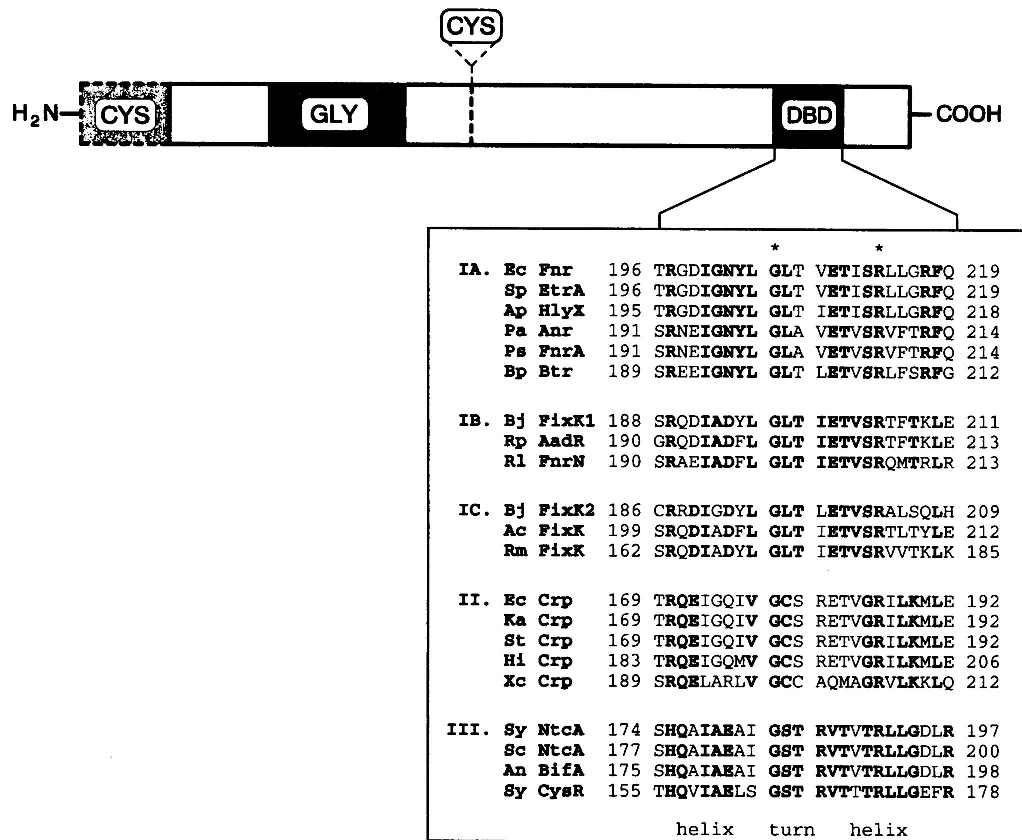


FIG. 5. Structural comparison of Crp-Fnr-like proteins belonging to different classes (IA, IB, IC, II, and III) as defined in the text and in Table 2. GLY and DBD in the model indicate a region containing five invariant glycine residues in the N-terminal half of the proteins and a C-terminal DNA-binding domain, respectively, which are conserved in all members of this protein family. The presence of at least three N-terminal cysteine residues and that of an additional cysteine residue in the central part, all characteristic for the proteins of classes IA and IB, is marked by CYS. The lower part of the figure shows an amino acid sequence alignment of the (putative) helix-turn-helix motif present in the DNA-binding domain. Numbers indicate the positions of the first and last amino acids shown within the respective protein. Amino acids residues conserved within at least one subclass are shown in boldface letters. The asterisks mark residues which are conserved among all proteins listed. Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; An, *Anabaena* sp. strain PCC 7120; Ac, *A. caulinodans*; Bp, *Bordetella pertussis*; Bj, *B. japonicum*; Ec, *E. coli*; Hi, *Haemophilus influenzae*; Ka, *Klebsiella aerogenes*; Pa, *Pseudomonas aeruginosa*; Ps, *Pseudomonas stutzeri*; Rl, *R. leguminosarum* bv. viciae; Rm, *R. meliloti*; Rp, *Rhodopseudomonas palustris*; St, *Salmonella typhimurium*; Sp, *Shewanella putrefaciens*; Sy, *Synechococcus* sp. strain PCC 7942; Sc, *Synechocystis* sp. strain PCC 6803; Xc, *Xanthomonas campestris* pv. campestris. For references, see Table 2.

reflected at the functional level. Both $FixK_1$ and FnrN can replace Fnr of *E. coli* with respect to the activation of Fnr-dependent promoters, and this activation is dependent on the presence of microaerobic or anaerobic conditions (15, 342). Conversely, Fnr can activate an *R. meliloti fixN'-'lacZ* fusion in *E. coli* and in *R. leguminosarum* bv. viciae lacking a functional *fnrN* gene (71, 342). In addition, homologous complementation tests have shown that $fixK_1$ of *B. japonicum* is able to substitute for $fixK_2$ with regard to regulation of anaerobic nitrate respiration and symbiotic nitrogen fixation (14, 15). However, the physiological significance of this result is uncertain since $fixK_1$ has been overexpressed from a constitutive promoter in this particular experiment. Nevertheless, these results are consistent with a model predicting that Fnr of *E. coli* and the rhizobial FixK proteins recognize similar DNA motifs in their target promoters. This idea is further corroborated by a comparison of the C-terminal helix-turn-helix motifs of the Crp-Fnr-like proteins (Fig. 5). Although no direct experimental data are available, genetic and model-building studies strongly suggest that this domain of the *E. coli* Fnr protein is indeed involved in DNA binding similarly to that in Crp (see reference 358 and references therein). Amino acids crucial for DNA binding of Fnr include Glu-209 and Ser-212. The latter is likely to contribute to the DNA-binding specificity of Fnr, and, remarkably, Ser-212 is conserved in all class I proteins.

Two Fnr-like proteins can be present in one and the same organism, as exemplified by $FixK_1$ and $FixK_2$ of *B. japonicum*. This situation may not be unique to *B. japonicum*, since there is evidence that *Shewanella putrefaciens*, *Pseudomonas stutzeri*, *A. caulinodans* and *R. leguminosarum* bv. viciae harbor Fnr-like proteins in addition to the already known EtrA, FnrA, FixK, and FrnN proteins, respectively (81, 89, 300a, 327). Apart from the future functional characterization of these new proteins, it will be of particular interest to analyze their N termini for the presence or absence of the cysteine motif and to determine the regulation of the respective genes. The biological significance of two Fnr-like proteins in a single organism remains speculative. It seems plausible that the Fnr-like proteins of classes IA and IB represent the "classic" Fnr homologs of the respective bacteria. Diazotrophic organisms may have additionally evolved the FixK proteins belonging to class IC for the control

TABLE 3. Putative FixK-binding sites in the promoter regions of *R. meliloti*, *B. japonicum*, and *A. caulinodans* genes or operons

Organism	Gene/operon	Location relative to putative translational (transcriptional) start site[a]	Sequence[a,b]	Reference(s)
R. meliloti	*fixNOQP*	−106	gcac**TTGA**t**C**tg**GATCAA**g**G**tg	28
	fixGHIS	−80	agac**TTGA**cgca**GATCAA**g**G**tg	194
B. japonicum	*fixNOQP*	−70	tatc**TTGA**tttca**ATCAA**ttcc	299
	fixGHIS	−71	ccgt**TTGA**g**C**tg**GATCAA**c**G**ga	299
	fixK$_1$	−118	agaa**TTGA**t**C**tg**G**g**TCAA**ccgc	15
	rpoN$_1$	−81	gctt**TTG**cg**C**gac**ATCAA**a**G**cg	217
	hemA	−147 (−46)	ttct**TTGA**t**C**gg**GATCAA**gttt	252
	cycB	−267	cgcc**TGG**cggca**GATCAA**g**G**cg	323
A. caulinodans	*nifA*	−147 (−130)	aaat**TTGA**t**C**ca**GATCAA**a**G**cc	278, 304
	fixNOQP	−90	tcct**TTGA**cttgt**ATCAA**g**G**tg	243
			*	
(Brady)rhizobium spp.	FixK consensus		**TTGA-C--GATCAA-G**	
			*	
E. coli	Fnr consensus	(∼−48)	**AAA-TTGAT----ATCAA-TTT**	118

[a] The numbers indicated refer to the location of the marked (*) first T residue in the rhizobial consensus sequence with respect to the translational (transcriptional) start site of the respective gene or operon. The transcriptional start site is known only for *B. japonicum hemA* and *A. caulinodans nifA*.

[b] Boldface capital letters indicate nucleotides which are conserved in at least 6 of the 10 putative FixK-binding sites shown.

of functions related directly or indirectly to the process of nitrogen fixation. FixK proteins of this type probably do not respond directly to varying oxygen conditions but, rather, are integrated into an oxygen-responsive cascade. However, it cannot be excluded that the FixK proteins are involved in the transduction of another physiological signal which has not been identified yet. On the other hand, it is conceivable that FixK$_1$ of *B. japonicum*, FnrN of *R. leguminosarum* bv. viciae, and (hypothetical) homologous class IB proteins from other rhizobia have specific functions that are not related to nitrogen fixation. By analogy with Anr of *P. aeruginosa*, FnrA of *P. stutzeri* and AadR of *Rhodopseudomonas palustris*, such functions may include the anaerobic degradation of arginine or of aromatic compounds (cf. Table 2).

Intriguingly, the FixLJ proteins in *B. japonicum* control the synthesis of both FixK$_2$ and FixK$_1$, the latter through FixK$_2$. Thus, a hypothetical target gene of FixK$_1$ is under dual control with respect to oxygen, a situation which is reminiscent of *R. meliloti* genes regulated by NifA (cf. Fig. 2A and B). It seems plausible that FixLJ and FixK$_1$ respond to different low levels of oxygen. The hierarchical organization suggests that FixK$_1$ exerts a more stringent oxygen control than FixLJ. By this means it may be possible to optimally control the synthesis of cellular components which differ in their oxygen sensitivity or whose proper functioning requires defined (low) oxygen concentrations.

Regulation by FixK. A common function of the FixK proteins of all three species (in *B. japonicum* FixK$_2$ is always meant here) is their control of *fixNOQP* expression (Fig. 2) (14, 28, 242, 243). Possibly, the FixK proteins of *R. meliloti* and FixK$_2$ of *B. japonicum* also control expression of the *fixGHIS* operon, which is situated immediately downstream of *fixNOQP* in both organisms (28, 29, 313). This is supported by the presence of a potential FixK-binding site upstream of *fixG* (Table 3) (194, 299). By contrast, transcription of *A. caulinodans fixG* is independent of FixK, whereas expression of another *A. caulinodans* gene (*orf140*), which is located downstream of *fixLJ*, is also reported to be *fixK* dependent (242). However, its product (of unknown function) is not essential for symbiotic or free-living nitrogen fixation.

As depicted in Fig. 2, the functions of the FixK proteins of *R. meliloti*, *B. japonicum*, and *A. caulinodans* differ most significantly with respect to their role in the control of *nifA* expression. FixK of *R. meliloti* exerts a negative effect on *nifA* expression, whereas FixK of *A. caulinodans* is required for positive regulation of *nifA* expression (28, 199). By contrast, the FixLJ-FixK$_2$ cascade of *B. japonicum* is not involved at all in the regulation of *nifA* expression but is responsible for oxygen control of *rpoN*$_1$ and *fixK*$_1$ (14).

Genetic and physiological evidence may show the existence of further candidates for possible FixLJ-FixK$_2$-controlled genes in *B. japonicum*. These are (i) *hemA*, encoding 5-aminolevulinic acid synthase (252, 353); (ii) *cycB*, coding for a soluble *c*-type cytochrome (c_{552}) which is more abundant in bacteroids than in free-living, aerobically grown cells (16, 323); and (iii) the genes involved in the synthesis of the uptake hydrogenase (*hup* genes) (204, 205, 392).

The growing number of (putatively) FixK-regulated genes or operons offers the opportunity to inspect the respective promoter regions for a potential FixK consensus binding site (Table 3). This approach, however, is restricted by the fact that only two of these genes have been transcriptionally mapped, namely *hemA* of *B. japonicum* and *nifA* of *A. caulinodans* (252, 304). Furthermore, very little information on the structure of rhizobial promoters other than those depending on σ^{54} is available. Despite these shortcomings, a considerably conserved motif (5′-TTGA-C--GATCAA-G-3′) is detectable within a reasonable distance upstream of all positively controlled target genes or operons for which relevant sequence information is available (Table 3). This motif is highly similar to the core of the binding site for *E. coli* Fnr ("Fnr box"; 5′-AAA-TTGAT----ATCAA-TTT-3′ [118]). This finding is not unexpected in view of the pronounced similarity between the putative DNA-binding domain of Fnr and those of the FixK proteins (Fig. 5). Unlike the Fnr box, the putative FixK-binding sites do not show a preference for adenine or thymi-

dine residues in the 5'- and 3'-flanking regions, respectively, and the 5' half-site shows more variability than the 3' half-site. A cytosine may be preferred between the two half-sites at position 6 of the FixK consensus motif defined in Table 3. Similarly, a guanine residue, which is present at position 16 in 7 of the 10 sequences compared, may contribute to the specificity for FixK binding. Among the genes that are positively controlled by FixK, *rpoN*$_1$ of *B. japonicum* and *nifA* of *A. caulinodans* are presently the only examples for which a functional role of the putative FixK-binding site has been demonstrated (216, 362).

The FixK proteins of *R. meliloti* and FixK$_2$ of *B. japonicum* also exert negative control on their own expression and, in the case of *R. meliloti*, also on *nifA* (Fig. 2A and B). Assuming that this regulation is mediated directly by FixK, one might postulate that FixK interferes with RNA polymerase by binding to FixK-binding sites located in or near the promoter region of the respective genes. Such a mechanism is known from promoters which are negatively regulated by Fnr (146, 348, 356, 359, 374). The promoter regions of *fixK* and *nifA* in *R. meliloti* have been defined by transcriptional mapping (28, 396). No evident potential FixK-binding site is detectable in the promoter region of *nifA*, but a motif is present around position −43 of the *fixK* promoter that shows a limited similarity to a FixK consensus binding site (5'-gTGA-C--accCAA-t-3'; nucleotides shown in capital letters match the FixK consensus binding sequence as proposed in Table 3 [397]). A DNA sequence element with more convincing similarity to the consensus is located around position −487 with respect to the *fixK* transcriptional start site (5'-TTGA-C--GcgCAA-G-3'). Mutational analysis revealed that this element does indeed participate in negative autoregulation of *fixK*. It is speculated that the repression mechanism may involve DNA loop formation by binding of FixK to the distant operator sites around positions −487 and −43, which would then interfere with FixJ-mediated activation of the *fixK* promoter (397). An analogous model may be proposed for the negative autoregulation of *fixK*$_2$ in *B. japonicum* based on the presence of putative FixK-binding sites in the 5' region (14). However, their functional role remains to be investigated by mutational analysis. Alternatively, it is possible that negative autoregulation of *fixK* expression in *R. meliloti* and *fixK*$_2$ expression in *B. japonicum* is mediated indirectly at the level of *fixLJ* transcription. This idea is supported by the presence of putative FixK-binding sites upstream of *fixLJ* in both organisms. A potential functional role of these elements, and the role of FixK in *fixLJ* expression in general, has not been investigated systematically.

Although further experimental work is clearly required to define more precisely the structural requirements for the interaction of FixK with its DNA target site, the analysis of the promoter regions of FixLJ- and FixK-controlled genes may contribute to the identification of rhizobial promoters other than those depending on σ^{54} and to the characterization of the appropriate RNA polymerase holoenzyme.

RpoN (σ^{54})

Structure and function of RpoN. The promoter specificity of bacterial RNA polymerases is determined by the σ subunit of the polymerase holoenzyme, which has an $\alpha_2\beta\beta'\sigma$ subunit composition (for reviews, see references 150 and 161). The major vegetative σ factor of *E. coli* is σ^{70}, and homologous σ factors are known in many other gram-negative and gram-positive bacteria. A gene (*glnF*) encoding the alternative σ factor, σ^{54} (now also termed σ^N [258]), was initially identified by genetic means in *S. typhimurium* because of its requirement for the synthesis of glutamine synthetase (138). Subsequently, it became evident that σ^{54} plays a general role in the control of nitrogen metabolism including nitrogen fixation (155, 241). Accordingly, the respective gene was named *ntrA* or *rpoN*, the latter term being the generally used designation today. Final proof that σ^{54} acts as a σ factor was obtained by in vitro transcription experiments with purified components (172, 180). In the meantime, it is well established that *rpoN*-like genes are present in a wide range of eubacteria (gram-negative and gram-positive species) and that they are involved in a large number of different cellular functions such as pilin synthesis, degradation of xylene and toluene, dicarboxylic acid transport, special fermentation pathways, and nitrogen fixation (for reviews, see references 223, 258, and 378).

From genetic evidence, the *rpoN* genes of 27 bacterial species have been identified to date, and the DNA sequences of 17 of them have been determined (see reference 258 and references therein). The length of the deduced proteins varies between 426 (*Rhodobacter capsulatus*) and 526 (*Rhizobium* sp. strain NGR234) amino acids. Sequence alignments indicate a high overall similarity unevenly distributed over three distinct regions. The conserved N-terminal region I of ca. 50 amino acids is rich in glutamine and leucine. Region II is of variable length (27 to 110 amino acids) and shows only very little sequence conservation apart from a high proportion of acidic residues. Remarkably, the two RpoN proteins of *B. japonicum* (217) differ most with respect to the length of region II (62 and 100 amino acids in RpoN$_1$ and RpoN$_2$, respectively, according to the model presented in reference 258). Region II is followed by a long, well-conserved C-terminal region III of ca. 400 residues containing two characteristic motifs in its distal portion. First, there is a potential helix-turn-helix motif that may be involved in the interaction with the −13 promoter region as inferred from studies with RpoN mutant proteins and promoter suppressor mutants (74, 259, 261). Second, there is an almost absolutely invariant stretch of 10 amino acids, located adjacent to the proposed helix-turn-helix motif, which appears to be unique to σ^{54} proteins. The function of this element, which has been designated the RpoN box (391), is not clear. Footprinting and gel shift experiments have demonstrated that σ^{54} can specifically bind to certain σ^{54}-dependent promoters in the absence of core RNA polymerase (52). Binding of σ^{54} is stabilized in the presence of the core enzyme possibly by induction of conformational changes to enhance DNA binding (64). Recent cross-linking experiments have provided direct evidence that the distal part of the C-terminal domain of σ^{54} makes contact with the *R. meliloti nifH* promoter (65). The site(s) where σ^{54} interacts with the RNA polymerase core enzyme is not known, and the absence of significant similarity between σ^{54} and σ^{70} could mean that these σ factors make different contacts with the core.

RpoN-dependent promoters. The promoter region of σ^{54}-dependent genes shows a characteristic structure (5'-TGGCAC-N$_5$-TTGCA/T-3' [268]) and is located between positions −26 and −11 relative to the transcription start site. The alternative designation "−24/−12 promoter" refers to the most highly conserved GG and GC doublets at these positions. This type of promoter, which differs markedly from σ^{70}-dependent −35/−10 promoters of *E. coli* (5'-TTGACA-N$_{17}$-TATAAT-3' [234]), was first recognized in the context of the analysis of *nif* gene promoters from numerous diazotrophs such as *K. pneumoniae* (38), *R. meliloti* (371) and *B. japonicum* (1, 137, 196). Since then, a large amount of data has accumulated which clearly demonstrates that, consistent with the wide distribution of *rpoN*-like genes, −24/−12-type promoters are ubiquitous among eubacteria, in which they control the expres-

sion of a wide variety of different genes (for compilations, see references 223, 258, and 378).

In contrast to RNA polymerase containing σ^{70}, the σ^{54} holoenzyme is unable to initiate transcription by itself. This reaction requires the presence of a transcriptional activator that catalyzes the isomerization of a closed to an open promoter complex. Consequently, expression of σ^{54}-dependent genes is always subject to positive control by an activator protein whose activity is modulated in response to specific physiological signals (223). NifA, NtrC, NtrX, and DctD represent transcriptional regulators that are relevant for the expression of σ^{54}-dependent genes involved in symbiotic nitrogen fixation (Fig. 2; see below). In vitro experiments with NtrC and σ^{54} RNA polymerase have shown that open-complex formation by phosphorylated NtrC requires ATP (21, 298, 411, 412). Typically, activator proteins bind to specific sites located around 100 bp upstream of the $-24/-12$ core promoter region (see references 223 and 378 and references therein). Interaction of the activator protein with the σ^{54} holoenzyme bound to the core promoter involves looping of the intervening DNA (370). This process can be facilitated by integration host factor (IHF) (174). On the basis of in vivo activation studies with mutant forms of RNA polymerase, it has been speculated that the activator may directly contact σ^{54} of the holoenzyme (228).

RpoN of rhizobia. The occurrence of $-24/-12$ consensus promoter sequences upstream of many *nif* and *fix* genes from various rhizobial species strongly suggested the presence of σ^{54}-like proteins in these organisms. In fact, *rpoN*-like genes have now been cloned and characterized from *R. meliloti* (319), *B. japonicum* (217), *A. caulinodans* (362), and *Rhizobium* sp. strain NGR234 (360, 391). The *rpoN* gene of *R. meliloti* is located on the chromosome and not on the Sym plasmid (173). Interestingly, two highly conserved *rpoN* genes (*rpoN*$_1$ and *rpoN*$_2$) are present in *B. japonicum*. *rpoN*$_1$ maps between the symbiotic clusters I and II, whereas *rpoN*$_2$ is located elsewhere on the *B. japonicum* chromosome at a distance of more than 1,000 kb away from *rpoN*$_1$ (219). Indirect genetic evidence implies that alternative *rpoN* genes also exist in *A. caulinodans* (see below) (362) and in the photosynthetic bacteria *Rhodobacter capsulatus* (72, 134) and *Rhodobacter sphaeroides* (253) in addition to the *rpoN* genes already identified in these organisms. An ORF (ORF1) located upstream of the *rpoN* genes of *R. meliloti*, *A. caulinodans*, and numerous other bacteria (for a compilation, see reference 362) encodes a protein which shares homology with a family of ATP-binding proteins involved in transport and other cellular functions (9). No relevant sequence information is available for the corresponding region upstream of the *rpoN* genes of *B. japonicum*. The function of the protein encoded by ORF1 is unknown, but at least in *R. meliloti* it may be essential for growth since attempts to isolate ORF1 mutants were unsuccessful (9). Homology between *rpoN* loci from different organisms is also observed with respect to the downstream regions (for compilations, see references 189 and 362). Mutations in two ORFs (ORF2 and ORF3) located downstream of *rpoN* in *K. pneumoniae* result in an enhanced expression from certain σ^{54}-dependent promoters (260). It is speculated that the products of ORF2 and ORF3 may modulate the activity of σ^{54}. Homologous ORFs are found downstream of *B. japonicum rpoN*$_2$ but not downstream of the *B. japonicum rpoN*$_1$ and *A. caulinodans rpoN* genes. In *R. meliloti*, only the 5′ portion of an ORF2-homologous region has been sequenced. Transposon insertions in ORF2 and ORF3 of *B. japonicum* do not affect symbiotic nitrogen fixation, but these mutants have not been analyzed further with regard to the activity of σ^{54}-dependent promoters (217). On the basis of sequence homology between the ORF3 product and a region spanning the active site of a protein (enzyme IIA) of the bacterial phosphotransferase system, it has been proposed that there may exist a regulatory link between carbon and nitrogen assimilation (307). However, in the absence of experimental evidence it is currently not possible to substantiate this idea.

TABLE 4. Phenotypic properties of *rpoN* mutants of *R. meliloti*, *B. japonicum*, and *A. caulinodans*

Phenotype	Organism[a]		
	R. meliloti	*B. japonicum*[b]	*A. caulinodans*
Nif[c]	NA[d]	ND[d]	–
nifH expression	–	–	–
nifA expression	ND	+[e]	+[f]
Fix[c]	–	–	–
Nod	+	+[g]	+[g]
Growth on N sources			
Ammonia	+	+	+
Nitrate	–	–	–
Arginine	+	+	±
Histidine	+	+	+
Proline	+	+	+
Growth on dicarboxylic acids			
Succinate	–	+	–
Fumarate	ND	+	–
Malate	ND	+	–
dctA expression	–	ND	ND

[a] References are as follows: *R. meliloti*, 319; *B. japonicum*, 217; *A. caulinodans*, 362.

[b] The phenotypic properties of a *B. japonicum rpoN*$_{1/2}$ double mutant are shown.

[c] Nif and Fix phenotypes refer to acetylene reduction activity of microaerobically and symbiotically grown cells, respectively.

[d] NA, not applicable; ND, not determined.

[e] Mutation of the *rpoN* genes does not affect aerobic *nifA* expression, whereas anaerobic induction is reduced by ca. 30%.

[f] Expression of *nifA* has been assayed under inducing (microaerobic, nitrogen-deficient) conditions. Aerobiosis and combined nitrogen repress *nifA* expression in the *rpoN* mutant strain similarly as observed in the wild type.

[g] The number of nodules is increased, and the nodules are smaller.

Consistent with the regulatory role of *rpoN*, the phenotypes of *rpoN* mutants of *R. meliloti*, *B. japonicum*, and *A. caulinodans* are highly pleiotropic (Table 4) (217, 319, 362). The analysis of *B. japonicum* strains carrying single *rpoN* mutations suggests that both genes are functional and that they can replace each other in nitrogen fixation and nitrate utilization, provided conditions that allow their expression have been met (see below). Common properties of *rpoN* mutants from *R. meliloti*, *B. japonicum*, and *A. caulinodans* include the lack of nitrogen fixation activity (Nif$^-$ Fix$^-$ phenotype; no expression of dependent *nif* genes) and the inability to utilize nitrate as the sole nitrogen source. The latter phenotype corresponds to that of *ntrC* mutants and is in agreement with the concept that RpoN and NtrC act in concert. In contrast to *rpoN* mutants of *K. pneumoniae*, the rhizobial mutants can assimilate ammonia by means of a σ^{54}-independent glutamine synthetase (216, 319, 362). Similarly, utilization of arginine, histidine, or proline as the nitrogen source is not affected by mutation of *rpoN*. Growth on dicarboxylic acids is prevented in *rpoN* mutants of *R. meliloti* and *A. caulinodans* but not in *B. japonicum* mutants. For *R. meliloti* and *R. leguminosarum* bv. viciae it is well documented that uptake of dicarboxylic acids which represent the major energy source for bacteroids requires the DctA carrier protein. Synthesis of this protein is induced in the presence of dicarboxylic acids via activation of the σ^{54}-dependent *dctA* promoter by the two-component regulatory pair

DctB and DctD (188, 226, 318, 400, 401). Accordingly, a *dctA'-'lacZ* fusion is not expressed in an *R. meliloti rpoN* mutant. A similar transport system may be operating in *A. caulinodans*. By contrast, two succinate transport systems have been characterized in *B. japonicum* strains (178), and since one of them appears to be expressed constitutively, it is likely that σ^{54} is not essential for dicarboxylic acid uptake in *B. japonicum* (332). It should be noted here that *dctA* regulation in *R. meliloti* and *R. leguminosarum* bv. viciae may be different under free-living and symbiotic conditions. In contrast to the free-living state, *dctA* expression is still observed in bacteroids of *dctBD* mutants, implying the existence of an alternative activating mechanism in symbiosis (188, 302, 317, 318).

As will be described in more detail further below, the absence of RpoN has little or no effect on *nifA* expression in *B. japonicum* and *A. caulinodans*, respectively. On the basis of this observation and preliminary hybridization data, it is postulated that *A. caulinodans* may contain at least one additional *rpoN*-like gene whose product (RpoN*) has a distinct specificity for the *nifA* promoter (Fig. 2C; also see Fig. 7C) (362). Expression of *R. meliloti nifA* has not been tested in an *rpoN* mutant background, but it is predicted that it is diminished because of the lack of readthrough transcription from the σ^{54}-dependent *fixABCX* promoter (see Fig. 7A).

In contrast to the function of their products, the *rpoN* genes of *B. japonicum* differ with respect to their regulation. Expression of $rpoN_2$ is not influenced by the cellular nitrogen or oxygen conditions; thus, it resembles the constitutively expressed *rpoN* genes of *E. coli* (68), *K. pneumoniae* (88, 263), and *R. meliloti* (319). Studies with *rpoN'-'lacZ* fusions in an $rpoN_2$ mutant background indicate a specific negative autoregulation of $rpoN_2$, but the mechanism and biological significance of this control are not known (217). Nevertheless, it is interesting that expression of *rpoN* in *Pseudomonas putida* may be autoregulated by a similar mechanism (210).

Expression of *B. japonicum* $rpoN_1$ is subject to oxygen control mediated by the FixLJ-$FixK_2$ cascade (Fig. 2B). Although regulation at the transcriptional level is well known for σ factors other than σ^{54} (e.g., σ^{32} of *E. coli* or σ factors required for sporulation of *Bacillus subtilis* [for reviews, see references 122 and 420]), there are only two other examples known for transcriptional control of a σ^{54}-encoding gene, namely *rpoN* (*nifR4*) of *Rhodobacter capsulatus*, which is subject to control by ammonia and oxygen (192), and the temporally controlled *rpoN* gene of *C. crescentus* (48). Interestingly, *nifR4* also resembles $rpoN_1$ of *B. japonicum* with respect to the absence of the conserved downstream ORFs (see above), and, most notably, it is transcriptionally linked to a *nifU*-like gene (*nifU2*) which allows *nifR4* to boost its low basal expression by positive autoregulation in a NifA-dependent manner (80, 300). On the basis of these observations and the suspected presence of alternative *rpoN* genes in *A. caulinodans* and *Rhodobacter sphaeroides*, it is speculated that these organisms may belong to a group of bacteria which harbor two *rpoN* genes, one which is constitutively expressed and required for housekeeping functions, and a second copy which is induced under specific physiological conditions that require increased expression of (specific?) σ^{54}-dependent genes, e.g., during nitrogen fixation (217). In the future it will be interesting to investigate whether the products of two *rpoN* genes from a given organism can have specific functions by discriminating different σ^{54}-dependent promoters as has been proposed for *A. caulinodans* (362). Apparently, this is not the case for the RpoN proteins of *B. japonicum* with regard to the phenotypes already tested, but they may have additional functions that are not known yet.

NifA

Identification of *nifA* genes. The role of NifA as transcriptional activator for *nif* gene expression was first described in *K. pneumoniae*, and the respective *nifA* gene was the first to be cloned and sequenced (50, 58, 110). Subsequently, it was used as a probe and prompted the identification of *nifA*-like genes in a large number of other diazotrophs. NifA-homologous proteins (VnfA, AnfA) are required for the regulation of alternative nitrogen fixation systems in *Azotobacter vinelandii* and *Rhodobacter capsulatus* (191, 201, 344). To date the DNA sequences have been reported of at least 15 *nifA* genes originating from 14 species including *K. pneumoniae*, *Klebsiella oxytoca* (206), *Azotobacter vinelandii* (34), *Azotobacter chroococcum* (124, 125), *Azospirillum brasilense* (233), *Azospirillum lipoferum* (350a), *Herbaspirillum seropedicae* (355), *R. leguminosarum* bv. viciae 3855 and PRE (149, 316), *R. leguminosarum* bv. trifolii (183), *R. leguminosarum* bv. phaseoli (263a), *R. meliloti* (58, 403), *A. caulinodans* (278, 304), *B. japonicum* (377), and *Rhodobacter capsulatus* (248).

In *R. meliloti*, the *nifA* locus and its regulatory function were originally identified by using Tn*5* insertion mutations which resulted in the absence of the *nifHDK* mRNA and the corresponding nitrogenase polypeptides (373, 422). The *nifA* genes of *B. japonicum* and *A. caulinodans* were identified from their homology to the *nifA* genes of *K. pneumoniae* and *R. meliloti*, and their regulatory function has been confirmed by subsequent mutational analysis (127, 296). Null mutations in the *nifA* genes of *R. meliloti*, *B. japonicum*, and *A. caulinodans* completely abolish symbiotic nitrogen fixation (Fix^- phenotype), and, similarly, no free-living nitrogenase activity is detectable in *nifA* mutants of *B. japonicum* and *A. caulinodans* (Nif^- phenotype). This reflects the common function of the NifA proteins as transcriptional activator of the structural genes for nitrogenase and genes encoding accessory functions. Interestingly, *K. pneumoniae nifA* cannot complement the Fix^- phenotype of *B. japonicum* and *R. meliloti nifA* mutants, although its product is able to activate the promoters of the nitrogenase structural genes of *R. meliloti* and *B. japonicum* in *E. coli* (11, 335, 339, 371). Although other explanations are possible, this finding may indicate some functional specificity of the rhizobial NifA proteins. Clearly, NifA in rhizobia is also involved in processes not directly related to nitrogen fixation (see below).

The *nifA* genes are located at different relative positions in the genomes of *R. meliloti*, *B. japonicum*, and *A. caulinodans* (Fig. 1; also see Fig. 7). In *R. meliloti*, the *nifA* gene is found in cluster I between the *fixABCX* operon and the *nifB* gene. The same genetic organization has also been described for other fast-growing rhizobia such as *R. leguminosarum* bv. viciae and bv. trifolii. In *B. japonicum*, *nifA* is the promoter-distal gene in the *fixRnifA* operon which is located in cluster II upstream of the *fixA* gene. Finally, in *A. caulinodans*, *nifA* is present in cluster I at a distance of ca. 8 kb upstream of the $nifH_1DKE$ operon.

Structure and function of NifA. The predicted sequences of all NifA proteins are roughly of similar lengths, varying between 519 (*R. leguminosarum* bv. viciae) and 626 (*Azospirillum brasilense*) amino acids, except that of *R. leguminosarum* bv. trifolii, which has only 353 amino acids. Sequence comparisons indicate that the sequences consist of different domains with variable conservation. This modular protein structure was proposed originally for NifA and NtrC of *K. pneumoniae* (110) and appears to be a common feature of many transcriptional activator proteins that regulate gene expression in concert with the σ^{54} RNA polymerase (for reviews, see references 272 and

286; also see below). The characteristic features of NifA proteins are outlined in Fig. 6, in which the structural and functional domains of representative NifA proteins from *R. meliloti*, *B. japonicum*, *A. caulinodans*, and *K. pneumoniae* are compared.

The N-terminal domains are of quite variable lengths (164 to 216 amino acids) and exhibit a rather low degree of amino acid sequence conservation (29 to 46% identity). So far no functional role could be attributed to this domain of the rhizobial NifA proteins. NifA is probably not a member of a two-component regulatory system. There is no evidence for NifA phosphorylation, and the conserved aspartic acid residue that is typically phosphorylated in response regulators by a cognate sensor kinase is missing in the N-terminal NifA domain. This agrees with the finding that this domain can be deleted without impairing the ability of Nif to activate *nif* target promoters (39, 129, 175). However, the effect of such deletions on nitrogen fixation in symbiosis is not known. Interestingly, the native NifA protein of *R. leguminosarum* bv. trifolii, as an exception, lacks the N-terminal domain entirely, which clearly indicates that it is dispensable, at least in this bacterium (183). Oxygen sensitivity, which is typical for the activity of the rhizobial NifA proteins (see below), is not affected by N-terminal deletions. NifA activity in *K. pneumoniae* is regulated by another regulatory protein, NifL, in response to the cellular oxygen and nitrogen conditions, and it is suggested that the N-terminal domain of the *K. pneumoniae* NifA protein may antagonize the inhibitory effect of NifL under derepressing conditions (111). Currently, there is no evidence for the presence of a NifL-like protein in rhizobia.

The N-terminal and central domains of NifA are separated by a short interdomain linker whose likely function is to tether two adjacent functional domains. Such linkers are commonly found in modular proteins of bacterial signal transduction systems and are named Q-linkers because of their striking content of glutamine residues and other hydrophilic amino acids (416). Deletions extending into the Q-linkers of NifA from *B. japonicum* or *R. meliloti* and insertion of four or eight amino acids into the Q-linker of *K. pneumoniae* NifA do not affect the activation function of the resulting mutant proteins (39, 129, 416).

The central domain of the NifA proteins consists of 240 amino acids and shows a high degree of sequence conservation along its entire length (pairwise comparisons of the NifA proteins from *R. meliloti*, *B. japonicum*, *A. caulinodans*, and *K. pneumoniae* reveal 53 to 72% identical amino acids). This domain is conserved not only in all NifA proteins but also in at least 13 additional transcriptional regulators from 20 different species (for a detailed compilation, see reference 272). Activation of gene expression by these proteins requires RNA polymerase containing the alternative sigma factor σ^{54}, and it is suggested that the conserved central domain is involved in the interaction with σ^{54} RNA polymerase (110, 223, 378). Other members of this protein family include, for example, the NtrC proteins from *K. pneumoniae* (58), *R. meliloti* (372), or *Bradyrhizobium* sp. (*Parasponia*) (282); the DctD proteins of *R. meliloti* (188) and *R. leguminosarum* bv. viciae (318); XylR from *P. putida* (184); HydG (365) and FhlA from *E. coli* (251, 341); and LevR and RocR from *Bacillus subtilis* (61, 208, 247). Deletion analysis and expression as separate domains have shown that the transcriptional activation function of the central domains of NifA from *K. pneumoniae* and *R. meliloti*, as well as that of DctD from *R. leguminosarum* bv. viciae, can be separated from the rest of the proteins, albeit with significantly reduced activation levels (175, 177, 269). Likewise, mutant proteins of *B. japonicum* NifA deleted for their C-terminal domain can weakly activate the *nifH* promoter of *R. meliloti* but not the *nifD* promoter of *B. japonicum* (339).

Similarly to NtrC, *K. pneumoniae* NifA has been shown to catalyze the isomerization of preexisting closed complexes to open forms (268). Recent in vitro experiments confirmed that open-complex formation by both complete *K. pneumoniae* NifA protein and the isolated central domain requires ATP or another nucleoside triphosphate (36, 227). Moreover, in vitro activity of the central domain is inhibited by NifL, and it is hypothesized that NifL interferes with protein-protein contacts between NifA and RNA polymerase (36). A putative binding site for ATP [GE(S/T)GTGKE (Fig. 6)], shown to be critical for *K. pneumoniae* NtrC and NifA activity, is almost perfectly conserved in the N-terminal part of the central domain from all NtrC- and NifA-like proteins (62, 318). It has been proposed that binding and hydrolysis of ATP is a common step during transcriptional activation by σ^{54}-dependent activators (223).

The C-terminal domain of the NifA proteins (DNA-binding domain) contains a highly conserved helix-turn-helix motif located within a segment of variable length (67, 78, and 86 residues in NifA of *R. meliloti*, *B. japonicum*, and *A. caulinodans*, respectively) and intermediate sequence similarity (41 to 56% identical amino acids in pairwise alignments). Remarkably, unlike the first helix, which shows a significant degree of conservation among all σ^{54}-dependent activators, the second helix of the DNA-binding motif (recognition helix) is specific for the NifA proteins. This is consistent with a NifA-specific DNA-binding site that differs from those of the other activators. In vivo methylation protection experiments demonstrated the interaction of *K. pneumoniae* and *B. japonicum* NifA with binding sites (see below) located in the 5′ regions upstream of NifA-regulated genes (267, 270). DNA binding of *K. pneumoniae* NifA is affected by mutations in the predicted helix-turn-helix motif and also by mutations in the DNA-binding site (269). Attempts to analyze DNA binding by intact NifA in vitro are hampered by the low solubility of overproduced NifA (22, 386; our unpublished results). However, in vitro binding was demonstrated by protection from DNase I digestion by using a purified fusion protein of the maltose-binding protein (MalE) with NifA of *K. pneumoniae* (MalE-NifA), which shows increased solubility over that of native NifA (227). Moreover, the C-terminal portion of *K. pneumoniae* NifA, including the DNA-binding domain, can be overproduced in a soluble form, and this peptide is sufficient to protect DNA from DNase I digestion in vitro (63, 227). These findings support a model that DNA binding of (*K. pneumoniae*) NifA can occur independently of the positive control function of the central domain.

The DNA-binding domain is linked directly to the central domain in the NifA proteins of *K. pneumoniae* and *A. vinelandii*, whereas these two domains are separated by a linker (interdomain linker) of 32 to 44 residues in all other NifA proteins (Fig. 6). The interdomain linker is characterized by two cysteine residues representing the distal residues of an absolutely invariant Cys-X_{11}-Cys-X_{19}-Cys-X_4-Cys motif that spans the boundary between the central domain and the interdomain linker in all NifA proteins except those of *K. pneumoniae* and *Azotobacter vinelandii*. In *B. japonicum*, individual replacement of the four cysteine residues by serine abolishes NifA activity (129). Moreover, the spacing between the two distal cysteine residues is critical but the identity of the intervening amino acids is not (130). Attempts to convert the *B. japonicum* NifA protein into a protein more closely resembling that of *K. pneumoniae* by deletion of the interdomain linker resulted in an inactive protein (our unpublished results). However, this result may not be meaningful, because the

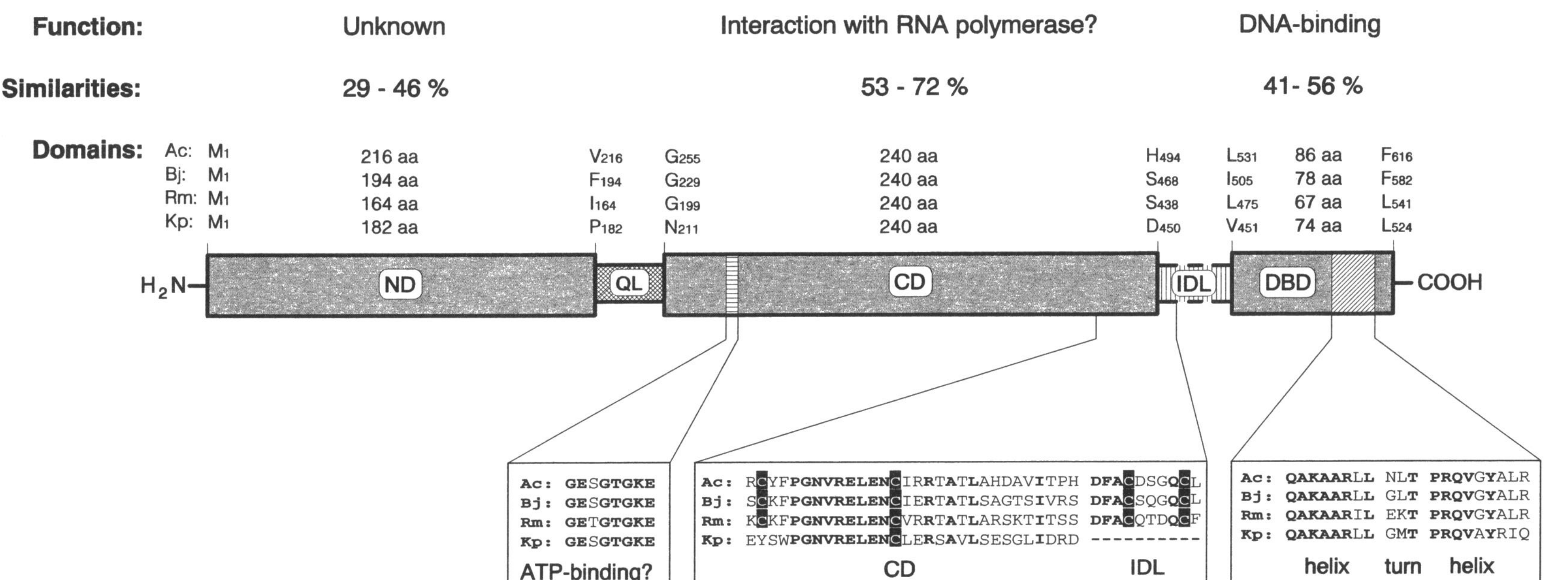


FIG. 6. Comparison of the structural and (proposed) functional domains of the NifA proteins from *A. caulinodans* (Ac), *B. japonicum* (Bj), *R. meliloti* (Rm), and *K. pneumoniae* (Kp). The amino acid (aa) sequences were aligned by using the Genetics Computer Group sequence analysis program PILEUP, and individual domains are indicated according to previous models (110, 129, 257, 272, 416). Common to all shown NifA proteins are the N-terminal domain of low sequence similarity (ND); a so-called Q-linker (QL) rich in glutamine, arginine, glutamate, serine, and proline; the highly conserved central domain (CD); and the C-terminal DNA-binding domain (DBD) containing a helix-turn-helix motif. The interdomain linker (IDL, 36 to 38 amino acids in length) is specific for the rhizobial NifA proteins. The similarity values indicated in the upper part of the figure refer to the percentage of identical amino acids found in pairwise alignments between individual domains of the NifA proteins. The amino acid sequences of characteristic motifs are shown in boxes in the lower part. Amino acids conserved in all aligned sequences are emphasized in boldface letters. Four conserved cysteine residues located at the end of the central domain and in the interdomain linker of all rhizobial NifA proteins are shown in reversed characters. For further details, see the text.

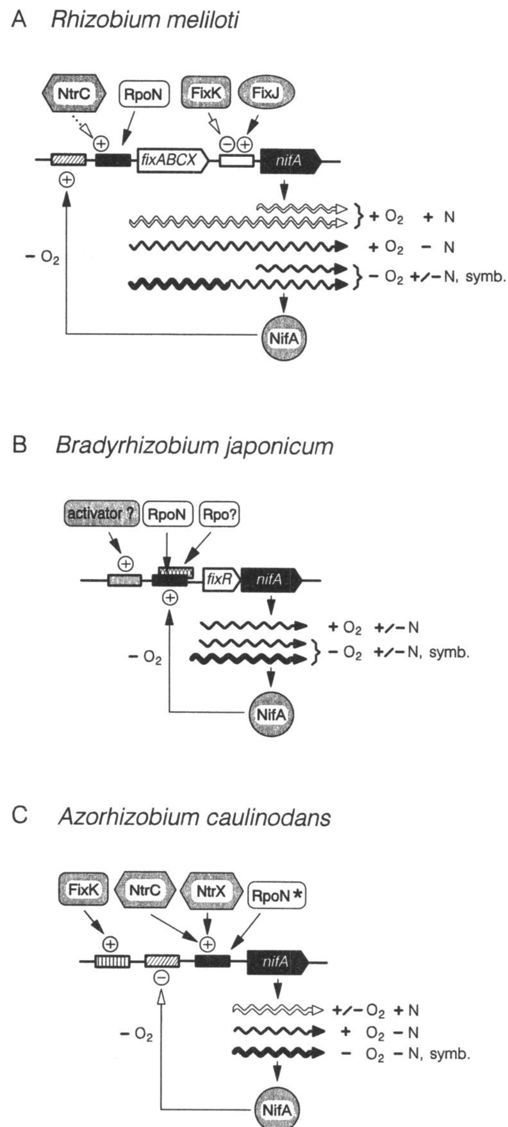


FIG. 7. Transcriptional organization and regulation of the *nifA* genes in *R. meliloti* (A), *B. japonicum* (B), and *A. caulinodans* (C). The schemes are not drawn to scale. Solid wavy lines of different thicknesses symbolize the size and relative abundance of (*fixABCX*) *nifA* transcripts present under the growth conditions described on the right, whereas open wavy lines refer to transcripts that are absent under the conditions indicated. Growth conditions are as follows: aerobic ($+O_2$), microaerobic or anaerobic ($-O_2$), nitrogen rich (+N), nitrogen limited (−N), and symbiotic (symb.); +/− means that the respective oxygen or nitrogen conditions are not relevant. Solid and open vertical arrowheads refer to the positive and negative control, respectively. The dotted line with the open arrowhead denotes the symbiotically irrelevant activation of the *R. meliloti fixABCX* promoter by NtrC under free-living conditions (see the legend to Fig. 2). Expression of *R. meliloti nifA* is additionally modulated by ammonia repression via an unknown mechanism involving FixL (not shown in panel A [283]). Similarly, expression of *A. caulinodans nifA* is additionally controlled by *nrfA* via a mechanism that is not yet understood in detail (not shown in panel C [197]). The open box marks the promoter region of *R. meliloti nifA* (p_{nifA}). Putative binding sites for NifA and FixK are indicated by diagonally and vertically hatched boxes, respectively. Solid boxes refer to −24/−12-type consensus promoters dependent on σ^{54} (RpoN) and the regulatory proteins NifA, NtrC, or NtrX. The proposed promoter which directs aerobic expression of *B. japonicum nifA* by means of an RNA polymerase containing an unidentified σ factor (Rpo?) and the binding site for a putative cognate activator protein are represented by a cross-hatched and a shaded box, respectively. RpoN* denotes a distinct σ^{54} factor which is postulated to be required for the specific recognition of the −24/−12-type promoter upstream of *nifA* in *A. caulinodans*. Key references describing the transcriptional regulation of *nifA* include references 2, 28, 82, 103, 203, and 396 for *R. meliloti*; 217, 271, 376, and 377 for *B. japonicum*; and 199, 278, 295, 304, and 362 for *A. caulinodans*. For additional references and further details, see the text.

remaining domains of this mutant form may fold incorrectly or may not be positioned correctly relative to each other.

The mechanism by which NifA activates transcription from σ^{54}-dependent target promoters has been elucidated predominantly with *K. pneumoniae* NifA, but it is very likely that this represents a universal model not only for NifA-mediated activation in rhizobia and other diazotrophs but generally also for other activators interacting with σ^{54}-RNA polymerase (for reviews, see references 223, 257, and 378). NifA binds to a conserved sequence motif, the upstream activator sequence (UAS) (5′-TGT-N_{10}-ACA-3′), which is present in most (but not all) NifA-dependent promoters at a distance of 80 to 150 nucleotides upstream of the transcriptional start site (10, 53, 55, 267, 270). The function of a UAS is probably to increase the local concentration of NifA in the vicinity of −24/−12 promoters and/or to correctly orientate NifA in order to facilitate a productive interaction with the σ^{54}-RNA polymerase complex (54). However, both NifA-mediated activation of promoters lacking a UAS and activity of mutant NifA proteins deleted for their DNA-binding domain indicate that NifA can also interact directly with the σ^{54}-RNA polymerase (51, 152, 175, 269). It was proposed that the number and location of UAS elements allow for an optimal fine-tuning of *nif* and *fix* gene expression (152).

Interaction of UAS-bound NifA with the σ^{54}-RNA polymerase complex involves loop formation of the DNA region between the UAS and the −24/−12 core promoter (54). As shown originally for the *K. pneumoniae nifH* promoter, this process is facilitated by DNA bending induced by IHF, which binds to a site between the UAS and the core promoter region (336). DNase I and hydroxyl radical protection experiments show that IHF binds to the promoter regions of NifA-dependent promoters from many nitrogen-fixing organisms including the *nifH* promoters from *R. meliloti* and *B. japonicum* (174). Accordingly, DNA sequence motifs resembling the IHF consensus binding site (5′-WATCAAN$_4$TTR-3′) (78, 231) are found in the regulatory regions of *nif* promoters from many organisms (174). Furthermore, IHF stimulates NifA-mediated in vitro transcription from the *nifH* and *nifD* promoters of *B. japonicum* and also from the *nifH* promoter of *R. meliloti* (337). Thus, it seems likely that IHF-like proteins play a regulatory role in the expression of NifA-dependent genes not only in enteric bacteria but also in rhizobia and other diazotrophs. In this context it will be of interest to investigate the effect on nitrogenase activity of null mutations in the recently identified *himA* and *hip* genes of *Rhodobacter capsulatus* which encode the α and β subunits of an IHF-like protein, respectively (383, 384). Furthermore, these genes could be helpful in the identification of homologous genes in related diazotrophs.

Control of NifA synthesis. Apart from their common structural and functional features, the NifA proteins of *R. meliloti*, *B. japonicum*, and *A. caulinodans* resemble each other in that all of them are regulated at the levels of both expression and activity, although the former control is brought about by different means.

(i) *R. meliloti*. In *R. meliloti*, transcription of *nifA* is controlled primarily by a FixJ-dependent promoter (p_{nifA}) situated between *fixABCX* and *nifA* (Fig. 7A). Comparatively little is known about the detailed structure of this promoter, which differs from σ^{54}-dependent promoters. Promoter activity is affected by point mutations introduced into the −39-to-−54 region relative to the transcriptional start site (2). However, attempts to functionally dissect the *nifA* promoter into a core promoter region and a binding site for FixJ have not been successful (2). DNA sequence comparison between the putative promoter regions of the *nifA* genes from *R. meliloti* and *R. leguminosarum* bv. trifolii suggests that an A+T-rich region between positions −30 and −40 may represent a critical promoter element (183).

Under free-living aerobic conditions, expression from p_{nifA} is very low (203). However, in bacteroids or in free-living cells growing under microaerobic conditions, FixJ-phosphate induces transcription at p_{nifA}, leading to the synthesis of NifA, which in turn activates transcription of *fixABCX* and other NifA-dependent genes or operons in concert with the σ^{54} RNA polymerase holoenzyme (Fig. 2A and 7A) (103). This induction is independent of the cellular nitrogen conditions. Transcription starting from the *fixABCX* promoter continues into *nifA* by readthrough, resulting in a further enhancement of *nifA* expression (58, 203). It is estimated that at least 50% of the transcripts starting at the *fixABCX* promoter include *nifA*. Results from complementation experiments indicate that readthrough transcription of *nifA* is not absolutely required for symbiotic nitrogen fixation (203, 326). In addition to positive (auto)regulation, *nifA* of *R. meliloti* is subject to negative regulation by FixK via an unknown mechanism (28). This mixed regulation, which is reminiscent of the regulation of *fixK*, prevents an unfavorable overexpression of *nifA*. Alternatively, *nifA* expression can be induced to some extent also in free-living, nitrogen-limited *R. meliloti* cells grown under aerobic or microaerobic conditions via activation of the *fixABCX* promoter by the *ntrC* gene product (103, 372). However, this regulatory pathway is probably not relevant during symbiosis since *ntrC* is not essential for symbiotic nitrogen fixation.

Recently, another component has been added to this complex regulatory network. By using β-galactosidase gene fusions, expression of *R. meliloti nifA* has been found to be negatively controlled by ammonia and nitrate (but not glutamate) under free-living, aerobic, and microaerobic conditions (283) (not shown in Fig. 2A and 7A). NtrC and FixK are not required for this type of control, and no effect of ammonia on *fixK* expression is observed; it is probably mediated through the FixL protein since no repression is observed in a *fixL* mutant background. However, it is not known by what means FixL is able to respond to ammonia or nitrate levels and whether transmission of the signal to the level of *nifA* expression involves FixJ or a separate pathway. The significance of this control during symbiosis is uncertain; it may be a mechanism to fine-tune *nif* gene expression in response to fluctuations of ammonia levels in nodules where *nif* gene expression is normally fully induced owing to the microaerobic environment.

(ii) *B. japonicum*. Regulation of *nifA* gene expression in *B. japonicum* is different but similarly complex. The *fixRnifA* operon is expressed at a significant basal level under aerobic conditions, and this expression requires a DNA element located between 50 and 86 nucleotides upstream of the transcriptional start site (Fig. 7B) (376, 377). Extracts of aerobically or anaerobically grown *B. japonicum* cells contain a protein that binds specifically to a 32-bp oligonucleotide spanning a critical adenine at position −66 of the *fixRnifA* promoter, and it is speculated that this protein functions as a positive regulator for the aerobic expression of this operon. Under microaerobic, anaerobic, or symbiotic conditions, expression of the *fixRnifA* operon is increased ca. fivefold by a process that requires NifA, but it is not known whether autoregulation occurs directly or indirectly. Notably, this type of regulation is detectable only when the *fixR* promoter is present on the chromosome but not when it is located on a plasmid (376). A similar effect, which may be caused by the copy number or differences in DNA topology, has been observed with the *B. japonicum fixA* and *fixB* promoters (152). The symbiotic phenotype of a *B. japonicum* mutant strain carrying a chromosomal deletion between positions −36 and −139 of the *fixRnifA* promoter region is identical to that of a *nifA* strain, suggesting a functional role of the putative upstream element in symbiosis (376). It cannot be excluded, however, that the 3′ end of this deletion also affects a core promoter element required for symbiotic expression of *nifA* (see below).

There is a well-conserved −24/−12-type promoter present upstream of the *fixRnifA* operon, which implies that expression of this operon depends on σ^{54}. Point mutations in the −12 region drastically reduce *fixRnifA* expression, but nucleotide exchanges in the −24 region interfere only marginally with aerobic promoter activity (377). Furthermore, in the absence of σ^{54}, aerobic *fixRnifA* expression is not altered, and expression is still elevated under anaerobic or symbiotic conditions, although to only ca. 60% of the level observed in the presence of σ^{54} (217). The absence of a well-conserved UAS for potential NifA binding could mean that in this case NifA activates the *fixRnifA* promoter directly or that autoregulation is indirect.

Transcript analyses performed in different backgrounds revealed the presence of two *fixRnifA* transcripts, one that is independent of NifA and σ^{54} and another that is 2 nucleotides longer, is most abundant under anaerobic or symbiotic conditions, and is dependent on NifA and σ^{54} (271). Both transcripts are present in anaerobically grown cells. These results are consistent with a model which predicts that expression of the *B. japonicum fixRnifA* operon is under dual control by two overlapping promoters recognized by different RNA polymerase holoenzymes (217, 271). Aerobic expression may depend on a σ factor different from σ^{54} (Rpo? in Fig. 7B) and on the postulated activator protein that binds to the upstream region around position −66. The same type of RNA polymerase also contributes to the expression under anaerobic conditions. It remains to be tested whether this σ factor corresponds to σ^{96} present in the RNA polymerase purified from free-living *B. japonicum* cells (305). Under microaerobic or symbiotic conditions, expression is achieved predominantly by the σ^{54} RNA polymerase, which interacts with the −24/−12-type promoter and whose activity is oxygen regulated via NifA (see below). Since this model does not readily explain why anaerobic induction of *fixRnifA* expression is NifA dependent but σ^{54} independent, it was proposed that *fixRnifA* expression may be kept at a basal level under aerobic conditions by a hypothetical repressor whose expression or activity is inhibited under anaerobic conditions by NifA (217). This hypothetical circuitry is not considered in Fig. 7B. However, such a double-negative control would eventually result in positive control and may thus contribute to the positive autoregulation of *nifA* under low-oxygen conditions. In this context it is noteworthy that Fnr of *E. coli* has been shown to mediate both positive and negative control depending on the location of its operator site (358).

In view of the oxygen sensitivity of *B. japonicum* NifA (129, 132), it seems futile to express *nifA* under conditions (aerobi-

osis) that are not compatible with the activator function of NifA. However, it cannot be excluded that *B. japonicum* affords a basal *nifA* expression to have NifA readily available when it is needed, i.e., during the switch from a free-living to a symbiotic lifestyle. Hypothetical activation of preformed, inactive NifA may involve the FixR protein. Both its similarity to NAD-dependent dehydrogenases and its coexpression with NifA would be in agreement with this presumptive function. Reactivation of NifA in *E. coli* has not been observed (218), but these experiments were done in the absence of FixR. It is also conceivable that FixR is involved in functions that are not related to NifA and that *fixRnifA* expression is regulated by physiological parameters other than oxygen. Such a type of control may be mediated by the putative regulatory protein required for aerobic *fixRnifA* expression, which implies that certain physiological conditions may exist under which an aerobic expression level of *fixRnifA* is lower than the values measured so far. Aerobic synthesis of an oxygen-sensitive NifA protein is not observed exclusively in *B. japonicum*. In *Azospirillum brasilense*, *nifA* is expressed from an unidentified promoter not only under conditions of nitrogen fixation but also in the presence of oxygen and ammonia (232, 233). Genetic evidence suggests the existence in *Azospirillum brasilense* of an as yet unidentified activator required for *nifA* expression similar to that in *B. japonicum*.

(iii) *A. caulinodans*. Expression of *nifA* in *A. caulinodans* is regulated in response to both the cellular nitrogen and oxygen concentrations under free-living and symbiotic conditions (Fig. 2C and 7C) (304, 362). The monocistronic *nifA* gene is preceded by a functional $-24/-12$-type promoter, as indicated by the loss of its activity upon mutation of highly conserved nucleotides at positions -25 and -13 (362). Nitrogen-rich conditions repress *nifA* expression completely, regardless of the oxygen status. In the presence of poor nitrogen sources (e.g., proline, arginine, or leucine), expression is induced to a low level under aerobiosis. Maximal *nifA* expression is observed under microaerobic conditions in the absence or presence of a poor nitrogen source. Nitrogen control of *nifA* expression is mediated by the combined action of two nitrogen-responsive systems, NtrBC and NtrYX, presumably via the $-24/-12$-type promoter (295, 296). It cannot be excluded that regulation by NtrBC works via NtrYX. On the basis of genetic data, it is postulated that transcription from the *nifA* promoter requires a distinct RpoN-like σ factor (RpoN*) since a mutation in a recently identified gene encoding a conventional σ^{54} protein has no effect on *nifA* expression (362). Oxygen control of *nifA* expression is brought about by the FixLJ-FixK cascade as described (Fig. 2C). An essential sequence motif in the 5′ *nifA* promoter region, which exhibits pronounced homology to the consensus binding site for *E. coli* Fnr, may constitute a binding site for FixK (Table 3; Fig. 7C). Both the nature of the corresponding RNA polymerase holoenzyme and the core promoter elements remain to be identified. It may turn out that two different RNA polymerase holoenzymes interacting with closely adjacent or overlapping promoters contribute to the overall expression of the *A. caulinodans nifA* gene similarly to the mechanism proposed for *B. japonicum fixRnifA* expression (see above). In addition, expression of *nifA* in *A. caulinodans* is negatively autoregulated. The mechanism of this control is not known, but it is speculated that it may involve a putative binding site (UAS) for the NifA protein located between the potential FixK-binding site and the $-24/-12$ core promoter (278, 304, 362). Finally, the DNA topology of the *nifA* promoter region may represent an additional regulatory factor, as deduced from the inhibitory effect of gyrase inhibitors under microaerobic conditions (304). A similar phenomenon has been reported previously for *nif* gene expression in *K. pneumoniae* and *Rhodobacter capsulatus* (100, 105, 214, 415).

Most recently, a novel *A. caulinodans* gene, *nfrA* (nitrogen regulation factor), which is required for expression of *nifA*, has been identified (197). Interestingly, the *nfrA* gene product is similar to the *hfq*-encoded HF-1 protein of *E. coli*, which is required for replication of bacteriophage Qβ RNA. HF-1 is a single-stranded RNA-binding protein and is able to complement an *A. caulinodans nrfA* mutant. This led to the hypothesis that NfrA may be required for *nifA* expression at the posttranscriptional level. Alternatively, NfrA may be involved in the expression of a factor essential for *nifA* transcription such as the postulated σ factor RpoN* (Fig. 7C). *nfrA*-like genes may also be present in *R. meliloti* and *B. japonicum*, as indicated by the presence of cross-hybridizing DNA fragments (197). Future work should elucidate the molecular basis of *nrfA*-mediated control in *A. caulinodans* and determine whether this new control level also plays a role in other diazotrophs.

Control of NifA activity. The structural difference between the NifA proteins of rhizobia and that of *K. pneumoniae* with respect to the presence or absence of the characteristic cysteine-rich motif (cf. Fig. 6) has an interesting functional counterpart. In the absence of its antagonist, NifL, the activity of NifA from *K. pneumoniae* is insensitive to oxygen both in vivo and in vitro (20, 50, 227, 262). By contrast, the in vivo activity of NifA from *R. meliloti*, *B. japonicum*, and *R. leguminosarum* bv. phaseoli is inhibited by high oxygen concentrations in the *E. coli* background, and the same observation has also been made for the homologous backgrounds with the two former proteins (39, 132, 159, 175). It is therefore tempting to predict that all NifA proteins of the rhizobial type (i.e., those containing the cysteine-rich motif) exhibit oxygen sensitivity. It remains to be investigated whether oxygen per se is required for NifA inactivation or whether positive redox potentials in general mediate this control, as has been proposed for *E. coli* Fnr (389).

On the basis of its similarity to (potential) metal-binding domains present in numerous regulatory proteins (35), the cysteine-rich motif of rhizobial NifA proteins has been proposed to be involved in the binding of a metal ion required for activity and redox sensing (129, 164). This idea is corroborated by the finding that the in vivo activity of the NifA proteins of *B. japonicum* and *R. meliloti*, but not that of *K. pneumoniae*, is sensitive to chelators such as *o*-phenanthroline or EDTA (129). Furthermore, the inhibitory effect of the chelators can be reversed by the addition of divalent metal ions, with Fe^{2+} showing the most pronounced restoration. As an attractive hypothesis, it follows that under low-oxygen conditions a metal ion in its reduced state (e.g., Fe^{2+}) may be coordinated by NifA via the cysteine motif, thereby properly positioning the central domain and the DNA-binding domain. Conversely, under aerobic conditions the metal ion may be oxidized, rendering NifA inactive (129). Alternatively, it is possible that the cellular oxygen conditions affect the NifA protein structure directly via the redox state of cysteine sulfhydryl groups which may participate in the formation of disulfide bridges. Finally, the switch from inactive NifA under aerobic conditions to active NifA under microaerobic conditions may involve a change in the oligomerization state similar to that has been proposed for *E. coli* Fnr (225). It appears as if parts of several of these models could be combined, as exemplified by MerR, which controls the transcription of genes conferring resistance to mercury ions in bacteria. Interestingly, the activator form of MerR is a homodimer in which the subunits are bridged by a mercury ion via three cysteine residues (see reference 164 and references therein).

In vivo DNA footprinting experiments in *E. coli* with *B. japonicum* NifA confirmed that aerobic conditions interfere with the positive control function of NifA as assessed by open-complex formation and its binding to the UAS (270). Similarly, exchange of one of the critical cysteines (Cys-472) for serine affects both open-complex formation and DNA binding. These studies also showed that inactive *B. japonicum* NifA is rapidly degraded in *E. coli* upon a shift from microaerobic to aerobic conditions. Furthermore, metal deprivation induced by chelators destabilizes NifA even under microaerobic conditions. Similarly, it has been reported that the NifA protein of *R. meliloti* is degraded in aerobically grown *E. coli* cells by a mechanism involving the Lon protease or the products of either of two uncharacterized *E. coli* loci, *snoB* and *snoC* (176). Mutations in *lon*, *snoB*, or *snoC* result in an increased aerobic activity of *R. meliloti* NifA. From these findings, it has been speculated that aerobic degradation may contribute to the posttranslational regulation of NifA activity. However, it is open whether degradation is the cause or the effect of NifA inactivation under aerobic conditions and whether similar mechanisms are operating in the rhizobial backgrounds.

In vitro mutagenesis of *R. meliloti nifA* and subsequent in vivo screening led to the isolation of oxygen-tolerant NifA mutant proteins which carry a defined Met-217–to–Ile exchange near the putative nucleotide-binding site within the central domain (cf. Fig. 6) (215). As an attractive model it has been proposed that this mutation locks the nucleotide-binding site in a conformation that allows binding or hydrolysis of ATP even under aerobic conditions whereas under the same conditions this interaction is prevented in the wild-type protein by a conformational change induced by oxidation of the postulated redox-sensing metal ion. However, in the light of a potential role for protein degradation in NifA regulation, it seems critical to compare the stability of the mutant protein with that of wild-type NifA, because it cannot be ruled out that enhanced resistance against degradation has contributed to the oxygen tolerance of the mutant protein.

Future progress toward answering the question of how the activity of rhizobial NifA proteins is regulated by oxygen will depend on the availability of purified NifA protein and an in vitro system to assay its activity under defined conditions. Unfortunately, overproduced *B. japonicum* NifA protein appears to be as poorly soluble as that of *K. pneumoniae*, and attempts to adapt an in vitro assay system originally developed for *K. pneumoniae* NifA have so far not been successful (22, 336, 386; our unpublished results). However, recent observations indicate that, unlike a previously studied glutathione *S*-transferase–NifA hybrid, a fusion of *B. japonicum* NifA to MalE shows a significantly increased solubility (23). These experiments had been prompted by similar observations made with *K. pneumoniae* NifA, which finally turned out to be amenable to in vitro studies as a MalE-NifA fusion protein (36, 222, 227, 229). Likewise, progress has been made recently with *E. coli* Fnr, which may serve as another model for the rhizobial NifA proteins. Biochemical studies revealed that purified Fnr may contain up to 1 mol of iron per monomer, and iron (though not anaerobiosis) is critical for in vitro activity (148, 348). Moreover, purified iron-deficient Fnr protein which is unable to activate transcription from a model promoter can be reactivated in vitro by preincubation with Fe^{2+} and β-mercaptoethanol (145). In the light of these advances, it seems promising to reenforce the attempts to study rhizobial NifA proteins in vitro.

NtrBC and NtrYX

The following discussion focuses on the (limited) role of the general nitrogen regulatory (*ntr*) system in nitrogen fixation by *R. meliloti*, *B. japonicum*, and *A. caulinodans*. The reader is referred to detailed reviews for a more general description of the *ntr* system and its role in fixation and assimilation of nitrogen (104, 256, 257, 306). Briefly, the cellular nitrogen status is sensed in *K. pneumoniae* by an uridylyltransferase (GlnD) which transmits the signal via the P_{II} protein (GlnB) to the sensor kinase NtrB. Under nitrogen-limited conditions, NtrB phosphorylates its cognate response regulator NtrC, which in turn activates transcription from the *nifLA* promoter and other σ^{54}-dependent promoters. Under conditions of nitrogen excess, transcriptional activation by NtrC is prevented via dephosphorylation by the phosphatase activity of NtrB, which is dominant under these conditions. Thus, in *K. pneumoniae*, the synthesis of NifA is regulated in response to the cellular nitrogen conditions via the level of phosphorylated NtrC which controls *nifLA* transcription. *K. pneumoniae ntrC* mutants show a characteristic pleiotropic *ntr* phenotype which includes glutamine auxotrophy, the inability to utilize nitrate or amino acids (e.g., histidine, proline, or arginine) as sole nitrogen sources, and the lack of nitrogen fixation.

Genes homologous to *K. pneumoniae ntrBC* have been identified in *R. meliloti* (372), *B. japonicum* (245), and *A. caulinodans* (296), as well as in other members of the *Rhizobiaceae* such as *Bradyrhizobium* sp. (*Parasponia*) (282, 387), *R. leguminosarum* bv. phaseoli (293), and *Agrobacterium tumefaciens* (324). In addition, an operon encoding a second regulatory pair, NtrYX, which is highly homologous to NtrBC, is present in *A. caulinodans* 4.5 kb downstream of the *ntrBC* genes (295). In contrast to NtrB, which is located in the cytoplasm, NtrY shows two putative transmembrane domains in its N-terminal end, suggesting that it may be involved in sensing the extracellular nitrogen concentration. Generally, the phenotypic properties of either *A. caulinodans ntrC* or *ntrX* mutants are somewhat leaky, possibly because of the presence of the alternative system substituting at least partially the mutated function. Also, it must be considered that distinct two-component regulators may interact by cross talk (281). Attempts to construct *ntrX ntrC* double mutants have apparently been unsuccessful (295).

R. meliloti and *B. japonicum ntrC* mutants are unable to grow on nitrate as the sole nitrogen source, and *A. caulinodans ntrX* or *ntrC* mutants show impaired growth with this substrate. Thus, *ntrC* (*ntrX*) may be required for the synthesis of assimilatory nitrate reductase in these bacteria, in a similar way to that demonstrated in *Azotobacter vinelandii* (382). Growth on amino acids as nitrogen sources is affected in *ntrC* mutants of *A. caulinodans* (and more severely in *ntrY ntrC* double mutants) but not in those of *R. meliloti* and *B. japonicum* (245, 295, 372). In *R. meliloti*, the synthesis of two enzymes involved in glutamine biosynthesis, glutamine synthetases II and III (GSII and GSIII, encoded by *glnII* and *glnT*, respectively), but not that of a third isoenzyme, glutamine synthetase I (GSI or GlnA, encoded by *glnA*), is controlled by NtrC (90). Similarly, expression of *glnII* but not that of *glnA* of *B. japonicum* is dependent on NtrC under aerobic conditions; however, under microaerobic or symbiotic conditions, NtrC can be replaced by NifA (67, 245, 246). On the other hand, *A. caulinodans* contains only one glutamine synthetase activity (GSI), whose expression is not affected by *ntrC* mutations (87, 295).

The functional role of the *ntr* system in *R. meliloti*, *B. japonicum*, and *A. caulinodans* differs most significantly with respect to symbiotic nitrogen fixation. Symbiotic properties of

TABLE 5. NifA-dependent genes or functions not directly related to nitrogen fixation

Organism	Target of regulation		Reference(s)
	Gene	Function or product(s)	
R. meliloti, B. japonicum, A. caulinodans	Unknown	Nodule development and bacteroid persistence[a]	87, 127, 171, 373, 422
R. meliloti	*nfe*	Nodulation competitiveness	354
R. meliloti	*mos*	Rhizopine synthesis	274
R. leguminosarum bv. phaseoli	*melA*	Melanin synthesis	159
B. japonicum	*groESL*$_3$	Chaperonins Cpn-10 and Cpn-60	128
B. japonicum	*ndp*	Unknown	406
B. japonicum	*glnII*	Glutamine synthetase	245

[a] In contrast to *R. meliloti* and *A. caulinodans*, these functions are affected specifically by mutations in *nifA* in *B. japonicum* but not by other mutations resulting in Fix$^-$ phenotypes (see the text).

R. meliloti and *B. japonicum* are not affected by mutations in *ntrC*, which is consistent with the NtrC-independent *nifA* expression under these conditions. As mentioned further above, the capacity of *R. meliloti* NtrC to activate transcription of *nifA* and *nifHDKE* under (free-living) nitrogen-limited conditions is physiologically probably not significant. In contrast, mutations in *ntrC* and/or *ntrX* of *A. caulinodans* severely impair both free-living and symbiotic nitrogen fixation, most probably via their effect on *nifA* expression. Moreover, nodule development is disturbed, although to different extents, in root and stem nodules. The last observation may reflect impaired proliferation of the infecting mutant bacteria as a result of a disturbed nitrogen metabolism (295).

In contrast the control of *nif* and *fix* gene expression in symbiosis, there is evidence that ammonia exerts a regulatory effect at the level of *nod* gene expression in *R. meliloti* and *B. japonicum*. According to a recently proposed model for *R. meliloti*, the nitrogen conditions sensed by the *ntr* system affect the expression of the nodulation regulatory genes *nodD*$_3$ and *syrM* by a mechanism involving NtrC and a repressor protein, NtrR (112, 113). However, ammonia control of *nod* gene expression in *B. japonicum* is likely to follow a different route since it is independent of NtrC (399).

REGULATION BY FixLJ-FixK AND NifA IS NOT RESTRICTED TO NITROGEN FIXATION GENES

Several lines of evidence accumulated over the last few years indicate that the roles of FixLJ-FixK and NifA in rhizobia are not limited to the regulation of the nitrogenase and accessory genes but include additional functions which are more generally related to the symbiosis.

FixLJ-FixK-Regulated Functions

Although the role of the FixLJ-FixK cascade of *R. meliloti*, *B. japonicum*, and *A. caulinodans* differs substantially with respect to the regulation of the central nitrogen fixation genes, the cascade is uniformly involved in the induction of the *fixNOQP* genes under microaerobic conditions in these species (Fig. 2). The *fixNOQP* operon codes for a cytochrome oxidase which is apparently required for respiration by oxygen-limited, free-living rhizobia or bacteroids living under microaerobic conditions in nodules. Moreover, mutational analyses indicate that the FixLJ-FixK$_2$ cascade of *B. japonicum* also controls anaerobic nitrate respiration. This could mean that FixK$_2$ of *B. japonicum* plays a similar role to that of Fnr of *E. coli* with regard to control of genes for nitrate respiration. Microaerobically grown *fixLJ* mutants of *B. japonicum* are further characterized by the lack of several *c*-type cytochromes, which is in perfect agreement with their respiratory defects (12). Thus, it appears as if, at least in *B. japonicum*, the FixLJ-FixK$_2$ cascade is concerned primarily with the regulation of anaerobic or microaerobic respiration, the latter being indirectly related to symbiotic nitrogen fixation. It may well be possible that there are additional cellular functions which are oxygen regulated by the FixLJ-FixK cascade. In *B. japonicum*, such hypothetical control functions may also include FixK$_1$, as has already been discussed (Fig. 2B).

NifA-Regulated Functions

Table 5 shows a list of rhizobial genes or functions which are not directly involved in nitrogen fixation even though they are under the control of NifA. Their discussion is the topic of the following paragraphs.

Nodule development and bacteroid persistence. In addition to their inability to fix nitrogen, *nifA* mutant strains of *R. meliloti*, *B. japonicum*, and *A. caulinodans* are characterized by the induction of nodules that show an altered morphology and ultrastructure (87, 127, 171, 373, 422). *nifA* mutants from all three species induce an increased number of small nodules containing fewer bacteroids which degenerate prematurely, compared with wild-type strains. This phenotype is most pronounced for *nifA* mutants of *B. japonicum* and, in contrast to *R. meliloti* and *A. caulinodans*, is not observed to such a drastic extent with other Fix$^-$ mutants of *B. japonicum* (e.g., *nifH* or *nifDK* mutants); i.e., the effect is not an indirect consequence of nitrogen starvation. Interestingly, wild-type nodulation is largely restored in *B. japonicum nifA* mutants by heterologous complementation with *nifA* from *K. pneumoniae*, yet no nitrogen fixation activity is detectable in these nodules (339). Leghemoglobin mRNA and protein are present only at very low levels in soybean nodules elicited by a *B. japonicum nifA* mutant, whereas other nodule-specific proteins (nodulins) appear to be expressed normally (369). These nodules are dark brown to black, and electron-microscopic studies reveal disintegrated bacteroids and peribacteroid membranes. Furthermore, a striking accumulation of the phytoalexin glyceollin I is detected in parallel with localized death of infected cells (291). These features are reminiscent of a hypersensitive response normally observed in incompatible plant-pathogen interactions (207). Thus, it appears as if *B. japonicum nifA* controls functions that help the bacterial symbiont to suppress to overcome potential plant defense reactions and to persist as a nitrogen-fixing bacteroid. This idea is consistent with earlier concepts that describe the *Rhizobium*-plant symbiosis as a controlled disease (106, 390). In fact, more recent analyses at the molecular level demonstrate that rhizobia induce the expression of two enzymes of the plant phenylpropanoid biosynthetic pathway, phenylalanine ammonia-lyase and chalcone synthase, which play key roles in the plant defense

response (123, 151). Moreover, the pattern of induction differs between wild-type rhizobia and ineffective mutants. Exopolysaccharides seem to play a critical role in the suppression of a defense reaction by plants forming indeterminate nodules. Evidence for this comes from the observation that both an exopolysaccharide-overproducing mutant of *Rhizobium* sp. strain NGR234 and an exopolysaccharide-deficient mutant of *R. meliloti* elicit typical plant defense responses on their hosts during the induction of pseudonodules (107, 280). By contrast, however, exopolysaccharides are apparently not essential for the infection of plants forming determinate nodules, such as soybean (see reference 292 and references therein). Future work will be challenged by the characterization of the rhizobial signal(s) whose presence or absence provokes the plant defense response. More specifically, it will be interesting to find the means by which *B. japonicum* NifA contributes to the generation of this hypothetical signal.

R. meliloti nfe. Some *R. meliloti* strains contain one or more cryptic plasmids in addition to the large megaplasmids harboring the symbiotic genes. In *R. meliloti* GR4, one of these plasmids (pRmeGR4b) has been reported to be involved in nodulation efficiency and competitiveness (380). The relevant locus has been identified and characterized by mutation, DNA sequence determination, and transcriptional analysis (333, 334, 354). It contains two contiguous genes, nfe_1 and nfe_2 (nodule formation efficiency), which encode proteins of predicted molecular masses of 16 and 34 kDa, respectively. Both genes are preceded by a functional σ^{54}- and NifA-dependent promoter, and, in addition, nfe_1 is transcribed from a NifA-independent promoter. Accordingly, nfe_1 is transcribed at basal level in free-living cells and expression of both nfe_1 and nfe_2 is probably activated during infection and nodule development by the switch to microaerobic conditions which induce NifA synthesis. Surprisingly, the region encoding nfe_1 is homologous to the 5′ region of *R. meliloti nifH* and to the noncoding region upstream of *fixABCX*, and it has been proposed that this structure may originate from a transposition event. By contrast, the predicted Nfe_2 protein shows no obvious similarity to any known protein. Thus, although the role of the *nfe* genes in nodulation remains unknown, they show that NifA can have a function in very early stages of the symbiosis, i.e., when nitrogen fixation has not yet started. This is consistent with the reduced competitiveness of a *R. meliloti nifA* mutant (335).

R. meliloti mos. Rhizopines are opine-like compounds that are synthesized in nodules by certain rhizobial strains. The same strains also have the distinct ability to catabolize these compounds (275). Genetic analysis of two rhizopine-producing strains of *R. meliloti* has revealed that the genes required for synthesis (*mos*) and catabolism (*moc*) of the respective rhizopines are closely linked and located on the megaplasmid pSym-a (273, 328). Expression of the *mos* genes but not of the *moc* genes is controlled by NifA. The 5′ region of the first ORF of the *mos* locus is highly homologous to DNA sequences from the *nifH* and *fixA* regions of *R. meliloti*, including two well-conserved σ^{54}-dependent promoters and NifA-binding sites (274, 276). Thus, similar to the *nfe* locus, the *mos* genes became subject to symbiotic control, probably as a consequence of DNA rearrangements involving the *nifH* promoter region. Although isogenic mos^+ and *mos* strains show no differences with respect to nodulation and symbiotic nitrogen fixation, the ability to synthesize and catabolize specific compounds may offer an advantage to the proficient strains with regard to persistence in the rhizosphere. Bacteroids in root nodules transform plant-derived photosynthates into rhizopines, which are then released by senescing nodules and used by its free-living relatives as a selective growth substrate (275).

R. leguminosarum* bv. phaseoli *melA. Melanin production is a common property of numerous strains of *R. leguminosarum* bv. viciae, bv. trifolii, and bv. phaseoli and also of *R. meliloti* but has not been found in *Bradyrhizobium* strains (79). Activity of the polyphenol oxidase tyrosinase, which catalyzes a step of melanin synthesis, can be detected in colonies of *R. leguminosarum* bv. phaseoli and in symbiotically grown cells. Genetic analysis indicates that melanin synthesis by *R. leguminosarum* bv. phaseoli, which is not essential for symbiotic nitrogen fixation, involves at least three distinct loci (43, 160). Two of them are located on the symbiotic megaplasmid and have been identified as the tyrosinase structural gene, *melA*, and *nifA*, which is required for *melA* expression (159). The third locus, *melC*, may correspond to *rpoN* as inferred from the pleiotropic phenotype of *melC* mutants (160). It is likely that NifA directly controls *melA* expression since a *melA′-′lacZ* fusion can be activated in *E. coli* by *K. pneumoniae* NifA in a σ^{54}-dependent manner (159). By contrast, melanin production by *R. meliloti* GR4 is NifA and RpoN independent (255). The biological significance of melanin production is uncertain. On the basis of its coregulation with symbiotic genes, it is speculated that tyrosinase might contribute to the detoxification of plant phenolic compounds during infection and nodule senescence (159).

***B. japonicum* $groESL_3$.** During searches for new *nifA*-dependent genes of *B. japonicum*, a multigene family which consists of five highly conserved *groESL* operons encoding the bacterial chaperonins Cpn-10 (GroES) and Cpn-60 (GroEL) has been discovered (128). Chaperonins represent a subgroup of ubiquitous molecular chaperones which prevent aggregation of newly synthesized proteins and assist their proper folding (for recent reviews, see references 33, 156, and 162). Unlike in other organisms studied so far, one of the five *groESL* operons of *B. japonicum*, $groESL_3$, is transcribed from a σ^{54}-dependent promoter under the control of NifA. Hence, the synthesis of the $GroESL_3$ chaperonins is specifically induced during symbiosis or in free-living bacteria growing under low-oxygen conditions. Although deletion of $groEL_3$ has no effect on the symbiotic properties of *B. japonicum*, a mutant with a Fix^- phenotype is obtained when the level of chaperonin synthesis is further affected by additional deletion of $groEL_4$, a homolog that is normally expressed in the wild type under all growth conditions tested (23). A specific chaperonin requirement for nitrogen fixation is consistent with genetic and biochemical data suggesting that both the folding of *K. pneumoniae* NifA and the assembly of newly synthesized nitrogenase proteins are stimulated by *E. coli* GroEL (143a, 144). A *groESL* multigene family is also found in *R. leguminosarum* bv. viciae and *R. meliloti* (325, 398). None of the multiple *groESL* operons in these bacteria appears to be controlled by NifA, yet one of the *R. meliloti* GroEL proteins is required for full activity of the $NodD_3$ protein, a transcriptional activator of nodulation genes (133). Thus, posttranslational control by chaperonins may represent another regulatory level in (symbiotic) nitrogen fixation. In the light of these findings, it appears sensible for *B. japonicum* to balance the ratio between Nif proteins and chaperonins by coregulation of the respective genes. This principle is reminiscent of the boost of σ^{54} synthesis by induction of $rpoN_1$ under microaerobic or symbiotic conditions (Fig. 2B). Moreover, $rpoN_1$ or $groESL_3$ alone is apparently not essential under the conditions applied in a laboratory plant infection test, but the function of these genes may be advantageous under the competitive conditions found in the soil.

B. japonicum ndp. A functional *B. japonicum* NifA-depen-

dent promoter (*ndp*) has been cloned in the course of a search for new NifA-dependent genes by using a promoter-probe vector system (406). If a functional gene is associated with *ndp*, however, it is not essential for symbiosis, as inferred from the wild-type phenotype of a *ndp* deletion mutant. Since no detectable phenotype could be attributed to this region, it may encode a functionally redundant gene or represent part of a pseudogene.

B. japonicum glnII. As mentioned above, *B. japonicum* has two genes encoding glutamine synthetase (*glnA* and *glnII*). Expression of *glnA* appears to be constitutive, whereas *glnII* is under dual control (66, 67, 245, 246). In free-living cells, *glnII* expression is activated by NtrC under aerobic, nitrogen-limiting conditions. By contrast, *glnII* expression is dependent on NifA in microaerobic, free-living cells or in bacteroids in which the presence of combined nitrogen prevents NtrC activity. Thus, this dual regulation allows *B. japonicum* to maintain a continuous level of *glnII* expression during the transition from the free-living state in the soil to the symbiotic state as a bacteroid. However, the necessity for this type of control is not obvious, because *glnII* is dispensable for symbiosis (67). Hence, *glnII* is another example of a rhizobial gene whose expression is coregulated with nitrogen fixation genes via NifA but which is apparently not essential for symbiosis.

CONCLUSIONS AND PERSPECTIVES

In this review I have attempted to provide an overview of the genetic components and principles which mediate the regulation of nitrogen fixation in three rhizobial model organisms. Evidently, control of the central nitrogen fixation genes (i.e., the nitrogenase and accessory genes) is brought about by the same means as in the free-living diazotrophic paradigm, *K. pneumoniae*. These include a specialized promoter type, the $-24/-12$ promoter, RNA polymerase containing a unique σ factor (σ^{54}), and an activator protein (NifA). In fact, the identification of $-24/-12$ promoters in rhizobia has contributed to the recognition of the fundamental significance of this promoter type, which has since been encountered in the context of numerous bacterial functions.

In contrast to this relative uniformity at the level of expression of σ^{54}-dependent, NifA-regulated *nif* and *fix* genes, free-living and symbiotic diazotrophs differ with respect to the regulatory effects of physiological conditions and/or the mechanisms by which these signals are transmitted to the level of *nif* and *fix* gene expression. All diazotrophs prevent the futile synthesis of the nitrogenase complex under aerobic conditions. To this end, both free-living and symbiotic diazotrophs regulate synthesis and activity of NifA, albeit by different means. In the rhizobial species discussed in this article aerobiosis directly interferes with NifA activity, whereas in *K. pneumoniae* an additional protein, NifL, is required for this control. At the same time, NifL regulates the activity of *K. pneumoniae* NifA in response to the nitrogen conditions, a control mechanism that is not found in rhizobia. This difference may explain the dissimilar strategies for controlling NifA activity.

Maximal divergence is found with respect to the regulation of *nifA* transcription among the three rhizobial diazotrophs compared in this review. Nitrogen control is absent in *B. japonicum* and probably not relevant in *R. meliloti*, but it plays a significant role in *A. caulinodans*, similar to its role in *K. pneumoniae*. Thus, the intermediate physiological position of *A. caulinodans* between being either a free-living or a symbiotic diazotroph is reflected at the level of *nifA* expression. Interestingly, the conventional nitrogen regulatory system NtrBC is accompanied by a novel two-component regulatory system, NtrYX, whose precise role has yet to be defined.

A variety of mechanisms have evolved to regulate *nifA* transcription with respect to the cellular oxygen conditions. In *R. meliloti* and *A. caulinodans* this control involves the FixLJ two-component regulatory system, whereas in *B. japonicum nifA* expression is stimulated under low-oxygen conditions by autoactivation. As a consequence, in *R. meliloti* and *A. caulinodans* oxygen control of nitrogen fixation genes is exerted at two levels (FixLJ and NifA) whereas in *B. japonicum* it is limited to one (NifA). In this light, aerobic expression of *B. japonicum nifA* may appear meaningful. Analogous to FixLJ in *R. meliloti* and *A. caulinodans*, a basal level of NifA synthesis is permanently required in *B. japonicum* to monitor the prevailing oxygen concentration and to eventually induce gene expression under appropriate low-oxygen conditions. However, considering that the FixLJ system is present also in *B. japonicum*, it is not evident why this organism has evolved a distinct type of *nifA* regulation.

The *cis* elements required for transcription and regulation of *nifA* are rather poorly defined in all three systems described here. Their further characterization is required not only in view of the fundamental role of *nifA* but also with regard to a better understanding of the structure, function, and control of rhizobial promoters other than those dependent on σ^{54}. Expression of *nifA* in *B. japonicum* appears to originate from two largely independent promoters, and the one which is σ^{54} independent, as well as the predicted activator protein, is essentially unknown. Similarly, the *nifA* promoters of *R. meliloti* and *A. caulinodans*, which are positively regulated by FixJ and FixK, respectively, await further dissection and functional analysis. The recent availability of an in vitro system will undoubtedly stimulate future analysis of the *cis* elements involved in transcriptional regulation of *R. meliloti nifA*. Ideally, similar systems should be developed for *B. japonicum* and *A. caulinodans*. These studies will also contribute to a better characterization of the rhizobial RNA polymerase holoenzyme involved in expression of genes that are not dependent on σ^{54}.

The identification of FixLJ and NifA in rhizobia has added two more members to a growing list of bacterial regulatory systems concerned with the control of gene expression in response to the cellular oxygen conditions. Alternative systems include Fnr of *E. coli* and homologous proteins of other bacteria (Table 2), as well as ArcBA (185), OxyR (367) and SoxRS of *E. coli* (92, 93); NifLA of *K. pneumoniae*; RegBA of *Rhodobacter capsulatus* (32, 346); and PrrA of *Rhodobacter sphaeroides* (121). Signal transduction is increasingly well understood at the molecular level in the OxyR, SoxRS, and FixLJ systems, particularly owing to the availability of reconstituted in vitro systems (3, 168, 312, 366). Recent progress achieved with respect to the design of in vitro systems for *E. coli* Fnr and *K. pneumoniae* NifA should stimulate analogous efforts concerning the analysis of rhizobial NifA proteins. It seems likely that future research on rhizobial nitrogen fixation will continue to contribute to an understanding of fundamental aspects of contemporary biology such as signal transduction and control of gene expression.

ACKNOWLEDGMENTS

I am most grateful to Professor Hennecke for generous support and valuable help during the preparation of the manuscript. I thank Michael Ibba for critical reading of the manuscript and helpful suggestions, and I thank all past and present coworkers from the laboratory for their contributions to the work cited in this article. I also thank C. Elmerich, A. Kaminski, U. Priefer, and M. Gilles-Gonzalez, who provided manuscripts or unpublished results.

REFERENCES

1. **Adams, T. H., and B. K. Chelm.** 1984. The *nifH* and *nifDK* promoter regions from *Rhizobium japonicum* share structural homologies with each other and with nitrogen-regulated promoters from other organisms. J. Mol. Appl. Genet. **2:**392–405.
2. **Agron, P. G., G. S. Ditta, and D. R. Helinski.** 1992. Mutational analysis of the *Rhizobium meliloti nifA* promoter. J. Bacteriol. **174:**4120–4129.
3. **Agron, P. G., G. S. Ditta, and D. R. Helinski.** 1993. Oxygen regulation of *nifA* transcription *in vitro*. Proc. Natl. Acad. Sci. USA **90:**3506–3510.
4. **Aguilar, O. M., D. Kapp, and A. Pühler.** 1985. Characterization of a *Rhizobium meliloti* fixation gene (*fixF*) located near the common nodulation region. J. Bacteriol. **164:**245–254.
5. **Aguilar, O. M., H. Reiländer, W. Arnold, and A. Pühler.** 1987. *Rhizobium meliloti nifN* (*fixF*) gene is part of an operon regulated by a *nifA*-dependent promoter and codes for a polypeptide homologous to the *nifK* gene product. J. Bacteriol. **169:**5393–5400.
6. **Aguilar, O. M., J. Taormino, B. Thöny, T. Ramseier, H. Hennecke, and A. A. Szalay.** 1990. The *nifEN* genes participating in FeMo cofactor biosynthesis and genes encoding dinitrogenase are part of the same operon in *Bradyrhizobium* species. Mol. Gen. Genet. **224:**413–420.
7. **Aiba, H., S. Fujimoto, and N. Ozaki.** 1982. Molecular cloning and nucleotide sequencing of the gene for *E. coli* cAMP receptor protein. Nucleic Acids Res. **10:**1345–1361.
8. **Albright, L. M., E. Huala, and F. M. Ausubel.** 1989. Prokaryotic signal transduction mediated by sensor and regulator protein pairs. Annu. Rev. Genet. **23:**311–336.
9. **Albright, L. M., C. W. Ronson, B. T. Nixon, and F. M. Ausubel.** 1989. Identification of a gene linked to *Rhizobium meliloti ntrA* whose product is homologous to a family of ATP-binding proteins. J. Bacteriol. **171:**1932–1941.
10. **Alvarez-Morales, A., M. Betancourt-Alvarez, K. Kaluza, and H. Hennecke.** 1986. Activation of the *Bradyrhizobium japonicum nifH* and *nifDK* operons is dependent on promoter-upstream DNA sequences. Nucleic Acids Res. **14:**4207–4227.
11. **Alvarez-Morales, A., and H. Hennecke.** 1985. Expression of *Rhizobium japonicum nifH* and *nifDK* operons can be activated by the *Klebsiella pneumoniae nifA* protein but not by the product of *ntrC*. Mol. Gen. Genet. **199:**306–314.
12. **Anthamatten, D.** 1992. Untersuchungen zur Regulation der symbiontischen N_2-Fixierung in *Bradyrhizobium japonicum*: Entdeckung und Charakterisierung der *fixJ*-, *fixK*- und *fixN*-Genregionen. Ph.D. dissertation ETH 9819. Eidgenössische Technische Hochschule, Zürich.
13. **Anthamatten, D., and H. Hennecke.** 1991. The regulatory status of the *fixL*-like and *fixJ*-like genes in *Bradyrhizobium japonicum* may be different from that in *Rhizobium meliloti*. Mol. Gen. Genet. **225:**38–48.
14. **Anthamatten, D., P. Rossi, M. Babst, H. M. Fischer, and H. Hennecke.** Unpublished data.
15. **Anthamatten, D., B. Scherb, and H. Hennecke.** 1992. Characterization of a *fixLJ*-regulated *Bradyrhizobium japonicum* gene sharing similarity with the *Escherichia coli fnr* and *Rhizobium meliloti fixK* genes. J. Bacteriol. **174:**2111–2120.
16. **Appleby, C. A., P. James, and H. Hennecke.** 1991. Characterization of three soluble *c*-type cytochromes isolated from soybean root nodule bacteroids of *Bradyrhizobium japonicum* strain CC705. FEMS Microbiol. Lett. **83:**137–144.
17. **Arico, B., J. F. Miller, C. Roy, S. Stibitz, D. Monack, S. Falkow, R. Gross, and R. Rappuoli.** 1989. Sequences required for expression of *Bordetella pertussis* virulence factors share homology with prokaryotic signal transduction proteins. Proc. Natl. Acad. Sci. USA **86:**6671–6675.
18. **Arigoni, F., P. A. Kaminski, H. Hennecke, and C. Elmerich.** 1991. Nucleotide sequence of the *fixABC* region of *Azorhizobium caulinodans* ORS571: similarity of the *fixB* product with eukaryotic flavoproteins, characterization of *fixX*, and identification of *nifW*. Mol. Gen. Genet. **225:**514–520.
19. **Arnold, W., A. Rump, W. Klipp, U. B. Priefer, and A. Pühler.** 1988. Nucleotide sequence of a 24,206-base-pair DNA fragment carrying the entire nitrogen fixation gene cluster of *Klebsiella pneumoniae*. J. Mol. Biol. **203:**715–738.
20. **Arnott, M., C. Sidoti, S. Hill, and M. J. Merrick.** 1989. Deletion analysis of the nitrogen fixation regulatory gene *nifL* of *Klebsiella pneumoniae*. Arch. Microbiol. **151:**180–182.
21. **Austin, S., and R. Dixon.** 1992. The prokaryotic enhancer binding protein NTRC has an ATPase activity which is phosphorylation and DNA dependent. EMBO J. **11:**2219–2228.
22. **Austin, S., N. Henderson, and R. Dixon.** 1990. Characterisation of the *Klebsiella pneumoniae* nitrogen-fixation regulatory proteins NIFA and NIFL *in vitro*. Eur. J. Biochem. **187:**353–360.
23. **Babst, M., and H. M. Fischer.** Unpublished data.
24. **Baker, M. E.** 1992. Similarities between legume-rhizobium communication and steroid-mediated intercellular communication in vertebrates. Can. J. Microbiol. **38:**541–547.
25. **Banfalvi, Z., V. Sakanyan, C. Koncz, A. Kiss, I. Dusha, and A. Kondorosi.** 1981. Location of nodulation and nitrogen fixation genes on a high molecular weight plasmid of *R. meliloti*. Mol. Gen. Genet. **184:**318–325.
26. **Bannan, J. D., M. J. Moran, J. I. Macinnes, G. A. Soltes, and R. L. Friedman.** 1993. Cloning and characterization of *btr*, a *Bordetella pertussis* gene encoding an FNR-like transcriptional regulator. J. Bacteriol. **175:**7228–7235.
27. **Barbour, W. M., S.-P. Wang, and G. Stacey.** 1992. Molecular genetics of *Bradyrhizobium* symbioses, p. 648–684. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
28. **Batut, J., M.-L. Daveran-Mingot, M. David, J. Jacobs, A. M. Garnerone, and D. Kahn.** 1989. *fixK*, a gene homologous with *fnr* and *crp* from *Escherichia coli*, regulates nitrogen fixation genes both positively and negatively in *Rhizobium meliloti*. EMBO J. **8:**1279–1286.
29. **Batut, J., P. de Philip, J. M. Reyrat, F. Waelkens, and P. Boistard.** 1993. Oxygen regulation of nitrogen fixation gene expression in *Rhizobium meliloti*, p. 193–197. *In* E. W. Nester and D. P. S. Verma (ed.), Advances in molecular genetics of plant-microbe interactions. Kluwer Academic Publishers, Dordrecht, The Netherlands.
30. **Batut, J., E. Santero, and S. Kustu.** 1991. In vitro activity of the nitrogen fixation regulatory protein FixJ from *Rhizobium meliloti*. J. Bacteriol. **173:**5914–5917.
31. **Batut, J., B. Terzaghi, M. Ghérardi, M. Huguet, E. Terzaghi, A. M. Garnerone, P. Boistard, and T. Huguet.** 1985. Localization of a symbiotic *fix* region on *Rhizobium meliloti* pSym megaplasmid more than 200 kilobases from the *nod-nif* region. Mol. Gen. Genet. **199:**232–239.
32. **Bauer, C., J. Buggy, and C. Mosley.** 1993. Control of photosystem genes in *Rhodobacter capsulatus*. Trends Genet. **9:**56–60.
33. **Becker, J., and E. A. Craig.** 1994. Heat-shock proteins as molecular chaperones. Eur. J. Biochem. **219:**11–23.
34. **Bennett, L. T., F. C. Cannon, and D. Dean.** 1988. Nucleotide sequence and mutagenesis of the *nifA* gene from *Azotobacter vinelandii*. Mol. Microbiol. **2:**315–321.
35. **Berg, J. M.** 1986. Potential metal-binding domains in nucleic acid binding proteins. Science **232:**485–487.
36. **Berger, D. K., F. Narberhaus, and S. Kustu.** 1994. The isolated catalytic domain of NIFA, a bacterial enhancer-binding protein, activates transcription *in vitro*: activation is inhibited by NIFL. Proc. Natl. Acad. Sci. USA **91:**103–107.
37. **Beynon, J., M. Cannon, V. Buchanan-Wollaston, A. Ally, R. Setterquist, D. Dean, and F. Cannon.** 1988. The nucleotide sequence of the *nifT*, *nifY*, *nifX* and *nifW* genes of *K. pneumoniae*. Nucleic Acids Res. **16:**9860.
38. **Beynon, J., M. Cannon, V. Buchanan-Wollaston, and F. Cannon.** 1983. The *nif* promoters of *Klebsiella pneumoniae* have a characteristic primary structure. Cell **34:**665–671.
39. **Beynon, J. L., M. K. Williams, and F. C. Cannon.** 1988. Expression and functional analysis of the *Rhizobium meliloti nifA* gene. EMBO J. **7:**7–14.
40. **Bishop, P. E., and R. Premakumar.** 1992. Alternative nitrogen fixation systems, p. 736–762. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.

41. **Blanco, G., M. Drummond, P. Woodley, and C. Kennedy.** 1993. Sequence and molecular analysis of the *nifL* gene of *Azotobacter vinelandii*. Mol. Microbiol. **9:**869–879.
42. **Boistard, P., J. Batut, M. David, J. Fourment, A. M. Garnerone, D. Kahn, P. de Philip, J. M. Reyrat, and F. Waelkens.** 1991. Regulation of nitrogen fixation genes in *Rhizobium meliloti*, p. 195–202. *In* H. Hennecke and D. P. S. Verma (ed.), Advances in molecular genetics of plant-microbe interactions. Kluwer Academic Publishers, Dordrecht, The Netherlands.
43. **Borthakur, D., J. W. Lamb, and A. W. Johnston.** 1987. Identification of two classes of *Rhizobium phaseoli* genes required for melanin synthesis, one of which is required for nitrogen fixation and activates the transcription of the other. Mol. Gen. Genet. **207:**155–160.
44. **Botsford, J. L., and J. G. Harman.** 1992. Cyclic AMP in prokaryotes. Microbiol. Rev. **56:**100–122.
45. **Bourret, R. B., K. A. Borkovich, and M. I. Simon.** 1991. Signal transduction pathways involving protein phosphorylation in prokaryotes. Annu. Rev. Biochem. **60:**401–441.
46. **Brewin, N. J.** 1991. Development of the legume root nodule. Annu. Rev. Cell Biol. **7:**191–226.
47. **Brigle, K. E., M. C. Weiss, W. E. Newton, and D. R. Dean.** 1987. Products of the iron-molybdenum cofactor-specific biosynthetic genes, *nifE* and *nifN*, are structurally homologous to the products of the nitrogenase molybdenum-iron protein genes, *nifD* and *nifK*. J. Bacteriol. **169:**1547–1553.
48. **Brun, Y. V., and L. Shapiro.** 1992. A temporally controlled σ-factor is required for polar morphogenesis and normal cell division in *Caulobacter*. Genes Dev. **6:**2395–2408.
49. **Brushi, M., and F. Guerlesquin.** 1988. Structure, function and evolution of bacterial ferredoxins. FEMS Microbiol. Rev. **54:** 155–176.
50. **Buchanan-Wollaston, V., M. C. Cannon, J. L. Beynon, and F. C. Cannon.** 1981. Role of the *nifA* gene product in the regulation of *nif* expression in *Klebsiella pneumoniae*. Nature (London) **294:** 776–778.
51. **Buck, M., and W. Cannon.** 1989. Mutations in the RNA polymerase recognition sequence of the *Klebsiella pneumoniae nifH* promoter permitting transcriptional activation in the absence of NifA binding to upstream sequences. Nucleic Acids Res. **17:** 2597–2611.
52. **Buck, M., and W. Cannon.** 1992. Specific binding of the transcription factor σ^{54} to promoter DNA. Nature (London) **358:** 422–424.
53. **Buck, M., W. Cannon, and J. Woodcock.** 1987. Mutational analysis of upstream sequences required for transcriptional activation of the *Klebsiella pneumoniae nifH* promoter. Nucleic Acids Res. **15:**9945–9956.
54. **Buck, M., W. Cannon, and J. Woodcock.** 1987. Transcriptional activation of the *Klebsiella pneumoniae* nitrogenase promoter may involve DNA loop formation. Mol. Microbiol. **1:**243–249.
55. **Buck, M., S. Miller, M. Drummond, and R. Dixon.** 1986. Upstream activator sequences are present in the promoters of nitrogen fixation genes. Nature (London) **320:**374–378.
56. **Buikema, W. J., and R. Haselkorn.** 1993. Molecular genetics of cyanobacterial development. Annu. Rev. Plant Physiol. Plant Mol. Biol. **44:**33–52.
57. **Buikema, W. J., J. A. Klingensmith, S. L. Gibbons, and F. M. Ausubel.** 1987. Conservation of structure and location of *Rhizobium meliloti* and *Klebsiella pneumoniae nifB* genes. J. Bacteriol. **169:**1120–1126.
58. **Buikema, W. J., W. W. Szeto, P. V. Lemley, W. H. Orme-Johnson, and F. M. Ausubel.** 1985. Nitrogen fixation specific regulatory genes of *Klebsiella pneumoniae* and *Rhizobium meliloti* share homology with the general nitrogen regulatory gene *ntrC* of *K. pneumoniae*. Nucleic Acids Res. **13:**4539–4555.
59. **Burkhardt, B., D. Schillik, and A. Pühler.** 1987. Physical characterization of *Rhizobium meliloti* megaplasmids. Plasmid **17:**13–25.
60. **Burris, R. H., and G. P. Roberts.** 1993. Biological nitrogen fixation. Annu. Rev. Nutr. **13:**317–335.
61. **Calogero, S., R. Gardan, P. Glaser, J. Schweizer, G. Rapoport, and M. Debarbouille.** 1994. RocR, a novel regulatory protein controlling arginine utilization in *Bacillus subtilis*, belongs to the NtrC/NifA family of transcriptional activators. J. Bacteriol. **176:** 1234–1241.
62. **Cannon, W., and M. Buck.** 1992. Central domain of the positive control protein NifA and its role in transcriptional activation. J. Mol. Biol. **225:**271–286.
63. **Cannon, W., W. Charlton, and M. Buck.** 1991. Organization and function of binding sites for the transcriptional activator NifA in the *Klebsiella pneumoniae nifE* and *nifU* promoters. J. Mol. Biol. **220:**915–931.
64. **Cannon, W., F. Claverie-Martin, S. Austin, and M. Buck.** 1993. Core RNA polymerase assists binding of the transcription factor σ^{54} to promoter DNA. Mol. Microbiol. **8:**287–298.
65. **Cannon, W., F. Claverie-Martin, S. Austin, and M. Buck.** 1994. Identification of a DNA-contacting surface in the transcription factor σ^{54}. Mol. Microbiol. **11:**227–236.
66. **Carlson, T. A., M. L. Guerinot, and B. K. Chelm.** 1985. Characterization of the gene encoding glutamine synthetase I (*glnA*) from *Bradyrhizobium japonicum*. J. Bacteriol. **162:**698–703.
67. **Carlson, T. A., G. B. Martin, and B. K. Chelm.** 1987. Differential transcription of the two glutamine synthetase genes of *Bradyrhizobium japonicum*. J. Bacteriol. **169:**5861–5866.
68. **Castano, I., and F. Bastarrachea.** 1984. *glnF-lacZ* fusions in *Escherichia coli*: studies on *glnF* expression and its chromosomal orientation. Mol. Gen. Genet. **195:**228–233.
69. **Chandler, M. S.** 1992. The gene encoding cAMP receptor protein is required for competence development in *Haemophilus influenzae* Rd. Proc. Natl. Acad. Sci. USA **89:**1626–1630.
70. **Chastain, C. J., J. S. Brusca, T. S. Ramasubramanian, T.-F. Wei, and J. W. Golden.** 1990. A sequence-specific DNA-binding factor (VF1) from *Anabaena* sp. strain PCC 7120 vegetative cells bind to three adjacent sites in the *xisA* upstream region. J. Bacteriol. **172:**5044–5051.
71. **Cherfils, J., J. F. Gibrat, J. Levin, J. Batut, and D. Kahn.** 1989. Model-building of Fnr and FixK DNA-binding domains suggests a basis for specific DNA recognition. J. Mol. Recognit. **2:**114–121.
72. **Colbeau, A., and P. M. Vignais.** 1992. Use of *hupS*::*lacZ* gene fusion to study regulation of hydrogenase expression in *Rhodobacter capsulatus*: stimulation by H_2. J. Bacteriol. **174:** 4258–4264.
73. **Colonna-Romano, S., W. Arnold, A. Schlüter, P. Boistard, A. Pühler, and U. B. Priefer.** 1990. An Fnr-like protein encoded in *Rhizobium leguminosarum* biovar *viciae* shows structural and functional homology to *Rhizobium meliloti* FixK. Mol. Gen. Genet. **223:**138–147.
74. **Coppard, J. R., and M. J. Merrick.** 1991. Cassette mutagenesis implicates a helix-turn-helix motif in promoter recognition by the novel RNA polymerase sigma factor σ^{54}. Mol. Microbiol. **5:**1309–1317.
75. **Corbin, D., L. Barran, and G. Ditta.** 1983. Organization and expression of *Rhizobium meliloti* nitrogen fixation genes. Proc. Natl. Acad. Sci. USA **80:**3005–3009.
76. **Cossart, P., and B. Gicquel-Sanzey.** 1982. Cloning and sequence of the *crp* gene of *Escherichia coli* K12. Nucleic Acids Res. **10:**1363–1378.
77. **Cossart, P., E. A. Groisman, M. C. Serre, M. J. Casadaban, and B. Gicquel-Sanzey.** 1986. *crp* genes of *Shigella flexneri*, *Salmonella typhimurium*, and *Escherichia coli*. J. Bacteriol. **167:**639–646.
78. **Craig, N. L., and H. A. Nash.** 1984. *E. coli* integration host factor binds to specific sites in DNA. Cell **39:**707–716.
79. **Cubo, M. T., A. M. Buendia-Claveria, J. E. Beringer, and J. E. Ruiz-Sainz.** 1988. Melanin production by *Rhizobium* strains. Appl. Environ. Microbiol. **54:**1812–1817.
80. **Cullen, P. J., D. Foster-Hartnett, K. K. Gabbert, and R. G. Kranz.** 1994. Structure and expression of the alternative sigma factor, RpoN, in *Rhodobacter capsulatus*: physiological relevance of an autoactivated *nifU2-rpoN* superoperon. Mol. Microbiol. **11:**51–65.
81. **Cuypers, H., and W. G. Zumft.** 1993. Anaerobic control of dentrification in *Pseudomonas stutzeri* escapes mutagenesis of an *fnr*-like gene. J. Bacteriol. **175:**7236–7246.

81a. **Da Re, S., S. Bertagnoli, J. Fourment, J.-M. Reyrat, and D. Kahn.** 1994. Intramolecular signal transduction within the FixJ tran-

scriptional activator: *in vitro* evidence for the inhibitory effect of the phosphorylatable regulatory domain. Nucleic Acids Res. **22:**1555–1561.

82. **David, M., M.-L. Daveran, J. Batut, A. Dedieu, O. Domergue, J. Ghai, C. Hertig, P. Boistard, and D. Kahn.** 1988. Cascade regulation of *nif* gene expression in *Rhizobium meliloti*. Cell **54:**671–683.
83. **David, M., O. Domergue, P. Pognonec, and D. Kahn.** 1987. Transcription patterns of *Rhizobium meliloti* symbiotic plasmid pSym; identification of *nifA*-independent *fix* genes. J. Bacteriol. **169:**2239–2244.
84. **Dean, D. R., J. T. Bolin, and L. Zheng.** 1993. Nitrogenase metalloclusters: structures, organization, and synthesis. J. Bacteriol. **175:**6737–6744.
85. **Dean, D. R., and M. R. Jacobson.** 1992. Biochemical genetics of nitrogenase, p. 763–834. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
86. **de Billy, F., D. G. Barker, P. Gallusci, and G. Truchet.** 1991. Leghaemoglobin gene transcription is triggered in a single cell layer in the indeterminate nitrogen-fixing root nodule of alfalfa. Plant J. **1:**27–35.
87. **de Bruijn, F. J.** 1989. The unusual symbiosis between the diazotrophic stem-nodulating bacterium *Azorhizobium caulinodans* ORS571 and its host, the tropical legume *Sesbania rostrata*, p. 457–504. *In* T. Kosuge and E. W. Nester (ed.), Plant-microbe interactions, vol. 3. McGraw-Hill Book Co., New York.
88. **de Bruijn, F. J., and F. Ausubel.** 1983. The cloning and characterization of the *glnF* (*ntrA*) gene of *Klebsiella pneumoniae*: role of *glnF* (*ntrA*) in the regulation of nitrogen fixation (*nif*) and other nitrogen assimilation genes. Mol. Gen. Genet. **192:**342–353.
89. **de Bruijn, F. J., U. Hilgert, J. Stigter, M. Schneider, H. Meyer, U. Klosse, and K. Pawlowski.** 1990. Regulation of nitrogen fixation and assimilation genes in the free-living versus symbiotic state, p. 33–44. *In* P. M. Gresshoff, L. E. Roth, G. Stacey, and W. E. Newton (ed.), Nitrogen fixation: achievements and objectives. Chapman & Hall, New York.
90. **de Bruijn, F. J., S. Rossbach, M. Schneider, P. Ratet, S. Messmer, W. W. Szeto, F. M. Ausubel, and J. Schell.** 1989. *Rhizobium meliloti* 1021 has three differentially regulated loci involved in glutamine biosynthesis, none of which is essential for symbiotic nitrogen fixation. J. Bacteriol. **171:**1673–1682.
91. **de Crecy-Lagard, V., P. Glaser, P. Lejeune, O. Sismeiro, C. E. Barber, M. J. Daniels, and A. Danchin.** 1990. A *Xanthomonas campestris* pv. *campestris* protein similar to catabolite activation factor is involved in regulation of phytopathogenicity. J. Bacteriol. **172:**5877–5883.
92. **Demple, B.** 1991. Regulation of bacterial oxidative stress genes. Annu. Rev. Genet. **25:**315–337.
93. **Demple, B., and C. F. Amábile-Cuevas.** 1991. Redox redux: the control of oxidative stress responses. Cell **67:**837–839.
94. **Denèfle, P., A. Kush, F. Norel, A. Paquelin, and C. Elmerich.** 1987. Biochemical and genetic analysis of the *nifHDKE* region of *Rhizobium* ORS571. Mol. Gen. Genet. **207:**280–287.
95. **de Philip, P., J. Batut, and P. Boistard.** 1990. *Rhizobium meliloti* FixL is an oxygen sensor and regulates *R. meliloti nifA* and *fixK* genes differently in *Escherichia coli*. J. Bacteriol. **172:**4255–4262.
96. **de Philip, P., P. Boistard, A. Schlüter, T. Patschkowski, U. B. Priefer, F. O'Gara, B. Boesten, B. Noonan, and A. Pühler.** 1992. Developmental and metabolic regulation of nitrogen fixation gene expression in *Rhizobium meliloti*. Can. J. Microbiol. **38:**467–474.
97. **de Philip, P., E. Soupène, J. Batut, and P. Boistard.** 1992. Modular structure of the FixL protein of *Rhizobium meliloti*. Mol. Gen. Genet. **235:**49–54.
98. **Dénarié, J., and J. Cullimore.** 1993. Lipo-oligosaccharide nodulation factors: a minireview. New class of signaling molecules mediating recognition and morphogenesis. Cell **74:**951–954.
99. **Dénarié, J., F. Debellé, and C. Rosenberg.** 1992. Signaling and host range variation in nodulation. Annu. Rev. Microbiol. **46:**497–531.
100. **Dimri, G. P., and H. K. Das.** 1988. Transcriptional regulation of nitrogen fixation genes by DNA supercoiling. Mol. Gen. Genet. **212:**360–363.
101. **Dispensa, M., C. T. Thomas, M. K. Kim, J. A. Perrotta, J. Gibson, and C. S. Harwood.** 1992. Anaerobic growth of *Rhodopseudomonas palustris* on 4-hydroxybenzoate is dependent on AadR, a member of the cyclic AMP receptor protein family of transcriptional regulators. J. Bacteriol. **174:**5803–5813.
102. **Ditta, G.** 1989. Regulation of *nif* genes in *Rhizobium*, p. 11–24. *In* T. Kosuge and E. W. Nester (ed.), Plant-microbe interactions, vol. 3. McGraw-Hill Book Co., New York.
103. **Ditta, G., E. Virts, A. Palomares, and K. Choong-Hyun.** 1987. The *nifA* gene of *Rhizobium meliloti* is oxygen regulated. J. Bacteriol. **169:**3217–3223.
104. **Dixon, R.** 1988. Genetic regulation of nitrogen fixation, p. 417–438. *In* J. A. Cole and S. J. Ferguson (ed.), The nitrogen and sulphur cycles. Cambridge University Press, Cambridge.
105. **Dixon, R., N. C. Henderson, and S. Austin.** 1988. DNA supercoiling and aerobic regulation of transcription from the *Klebsiella pneumoniae nifLA* promoter. Nucleic Acids Res. **16:**9933–9946.
106. **Djordjevic, M. A., D. W. Gabriel, and B. G. Rolfe.** 1987. *Rhizobium*—the redefined parasite of legumes. Annu. Rev. Phytopathol. **25:**145–168.
107. **Djordjevic, S. P., R. W. Ridge, H. Chen, J. W. Redmond, M. Batley, and B. G. Rolfe.** 1988. Induction of pathogenic-like responses in the legume *Macroptilium atropurpureum* by a transposon-induced mutant of the fast-growing, broad-host-range *Rhizobium* strain NGR234. J. Bacteriol. **170:**1848–1857.
108. **Donald, R. G. K., D. W. Nees, C. K. Raymond, A. I. Loroch, and R. A. Ludwig.** 1986. Characterization of three genomic loci encoding *Rhizobium* sp. strain ORS571 N_2 fixation genes. J. Bacteriol. **165:**72–81.
109. **Dreyfus, B. L., C. Elmerich, and Y. R. Dommergues.** 1983. Free-living *Rhizobium* strain able to grow on N_2 as the sole nitrogen source. Appl. Environ. Microbiol. **45:**711–713.
110. **Drummond, M., P. Whitty, and J. Wootton.** 1986. Sequence and domain relationships of *ntrC* and *nifA* from *Klebsiella pneumoniae*: homologies to other regulatory proteins. EMBO J. **5:**441–447.
111. **Drummond, M. H., A. Contreras, and L. Mitchenall.** 1990. The function of isolated domains and chimaeric proteins constructed from NifA and NtrC proteins of *Klebsiella pneumoniae*. Mol. Microbiol. **4:**29–37.
112. **Dusha, I., A. Bakos, A. Kondorosi, F. J. de Bruijn, and J. Schell.** 1989. The *Rhizobium meliloti* early nodulation genes *nodABC* are nitrogen-regulated: isolation of a mutant with efficient nodulation capacity on alfalfa in the presence of ammonium. Mol. Gen. Genet. **219:**89–96.
113. **Dusha, I., and A. Kondorosi.** 1993. Genes at different regulatory levels are required for the ammonia control of nodulation in *Rhizobium meliloti*. Mol. Gen. Genet. **240:**435–444.
114. **Dylan, T., S. Ielpi, S. Stanfield, L. Kashyap, C. Douglas, M. Yanofsky, E. W. Nester, D. R. Helinski, and G. Ditta.** 1986. *Rhizobium meliloti* genes required for nodule development are related to chromosomal virulence genes in *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. USA **83:**4403–4407.
115. **Earl, C. D., C. W. Ronson, and F. M. Ausubel.** 1987. Genetic and structural analysis of the *Rhizobium meliloti fixA*, *fixB*, *fixC*, and *fixX* genes. J. Bacteriol. **169:**1127–1136.
116. **Ebeling, S., M. Hahn, H. M. Fischer, and H. Hennecke.** 1987. Identification of *nifE*-, *nifN*- and *nifS*-like genes in *Bradyrhizobium japonicum*. Mol. Gen. Genet. **207:**503–508.
117. **Ebeling, S., J. D. Noti, and H. Hennecke.** 1988. Identification of a new *Bradyrhizobium japonicum* gene (*frxA*) encoding a ferredoxin-like protein. J. Bacteriol. **170:**1999–2001.
118. **Eiglmeier, K., N. Honoré, S. Iuchi, E. C. C. Lin, and S. T. Cole.** 1989. Molecular genetic analysis of FNR-dependent promoters. Mol. Microbiol. **3:**869–878.
119. **Elkan, G. H., and C. R. Bunn.** 1992. The rhizobia, p. 2197–2213. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes. Springer-Verlag, New York.
120. **Elmerich, C., W. Zimmer, and C. Vieille.** 1992. Associative nitrogen-fixing bacteria, p. 212–258. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman &

Hall, New York.

121. **Eraso, J. M., and S. Kaplan.** 1994. *prrA*, a putative response regulator involved in oxygen regulation of photosynthesis gene expression in *Rhodobacter sphaeroides*. J. Bacteriol. **176:**32–43.
122. **Errington, J.** 1993. *Bacillus subtilis* sporulation: regulation of gene expression and control of morphogenesis. Microbiol. Rev. **57:**1–33.
123. **Estabrook, E. M., and C. Sengupta-Gopalan.** 1991. Differential expression of phenylalanine ammonia-lyase and chalcone synthase during nodule development. Plant Cell **3:**299–308.
124. **Evans, D.** 1987. The molecular genetics of nitrogen fixation in *Azotobacter chroococcum*, Ph.D. thesis. University of Sussex, Brighton, England.
125. **Evans, D., R. Jones, P. Woodley, and R. Robson.** 1988. Further analysis of nitrogen fixation (*nif*) genes in *Azotobacter chroococcum*: identification and expression in *Klebsiella pneumoniae* of *nifS*, *nifV*, *nifM* and *nifB* genes and localization of *nifE/N*-, *nifU*-, *nifA*- and *fixABC*-like genes. J. Gen. Microbiol. **134:**931–942.
126. **Evans, H. J., and R. H. Burris.** 1992. Highlights in biological nitrogen fixation during the last 50 years, p. 1–42. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
127. **Fischer, H. M., A. Alvarez-Morales, and H. Hennecke.** 1986. The pleiotropic nature of symbiotic regulatory mutants: *Bradyrhizobium japonicum nifA* gene is involved in control of *nif* gene expression and formation of determinate symbiosis. EMBO J. **5:**1165–1173.
128. **Fischer, H. M., M. Babst, T. Kaspar, G. Acuña, F. Arigoni, and H. Hennecke.** 1993. One member of a *groESL*-like chaperonin multigene family in *Bradyrhizobium japonicum* is co-regulated with symbiotic nitrogen fixation genes. EMBO J. **12:**2901–2912.
129. **Fischer, H. M., T. Bruderer, and H. Hennecke.** 1988. Essential and non-essential domains in the *Bradyrhizobium japonicum* NifA protein: identification of indispensible cysteine residues potentially involved in redox reactivity and/or metal binding. Nucleic Acids Res. **16:**2207–2224.
130. **Fischer, H. M., S. Fritsche, B. Herzog, and H. Hennecke.** 1989. Critical spacing between two essencial cysteine residues in the interdomain linker of the *Bradyrhizobium japonicum* NifA protein. FEBS Lett. **255:**167–171.
131. **Fischer, H. M., and H. Hennecke.** 1984. Linkage map of the *Rhizobium japonicum nifH* and *nifDK* operons encoding the polypeptides of the nitrogenase enzyme complex. Mol. Gen. Genet. **196:**537–540.
132. **Fischer, H. M., and H. Hennecke.** 1987. Direct response of *Bradyrhizobium japonicum nifA*-mediated *nif* gene regulation to cellular oxygen status. Mol. Gen. Genet. **209:**621–626.
133. **Fisher, R. F., and S. R. Long.** 1992. *Rhizobium*-plant signal exchange. Nature (London) **357:**655–660.
134. **Foster-Hartnett, D., and R. G. Kranz.** 1992. Analysis of the promoters and upstream sequences of *nifA1* and *nifA2* in *Rhodobacter capsulatus*: activation requires *ntrC* but not *rpoN*. Mol. Microbiol. **6:**1049–1060.
135. **Franssen, H. J., I. Vijn, W. C. Yang, and T. Bisseling.** 1992. Developmental aspects of the *Rhizobium*-legume symbiosis. Plant Mol. Biol. **19:**89–107.
136. **Fuhrmann, M., H. M. Fischer, and H. Hennecke.** 1985. Mapping of *Rhizobium japonicum nifB*-, *fixBC*-, and *fixA*-like genes and identification of the *fixA* promoter. Mol. Gen. Genet. **199:**315–322.
137. **Fuhrmann, M., and H. Hennecke.** 1984. *Rhizobium japonicum* nitrogenase Fe protein gene (*nifH*). J. Bacteriol. **158:**1005–1011.
138. **Garcia, E., S. Bancroft, S. G. Rhee, and S. Kustu.** 1977. The product of a newly identified gene, *glnF*, is required for synthesis of glutamine synthetase in *Salmonella*. Proc. Natl. Acad. Sci. USA **74:**1662–1666.
139. **Gilles-Gonzalez, M. A., G. S. Ditta, and D. R. Helinski.** 1991. A haemoprotein with kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*. Nature (London) **350:**170–172.
140. **Gilles-Gonzalez, M. A., and G. Gonzalez.** 1993. Regulation of the kinase activity of heme protein FixL from the two-component system FixL/FixJ of *Rhizobium meliloti*. J. Biol. Chem. **268:**16293–16297.

140a. **Gilles-Gonzalez, M. A., G. Gonzalez, M. F. Perutz, L. Kiger, M. C. Marden, and C. Poyart.** Heme-based sensors, exemplified by the kinase FixL, are a new class of heme protein with distinctive ligand binding and autoxidation. Biochemistry, in press.
141. **Goodman, S. I., K. M. Axtell, L. A. Bindoff, S. E. Beard, R. E. Gill, and F. E. Frerman.** 1994. Molecular cloning and expression of a cDNA encoding human electron transfer flavoprotein-ubiquinone oxidoreductase. Eur. J. Biochem. **219:**277–286.
142. **Göttfert, M.** 1993. Regulation and function of rhizobial nodulation genes. FEMS Microbiol. Rev. **104:**39–63.
143. **Göttfert, M., P. Grob, and H. Hennecke.** 1990. Proposed regulatory pathway encoded by the *nodV* and *nodW* genes, determinants of host specificity in *Bradyrhizobium japonicum*. Proc. Natl. Acad. Sci. USA **87:**2680–2684.

143a. **Govezensky, D., E. S. Bochkareva, A. Zamir, and A. S. Girshovich.** 1994. Chaperonins as potential gene regulatory factors: *in vitro* interaction and solubilization of NifA, the *nif* transcriptional activator, with GroEL. J. Biol. Chem. **269:**14003–14006.
144. **Govezensky, D., T. Greener, G. Segal, and A. Zamir.** 1991. Involvement of GroEL in *nif* gene regulation and nitrogenase assembly. J. Bacteriol. **173:**6339–6346.
145. **Green, J., and J. R. Guest.** 1993. Activation of FNR-dependent transcription by iron: an *in vitro* switch for FNR. FEMS Microbiol. Lett. **113:**219–222.
146. **Green, J., and J. R. Guest.** 1994. Regulation of transcription at the *ndh* promoter of *Escherichia coli* by FNR and novel factors. Mol. Microbiol. **12:**433–444.
147. **Green, J., A. D. Sharrocks, B. Green, M. Geisow, and J. R. Guest.** 1993. Properties of FNR proteins substituted at each of the five cysteine residues. Mol. Microbiol. **8:**61–68.
148. **Green, J., M. Trageser, S. Six, G. Unden, and J. R. Guest.** 1991. Characterization of the FNR protein of *Escherichia coli*, an iron-binding transcriptional regulator. Proc. R. Soc. London Ser. B **244:**137–144.
149. **Grönger, P., S. S. Manian, H. Reiländer, M. O'Connell, U. B. Priefer, and A. Pühler.** 1987. Organisation and partial sequence of a DNA region of the *Rhizobium leguminosarum* symbiotic plasmid pRL6JI containing the genes *fixABC*, *nifA*, *nifB* and a novel open reading frame. Nucleic Acids Res. **15:**31–49.
150. **Gross, C. A., M. A. Lonetto, and R. Losick.** 1992. Bacterial sigma factors, p. 129–176. *In* S. L. McKnight and K. R. Yamamoto (ed.), Transcriptional regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
151. **Grosskopf, E., D. T. C. Ha, R. Wingender, H. Röhrig, J. Szecsi, E. Kondorosi, J. Schell, and A. Kondorosi.** 1993. Enhanced levels of chalcone synthase in alfalfa nodules induced by a Fix$^-$ mutant of *Rhizobium meliloti*. Mol. Plant-Microbe Interact. **6:**173–181.
152. **Gubler, M.** 1989. Fine-tuning of *nif* and *fix* gene expression by upstream activator sequences in *Bradyrhizobium japonicum*. Mol. Microbiol. **3:**149–159.
153. **Gubler, M., and H. Hennecke.** 1986. *FixA*, *B* and *C* genes are essential for symbiotic and free-living, microaerobic nitrogen fixation. FEBS Lett. **200:**186–192.
154. **Gubler, M., T. Zürcher, and H. Hennecke.** 1989. The *Bradyrhizobium japonicum fixBCX* operon: identification of *fixX* and of a 5′ mRNA region affecting the level of the *fixBCX* transcript. Mol. Microbiol. **3:**141–148.
155. **Gussin, G. N., C. W. Ronson, and F. M. Ausubel.** 1986. Regulation of nitrogen fixation genes. Annu. Rev. Genet. **20:**567–591.
156. **Hartl, F.-U., R. Hlodan, and T. Langer.** 1994. Molecular chaperones in protein folding: the art of avoiding sticky situations. Trends Biochem. Sci. **19:**20–25.
157. **Haselkorn, R.** 1992. Developmentally regulated gene rearrangements in prokaryotes. Annu. Rev. Genet. **26:**113–130.
158. **Haselkorn, R., and W. J. Buikema.** 1992. Nitrogen fixation in cyanobacteria, p. 166–190. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
159. **Hawkins, F. K. L., and A. W. B. Johnston.** 1988. Transcription of a *Rhizobium leguminosarum* biovar *phaseoli* gene needed for melanin synthesis is activated by *nifA* of *Rhizobium* and *Klebsiella pneumoniae*. Mol. Microbiol. **2:**331–337.

160. **Hawkins, F. K. L., C. Kennedy, and A. W. B. Johnston.** 1991. A *Rhizobium leguminosarum* gene required for symbiotic nitrogen fixation, melanin synthesis and normal growth on certain growth media. J. Gen. Microbiol. **137**:1721–1728.
161. **Helmann, J. D., and M. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57**:839–872.
162. **Hendrick, J. P., and F. U. Hartl.** 1993. Molecular chaperone functions of heat-shock proteins. Annu. Rev. Biochem. **62**:349–384.
163. **Hennecke, H.** 1990. Nitrogen fixation genes involved in the *Bradyrhizobium japonicum*-soybean symbiosis. FEBS Lett. **268**: 422–426.
164. **Hennecke, H.** 1990. Regulation of bacterial gene expression by metal-protein complexes. Mol. Microbiol. **4**:1621–1628.
165. **Hennecke, H.** 1993. The role of respiration in symbiotic nitrogen fixation, p. 55–64. *In* R. Palacios, J. Mora, and W. E. Newton (ed.), New horizons in nitrogen fixation. Kluwer Academic Publishers, Dordrecht, The Netherlands.
166. **Henner, D. J., M. Yang, and E. Ferrari.** 1988. Localization of *Bacillus subtilis sacU*(Hy) mutations to two linked genes with similarities to the conserved procaryotic family of two-component signaling systems. J. Bacteriol. **170**:5102–5109.
167. **Hertig, C., R. Y. Li, A.-M. Louarn, A.-M. Garnerone, M. David, J. Batut, D. Kahn, and P. Boistard.** 1989. *Rhizobium meliloti* regulatory gene *fixJ* activates transcription of *R. meliloti nifA* and *fixK* genes in *Escherichia coli*. J. Bacteriol. **171**:1736–1738.
168. **Hidalgo, E., and B. Demple.** 1994. An iron-sulfur center essential for transcriptional activation by the redox-sensing SoxR protein. EMBO J. **13**:138–146.
169. **Hill, S.** 1992. Physiology of nitrogen fixation in free-living heterotrophs, p. 87–134. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
170. **Hirsch, A. M.** 1992. Developmental biology of nodulation. New Phytol. **122**:211–237.
171. **Hirsch, A. M., and C. A. Smith.** 1987. Effects of *Rhizobium meliloti nif* and *fix* mutants on alfalfa root nodule development. J. Bacteriol. **169**:1137–1146.
172. **Hirschman, J., P. K. Wong, K. Sei, J. Keener, and S. Kustu.** 1985. Products of nitrogen regulatory genes *ntrA* and *ntrC* of enteric bacteria activate *glnA* transcription *in vitro*: evidence that the *ntrA* product is a sigma factor. Proc. Natl. Acad. Sci. USA **82**:7525–7529.
173. **Honeycutt, R. J., M. McClelland, and B. W. S. Sobral.** 1993. Physical map of the genome of *Rhizobium meliloti* 1021. J. Bacteriol. **175**:6945–6952.
174. **Hoover, T. R., E. Santero, S. Porter, and S. Kustu.** 1990. The integration host factor stimulates interaction of RNA polymerase with NifA, the transcriptional activator for nitrogen fixation operons. Cell **63**:11–22.
175. **Huala, E., and F. M. Ausubel.** 1989. The central domain of *Rhizobium meliloti* NifA is sufficient to activate transcription from the *R. meliloti nifH* promoter. J. Bacteriol. **171**:3354–3365.
176. **Huala, E., A. L. Moon, and F. M. Ausubel.** 1991. Aerobic inactivation of *Rhizobium meliloti* NifA in *Escherichia coli* is mediated by *lon* and two newly identified genes, *snoB* and *snoC*. J. Bacteriol. **173**:382–390.
177. **Huala, E., J. Stigter, and F. M. Ausubel.** 1992. The central domain of *Rhizobium leguminosarum* DctD functions independently to activate transcription. J. Bacteriol. **174**:1428–1431.
178. **Humbeck, C., and D. Werner.** 1987. Two succinate uptake systems in *Bradyrhizobium japonicum*. Curr. Microbiol. **14**:259–262.
179. **Hunt, S., and D. B. Layzell.** 1993. Gas exchange of legume nodules and the regulation of nitrogenase activity. Annu. Rev. Plant Physiol. Plant Mol. Biol. **44**:483–511.
180. **Hunt, T. P., and B. Magasanik.** 1985. Transcription of *glnA* by purified *Escherichia coli* components: core RNA polymerase and the products of *glnF*, *glnG*, and *glnL*. Proc. Natl. Acad. Sci. USA **82**:8453–8457.
180a. **Hynes, M. F., J. Quandt, A. Schlüter, T. Paschkowski, S. Weidner, and U. B. Priefer.** 1992. Abstr. Book Ninth Int. Congr. Nitrogen Fixation, abstr. 464.
181. **Hynes, M. F., R. Simon, P. Müller, K. Niehaus, M. Labes, and A. Pühler.** 1986. The two megaplasmids of *Rhizobium meliloti* are involved in the effective nodulation of alfalfa. Mol. Gen. Genet. **202**:356–362.
182. **Iismaa, S. E., P. M. Ealing, K. F. Scott, and J. M. Watson.** 1989. Molecular linkage of the *nif*/*fix* and *nod* gene regions in *Rhizobium leguminosarum* biovar *trifolii*. Mol. Microbiol. **3**:1753–1764.
183. **Iismaa, S. E., and J. M. Watson.** 1989. The *nifA* gene product from *Rhizobium leguminosarum* biovar *trifolii* lacks the N-terminal domain found in other NifA proteins. Mol. Microbiol. **3**:943–955.
184. **Inouye, S., A. Nakazawa, and T. Nakazawa.** 1988. Nucleotide sequence of the regulatory gene *xylR* of the TOL plasmid from *Pseudomonas putida*. Gene **66**:301–306.
185. **Iuchi, S., and E. C. C. Lin.** 1993. Adaptation of *Escherichia coli* to redox environments by gene expression. Mol. Microbiol. **9**:9–15.
186. **Jacobson, M. R., K. V. Brigle, L. T. Bennett, R. A. Setterquist, M. S. Wilson, V. L. Cash, J. Beynon, W. E. Newton, and D. R. Dean.** 1989. Physical and genetic map of the major *nif* gene cluster from *Azotobacter vinelandii*. J. Bacteriol. **171**:1017–1027.
187. **Jacobson, M. R., V. L. Cash, M. C. Weiss, N. F. Laird, W. E. Newton, and D. R. Dean.** 1989. Biochemical and genetic analysis of the *nifUSVWZM* cluster from *Azotobacter vinelandii*. Mol. Gen. Genet. **219**:49–57.
188. **Jiang, J., B. Gu, L. M. Albright, and B. T. Nixon.** 1989. Conservation between coding and regulatory elements of *Rhizobium meliloti* and *Rhizobium leguminosarum dct* genes. J. Bacteriol. **171**:5244–5253.
189. **Jin, S. G., K. Ishimoto, and S. Lory.** 1994. Nucleotide sequence of the *rpoN* gene and characterization of two downstream open reading frames in *Pseudomonas aeruginosa*. J. Bacteriol. **176**: 1316–1322.
190. **Jin, S. G., R. K. Prusti, T. Roitsch, R. G. Ankebauer, and E. W. Nester.** 1990. Phosphorylation of the VirG protein of *Agrobacterium tumefaciens* by the autophosphorylated VirA protein: essential role in biological activity of VirG. J. Bacteriol. **172**:4945–4950.
191. **Joerger, R. D., R. Premakumar, and P. E. Bishop.** 1986. Tn*5*-induced mutants of *Azotobacter vinelandii* affected in nitrogen fixation under Mo-deficient and Mo-sufficient conditions. J. Bacteriol. **168**:673–682.
192. **Jones, R., and R. Haselkorn.** 1989. The DNA sequence of the *Rhodobacter capsulatus ntrA*, *ntrB* and *ntrC* gene analogues required for nitrogen fixation. Mol. Gen. Genet. **215**:507–516.
193. **Kahn, D., J. Batut, M.-L. Daveran, and J. Fourment.** 1993. Structure and regulation of the *fixNOQP* operon from *Rhizobium meliloti*, p. 474. *In* R. Palacios, J. Mora, and W. E. Newton (ed.), New horizons in nitrogen fixation. Kluwer Academic Publishers, Dordrecht, The Netherlands.
194. **Kahn, D., M. David, O. Domergue, M.-L. Daveran, J. Ghai, P. R. Hirsch, and J. Batut.** 1989. *Rhizobium meliloti fixGHI* sequence predicts involvement of a specific cation pump in symbiotic nitrogen fixation. J. Bacteriol. **171**:929–939.
195. **Kahn, D., and G. Ditta.** 1991. Modular structure of FixJ: homology of the transcriptional activator domain with the −35 binding domain of sigma factors. Mol. Microbiol. **5**:987–997.
196. **Kaluza, K., and H. Hennecke.** 1984. Fine structure analysis of the *nifDK* operon encoding the α and β subunits of dinitrogenase from *Rhizobium japonicum*. Mol. Gen. Genet. **196**:35–42.
197. **Kaminski, P. A., N. Desnoues, and C. Elmerich.** 1994. The expression of *nifA* in *Azorhizobium caulinodans* requires a gene product homologous to *Escherichia coli* HF-I, a RNA-binding protein involved in the replication of the phage Qβ. Proc. Natl. Acad. Sci. USA **91**:4663–4667.
198. **Kaminski, P. A., and C. Elmerich.** 1991. Involvement of *fixLJ* in the regulation of nitrogen fixation in *Azorhizobium caulinodans*. Mol. Microbiol. **5**:665–673.
199. **Kaminski, P. A., K. Mandon, F. Arigoni, N. Desnoues, and C. Elmerich.** 1991. Regulation of nitrogen fixation in *Azorhizobium caulinodans*: identification of a *fixK*-like gene, a positive regulator of *nifA*. Mol. Microbiol. **5**:1983–1991.
200. **Kaminski, P. A., F. Norel, N. Desnoues, A. Kush, G. Salzano, and C. Elmerich.** 1988. Characterization of the *fixABC* region of *Azorhizobium caulinodans* ORS571 and identification of a new nitrogen fixation gene. Mol. Gen. Genet. **214**:496–502.

201. **Kennedy, C., A. Bali, G. Blanco, A. Contreras, M. Drummond, M. J. Merrick, J. Walmsley, and P. Woodley.** 1991. Regulation of expression of genes for three nitrogenases in *Azotobacter vinelandii*, p. 13–23. *In* M. Polsinelli, R. Materassi, and M. Vincenzini (ed.), Nitrogen fixation. Proceedings of the Fifth International Symposium on Nitrogen Fixation with Non-Legumes. Kluwer Academic Publishers, Dordrecht, The Netherlands.
202. **Kijne, J. W.** 1992. The *Rhizobium* infection process, p. 349–398. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
203. **Kim, C.-H., D. R. Helinski, and G. Ditta.** 1986. Overlapping transcription of the *nifA* regulatory gene in *Rhizobium meliloti*. Gene **50:**141–148.
204. **Kim, H., C. Gabel, and R. J. Maier.** 1993. Expression of hydrogenase in Hup^c strains of *Bradyrhizobium japonicum*. Arch. Microbiol. **160:**43–50.
205. **Kim, H., C. B. Yu, and R. J. Maier.** 1991. Common *cis*-acting region responsible for transcriptional regulation of *Bradyrhizobium japonicum* hydrogenase by nickel, oxygen, and hydrogen. J. Bacteriol. **173:**3993–3999.
206. **Kim, Y. M., K. J. Ahn, T. Beppu, and T. Uozomi.** 1986. Nucleotide sequence of the *nifLA* operon of *Klebsiella oxytoca* NG13 and characterisation of the gene products. Mol. Gen. Genet. **205:**253–259.
207. **Klement, Z.** 1982. Hypersensitivity, p. 149–177. *In* M. S. Mount and G. H. Lacy (ed.), Phytopathogenic prokaryotes. Academic Press, Inc., New York.
208. **Klier, A., T. Msadek, and G. Rapoport.** 1992. Positive regulation in the gram-positive bacterium: *Bacillus subtilis*. Annu. Rev. Microbiol. **46:**429–459.
209. **Klipp, W., H. Reiländer, A. Schlüter, R. Krey, and A. Pühler.** 1989. The *Rhizobium meliloti fdxN* gene encoding a ferredoxin-like protein is necessary for nitrogen fixation and is cotranscribed with *nifA* and *nifB*. Mol. Gen. Genet. **216:**293–302.
210. **Köhler, T., J. F. Alvarez, and S. Harayama.** 1994. Regulation of the *rpoN*, ORF102 and ORF154 genes in *Pseudomonas putida*. FEMS Microbiol. Lett. **115:**177–184.
211. **Kolb, A., S. Busby, H. Buc, S. Garges, and S. Adhya.** 1993. Transcriptional regulation by cAMP and its receptor protein. Annu. Rev. Biochem. **62:**749–795.
212. **Kondorosi, A., E. Kondorosi, M. John, J. Schmidt, and J. Schell.** 1991. The role of nodulation genes in bacterium-plant communication. Genet. Eng. **13:**115–136.
213. **Kranz, R. G., and D. Foster-Hartnett.** 1990. Transcriptional regulatory cascade of nitrogen-fixation genes in anoxygenic photosynthetic bacteria: oxygen- and nitrogen-responsive factors. Mol. Microbiol. **4:**1793–1800.
214. **Kranz, R. G., and R. Haselkorn.** 1986. Anaerobic regulation of nitrogen-fixation genes in *Rhodopseudomonas capsulata*. Proc. Natl. Acad. Sci. USA **83:**6805–6809.
215. **Krey, R., A. Pühler, and W. Klipp.** 1992. A defined amino acid exchange close to the putative nucleotide binding site is responsible for an oxygen-tolerant variant of the *Rhizobium meliloti* NifA protein. Mol. Gen. Genet. **234:**433–441.
216. **Kullik, I.** 1992. Untersuchungen zur Funktion und Regulation der beiden *rpoN* Gene von *Bradyrhizobium japonicum*. Ph.D. dissertation ETH 9895. Eidgenössische Technische Hochschule, Zürich.
217. **Kullik, I., S. Fritsche, H. Knobel, J. Sanjuan, H. Hennecke, and H. M. Fischer.** 1991. *Bradyrhizobium japonicum* has two differentially regulated, functional homologs of the σ^{54} gene (*rpoN*). J. Bacteriol. **173:**1125–1138.
218. **Kullik, I., H. Hennecke, and H. M. Fischer.** 1989. Inhibition of *Bradyrhizobium japonicum nifA*-dependent *nif* gene activation by oxygen occurs at the NifA protein level and is irreversible. Arch. Microbiol. **151:**191–197.
219. **Kündig, C., H. Hennecke, and M. Göttfert.** 1993. Correlated physical and genetic map of the *Bradyrhizobium japonicum* 110 genome. J. Bacteriol. **175:**613–622.
220. **Kunst, F., M. Debarbouille, T. Msadek, M. Young, C. Mauel, D. Karamata, A. Klier, G. Rapoport, and R. Dedonder.** 1988. Deduced polypeptides encoded by the *Bacillus subtilis sacU* locus share homology with two-component sensor regulator systems. J. Bacteriol. **170:**5093–5101.
221. **Kush, A., C. Elmerich, and J. P. Aubert.** 1985. Nitrogenase of *Sesbania Rhizobium* strain ORS571: purification, properties and 'switch-off' by ammonia. J. Gen. Microbiol. **131:**1765–1777.
222. **Kustu, S., D. K. Berger, and H.-S. Lee.** 1993. Strategy for purifying the NIFA protein, a major regulator of biological nitrogen fixation, p. 3–10. *In* R. H. Baltz, G. D. Hegeman, and P. L. Skatrud (ed.), Industrial microorganisms: basic and applied molecular genetics. American Society for Microbiology, Washington, D.C.
223. **Kustu, S., E. Santero, D. Popham, J. Keener, and D. Weiss.** 1989. Expression of σ^{54} (*ntrA*)-dependent genes is probably united by a common mechanism. Microbiol. Rev. **53:**367–376.
224. **Laudenbach, D. E., and A. R. Grossman.** 1991. Characterization and mutagenesis of sulfur-regulated genes in a cyanobacterium: evidence for function in sulfate transport. J. Bacteriol. **173:**2739–2750.
225. **Lazazzera, B. A., D. M. Bates, and P. J. Kiley.** 1993. The activity of the *Escherichia coli* transcription factor Fnr is regulated by a change in oligomeric state. Genes Dev. **7:**1993–2005.
226. **Ledebur, H., B. Gu, J. Sojda III, and B. T. Nixon.** 1990. *Rhizobium meliloti* and *Rhizobium leguminosarum dctD* gene products bind to tandem sites in an activation sequence located upstream of σ^{54}-dependent *dctA* promoters. J. Bacteriol. **172:**3888–3897.
227. **Lee, H.-S., D. K. Berger, and S. Kustu.** 1993. Activity of purified NIFA, a transcriptional activator of nitrogen fixation genes. Proc. Natl. Acad. Sci. USA **90:**2266–2270.
228. **Lee, H.-S., A. Ishihama, and S. Kustu.** 1993. The C-terminus of the α subunit of RNA polymerase is not essential for transcriptional activation of σ^{54} holoenzyme. J. Bacteriol. **175:**2479–2482.
229. **Lee, H.-S., F. Narberhaus, and S. Kustu.** 1993. In vitro activity of NifL, a signal transduction protein for biological nitrogen fixation. J. Bacteriol. **175:**7683–7688.
230. **Leigh, J. A., and G. C. Walker.** 1994. Exopolysaccharides of *Rhizobium*: synthesis, regulation and symbiotic function. Trends. Genet. **10:**63–67.
231. **Leong, J. M., S. Nunes-Duby, C. F. Lesser, P. Youderian, M. M. Susskind, and A. Landy.** 1985. The psi80 and P22 attachment site: primary structure and interaction with *Escherichia coli* integration host factor. J. Biol. Chem. **260:**4468–4477.
232. **Liang, Y. Y., M. Dezamaroczy, F. Arsène, A. Paquelin, and C. Elmerich.** 1992. Regulation of nitrogen fixation in *Azospirillum brasilense* Sp7: involvement of *nifA*, *glnA* and *glnB* gene products. FEMS Microbiol. Lett. **100:**113–120.
233. **Liang, Y. Y., P. A. Kaminski, and C. Elmerich.** 1991. Identification of a *nifA*-like regulatory gene of *Azospirillum brasilense* Sp7 expressed under conditions of nitrogen fixation and in the presence of air and ammonia. Mol. Microbiol. **5:**2735–2744.
234. **Lisser, S., and H. Margalit.** 1993. Compilation of *E. coli* mRNA promoter sequences. Nucleic Acids Res. **21:**1507–1516.
235. **Lobo, A. L., and S. H. Zinder.** 1992. Nitrogen fixation by methanogenic bacteria, p. 191–211. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
236. **Lois, A. F., G. S. Ditta, and D. R. Helinski.** 1993. The oxygen sensor FixL of *Rhizobium meliloti* is a membrane protein containing four possible transmembrane segments. J. Bacteriol. **175:**1103–1109.
237. **Lois, A. F., M. Weinstein, G. S. Ditta, and D. R. Helinski.** 1993. Autophosphorylation and phosphatase activities of the oxygen-sensing protein FixL of *Rhizobium meliloti* are coordinately regulated by oxygen. J. Biol. Chem. **268:**4370–4375.
238. **Long, S. R.** 1989. *Rhizobium*-legume nodulation: life together in the underground. Cell **56:**203–214.
239. **Ludden, P. W., and G. P. Roberts.** 1989. Regulation of nitrogenase activity by reversible ADP-ribosylation. Curr. Top. Cell. Regul. **30:**23–55.
240. **MacInnes, J. I., J. E. Kim, C.-J. Lian, and G. A. Soltes.** 1990. *Actinobacillus pleuropneumoniae hlyX* gene homology with the *fnr* gene of *Escherichia coli*. J. Bacteriol. **172:**4587–4592.
241. **Magasanik, B.** 1982. Genetic control in nitrogen assimilation in bacteria. Annu. Rev. Genet. **16:**135–168.

242. **Mandon, K., H. Hillebrand, C. Mougel, N. Desnoues, B. Dreyfus, P. A. Kaminski, and C. Elmerich.** 1993. Characterization of *fixK*-regulated *Azorhizobium caulinodans* genes, p. 478. *In* R. Palacios, J. Mora, and W. E. Newton (ed.), New horizons in nitrogen fixation. Kluwer Academic Publishers, Dordrecht, The Netherlands.

243. **Mandon, K., P. A. Kaminski, and C. Elmerich.** 1994. Functional analysis of the *fixNOQP* region of *Azorhizobium caulinodans*. J. Bacteriol. **176:**2560–2568.

244. **Mandon, K., P. A. Kaminski, C. Mougel, N. Desnoues, B. Dreyfus, and C. Elmerich.** 1993. Role of the *fixGHI* region of *Azorhizobium caulinodans* in free-living and symbiotic nitrogen fixation. FEMS Microbiol. Lett. **114:**185–190.

245. **Martin, G. B., K. A. Chapman, and B. K. Chelm.** 1988. Role of the *Bradyrhizobium japonicum ntrC* gene product in differential regulation of the glutamine synthetase II gene (*glnII*). J. Bacteriol. **170:**5452–5459.

246. **Martin, G. B., and B. K. Chelm.** 1991. *Bradyrhizobium japonicum ntrBC/glnA* and *nifA/glnA* mutants: further evidence that separate regulatory pathways govern *glnII* expression in free-living and symbiotic cells. Mol. Plant-Microbe Interact. **4:**254–261.

247. **Martin-Verstraete, I., M. Débarbouille, A. Klier, and G. Rapoport.** 1992. Mutagenesis of the *Bacillus subtilis* "−12, −24" promoter of the levanase operon and evidence for the existence of an upstream activating sequence. J. Mol. Biol. **226:**85–99.

248. **Masepohl, B., W. Klipp, and A. Pühler.** 1988. Genetic characterization and sequence analysis of the duplicated *nifA/nifB* gene region of *Rhodobacter capsulatus*. Mol. Gen. Genet. **212:**27–37.

249. **Masepohl, B., R. Krey, K.-U. Riedel, H. Reiländer, D. Jording, H. Prechel, W. Klipp, and A. Pühler.** 1989. Analysis of the *Rhizobium meliloti* main *nif* and *fix* gene cluster, p. 63–68. *In* P. Nardon, V. Gianinazzi-Pearson, A. M. Grenier, L. Margulis, and D. C. Smith (ed.), Endocytobiology IV. Institut National de la Recherche Agronomique, Paris.

250. **Masepohl, B., M. Kutsche, K. U. Riedel, M. Schmehl, W. Klipp, and A. Pühler.** 1992. Functional analysis of the cysteine motifs in the ferredoxin-like protein FdxN of *Rhizobium meliloti* involved in symbiotic nitrogen fixation. Mol. Gen. Genet. **233:**33–41.

251. **Maupin, J. A., and K. T. Shanmugam.** 1990. Genetic regulation of formate hydrogenlyase of *Escherichia coli*: role of the *fhlA* gene product as a transcriptional activator for a new regulatory gene, *fhlB*. J. Bacteriol. **172:**4798–4806.

252. **McClung, C. R., J. E. Somerville, M. L. Guerinot, and B. K. Chelm.** 1987. Structure of the *Bradyrhizobium japonicum* gene *hemA* encoding 5-aminolevulinic acid synthase. Gene **54:**133–139.

253. **Meijer, W. G., and F. R. Tabita.** 1992. Isolation and characterization of the *nifUSVW-rpoN* gene cluster from *Rhodobacter sphaeroides*. J. Bacteriol. **174:**3855–3866.

254. **Melville, S. B., and R. P. Gunsalus.** 1990. Mutations in *fnr* that alter the anaerobic regulation of electron transport-associated genes in *Escherichia coli*. J. Biol. Chem. **265:**18733–18736.

255. **Mercado-Blanco, J., F. García, M. Fernández-López, and J. Olivares.** 1993. Melanin production by *Rhizobium meliloti* GR4 is linked to nonsymbiotic plasmid pRmeGR4b: cloning, sequencing, and expression of the tyrosinase gene *mepA*. J. Bacteriol. **175:**5403–5410.

256. **Merrick, M. J.** 1988. Regulation of nitrogen assimilation by bacteria, p. 331–361. *In* J. A. Cole and S. Ferguson (ed.), The nitrogen and sulphur cycles. Cambridge University Press, Cambridge.

257. **Merrick, M. J.** 1992. Regulation of nitrogen fixation genes in free-living and symbiotic bacteria, p. 835–876. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.

258. **Merrick, M. J.** 1993. In a class of its own—the RNA polymerase sigma factor σ^{54} (σ^N). Mol. Microbiol. **10:**903–909.

259. **Merrick, M. J., and S. Chambers.** 1992. The helix-turn-helix motif of σ^{54} is involved in recognition of the −13 promoter region. J. Bacteriol. **174:**7221–7226.

260. **Merrick, M. J., and J. R. Coppard.** 1989. Mutations in genes downstream of the *rpoN* gene (encoding σ^{54}) of *Klebsiella pneumoniae* affect expression from σ^{54}-dependent promoters. Mol. Microbiol. **3:**1765–1775.

261. **Merrick, M. J., J. Gibbins, and A. Toukdarian.** 1987. The nucleotide sequence of the sigma factor gene *ntrA* (*rpoN*) of *Azotobacter vinelandii*: analysis of conserved sequences in NtrA proteins. Mol. Gen. Genet. **210:**323–330.

262. **Merrick, M. J., S. Hill, H. Hennecke, M. Hahn, R. Dixon, and C. Kennedy.** 1982. Repressor properties of the *nifL* gene product of *Klebsiella pneumoniae*. Mol. Gen. Genet. **185:**75–81.

263. **Merrick, M. J., and W. D. P. Stewart.** 1985. Studies on the regulation and function of the *Klebsiella pneumoniae ntrA* gene. Gene **35:**297–303.

263a. **Michiels, J., I. d'Hooghe, C. Verreth, H. Pelemans, and J. Vanderleyden.** 1994. Characterization of the *Rhizobium leguminosarum* biovar *phaseoli nifA* gene, a positive regulator of *nif* gene expression. Arch. Microbiol. **161:**404–408.

264. **Michiels, J., and J. Vanderleyden.** 1993. Cloning and sequence of the *Rhizobium leguminosarum* biovar *phaseoli fixA* gene. Biochim. Biophys. Acta **1144:**232–233.

265. **Monson, E. K., A. F. Lois, P. G. Agron, M. Weinstein, S. W. Stanfield, G. S. Ditta, and D. R. Helinski.** 1993. Oxygen sensing by the *Rhizobium meliloti* two-component regulatory system, FixLJ, p. 405–410. *In* R. Palacios, J. Mora, and W. E. Newton (ed.), New horizons in nitrogen fixation. Kluwer Academic Publishers, Dordrecht, The Netherlands.

266. **Monson, E. K., M. Weinstein, G. S. Ditta, and D. R. Helinski.** 1992. The FixL protein of *Rhizobium meliloti* can be separated into a heme-binding oxygen-sensing domain and a functional C-terminal kinase domain. Proc. Natl. Acad. Sci. USA **89:**4280–4284.

267. **Morett, E., and M. Buck.** 1988. NifA-dependent *in vivo* protection demonstrates that the upstream activator sequence of *nif* promoters is a protein binding site. Proc. Natl. Acad. Sci. USA **85:**9401–9405.

268. **Morett, E., and M. Buck.** 1989. *In vivo* studies on the interaction of RNA polymerase-σ^{54} with the *Klebsiella pneumoniae* and *Rhizobium meliloti nifH* promoters: the role of NifA in the formation of an open promoter complex. J. Mol. Biol. **210:**65–77.

269. **Morett, E., W. Cannon, and M. Buck.** 1988. The DNA-binding domain of the transcriptional activator protein NifA resides in its carboxy terminus, recognises the upstream activator sequences of *nif* promoters and can be separated from the positive control function of NifA. Nucleic Acids Res. **16:**11469–11488.

270. **Morett, E., H. M. Fischer, and H. Hennecke.** 1991. Influence of oxygen on DNA binding, positive control, and stability of the *Bradyrhizobium japonicum* NifA regulatory protein. J. Bacteriol. **173:**3478–3487.

271. **Morett, E., L. Olvera, and H. Hennecke.** 1993. Overlapping promoters for two polymerase holoenzymes regulate transcription of *nifA* in *Bradyrhizobium japonicum*, p. 483. *In* R. Palacios, J. Mora, and W. E. Newton (ed.), New horizons in nitrogen fixation. Kluwer Academic Publishers, Dordrecht, The Netherlands.

272. **Morett, E., and L. Segovia.** 1993. The σ^{54} bacterial enhancer-binding protein family: mechanism of action and phylogenetic relationship of their functional domains. J. Bacteriol. **175:**6067–6074.

273. **Murphy, P. J., N. Heycke, Z. Banfalvi, M. E. Tate, F. J. de Bruijn, A. Kondorosi, J. Tempe, and J. Schell.** 1987. Genes for the catabolism and synthesis of an opine-like compound in *Rhizobium meliloti* are closely linked and on the sym plasmid. Proc. Natl. Acad. Sci. USA **84:**493–497.

274. **Murphy, P. J., N. Heycke, S. T. Trenz, P. Ratet, F. J. de Bruijn, and J. Schell.** 1988. Synthesis of an opine-like compound, a rhizopine, in alfalfa nodules is symbiotically regulated. Proc. Natl. Acad. Sci. USA **85:**9133–9137.

275. **Murphy, P. J., and C. P. Saint.** 1992. Rhizopines in the legume-*Rhizobium* symbiosis, p. 377–390. *In* D. P. S. Verma (ed.), Molecular signals in plant-microbe communications. CRC Press, Inc., Boca Raton, Fla.

276. **Murphy, P. J., S. P. Trenz, W. Grzemski, F. J. de Bruijn, and J. Schell.** 1993. The *Rhizobium meliloti* rhizopine *mos* locus is a mosaic structure facilitating its symbiotic regulation. J. Bacteriol. **175:**5193–5204.

277. **Murry, M. A., J. Stigter, and F. J. de Bruijn.** 1993. PCR-based

cloning and sequence analysis of a *fixL* homologue from *Frankia* species strain CeS15, p. 485. *In* R. Palacios, J. Mora, and W. E. Newton (ed.), New horizons in nitrogen fixation, Kluwer Academic Publishers, Dordrecht, The Netherlands.

278. **Nees, D. W., P. A. Stein, and R. A. Ludwig.** 1988. The *Azorhizobium caulinodans nifA* gene: identification of upstream-activating sequences including a new element, the "anaerobox." Nucleic Acids Res. **16:**9839–9853.
279. **Newton, A., and N. Ohta.** 1990. Regulation of the cell division cycle and differentiation in bacteria. Annu. Rev. Microbiol. **44:**689–719.
280. **Niehaus, K., D. Kapp, and A. Pühler.** 1993. Plant defence and delayed infection of alfalfa pseudonodules induced by an exopolysaccharide (EPS I)-deficient *Rhizobium meliloti* mutant. Planta **190:**415–425.
281. **Ninfa, A. J., E. G. Ninfa, A. N. Lupas, A. Stock, B. Magasanik, and J. Stock.** 1988. Crosstalk between bacterial chemotaxis signal transduction proteins and regulators of transcription of the Ntr regulon: evidence that nitrogen assimilation and chemotaxis are controlled by a common phosphotransfer mechanism. Proc. Natl. Acad. Sci. USA **85:**5492–5496.
282. **Nixon, B. T., C. W. Ronson, and F. M. Ausubel.** 1986. Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes *ntrB* and *ntrC*. Proc. Natl. Acad. Sci. USA **83:**7850–7854.
283. **Noonan, B., M. Motherway, and F. O'Gara.** 1992. Ammonia regulation of the *Rhizobium meliloti* nitrogenase structural and regulatory genes under free-living conditions: involvement of the *fixL* gene product? Mol. Gen. Genet. **234:**423–428.
284. **Norel, F., N. Desnoues, and C. Elmerich.** 1985. Characterization of DNA sequences homologous to *Klebsiella pneumoniae nifH,D,K* and *E* in the tropical *Rhizobium* ORS571. Mol. Gen. Genet. **199:**352–356.
285. **Norel, F., and C. Elmerich.** 1987. Nucleotide sequence and functional analysis of the two *nifH* copies of *Rhizobium* ORS571. J. Gen. Microbiol. **133:**1563–1576.
286. **North, A. K., K. E. Klose, K. M. Stedman, and S. Kustu.** 1993. Prokaryotic enhancer-binding proteins reflect eukaryote-like modularity: the puzzle of nitrogen regulatory protein C. J. Bacteriol. **175:**4267–4273.
287. **Noti, J. D., O. Folkerts, A. N. Turken, and A. A. Szalay.** 1986. Organization and characterization of genes essential for symbiotic nitrogen fixation from *Bradyrhizobium japonicum* I110. J. Bacteriol. **167:**774–783.
288. **Osuna, R., and R. A. Bender.** 1991. *Klebsiella aerogenes* catabolite gene activator protein and the gene encoding it (*crp*). J. Bacteriol. **173:**6626–6631.
289. **Parkinson, J. S.** 1993. Signal transduction schemes of bacteria. Cell **73:**857–871.
290. **Parkinson, J. S., and E. C. Kofoid.** 1992. Communication modules in bacterial signaling proteins. Annu. Rev. Genet. **26:**71–112.
291. **Parniske, M., H. M. Fischer, H. Hennecke, and D. Werner.** 1991. Accumulation of the phytoalexin glyceollin I in soybean nodules infected by a *Bradyrhizobium japonicum nifA* mutant. Z. Naturforsch. Sect. C **46:**318–320.
292. **Parniske, M., K. Kosch, D. Werner, and P. Müller.** 1993. ExoB mutants of *Bradyrhizobium japonicum* with reduced competitiveness for nodulation of *Glycine max*. Mol. Plant-Microbe Interact. **6:**99–106.
293. **Patriarca, E. J., A. Riccio, R. Taté, S. Colonna-Romano, M. Iaccarino, and R. Defez.** 1993. The *ntrBC* genes of *Rhizobium leguminosarum* are part of a complex operon subject to negative regulation. Mol. Microbiol. **9:**569–577.
294. **Paul, W., and M. J. Merrick.** 1989. The roles of the *nifW*, *nifZ* and *nifM* genes of *Klebsiella pneumoniae* in nitrogenase biosynthesis. Eur. J. Biochem. **178:**675–682.
295. **Pawlowski, K., U. Klosse, and F. J. de Bruijn.** 1991. Characterization of a novel *Azorhizobium caulinodans* ORS571 two-component regulatory system, NtrY/NtrX, involved in nitrogen fixation and metabolism. Mol. Gen. Genet. **231:**124–138.
296. **Pawlowski, K., P. Ratet, J. Schell, and F. J. de Bruijn.** 1987. Cloning and characterisation of *nifA* and *ntrC* genes of the stem nodulating bacterium ORS571, the nitrogen fixing symbiont of *Sesbania rostrata*: regulation of nitrogen fixation (*nif*) genes in the free-living versus symbioyic state. Mol. Gen. Genet. **206:**207–219.
297. **Phillips-Jones, M. K., and C. N. Hunter.** 1994. Cloning and nucleotide sequence of *regA*, a putative response regulator gene of *Rhodobacter sphaeroides*. FEMS Microbiol. Lett. **116:**269–276.
298. **Popham, D. L., D. Szeto, J. Keener, and S. Kustu.** 1989. Function of a bacterial activator protein that binds to transcriptional enhancers. Science **243:**629–635.
299. **Preisig, O., D. Anthamatten, and H. Hennecke.** 1993. Genes for a microaerobically induced oxidase complex in *Bradyrhizobium japonicum* are essential for a nitrogen-fixing endosymbiosis. Proc. Natl. Acad. Sci. USA **90:**3309–3313.
300. **Preker, P., P. Hübner, M. Schmehl, W. Klipp, and T. A. Bickle.** 1992. Mapping and characterization of the promoter elements of the regulatory *nif* genes *rpoN*, *nifA1* and *nifA2* in *Rhodobacter capsulatus*. Mol. Microbiol. **6:**1035–1047.

300a. **Priefer, U.** Personal communication.

301. **Pühler, A., M. O. Aguilar, M. Hynes, P. Müller, W. Klipp, U. Priefer, R. Simon, and G. Weber.** 1984. Advances in the genetics of free-living and symbiotic nitrogen fixing bacteria, p. 609–619. *In* C. Veeger and W. E. Newton (ed.), Advances in nitrogen fixation research. Martinus Nijhoff, The Hague, The Netherlands.
302. **Pühler, A., T. Engelke, D. Jording, and P. K. Sharma.** 1990. Regulation of the C_4-dicarboxylate transport in free-living and symbiotic *Rhizobium meliloti*, p. 449–450. *In* P. M. Gresshoff, L. E. Roth, G. Stacey, and W. E. Newton (ed.), Nitrogen fixation: achievements and objectives. Chapman & Hall, New York.
303. **Quinto, C., H. de la Vega, M. Flores, J. Leemans, M. A. Cevallos, M. A. Pardo, R. Azpiroz, M. L. Girard, E. Calva, and R. Palacios.** 1985. Nitrogenase reductase: a functional multigene family in *Rhizobium phaseoli*. Proc. Natl. Acad. Sci. USA **82:**1170–1174.
304. **Ratet, P., K. Pawlowski, J. Schell, and F. J. de Bruijn.** 1989. The *Azorhizobium caulinodans* nitrogen-fixation regulatory gene, *nifA*, is controlled by the cellular nitrogen and oxygen status. Mol. Microbiol. **3:**825–838.
305. **Regensburger, B., and H. Hennecke.** 1983. RNA polymerase from *Rhizobium japonicum*. Arch. Microbiol. **135:**103–109.
306. **Reitzer, L. J., and B. Magasanik.** 1987. Ammonia assimilation and the biosynthesis of glutamine, glutamate, aspartate, asparagine, L-alanine, and D-alanine, p. 302–320. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
307. **Reizer, J., A. Reizer, M. H. Saier, and G. R. Jacobson.** 1992. A proposed link between nitrogen and carbon metabolism involving protein phosphorylation in bacteria. Protein Sci. **1:**722–726.
308. **Renalier, M.-H., J. Batut, J. Ghai, B. Terzaghi, M. Ghérardi, M. David, A.-M. Garnerone, J. Vasse, G. Truchet, T. Huguet, and P. Boistard.** 1987. A new symbiotic cluster on the pSym megaplasmid of *Rhizobium meliloti* 2011 carries a functional *fix* gene repeat and a *nod* locus. J. Bacteriol. **169:**2231–2238.
309. **Reuber, T. L., J. Reed, J. Glazebrook, M. A. Glucksmann, D. Ahmann, A. Marra, and G. C. Walker.** 1991. *Rhizobium meliloti* exopolysaccharides: genetic analyses and symbiotic importance. Biochem. Soc. Trans. **19:**636–641.
310. **Reuber, T. L., and G. C. Walker.** 1993. Biosynthesis of succinoglycan, a symbiotically important exopolysaccharide of *Rhizobium meliloti*. Cell **74:**269–280.
311. **Reyrat, J.-M., M. David, J. Batut, and P. Boistard.** 1994. FixL of *Rhizobium meliloti* enhances the transcriptional activity of a mutant FixD54N protein by phosphorylation of an alternate residue. J. Bacteriol. **176:**1969–1976.
312. **Reyrat, J.-M., M. David, C. Blonski, P. Boistard, and P. Batut.** 1993. Oxygen-regulated in vitro transcription of *Rhizobium meliloti nifA* and *fixK* genes. J. Bacteriol. **175:**6867–6872.
313. **Reyrat, J. M., M. David, P. de Philip, A. M. Garnerone, J. Batut, and P. Boistard.** 1993. *In vivo* and *in vitro* oxygen control of nitrogen fixation gene expression in *Rhizobium meliloti*, p. 399–403. *In* R. Palacios, J. Mora, and W. E. Newton (ed.), New horizons in nitrogen fixation. Kluwer Academic Publishers, Dor-

drecht, The Netherlands.

314. **Roberts, G. P., and P. W. Ludden.** 1992. Nitrogen fixation by photosynthetic bacteria, p. 135–165. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
315. **Roche, P., F. Debellé, P. Lerouge, J. Vasse, G. Truchet, J. C. Promé, and J. Dénarié.** 1992. The lipo-oligosaccharide symbiotic signals of *Rhizobium meliloti*. Biochem. Soc. Trans. **20:**288–291.
316. **Roelvink, P. W., J. G. J. Hontelez, A. van Kammen, and R. C. van den Bos.** 1989. Nucleotide sequence of the regulatory *nifA* gene of *Rhizobium leguminosarum* PRE: transcriptional control sites and expression in *Escherichia coli*. Mol. Microbiol. **3:**1441–1447.
317. **Ronson, C. W.** 1988. Genetic regulation of C_4-dicarboxylate transport in rhizobia, p. 547–551. *In* H. Bothe, F. J. de Bruijn, and W. E. Newton (ed.), Nitrogen fixation: hundred years after. Gustav Fischer Verlag, Stuttgart, Germany.
318. **Ronson, C. W., P. M. Astwood, B. T. Nixon, and F. M. Ausubel.** 1987. Deduced products of C_4-dicarboxylate transport regulatory genes of *Rhizobium leguminosarum* are homologous to nitrogen regulatory gene products. Nucleic Acids Res. **15:**7921–7934.
319. **Ronson, C. W., B. T. Nixon, L. M. Albright, and F. M. Ausubel.** 1987. *Rhizobium meliloti ntrA* (*rpoN*) gene is required for diverse metabolic functions. J. Bacteriol. **169:**2424–2431.
320. **Ronson, C. W., B. T. Nixon, and F. M. Ausubel.** 1987. Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. Cell **49:**579.
321. **Rosenberg, C., P. Boistard, J. Dénarié, and F. Casse-Delbart.** 1981. Genes controlling early and late functions in symbiosis are located on a megaplasmid in *Rhizobium meliloti*. Mol. Gen. Genet. **184:**326–333.
322. **Rossbach, S., and H. Hennecke.** 1991. Identification of *glyA* as a symbiotically essential gene in *Bradyrhizobium japonicum*. Mol. Microbiol. **5:**39–47.
323. **Rossbach, S., H. Loferer, G. Acuña, C. A. Appleby, and H. Hennecke.** 1991. Cloning, sequencing and mutational analysis of the cytochrome c_{552} gene (*cycB*) from *Bradyrhizobium japonicum* strain 110. FEMS Microbiol. Lett. **83:**145–152.
324. **Rossbach, S., J. Schell, and F. J. de Bruijn.** 1987. The *ntrC* gene of *Agrobacterium tumefaciens* C58 controls glutamine synthetase (GSII) activity, growth on nitrate and chromosomal but not Ti-encoded arginine catabolism pathways. Mol. Gen. Genet. **209:**419–426.
325. **Rusanganwa, E., and R. S. Gupta.** 1993. Cloning and characterization of multiple *groEL* chaperonin-encoding genes in *Rhizobium meliloti*. Gene **126:**67–75.
326. **Ruvkun, G. B., V. Sundaresan, and F. Ausubel.** 1982. Directed transposon Tn*5* mutagenesis and complementation analysis of *Rhizobium meliloti* symbiotic nitrogen fixation genes. Cell **29:**551–559.
327. **Saffarini, D. A., and K. H. Nealson.** 1993. Sequence and genetic characterization of *etrA*, an *fnr* analog that regulates anaerobic respiration in *Shewanella putrefaciens* MR-1. J. Bacteriol. **175:**7938–7944.
328. **Saint, C. P., M. Wexler, P. J. Murphy, J. Tempé, and M. E. Tate.** 1993. Characterization of genes for synthesis and catabolism of a new rhizopine induced in nodules by *Rhizobium meliloti* Rm220-3: extension of the rhizopine concept. J. Bacteriol. **175:**5205–5215.
329. **Sanchez, F., J. E. Padilla, H. Perez, and M. Lara.** 1991. Control of nodulin genes in root-nodule development and metabolism. Annu. Rev. Plant. Physiol. Plant Mol. Biol. **42:**507–528.
330. **Sanders, D. A., B. L. Gillece-Castro, A. L. Burlingame, and D. E. Koshland.** 1992. Phosphorylation site of NtrC, a protein phosphatase whose covalent intermediate activates transcription. J. Bacteriol. **174:**5117–5122.
331. **Sanders, D. A., B. L. Gillece-Castro, A. M. Stock, A. L. Burlingame, and D. E. Koshland.** 1989. Identification of the site of phosphorylation of the chemotaxis response regulator protein, CheY. J. Biol. Chem. **264:**21770–21778.
332. **San Francisco, M. J. D., and G. J. Jacobson.** 1985. Uptake of succinate and malate in cultured cells and bacteroids of two slow-growing species of *Rhizobium*. J. Gen. Microbiol. **131:**765–773.
333. **Sanjuan, J., and J. Olivares.** 1989. Implication of *nifA* in regulation of genes located on a *Rhizobium meliloti* cryptic plasmid that affect nodulation efficiency. J. Bacteriol. **171:**4154–4161.
334. **Sanjuan, J., and J. Olivares.** 1991. NifA-NtrA regulatory system activates transcription of *nfe*, a gene locus involved in nodulation competitiveness of *Rhizobium meliloti*. Arch. Microbiol. **155:**543–548.
335. **Sanjuan, J., and J. Olivares.** 1991. Multicopy plasmids carrying the *Klebsiella pneumoniae nifA* gene enhance *Rhizobium meliloti* nodulation competitiveness on alfalfa. Mol. Plant-Microbe Interact. **4:**365–369.
336. **Santero, E., T. Hoover, J. Keener, and S. Kustu.** 1989. *In vitro* activity of the nitrogen fixation regulatory protein NifA. Proc. Natl. Acad. Sci. USA **86:**7346–7350.
337. **Santero, E., T. R. Hoover, A. K. North, D. K. Berger, S. C. Porter, and S. Kustu.** 1992. Role of integration host factor in stimulating transcription from the σ^{54}-dependent *nifH* promoter. J. Mol. Biol. **227:**602–620.
338. **Sawers, R. G.** 1991. Identification and molecular characterization of a transcriptional regulator from *Pseudomonas aeruginosa* PAO1 exhibiting structural and functional similarity to the FNR protein of *Escherichia coli*. Mol. Microbiol. **5:**1469–1481.
339. **Schaffner, E., C. Walther, and H. M. Fischer.** Unpublished data.
340. **Schlaman, H. R. M., R. J. H. Okker, and B. J. J. Lugtenberg.** 1992. Regulation of nodulation gene expression by NodD in rhizobia. J. Bacteriol. **174:**5177–5182.
341. **Schlensog, V., and A. Böck.** 1990. Identification and sequence analysis of the gene encoding the transcriptional activator of the formate hydrogenlyase system of *Escherichia coli*. Mol. Microbiol. **4:**1319–1327.
342. **Schlüter, A., T. Patschkowski, G. Unden, and U. B. Priefer.** 1992. The *Rhizobium leguminosarum* FnrN protein is functionally similar to *Escherichia coli* Fnr and promotes heterologous oxygen-dependent activation of transcription. Mol. Microbiol. **6:**3395–3404.
343. **Schroeder, C. J., and W. J. Dobrogosz.** 1986. Cloning and DNA sequence analysis of the wild-type and mutant cyclic AMP receptor protein genes from *Salmonella typhimurium*. J. Bacteriol. **167:**616–622.
344. **Schüddekopf, K., S. Hennecke, U. Liese, M. Kutsche, and W. Klipp.** 1993. Characterization of *anf* genes specific for the alternative nitrogenase and identification of *nif* genes required for both nitrogenases in *Rhodobacter capsulatus*. Mol. Microbiol. **8:**673–684.
345. **Schultz, S. C., G. C. Shields, and T. A. Steitz.** 1991. Crystal structure of a CAP-DNA complex: the DNA is bent by 90°. Science **253:**1001–1007.
346. **Sganga, M. W., and C. E. Bauer.** 1992. Regulatory factors controlling photosynthetic reaction center and light harvesting gene expression in *Rhodobacter capsulatus*. Cell **68:**945–954.
347. **Sharrocks, A. D., J. Green, and J. R. Guest.** 1990. In vivo and in vitro mutants of FNR the anaerobic transcriptional regulator of *E. coli*. FEBS Lett. **270:**119–122.
348. **Sharrocks, A. D., J. Green, and J. R. Guest.** 1991. FNR activates and represses transcription *in vitro*. Proc. R. Soc. London Ser. B **245:**219–226.
349. **Shaw, D. J., and J. R. Guest.** 1982. Nucleotide sequence of the *fnr* gene and primary structure of the Fnr protein of *Escherichia coli*. Nucleic Acids Res. **10:**6119–6130.
350. **Shaw, D. J., D. W. Rice, and J. R. Guest.** 1983. Homology between CAP and FNR, a regulator of anaerobic respiration in *Escherichia coli*. J. Mol. Biol. **166:**241–247.

350a. **Shigematsu, T., M. Hidaka, H. Masaki, and T. Uozumi.** 1992. EMBL database accession no. D13799.

351. **Smit, G., S. Swart, B. J. J. Lugtenberg, and J. W. Kijne.** 1992. Molecular mechanisms of attachment of *Rhizobium* bacteria to plant roots. Mol. Microbiol. **6:**2897–2903.
352. **Sobral, B. W. S., R. J. Honeycutt, A. G. Atherly, and M. McClelland.** 1991. Electrophoretic separation of the three *Rhizobium meliloti* replicons. J. Bacteriol. **173:**5173–5180.
353. **Somerville, J. E., and B. K. Chelm.** 1988. Regulation of heme biosynthesis in *Bradyrhizobium japonicum*, p. 111–112. *In* R. Palacios and D. P. S. Verma (ed.), Molecular genetics of

plant-microbe interactions. APS Press, St. Paul, Minn.

354. **Soto, M. J., A. Zorzano, J. Mercadoblanco, V. Lepek, J. Olivares, and N. Toro.** 1993. Nucleotide sequence and characterization of *Rhizobium meliloti* nodulation competitiveness genes *nfe*. J. Mol. Biol. **229:**570–576.
355. **Souza, E. M., S. Funayama, L. U. Rigo, M. G. Yates, and F. O. Pedrosa.** 1991. Sequence and structural organization of a *nifA*-like gene and part of a *nifB*-like gene of *Herbaspirillum seropedicae* strain Z78. J. Gen. Microbiol. **137:**1511–1522.
356. **Spiro, S., and J. R. Guest.** 1987. Regulation and over-expression of the *fnr* gene of *Escherichia coli*. J. Gen. Microbiol. **133:**3279–3288.
357. **Spiro, S., and J. R. Guest.** 1988. Inactivation of the FNR protein of *Escherichia coli* by targeted mutagenesis in the *N*-terminal region. Mol. Microbiol. **701:**707.
358. **Spiro, S., and J. R. Guest.** 1990. FNR and its role in oxygen-regulated gene expression in *Escherichia coli*. FEMS Microbiol. Rev. **75:**399–428.
359. **Spiro, S., R. E. Roberts, and J. R. Guest.** 1989. FNR-dependent repression of the *ndh* gene of *Escherichia coli* and metal ion requirement for FNR-regulated gene expression. Mol. Microbiol. **3:**601–608.
360. **Stanley, J., J. van Slooten, D. N. Dowling, T. Finan, and W. J. Broughton.** 1989. Molecular cloning of the *ntrA* gene of the broad host-range *Rhizobium* sp. NGR234, and phenotypes of a site-directed mutant. Mol. Gen. Genet. **217:**528–532.
361. **Stewart, V., and J. Parales.** 1988. Identification and expression of genes *narL* and *narX* of the *nar* (nitrate reductase) locus in *Escherichia coli* K-12. J. Bacteriol. **170:**1589–1597.
362. **Stigter, J., M. Schneider, and F. J. de Bruijn.** 1993. *Azorhizobium caulinodans* nitrogen fixation (*nif*/*fix*) gene regulation: mutagenesis of the *nifA* −24/−12 promoter element, characterization of a *ntrA* (*rpoN*) gene, and derivation of a model. Mol. Plant-Microbe Interact. **6:**238–252.
363. **Stock, J., A. J. Ninfa, and A. M. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53:**450–490.
364. **Stock, J. B., A. M. Stock, and J. M. Mottonen.** 1990. Signal transduction in bacteria. Nature (London) **344:**395–400.
365. **Stoker, K., W. N. M. Reijnders, L. F. Oltman, and A. H. Stouthamer.** 1989. Initial cloning and sequencing of *hydHG*, an operon homologous to *ntrBC* and regulating the labile hydrogenase activity in *Escherichia coli* K-12. J. Bacteriol. **171:**4448–4456.
366. **Storz, G., L. A. Tartaglia, and B. N. Ames.** 1990. Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. Science **248:**189–194.
367. **Storz, G., L. A. Tartaglia, S. B. Farr, and B. N. Ames.** 1990. Bacterial defenses against oxidative stress. Trends Genet. **6:**363–368.
368. **Stout, V., and S. Gottesman.** 1990. RscB and RscC: a two-component regulator of capsule synthesis in *Escherichia coli*. J. Bacteriol. **172:**659–669.
369. **Studer, D., T. Gloudemans, H. J. Franssen, H. M. Fischer, T. Bisseling, and H. Hennecke.** 1987. Involvement of the bacterial nitrogen fixation regulatory gene (*nifA*) in control of nodule-specific host-plant gene expression. Eur. J. Cell Biol. **45:**177–184.
370. **Su, W., S. Porter, S. Kustu, and H. Echols.** 1990. DNA-looping and enhancer activity: association between DNA-bound NtrC activator and RNA polymerase at the bacterial *glnA* promoter. Proc. Natl. Acad. Sci. USA **87:**5504–5508.
371. **Sundaresan, V., J. D. Jones, D. W. Ow, and F. Ausubel.** 1983. *Klebsiella pneumoniae nifA* product activates the *Rhizobium meliloti* nitrogenase promoter. Nature (London) **301:**728–732.
372. **Szeto, W. W., B. T. Nixon, C. W. Ronson, and F. M. Ausubel.** 1987. Identification and characterization of the *Rhizobium meliloti ntrC* gene: *R. meliloti* has separate regulatory pathways for activating nitrogen fixation genes in free-living and symbiotic cells. J. Bacteriol. **169:**1423–1432.
373. **Szeto, W. W., J. L. Zimmerman, V. Sundaresan, and F. M. Ausubel.** 1984. A *Rhizobium meliloti* symbiotic regulatory gene. Cell **36:**1035–1043.
374. **Takahashi, K., T. Hattori, T. Nakanishi, T. Nohno, N. Fujita, A. Ishihama, and S. Taniguchi.** 1994. Repression of in vitro transcription of the *Escherichia coli fnr* and *narX* genes by Fnr protein. FEBS Lett. **340:**59–64.
375. **Thöny, B.** 1989. Structure and genetic control of a regulatory operon (*fixRnifA*) essential for symbiotic nitrogen fixation in *Bradyrhizobium japonicum*. Ph.D. dissertation ETH 8859. Eidgenössische Technische Hochschule, Zürich.
376. **Thöny, B., D. Anthamatten, and H. Hennecke.** 1989. Dual control of the *Bradyrhizobium japonicum* symbiotic nitrogen fixation regulatory operon *fixRnifA*: analysis of *cis*- and *trans*-acting elements. J. Bacteriol. **171:**4162–4169.
377. **Thöny, B., H. M. Fischer, D. Anthamatten, T. Bruderer, and H. Hennecke.** 1987. The symbiotic nitrogen fixation regulatory operon (*fixRnifA*) of *Bradyrhizobium japonicum* is expressed aerobically and is subject to a novel, *nifA*-independent type of activation. Nucleic Acids Res. **15:**8479–8499.
378. **Thöny, B., and H. Hennecke.** 1989. The −24/−12 promoter comes of age. FEMS Microbiol. Rev. **63:**341–358.
379. **Thöny, B., K. Kaluza, and H. Hennecke.** 1985. Structural and functional homology between the α and β subunits of the nitrogenase MoFe protein as revealed by sequencing the *Rhizobium japonicum nifK* gene. Mol. Gen. Genet. **198:**441–448.
380. **Toro, N., M. A. Herrera, and J. Olivares.** 1986. Characterization of a large plasmid of *Rhizobium meliloti* involved in enhancing nodulation. Mol. Gen. Genet. **202:**331–335.
381. **Török, I., and A. Kondorosi.** 1981. Nucleotide sequence of the *R. meliloti* nitrogenase reductase (*nifH*) gene. Nucleic Acids Res. **9:**5711–5723.
382. **Toukdarian, A., and C. Kennedy.** 1986. Regulation of nitrogen metabolism in *Azotobacter vinelandii*: isolation of *ntr* and *glnA* genes and construction of *ntr* mutants. EMBO J. **5:**399–407.
383. **Toussaint, B., C. Bosc, P. Richaud, A. Colbeau, and P. M. Vignais.** 1991. A mutation in a *Rhodobacter capsulatus* gene encoding an integration host factor-like protein impairs *in vivo* hydrogenase expression. Proc. Natl. Acad. Sci. USA **88:**10749–10753.
384. **Toussaint, B., I. Delic-Attree, R. de Sury d'Aspremont, L. David, M. Vinçon, and P. M. Vignais.** 1993. Purification of the integration host factor homolog of *Rhodobacter capsulatus*: cloning and sequencing of the *hip* gene, which encodes the β subunit. J. Bacteriol. **175:**6499–6504.
385. **Trageser, M., S. Spiro, A. Duchêne, E. Kojro, F. Fahrenholz, J. R. Guest, and G. Unden.** 1990. Isolation of intact FNR protein (M_r 30,000) of *Escherichia coli*. Mol. Microbiol. **4:**21–27.
386. **Tuli, R., and M. J. Merrick.** 1988. Over-production and characterisation of the *nifA* gene product of *Klebsiella pneumoniae*—the transcription activator of *nif* gene expression. J. Gen. Microbiol. **134:**425–432.
387. **Udvardi, M. K., D. L. Lister, and D. A. Day.** 1992. Isolation and characterization of a *ntrC* mutant of *Bradyrhizobium* (*Parasponia*) sp. ANU289. J. Gen. Microbiol. **138:**1019–1025.
388. **Unden, G., and M. Tragesser.** 1991. Oxygen regulated gene expression in *Escherichia coli*: control of anaerobic respiration by the FNR protein. Antonie Leeuwenhoek **59:**65–76.
389. **Unden, G., M. Tragesser, and A. Duchêne.** 1990. Effect of positive redox potentials (>+400 mV) on the expression of anaerobic respiratory enzymes in *Escherichia coli*. Mol. Microbiol. **4:**315–319.
390. **Vance, C. P.** 1983. *Rhizobium* infection and nodulation: a beneficial plant disease? Annu. Rev. Microbiol. **37:**399–424.
391. **van Slooten, J. C., E. Cervantes, W. J. Broughton, C. H. Wong, and J. Stanley.** 1990. Sequence and analysis of the *rpoN* sigma factor gene of *Rhizobium* sp. strain NGR234, a primary coregulator of symbiosis. J. Bacteriol. **172:**5563–5574.
392. **Van Soom, C., C. Verreth, M. J. Sampaio, and J. Vanderleyden.** 1993. Identification of a potential transcriptional regulator of hydrogenase activity in free-living *Bradyrhizobium japonicum* strains. Mol. Gen. Genet. **239:**235–240.
393. **Vasse, J., F. de Billy, S. Camut, and G. Truchet.** 1990. Correlation between ultrastructural differentiation of bacteroids and nitrogen fixation in alfalfa nodules. J. Bacteriol. **172:**4295–4306.
394. **Vega-Palas, M. A., E. Flores, and A. Herrero.** 1992. NtcA, a global nitrogen regulator from the cyanobacterium *Synechococcus* that belongs to the Crp family of bacterial regulators. Mol.

Microbiol. **6**:1853–1859.
395. **Verma, D. P. S., A. Hu, and M. Zhang.** 1992. Root nodule development: origin, function and regulation of nodulin genes. Physiol. Plant. **85**:253–265.
396. **Virts, E. L., S. W. Stanfield, D. R. Helinski, and G. S. Ditta.** 1988. Common regulatory elements control symbiotic and microaerobic induction of *nifA* in *Rhizobium meliloti*. Proc. Natl. Acad. Sci. USA **85**:3062–3065.
397. **Waelkens, F., A. Foglia, J. B. Morel, J. Fourment, J. Batut, and P. Boistard.** 1992. Molecular genetic analysis of the *Rhizobium meliloti fixK* promoter: identification of sequences involved in positive and negative regulation. Mol. Microbiol. **6**:1447–1456.
398. **Wallington, E. J., and P. A. Lund.** 1994. *Rhizobium leguminosarum* contains multiple chaperonin (*cpn60*) genes. Microbiology **140**:113–122.
399. **Wang, S.-P., and G. Stacey.** 1990. Ammonia regulation of *nod* genes in *Bradyrhizobium japonicum*. Mol. Gen. Genet. **223**:329–331.
400. **Wang, Y.-P., K. Birkenhead, B. Boesten, S. Manian, and F. O'Gara.** 1989. Genetic analysis and regulation of the *Rhizobium meliloti* genes controlling C_4-dicarboxylic acid transport. Gene **85**:135–144.
401. **Watson, R. J.** 1990. Analysis of the C_4-dicarboxylate transport genes of *Rhizobium meliloti*: nucleotide sequence and deduced products of *dctA*, *dctB*, and *dctD*. Mol. Plant-Microbe Interact. **3**:174–181.
402. **Watson, R. J., Y.-K. Chan, R. Wheatcroft, A.-F. Yang, and S. Han.** 1988. *Rhizobium meliloti* genes required for C_4-dicarboxylate transport and symbiotic nitrogen fixation are located on a megaplasmid. J. Bacteriol. **170**:927–934.
403. **Weber, G., H. Reiländer, and A. Pühler.** 1985. Mapping and expression of a regulatory nitrogen fixation gene (*fixD*) of *Rhizobium meliloti*. EMBO J. **4**:2751–2756.
404. **Weber, I. T., and T. A. Steitz.** 1987. Structure of a complex of catabolite gene activator protein and cyclic AMP refined at 2.5 angstrom resolution. J. Mol. Biol. **198**:311–326.
405. **Wei, T. F., T. S. Ramasubramanian, F. Pu, and J. W. Golden.** 1993. *Anabaena* sp. strain PCC 7120 *bifA* gene encoding a sequence-specific DNA-binding protein cloned by in vivo transcriptional interference selection. J. Bacteriol. **175**:4025–4035.
406. **Weidenhaupt, M., H. M. Fischer, G. Acuña, J. Sanjuan, and H. Hennecke.** 1993. Use of a promoter-probe vector system in the cloning of a new NifA-dependent promoter (*ndp*) from *Bradyrhizobium japonicum*. Gene **129**:33–40.
407. **Weinrauch, Y., N. Guillen, and D. Dubnau.** 1989. Sequence and transcription mapping of *Bacillus subtilis* competence genes *comB* and *comA*, one of which is related to a family of bacterial regulatory determinants. J. Bacteriol. **171**:5362–5375.
408. **Weinrauch, Y., R. Penchev, E. Dubnau, I. Smith, and D. Dubnau.** 1990. A *Bacillus subtilis* regulatory gene product for genetic competence and sporulation resembles sensor protein members of the bacterial two-component signal-transduction systems. Genes Dev. **4**:860–872.
409. **Weinstein, M., A. F. Lois, G. S. Ditta, and D. R. Helinski.** 1993. Mutants of the two-component regulatory protein FixJ of *Rhizobium meliloti* that have increased activity at the *nifA* promoter. Gene **134**:145–152.
410. **Weinstein, M., A. F. Lois, E. K. Monson, G. S. Ditta, and D. R. Helinski.** 1992. Isolation of phosphorylation-deficient mutants of the *Rhizobium meliloti* two-component regulatory protein, FixJ. Mol. Microbiol. **6**:2041–2049.
411. **Weiss, D. S., J. Batut, K. E. Klose, J. Keener, and S. Kustu.** 1991. The phosphorylated form of the enhancer-binding protein NTRC has an ATPase activity that is essential for activation of transcription. Cell **67**:155–167.
412. **Weiss, D. S., K. E. Klose, T. R. Hoover, A. K. North, S. C. Porter, A. B. Wedel, and S. Kustu.** 1992. Prokaryotic transcriptional enhancers, p. 667–694. *In* S. L. McKnight and K. R. Yamal (ed.), Transcriptional regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
413. **Werner, D.** 1992. Physiology of nitrogen-fixing legume nodules: compartments and functions, p. 399–431. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
414. **Weston, L. A., and R. J. Kadner.** 1988. Role of *uhp* genes in expression of the *Escherichia coli* sugar-phosphate transport system. J. Bacteriol. **170**:3375–3383.
415. **Whitehall, S., S. Austin, and R. Dixon.** 1993. The function of the upstream region of the σ^{54}-dependent *Klebsiella pneumoniae nifL* promoter is sensitive to DNA supercoiling. Mol. Microbiol. **9**:1107–1117.
416. **Wootton, J. C., and M. Drummond.** 1989. The Q-linker: a class of interdomain sequences found in bacterial multidomain regulatory proteins. Protein Eng. **2**:535–543.
417. **Yang, W. C., B. Horvath, J. Hontelez, A. van Kammen, and T. Bisseling.** 1991. *In situ* localization of *Rhizobium* mRNAs in pea nodules: *nifA* and *nifH* localization. Mol. Plant-Microbe Interact. **4**:464–468.
418. **Young, J. P. W.** 1992. Phylogenetic classification of nitrogen-fixing organisms, p. 43–86. *In* G. Stacey, R. H. Burris, and H. J. Evans (ed.), Biological nitrogen fixation. Chapman & Hall, New York.
419. **Yura, T., H. Mori, H. Nagai, T. Nagata, A. Ishihama, N. Fujita, K. Isono, K. Mizobuchi, and A. Nakata.** 1992. Systematic sequencing of the *Escherichia coli* genome: analysis of the 0-2.4 min region. Nucleic Acids Res. **13**:3305–3308.
420. **Yura, T., H. Nagai, and H. Mori.** 1993. Regulation of the heat-shock response in bacteria. Annu. Rev. Microbiol. **47**:321–350.
421. **Zheng, L. M., R. H. White, V. L. Cash, R. F. Jack, and D. R. Dean.** 1993. Cysteine desulfurase activity indicates a role for NIFS in metallocluster biosynthesis. Proc. Natl. Acad. Sci. USA **90**:2754–2758.
422. **Zimmerman, J. L., W. W. Szeto, and F. Ausubel.** 1983. Molecular characterization of Tn*5*-induced symbiotic (Fix$^-$) mutants of *Rhizobium meliloti*. J. Bacteriol. **156**:1025–1034.
423. **Zimmermann, A., C. Reimann, M. Galimand, and D. Haas.** 1991. Anaerobic growth and cyanide synthesis of *Pseudomonas aeruginosa* depend on *anr*, a regulatory gene homologous with *fnr* of *Escherichia coli*. Mol. Microbiol. **5**:1483–1490.