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Expression of σ^{54} (*ntrA*)-Dependent Genes Is Probably United by a Common Mechanism

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σ^{54} IS REQUIRED TO TRANSCRIBE A DIVERSE SET OF GENES

In addition to the most abundant sigma factor, both gram-negative and gram-positive eubacteria employ alternative sigma factors that confer different promoter specificities on the core form of RNA polymerase (reviewed in reference 49). Several bacteriophages that infect each of these groups also encode sigma factors. Some of the alternative sigma factors allow transcription of genes whose products contribute to a common physiological response. For example, σ^{32} of enteric bacteria allows transcription of genes whose products are needed for protection from heat shock and certain other stresses (42, 76). Sigma F of enteric bacteria (2) and σ^{28} of *Bacillus subtilis* (47, 48), both of which confer the same promoter specificity on core polymerase, allow transcription of genes whose products are required for motility and chemotaxis. Some sigma factors allow transcription of genes whose products are required at a precise time, for example, at a particular time after bacteriophage infection (40, 64) or during the sporulation process of *B. subtilis* (73, 77). σ^{54} differs from other alternative sigma factors in that it is needed for transcription of genes whose products have diverse physiological roles (9, 27, 62, 111).

σ^{54} (encoded by *ntrA* [*glnF*, *rpoN*]) was identified as a positive regulatory factor needed for expression of the gene encoding glutamine synthetase, *glnA*, in enteric bacteria (39; reviewed in reference 74). It was later found to be required for expression of other genes whose products function in the assimilation of nitrogen. For example, σ^{54} is required for transcription of genes encoding amino acid transport components and degradative enzymes (1, 117; reviewed in reference 78) and genes whose products are needed for biological nitrogen fixation (75; reviewed in references 28 and 44). It is required for transcription of the nitrogen fixation (*nif*) genes from a number of bacteria, including *Klebsiella pneumoniae* (28, 44), *Rhodobacter capsulatus* (63a, 79; reviewed in reference 45), and members of the genera *Azotobacter* (114, 125), *Azospirillum* (99), *Rhizobium* (44, 111), and *Bradyrhizobium* (31).

Recently, it has become apparent that σ^{54}-holoenzyme transcribes genes whose products have different physiological functions (Table 1). Examples of such genes are (i) the *dctA* gene of rhizobia (111), which encodes a transport component for dicarboxylic acids; (ii) genes on the TOL (toluene) plasmid of *Pseudomonas putida* that encode proteins required for catabolism of toluene and xylenes (27, 57, 101); (iii) genes encoding two of the components of a formate-degradative pathway in *Escherichia coli* (9); (iv) genes encoding hydrogenases responsible for the oxidation of molecular hydrogen in *Alcaligenes eutrophus* and *Pseudomonas facilis* (52, 105, 106, 127; J. Warrelmann, D. Romermann, and B. Friedrich, personal communication); (v) genes encoding the hook and filament proteins of *Caulobacter* flagella (85, 89, 94); and (vi) genes encoding pilins in *Pseudomonas aeruginosa* (59, 61) and *Neisseria gonorrhoeae* (84) that allow these organisms to adhere to human epithelial cells (121, 128, 131). In the first four cases and in the case of the *Pseudomonas* pilin gene, the requirement for σ^{54} has been demonstrated directly by showing that transcription does not occur in mutant strains that lack this sigma factor (9, 27, 59, 106, 111; Table 1). In the latter two cases, the evidence for dependence on σ^{54} is less direct (Table 1). Although it has been proposed that transcription from the major promoter for the *puf* photosynthetic operon of *Rhodobacter capsulatus* is σ^{54} dependent (4), we think that this is unlikely because the proposed promoter lacks the minimal conserved features common to σ^{54}-dependent promoters (see below; reviewed in references 28 and 44). Moreover, disruption of the *nifR4* gene, which was recently demonstrated to encode a σ^{54} homolog in *Rhodobacter capsulatus* (63a), does not cause defects in photosynthesis (45; R. Kranz, personal communication).

INITIATION OF TRANSCRIPTION BY σ^{54}-HOLOENZYME DEPENDS ON ACTIVATOR PROTEINS

σ^{54}-Holoenzyme (σ^{54} associated with core RNA polymerase) recognizes and binds to the major *glnA* promoter. (All promoters that are recognized by σ^{54}-holoenzyme are characterized minimally by a conserved GC doublet that lies

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TABLE 1. Examples of σ^{54}-dependent genes[a]

Gene	Gene product(s)	Organisms[b]	Activator	Evidence[c]	Selected reference(s)[d]	Comments
glnA	Glutamine synthetase	Enteric bacteria, including *Escherichia coli*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*	NTRC	(i), (iii), (v), (vi), (vii)	38, 39, 51, 54, 66, 74, 75, 78	In *Azotobacter vinelandii*, synthesis of glutamine synthetase appears to be constitutive and is normal in *ntrA*$^-$ mutants (114, 125). Similarly, synthesis of glutamine synthetase is normal in *hno*$^-$ (*ntrA*$^-$) mutants of *Alcaligenes eutrophus* (105) and in *nifR4*$^-$ (*ntrA*$^-$) mutants of *Rhodobacter capsulatus* (45; Kranz, personal communication). In *Rhizobium meliloti* and *Agrobacterium tumefaciens*, synthesis of glutamine synthetase II (the plantlike or eucaryotic form) appears to be under σ^{54} control because *ntrC*$^-$ mutants lack this enzyme (113). By contrast, synthesis of glutamine synthetase I (the bacterial enzyme, product of the *glnA* gene) is not affected in *ntrC*$^-$ mutants (113). In *Bradyrhizobium japonicum*, synthesis of glutamine synthetase I does not appear to be σ^{54} dependent because there is not a σ^{54}-dependent promoter upstream of the *glnA* transcriptional start site (18).
		Pseudomonas aeruginosa	Unknown	(iii)	59	
nas	Assimilatory nitrate and nitrite reductases	*Klebsiella pneumoniae*	NTRC	(vi)	17	Since promoter sequences for *nas* genes (17) have not yet been identified, the possibility remains that the σ^{54} requirement for their transcription is indirect.
		Azotobacter vinelandii	NTRC	(iii), (vi)	114, 125	
		Rhizobium meliloti	NTRC	(iii), (vi)	111, 122	
		Agrobacterium tumefaciens	NTRC	(vi)	113	
		Azospirillum brasilense	NTRC	(vi)	99	
		Alcaligenes eutrophus	Unknown	(iii)	105, 106	
nifHDK, other *nif*	Dinitrogenase (*nifDK*) and dinitrogenase reductase (*nifH*), proteins required for synthesis of the iron-molybdenum cofactor of dinitrogenase (e.g., *nifBENV*) and for processing of dinitrogenase reductase to the active form (e.g., *nifM*)[e]	*Klebsiella pneumoniae*	NIFA	(i)–(iii), (v)–(vii)	75, 130; reviewed in ref. 28 and 44	NIFA is inactivated by oxygen. In *K. pneumoniae*, this inactivation appears to require the NIFL product (50, 81), whereas in rhizobia (8, 53) and bradyrhizobia (32, 33) it does not require a *nif*-specific product and in this sense appears to be direct.
		Azotobacter vinelandii	NIFA	(i), (iii), (vi)	5, 114, 115, 125	
		Azospirillum brasilense	NIFA	(vi)	99	
		Rhodobacter capsulatus	NIFA	(i), (iii), (vi), (vii)	63, 63a, 79; reviewed in ref. 45	
		Rhizobium meliloti	NIFA	(i)–(iii), (vi), (vii)[f]	111; reviewed in ref. 44	
		Bradyrhizobium japonicum	NIFA	(i), (ii), (iv), (vi), (vii)	31	
		Azorhizobium sesbaniae ORS571	NIFA	(vi)	98	
nifA	Activator of transcription for *nif* and some *fix* genes (see below); also required for the formation of determinate symbiosis (production of normal, healthy nodules in normal number) by *Bradyrhizobium japonicum* (31) and probably by *Rhizobium meliloti* (123, 133)	*Klebsiella pneumoniae*	NTRC	(i)–(vii)	3, 130; reviewed in ref. 28 and 44	Interestingly, transcription of *nifA* is dependent on σ^{54} in some organisms but not others. In *K. pneumoniae*, and probably in *A. brasilense* and *R. capsulatus*, *nifA* transcription is dependent on σ^{54} and NTRC and occurs under nitrogen-limiting conditions. Transcription of *nifA* in *B. japonicum* under symbiotic conditions also appears to be σ^{54} dependent, but NTRC is not the activator; neither the activator nor the relevant physiological signal for *nifA* transcription is known (124). In *A. vinelandii*, a free-living diazotroph, *nifA* transcription is not dependent on NTRC, and it is not known whether it is σ^{54} dependent (115, 125).
		Azospirillum brasilense	NTRC	—[g]	99	
		Rhodobacter capsulatus	NTRC	—[g]	45	
		Bradyrhizobium japonicum	Unknown, but not NTRC	(i), (ii), (vii)	124	

						In *Rhizobium meliloti*, the initial transcription of *nifA* under symbiotic conditions, which appears to be equivalent to transcription under microaerobic conditions in the free-living state, is independent of both NTRC and σ^{54} (25, 44, 126). Continued transcription of *nifA* occurs partly from a different promoter (the upstream *fixABCX* promoter [see below]); transcription from this promoter is autogenously activated by NIFA and is σ^{54} dependent (69).
fixABCX	The *fixX* gene of *Rhizobium trifolii* encodes a ferrodoxinlike protein (55). Although the products of the other genes are not known, there is circumstantial evidence that they are involved in electron transport to dinitrogenase in aerobic diazotrophs, presumably under microaerobic conditions (discussed in ref. 43).	*Rhizobium meliloti*	NIFA	(i), (vi)	123; reviewed in ref. 44	*fixABCX* is a single operon in *R. meliloti*. In *B. japonicum*, *fixA* and *fixBC* constitute separate operons (43).
		Bradyrhizobium japonicum	NIFA	(i), (vi)	43	
dctA	C4-dicarboxylate transport component; required for growth on dicarboxylates in free-living state and apparently also symbiotically (110; reviewed in ref. 107)	Rhizobia (*Rhizobium leguminosarum*, *Rhizobium meliloti*, *Rhizobium trifolii*)	DCTD (free living); unknown (symbiotic)[h]	(i), (iii), (vi), (vii)	107–109, 111; J. Jiang, B. Gu, L. M. Albright, and B. T. Nixon, J. Bacteriol., submitted for publication)	The nomenclature is for *Rhizobium* spp. In the free-living state, induction apparently occurs in response to dicarboxylates external to the cell. Although the DCTB protein, which would be membrane bound on the basis of its amino acid sequence, is the primary sensor for the system; the DCTA protein itself appears to be involved because a haploid *dctA-lacZ* fusion is expressed at high levels in the absence of inducer (107, 109). Mutants *hno*$^-$ (*ntrA*$^-$) of *A. eutrophus* have defects in dicarboxylate transport.
		Alcaligenes eutrophus (see Comments)		(iii)	105, 106	
melA	Tyrosinase, required for synthesis of the pigment melanin	*Rhizobium leguminosarum* biovar *phaseoli* (the *melA* gene is found only in this biovar)	NIFA	(iv), (vi)	46	It is speculated that this tyrosinase may play a role in detoxification of plant phenolic compounds during senescence of root nodules (46).
fdhF and *hyd-3* (unlinked)	The selenopeptide of formate dehydrogenase F and the gas-evolving hydrogenase isoenzyme 3. These proteins are components of formate hydrogen lyase, which degrades formate to CO_2 and H_2 under anaerobic conditions (and in the absence of alternative electron acceptors such as nitrate).	*Escherichia coli*, probably *Salmonella typhimurium*	Unknown; requirement for *cis*-acting sequences upstream of the *fdhF* promoter (between −144 and −100). Upstream sequences mediating formate induction could not be separated physically from those mediating oxygen or nitrate repression (10).	(i)–(iii), (vii)	9, 10	Although the physiological function(s) of the formate hydrogen lyase pathway is not clear, a primary function may be deacidification of formate (reviewed in ref. 119).
hox	Soluble and membrane-bound hydrogenases that oxidize molecular hydrogen as a source of energy and/or reducing power. Oxygen is the terminal electron acceptor.	*Alcaligenes eutrophus*	HOXA, the product of a gene in the *hoxC* cluster; induction under conditions of energy limitation	(i), (iii)	30, 35, 36, 52, 71, 105, 106; Warrelmann, Romermann, and Friedrich, personal communication	The nomenclature is for the genus *Alcaligenes*. *hox* genes are clustered on a 450-kilobase megaplasmid, whereas the *hno* (*ntrA*) gene is chromosomal. Expression of *hox* genes encoding a membrane-bound hydrogenase in *P. facilis* is σ^{54} dependent.
		Pseudomonas facilis (see Comments)			106, 127	

Continued on following page

TABLE 1—*Continued*

Gene	Gene product(s)	Organisms[b]	Activator	Evidence[c]	Selected reference(s)[d]	Comments
Structural gene encoding pilin (designated *pilA* in *Pseudomonas aeruginosa* and *pilE* in *Neisseria gonorrhoeae*).	A pilin of the *N*-methylphenylalanine class, the monomeric subunit of pili; synthesized as a precursor called prepilin	*Pseudomonas aeruginosa* *Neisseria gonorrhoeae* *Bacteroides nodosus* *Moraxella bovis*	Unknown. The only condition known to result in greatly increased transcription of the *pilA* gene of *P. aeruginosa* appears to be the inability to retract (depolymerize) pili (60).	(i), (iii), (vii) (i) (i) (i)	59–61, 97a, 131 67, 68, 84, 121, 128	Gonococcal pili greatly enhance adherence of the bacterium to host epithelial cells (121, 128), and this increased adherence apparently accounts for the increased virulence of piliated strains (67, 68). Similarly for the pili of *P. aeruginosa* (131).
xylCAB and *xylS*	Enzymes (upper pathway) that catabolize toluene to benzoate and xylenes to the corresponding methylbenzoates (*xylCAB* operon) and activator of transcription for the operon encoding enzymes (lower or meta pathway) that further catabolize benzoate and methylbenzoates to products that can enter the tricarboxylic acid cycle (*xylS*)	*Pseudomonas putida* (TOL plasmid pWWO)	XYLR; induction by toluenes, xylenes, and their alcohol catabolic products	(i), (iv), (vi)	27, 34, 56–58, 62, 91, 101, 118, 132	These pathways are being manipulated for the degradation of toxic aromatic compounds (102).
Gene encoding carboxypeptidase G2	Carboxypeptidase G2, which hydrolyzes the C-terminal glutamate moiety from folic acid and analogs such as methotrexate	*Pseudomonas putida*	Unknown. When the organism is grown in the presence of glutamate and folate as carbon sources, enzyme synthesis does not occur until the glutamate has been depleted (unpublished results cited in ref. 87).	(i)	86, 87	This enzyme is used in cancer chemotherapy (86).
flbG operon (*flaK*), *flaN*, *flgK*, *flgL*	Hook and filament proteins of the flagellum *flaK*: Major structural protein of flagellar hook *flaN*: Unknown, loss of function results in loss of motility *flgK* and *flgL*: 25K and 27K flagellins, respectively, which are the major distal and proximal components of the flagellar filament	*Caulobacter crescentus* *Pseudomonas aeruginosa* (see Comments)	Unknown; requirement for upstream sequences (designated *ftr*) at *flbG* (at ~−100); similar sequences also occur upstream (at ~−100) of both *flgK* and *flgL*	(i), (ii), (v), (vii) (iii)	21, 85, 89, 90, 94, 97 S. Lory, personal communication	The nomenclature is for *C. crescentus*. A single flagellum is synthesized by the *Caulobacter* predivisional cell at the pole opposite to the stalk; it is inherited by the daughter swarmer cell (reviewed in ref. 20 and 92). Transcription of each of the genes listed depends on expression of flagellar regulatory and structural components in a hierarchical manner and occurs at a specific time in the cell cycle (21, 85, 97). It is not yet clear whether activators for σ^{54}-holoenzyme, which would be expected to play a role in the regulatory hierarchy, also play a role in cell cycle control or whether this control occurs by an independent mechanism. It is not clear whether σ^{28} (sigma F), which is required for transcription of flagellar and chemotaxis genes in *B. subtilis* and *E. coli* (2, 47, 48), is required for transcription of any of the *Caulobacter* flagellar or chemotaxis genes. Mutants *rpoN*$^-$ (*ntrA*$^-$) of *P. aeruginosa* lack flagella and lack flagellin as detected immunologically.

mbhA	Hemagglutinin. The *mbhA* gene is transcribed at high levels early in the process of fruiting body formation (24). Strains lacking the hemagglutinin show a delay in fruiting upon abrupt deprivation for nutrients, particularly if Mg^{2+} is also withheld (104).	*Myxococcus xanthus*	Unknown	(i), (ii), (vii)	104; J. Romeo, K. O'Connor, and D. Zusman, personal communication	Transcription of *mbhA* is controlled not only temporally during development but spatially: transcription occurs in peripheral cells but not in cells that have entered aggregates (K. O'Connor and D. Zusman, personal communication).

[a] We use the designation σ^{54}, which reflects the molecular weight of the protein in enteric bacteria (82; D. Popham, manuscript in preparation), even though homologs in other organisms have different molecular weights (80, 63a, 111). The gene encoding this alternate sigma factor has been variously designated *glnF*, *ntrA*, *rpoN*, and *rpoE* and could logically be given a *sig* designation. In the absence of agreement on terminology, we have not emphasized the gene designation.

[b] Although we cite a single species within a genus in many instances, it is likely that similar control is found in other species, and in some cases there is direct evidence for this.

[c] The lines of evidence are as follows: (i) presence of a σ^{54}-dependent promoter (reviewed in references 28 and 44) upstream of the transcriptional start site for the gene (such promoters are characterized minimally by a conserved GC doublet that lies between 11 and 14 bp upstream of the start site in different promoters [Popham and Kustu, in preparation] and a GG doublet that lies exactly 10 bp farther upstream); (ii) mutational analysis of the promoter, particularly by alteration of the conserved doublets or the spacing between them; (iii) lack of transcription in a strain carrying a disruption of the gene encoding σ^{54}; (iv) lack of transcription when the gene is transferred to an *E. coli* strain lacking σ^{54}; (v) in vitro transcription by σ^{54}-holoenzyme; (vi) dependence on a transcriptional activator that is known to work in conjunction with σ^{54}-holoenzyme; and (vii) requirement for upstream sequences (at ~−100 or farther) that have been demonstrated to function as activator-binding sites or are inferred to do so.

[d] When criteria (iii) and (v) of footnote *c* have been fulfilled, we cite references for these. We also attempt to cite reviews and recent references that will allow the reader to trace other, earlier references.

[e] In *K. pneumoniae*, the *nif* products include a flavodoxin (*nifF*) and pyruvate:flavodoxin oxidoreductase (*nifJ*), which are involved in electron transport to dinitrogenase reductase.

[f] Although upstream sequences were required for NIFA-mediated activation of transcription from the *R. meliloti nifHDK* promoter in *E. coli* (using the NIFA protein from *K. pneumoniae*), no such requirement could be demonstrated in mature alfalfa root nodules (7).

[g] In *Azospirillum* and *Rhodobacter* spp., the ability to fix nitrogen is dependent on both NTRC and NIFA; although we infer that NTRC is required for *nifA* transcription, to our knowledge there is no direct evidence for this. The designation for *ntrC* in *R. capsulatus* is *nifR1*, and the designation for *ntrA* is *nifR4* (63a).

[h] It is not known whether transcription of *dctA* is dependent on σ^{54} in the symbiotic state.

between 11 and 14 base pairs [bp] upstream of the transcriptional start and a conserved GG doublet that lies exactly 10 bp farther upstream [reviewed in references 28, 44, and 62; D. Popham and S. Kustu, manuscript in preparation].) However, recognition complexes between σ^{54}-holoenzyme and the *glnA* promoter (closed complexes) are nonproductive transcriptionally because the DNA remains double stranded (74, 100, 116). Initiation of transcription requires an activator protein called NTRC, also known as NRI (encoded by *ntrC* [*glnG*] [reviewed in reference 66]). NTRC binds upstream of the promoter to sites that have the properties of transcriptional enhancers (95, 103). It catalyzes the isomerization of closed complexes between σ^{54}-holoenzyme and the *glnA* promoter to transcriptionally productive open complexes in which the DNA strands are locally denatured in the region of the transcription start site (100, 116). The isomerization reaction requires ATP (100).

As is true for *glnA*, transcription of a number of other σ^{54}-dependent genes requires an activator. For example, transcription of *nif* genes in a variety of bacteria requires the activator protein NIFA (5, 13, 31, 79, 115, 123; reviewed in reference 44). Similarly, transcription of the *dctA* gene is dependent on DCTD (108, 109), transcription of the *xylCAB* and *xylS* genes requires XYLR (references 27, 57, 101, and 118 and references cited therein), and transcription of the *hox* (hydrogen oxidation) genes is dependent on the product of a gene in the *hoxC* locus (30, 36, 71, 106).

Like NTRC, other activators of σ^{54}-holoenzyme (NIFA [6, 7, 11, 14, 15, 88; reviewed in reference 44] and DCTD [B. T. Nixon, personal communication]) bind to sites located at least 80 bp away from the promoters they regulate. In several systems for which the specific activator has not yet been identified (*E. coli* formate hydrogen lyase, *C. crescentus* hook protein, and *P. aeruginosa* pilin), a requirement for upstream sequences that could serve as activator binding sites has nevertheless been demonstrated (10, 89, 90, 97a). It therefore seems likely that transcription by σ^{54}-holoenzyme will prove to depend on an activator in these other cases, and it is an attractive speculation that this will be true in every case (9, 100, 111).

The activator proteins whose sequences are known (NTRC, NIFA, DCTD, and XYLR) show a high degree of sequence similarity within their central domains (domain D of reference 29), each of which contains a putative ATP-binding site (regions 1 and 3 in reference 37; 58, 109). (The central domain spans ~240 amino acid residues [16, 29], of which 30% are identical in each of the four activators—NTRC and NIFA from *K. pneumoniae*, DCTD from *R. leguminosarum*, and XYLR from *P. putida* [58, 109].) Eleven independent mutant forms of *Salmonella* NTRC that are specifically defective in the ability to activate transcription (129) have amino acid substitutions within the central domain; moreover, the substitutions affect residues that are identical in the four activators, including residues that constitute the proposed ATP-binding site (D. Weiss and S. Kustu, unpublished data). These results indicate that the central domain of NTRC is specifically required for formation of open complexes between σ^{54}-holoenzyme and the *glnA* promoter. Sequence similarity among the activators is consistent with the simple hypothesis that they all function in a similar manner. Interestingly, truncated forms of the NIFA protein from *Rhizobium meliloti* that retain only the central domain appear to retain the ability to activate transcription from the *Rhizobium nifH* promoter (53).

FUNCTION OF ACTIVATOR PROTEINS IS CONTROLLED BY DIFFERENT PHYSIOLOGICAL SIGNALS AND DIFFERENT MECHANISMS

Control of transcription at σ^{54}-dependent promoters appears to be accomplished primarily by modulation of the activity state of activator proteins (see below; Table 1) and their abundance (57; reviewed in references 28, 44, and 74). The amount of σ^{54} does not vary much, at least under different conditions of nitrogen availability (19, 26, 83, 111). Each activator allows σ^{54}-holoenzyme to initiate transcription in response to a distinct physiological signal, such as (i) limitation of combined nitrogen (NTRC as activator [reviewed in references 66, 78]); (ii) low oxygen tension (NIFA as activator [8, 25, 28, 32, 33, 44, 50, 72, 81, 126]); (iii) availability of dicarboxylic acids, presumably external to the cell (DCTD as activator [107, 109]); (iv) availability of toluene, xylenes, or their alcohol catabolic products (XYLR as activator [34, 57, 91, 101, 132]); (v) energy limitation (product of a gene in the *hoxC* locus as activator [30, 35, 36, 71]); and (vi) presence of formate under anaerobic conditions (activator unknown [9, 10]). It will be of interest to understand the nature of the signals that regulate transcription of the *Caulobacter* hook and flagellin genes, expression of which is controlled by a complex regulatory hierarchy (12, 20, 21, 22, 92, 97) and occurs in a cell cycle-dependent manner (85, 97).

Function of the activators themselves is apparently controlled by a variety of mechanisms. NTRC is synthesized in an inactive form, and its activity is regulated positively and negatively by phosphorylation and dephosphorylation, respectively, of its NH_2-terminal domain (65, 93). This domain (~120 amino acids) appears to control the ability of the central domain to activate transcription by σ^{54}-holoenzyme (65, 96, 112). The degree of phosphorylation of NTRC is increased under nitrogen-limiting conditions. NTRC is a member of a two-component regulatory system (70, 96, 112) and is phosphorylated by its partner NTRB, also known as NRII (encoded by *ntrB* [*glnL*]). Like NTRC, the DCTD protein of rhizobia is a member of a two-component regulatory system, and therefore it is probably activated by phosphorylation by its partner DCTB (109); phosphorylation is thought to increase in response to availability of external dicarboxylates. Unlike NTRC and DCTD, the NIFA protein is apparently synthesized in an active form (8, 13, 33). In *K. pneumoniae*, it is inactivated by the NIFL protein (mechanism unknown) in response to molecular oxygen or combined nitrogen (50, 81; reviewed in references 28 and 44). In *Bradyrhizobium japonicum* (32, 33) and *Rhizobium meliloti* (8), NIFA is inactivated at high oxygen tensions by a mechanism(s) that does not involve NIFL or any other *nif*-specific protein. Finally, the XYLR protein of *P. putida* appears to be activated directly by binding low-molecular-weight substrates of the xylene catabolic pathway (27, 34, 57, 91, 101, 132). Thus, function of the activator proteins that control transcription by σ^{54}-holoenzyme is highly regulated. For different activator proteins, it is regulated by different mechanisms.

THE SEQUENCE OF σ^{54} HAS UNIQUE FEATURES

As discussed above, σ^{54} confers on core RNA polymerase the ability to bind specifically to a promoter (a minimal definition of a sigma factor) but it apparently does not confer the ability to form open complexes. In this regard it is interesting that σ^{54} shows little amino acid sequence similarity to other sigma factors (63, 80, 82, 111) (of which ~15 have now been identified [49]), whereas these share several regions of amino acid sequence similarity with each other (41, 49, 120). Rather, σ^{54} has a glutamine-rich region at its amino terminus that resembles the glutamine-rich region required for activation of transcription by mammalian transcription factor Sp1 (23). (In different organisms, between 15 and 25% of the first 50 residues of σ^{54} are glutamine [63a, 80, 82, 114].) S. Sasse-Dwight and J. D. Gralla have determined that the glutamine-rich region of σ^{54} is specifically required for NTRC-dependent isomerization of closed to open complexes at the *glnA* promoter (personal communication). Small deletions in this region allow the formation of closed recognition complexes at *glnA* but prevent the formation of open complexes.

CONCLUSIONS

σ^{54} has physiologically diverse roles and in this way resembles the most abundant sigma factor in eubacterial cells (σ^{70} and its homologs) rather than other alternative sigma factors. A direct line of evidence for physiological diversity is that mutant strains which lack σ^{54} have pleiotropic phenotypes. For example, such mutant strains of *A. eutrophus* (105; Warrelmann, Romermann, and Friedrich, personal communication), which were designated *hno*$^-$, for "hydrogen, nitrate and other things" (52, 105), fail to express not only hydrogenases required for utilization of molecular hydrogen as an energy source but also enzymes required for utilization of urea and formamide as nitrogen sources and a dicarboxylate transport system; they have defects in several additional functions as well. σ^{54}-Deficient strains of *E. coli*, *R. meliloti*, and *P. aeruginosa* also have physiologically pleiotropic phenotypes (9, 59, 111; Table 1).

The properties of mutant strains that lack σ^{54} indicate that this sigma factor is not essential for bacterial viability under all conditions (39, 59, 105, 111, 114, 125). As discussed above, however, σ^{54} is required for several important biological processes. It is required for the autotrophic growth of *P. facilis* (106, 127) and for that of *A. eutrophus* with molecular hydrogen as an electron donor (52, 105, 106). σ^{54} is required for biological nitrogen fixation in a variety of gram-negative bacteria (Table 1) and, in addition, is needed for the establishment of stable symbiotic relationships between bradyrhizobia (31), rhizobia (123, 133), and their plant hosts (Table 1). σ^{54} would appear to be required for the formation of functional swarmer cells in *C. crescentus* (Table 1) and for virulence of *N. gonorrhoeae* (67, 68, 121, 128) and *P. aeruginosa* (59, 131).

Transcription by σ^{54}-holoenzyme appears to be controlled by a common mechanism: use of an activator protein and ATP to catalyze formation of transcriptionally productive open complexes (100). It is the activator proteins that allow σ^{54}-holoenzyme to respond to diverse physiological signals. The selective advantage to use of σ^{54} and this mechanism, if any, remains to be determined (27, 111).

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LITERATURE CITED

1. **Ames, G. F.-L., and K. Nikaido.** 1985. Nitrogen regulation in *Salmonella typhimurium*. Identification of an *ntrC* protein-

binding site and definition of a consensus. EMBO J. **4**:539–547.
2. **Arnosti, D. N., and M. J. Chamberlin.** 1989. Secondary σ factor controls transcription of flagellar and chemotaxis genes in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **86**:830–834.
3. **Austin, S., N. Henderson, and R. Dixon.** 1987. Requirements for transcriptional activation in vitro of the nitrogen-regulated *glnA* and *nifLA* promoters from *Klebsiella pneumoniae*: dependence on activator concentration. Mol. Microbiol. **1**:92–100.
4. **Bauer, C. E., D. A. Young, and B. L. Marrs.** 1988. Analysis of the *Rhodobacter capsulatus puf* operon. Location of the oxygen-regulated promoter region and the identification of an additional *puf*-encoded gene. J. Biol. Chem. **263**:4820–4827.
5. **Bennet, L. T., F. Cannon, and D. Dean.** 1988. Nucleotide sequence and mutagenesis of the *nifA* gene from *Azotobacter vinelandii*. Mol. Microbiol. **2**:315–321.
6. **Betancourt-Alvarez, M., 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.
7. **Better, M., G. Ditta, and D. R. Helinski.** 1985. Deletion analysis of *Rhizobium meliloti* symbiotic promoters. EMBO J. **4**:2419–2424.
8. **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.
9. **Birkmann, A., R. G. Sawers, and A. Bock.** 1987. Involvement of the *ntrA* gene product in the anaerobic metabolism of *Escherichia coli*. Mol. Gen. Genet. **210**:535–542.
10. **Birkmann, A., F. Zinoni, G. Sawers, and A. Bock.** 1987. Factors affecting transcriptional regulation of the formate-hydrogen-lyase pathway of *Escherichia coli*. Arch. Microbiol. **148**:44–51.
11. **Brown, S. E., and F. M. Ausubel.** 1984. Mutations affecting regulation of the *Klebsiella pneumoniae nifH* (nitrogenase reductase) promoter. J. Bacteriol. **157**:143–147.
12. **Bryan, R., R. Champer, S. Gomes, B. Ely, and L. Shapiro.** 1987. Separation of temporal control and trans-acting modulation of flagellin and chemotaxis genes in *Caulobacter*. Mol. Gen. Genet. **206**:300–306.
13. **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.
14. **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.
15. **Buck, M., S. Miller, M. Drummond, and R. Dixon.** 1986. Upstream activators are present in the promoters of nitrogen fixation genes. Nature (London) **320**:374–378.
16. **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.
17. **Cali, B. M., J. L. Micca, and V. Stewart.** 1989. Genetic regulation of nitrate assimilation in *Klebsiella pneumoniae* M5A1. J. Bacteriol. **171**:2666–2672.
18. **Carlson, R. 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.
19. **Castano, I., and F. Bastarachea.** 1984. *glnF-lacZ* fusions in *Escherichia coli*: studies on *glnF* expression and its chromosomal orientation. Mol. Gen. Genet. **195**:228–233.
20. **Champer, R., R. Bryan, S. L. Gomes, M. Purucker, and L. Shapiro.** 1985. Temporal and spacial control of flagellar and chemotaxis gene expression during *Caulobacter* cell differentiation. Cold Spring Harbor Symp. Quant. Biol. **50**:831–840.
21. **Champer, R., A. Dingwall, and L. Shapiro.** 1987. Cascade regulation of *Caulobacter* flagellar and chemotaxis genes. J. Mol. Biol. **194**:71–80.
22. **Chen, L.-S., D. Mullin, and A. Newton.** 1986. Identification, nucleotide sequence, and control of developmentally regulated promoters in the hook operon region of *Caulobacter crescentus*. Proc. Natl. Acad. Sci. USA **83**:2860–2864.
23. **Courey, A. J., and R. Tjian.** 1988. Analysis of Sp1 in vivo reveals multiple transcriptional domains, including a novel, glutamine-rich activation motif. Cell **55**:887–898.
24. **Cumsky, M., and D. R. Zusman.** 1979. Myxobacterial hemagglutinin: a development-specific lectin of *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **76**:5505–5509.
25. **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.
26. **de Bruijn, F. J., and F. M. 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.
27. **Dixon, R.** 1986. The *xylABC* promoter from the *Pseudomonas putida* TOL plasmid is activated by nitrogen regulatory genes in *Escherichia coli*. Mol. Gen. Genet. **203**:129–136.
28. **Dixon, R. A.** 1984. The genetic complexity of nitrogen fixation. J. Gen. Microbiol. **130**:2745–2755.
29. **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.
30. **Eberz, G., C. Hogrefe, C. Kortluke, A. Kamienski, and B. Friedrich.** 1986. Molecular cloning of structural and regulatory hydrogenase (*hox*) genes of *Alcaligenes eutrophus* H16. J. Bacteriol. **168**:636–641.
31. **Fischer, H.-M., A. Alvarez-Morales, and J. 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.
32. **Fischer, H.-M., T. Bruderer, and H. Hennecke.** 1988. Essential and non-essential domains in the *Bradyrhizobium japonicum* NifA protein: identification of indispensable cysteine residues potentially involved in redox reactivity and/or metal binding. Nucleic Acids Res. **16**:2207–2224.
33. **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.
34. **Franklin, F. C. H., and P. A. Williams.** 1980. Construction of a partial diploid for the degradative pathway encoded by the TOL plasmid (pWWO) from *Pseudomonas putida* mt-2: evidence for the positive nature of the regulation by the *xylR* gene. Mol. Gen. Genet. **177**:321–328.
35. **Friedrich, C. G.** 1982. Derepression of hydrogenase during limitation of electron donors and derepression of ribulose bis-phosphate carboxylase. J. Bacteriol. **149**:203–210.
36. **Friedrich, C. G., and B. Friedrich.** 1983. Regulation of hydrogenase formation is temperature sensitive and plasmid coded in *Alcaligenes eutrophus*. J. Bacteriol. **153**:176–181.
37. **Fry, D. C., S. A. Kuby, and A. S. Mildvan.** 1986. ATP-binding site of adenylate kinase: mechanistic implications of its homology with *ras*-encoded p21, F_1-ATPase, and other nucleotide-binding proteins. Proc. Natl. Acad. Sci. USA **83**:907–911.
38. **Gaillardin, C. M., and B. Magasanik.** 1978. Involvement of the product of the *glnF* gene in the autogenous regulation of glutamine synthetase formation in *Klebsiella aerogenes*. J. Bacteriol. **133**:1329–1338.
39. **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.
40. **Geiduschek, E. P., and J. Ito.** 1982. Regulatory mechanisms in the development of lytic bacteriophages in *Bacillus subtilis*, p. 203–245. *In* D. A. Dubnau (ed.), The molecular biology of the bacilli. Academic Press, Inc., New York.
41. **Gribskov, M., and R. R. Burgess.** 1986. Sigma factors from *E.*

coli, *B. subtilis*, phage *SP01*, and phage *T4* are homologous proteins. Nucleic Acids Res. **14**:6745–6763.
42. **Grossman, A. D., J. E. Erickson, and C. A. Gross.** 1984. The *htpR* gene product of *E. coli* is a sigma factor for heat-shock promoters. Cell **38**:383–390.
43. **Gubler, M., and H. Hennecke.** 1988. Regulation of the *fixA* gene and *fixBC* operon in *Bradyrhizobium japonicum*. J. Bacteriol. **170**:1205–1214.
44. **Gussin, G. N., C. W. Ronson, and F. M. Ausubel.** 1986. Regulation of nitrogen fixation genes. Annu. Rev. Genet. **20**:567–591.
45. **Haselkorn, R.** 1986. Organization of the genes for nitrogen fixation in photosynthetic bacteria and cyanobacteria. Annu. Rev. Microbiol. **40**:527–547.
46. **Hawkins, F. K., and A. W. 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.
47. **Hellman, J. D., L. M. Marquez, and M. J. Chamberlin.** 1988. Cloning, sequencing and disruption of the *Bacillus subtilis* σ^{28} gene. J. Bacteriol. **170**:1568–1574.
48. **Helmann, J. D., and M. J. Chamberlin.** 1987. DNA sequence analysis suggests that expression of flagellar and chemotaxis genes in *Escherichia coli* and *Salmonella typhimurium* is controlled by an alternative σ factor. Proc. Natl. Acad. Sci. USA **84**:6422–6424.
49. **Helmann, J., and M. J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57**:839–872.
50. **Hill, S., C. Kennedy, E. Kavanagh, R. B. Goldberg, and R. Hanau.** 1981. Nitrogen fixation gene (*nifL*) involved in oxygen regulation of nitrogenase synthesis in *Klebsiella pneumoniae*. Nature (London) **290**:424–426.
51. **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.
52. **Hogrefe, C., D. Romermann, and B. Friedrich.** 1984. *Alcaligenes eutrophus* hydrogenase genes (*hox*). J. Bacteriol. **158**: 43–48.
53. **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.
54. **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.
55. **Iismaa, S. E., and J. M. Watson.** 1987. A gene upstream of the *Rhizobium trifolii nifA* gene encodes a ferredoxin-like protein. Nucleic Acids Res. **15**:3180.
56. **Inouye, S., Y. Ebina, A. Nakazawa, and T. Nakazawa.** 1984. Nucleotide sequence surrounding transcription initiation site of *xylABC* operon on TOL plasmid of *Pseudomonas putida*. Proc. Natl. Acad. Sci. USA **81**:1688–1691.
57. **Inouye, S., A. Nakazawa, and T. Nakazawa.** 1987. Expression of the regulatory gene *xylS* on the TOL plasmid is positively controlled by the *xylR* gene product. Proc. Natl. Acad. Sci. USA **84**:5182–5186.
58. **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.
59. **Ishimoto, K., and S. Lory.** 1989. Formation of pilin in *Pseudomonas aeruginosa* requires the RpoN subunit of RNA polymerase. Proc. Natl. Acad. Sci. USA **86**:1954–1957.
60. **Johnson, K., and S. Lory.** 1987. Characterization of *Pseudomonas aeruginosa* mutants with altered piliation. J. Bacteriol. **169**:5663–5667.
61. **Johnson, K., M. L. Parker, and S. Lory.** 1986. Nucleotide sequence and transcriptional initiation site of two *Pseudomonas aeruginosa* pilin genes. J. Biol. Chem. **261**:15703–15708.
62. **Johnston, A. W. B., and J. A. Downie.** 1984. What is a *nif* promoter? Trends Biochem. Sci. **9**:367–368.
63. **Jones, R., and R. Haselkorn.** 1988. The DNA sequence of the *Rhodobacter capsulatus nifH* gene. Nucleic Acids Res. **16**: 8735.
63a. **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.
64. **Kassavetis, G. A., and E. P. Geiduschek.** 1984. Defining a bacteriophage T4 late promoter: bacteriophage T4 gene 55 protein suffices for directing late promoter recognition. Proc. Natl. Acad. Sci. USA **81**:5101–5105.
65. **Keener, J., and S. Kustu.** 1988. Protein kinase and phosphoprotein phosphatase activities of nitrogen regulatory proteins NTRB and NTRC of enteric bacteria: roles of the conserved amino-terminal domain of NTRC. Proc. Natl. Acad. Sci. USA **85**:4976–4980.
66. **Keener, J., P. Wong, D. Popham, J. Wallis, and S. Kustu.** 1987. A sigma factor and auxiliary proteins required for nitrogen-regulated transcription in enteric bacteria, p. 159–175. *In* W. S. Reznikoff et al. (ed.), RNA polymerase and the regulation of transcription. Elsevier Science Publishing Co., New York.
67. **Kellogg, D. S., Jr., I. R. Cohen, L. C. Norins, A. L. Schroeter, and G. Reising.** 1968. *Neisseria gonorrhoeae*. II. Colonial variation and pathogenicity during 35 months in vitro. J. Bacteriol. **96**:596–605.
68. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. T. Pirkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85**:1274–1279.
69. **Kim, C. H., D. R. Helinski, and G. Ditta.** 1986. Overlapping transcription of the *nifA* regulatory gene in *Rhizobium meliloti*. Gene **50**:141–148.
70. **Kofoid, E. C., and J. S. Parkinson.** 1988. Transmitter and receiver modules in bacterial signaling proteins. Proc. Natl. Acad. Sci. USA **85**:4981–4985.
71. **Kortluke, C., C. Hogrefe, G. Eberz, A. Puhler, and B. Friedrich.** 1987. Genes of lithoautotrophic metabolism are clustered on the megaplasmid pHG1 in *Alcaligenes eutrophus*. Mol. Gen. Genet. **210**:122–128.
72. **Kranz, R. G., and R. Haselkorn.** 1986. Anaerobic regulation of nitrogen fixation genes in *Rhodopseudomonas capsulata*. Proc. Natl. Acad. Sci. USA **83**:6805–6809.
73. **Kroos, L., B. Kunkel, and R. Losick.** 1989. Switch protein alters specificity of RNA polymerase containing a compartment-specific sigma factor. Science **243**:526–528.
74. **Kustu, S., K. Sei, and J. Keener.** 1986. Nitrogen regulation in enteric bacteria, p. 139–154. *In* I. Booth and C. Higgins (ed.), Regulation of gene expression. Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge.
75. **Leonardo, J. M., and R. B. Goldberg.** 1980. Regulation of nitrogen metabolism in glutamine auxotrophs of *Klebsiella pneumoniae*. J. Bacteriol. **142**:99–110.
76. **Lindquist, S., and E. A. Craig.** 1988. The heat-shock proteins. Annu. Rev. Genet. **22**:631–677.
77. **Losick, R., P. Youngman, and P. J. Piggot.** 1986. Genetics of endospore formation in *Bacillis subtilis*. Annu. Rev. Genet. **20**:625–670.
78. **Magasanik, B.** 1982. Genetic control of nitrogen assimilation in bacteria. Annu. Rev. Genet. **16**:135–168.
79. **Masepohl, B., W. Klipp, and A. Puhler.** 1988. Genetic characterization and sequence analysis of the duplicated *nifA*/*nifB* gene region of *Rhodobacter capsulatus*. Mol. Gen. Genet. **212**:27–37.
80. **Merrick, M., 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.
81. **Merrick, M., S. Hill, H. Hennecke, M. Hahn, R. Dixon, and C. Kennedy.** 1982. Repressor properties of the *nifL* gene product in *Klebsiella pneumoniae*. Mol. Gen. Genet. **185**:75–81.
82. **Merrick, M. J., and J. R. Gibbins.** 1985. The nucleotide sequence of the nitrogen regulation gene *ntrA* of *Klebsiella*

pneumoniae and comparison with conserved features in bacterial sigma factors. Nucleic Acids Res. **13**:7607–7620.
83. **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.
84. **Meyer, T. F., E. Billyard, R. Haas, S. Storzbach, and M. So.** 1984. Pilus genes of *Neisseria gonorrhoeae*: chromosomal organization and DNA sequence. Proc. Natl. Acad. Sci. USA **81**:6110–6114.
85. **Minnich, S. A., and A. Newton.** 1987. Promoter mapping and cell cycle regulation of flagellin gene transcription in *Caulobacter crescentus*. Proc. Natl. Acad. Sci. USA **84**:1142–1146.
86. **Minton, N. P., T. Atkinson, and R. F. Sherwood.** 1983. Molecular cloning of the *Pseudomonas* carboxypeptidase G_2 gene and its expression in *Escherichia coli* and *Pseudomonas putida*. J. Bacteriol. **156**:1222–1227.
87. **Minton, N. P., and L. E. Clarke.** 1985. Identification of the promoter of the *Pseudomonas* gene coding for carboxypeptidase G2. J. Mol. Appl. Genet. **3**:26–35.
88. **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.
89. **Mullin, D., S. Minnich, L.-S. Chen, and A. Newton.** 1987. A set of positively regulated flagellar gene promoters in *Caulobacter crescentus* with sequence homology to the *nif* gene promoters of *Klebsiella pneumoniae*. J. Mol. Biol. **195**:939–943.
90. **Mullin, D. A., and A. Newton.** 1989. Ntr-like promoters and upstream regulatory sequence *ftr* are required for transcription of a developmentally regulated *Caulobacter crescentus* flagellar gene. J. Bacteriol. **171**:3218–3227.
91. **Nakazawa, T., S. Inouye, and A. Nakazawa.** 1980. Physical and functional mapping of RP4-TOL plasmid recombinants: analysis of insertion and deletion mutants. J. Bacteriol. **144**:222–231.
92. **Newton, A.** 1989. Differentiation in *Caulobacter*: flagellum development, motility and chemotaxis, p. 199–219. *In* D. A. Hopwood and K. F. Chater (ed.), Genetics of bacterial diversity. Academic Press, Inc., London.
93. **Ninfa, A. J., and B. Magasanik.** 1986. Covalent modification of the *glnG* product NRI, by the *glnL* product, NRII, regulates the transcription of the *glnALG* operon in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83**:5909–5913.
94. **Ninfa, A. J., D. A. Mullin, G. Ramakrishnan, and A. Newton.** 1989. *Escherichia coli* σ^{54} RNA polymerase recognizes *Caulobacter crescentus flbG* and *flaN* flagellar gene promoters in vitro. J. Bacteriol. **171**:383–391.
95. **Ninfa, A. J., L. J. Reitzer, and B. Magasanik.** 1987. Initiation of transcription at the bacterial glnAp2 promoter by purified *E. coli* components is facilitated by enhancers. Cell **50**:1039–1046.
96. **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.
97. **Ohta, N., L.-S. Chen, E. Swanson, and A. Newton.** 1985. Transcriptional regulation of a periodically controlled flagellar gene operon in *Caulobacter crescentus*. J. Mol. Biol. **186**: 107–115.
97a. **Pasloske, B. L., D. S. Drummond, L. S. Frost, and W. Paranchych.** 1989. Gene **81**:25–34.
98. **Pawlowski, K., P. Ratet, J. Schell, and F. J. de Bruijn.** 1987. Cloning and characterization 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 symbiotic state. Mol. Gen. Genet. **206**:207–219.
99. **Pederosa, F. O., and M. G. Yates.** 1984. Regulation of nitrogen fixation (*nif*) genes of *Azospirillum brasilense* by *nifA* and *ntr* (*gln*) type gene products. FEMS Microbiol. Lett. **23**:95–101.
100. **Popham, D., D. Szeto, J. Keener, and S. Kustu.** 1989. Function of a bacterial activator protein that binds to transcriptional enhancers. Science **243**:629–635.
101. **Ramos, J. L., N. Mermod, and K. N. Timmis.** 1987. Regulatory circuits controlling transcription of TOL plasmid operon encoding meta-cleavage pathway for degradation of alkylbenzoates by *Pseudomonas*. Mol. Microbiol. **1**:293–300.
102. **Ramos, J. L., A. Wasserfallen, L. Rose, and K. N. Timmis.** 1987. Redesigning metabolic routes: manipulation of the TOL plasmid pathway for catabolism of alkyl benzoates. Science **235**:593–596.
103. **Reitzer, L. J., and B. Magasanik.** 1986. Transcription of *glnA* in *E. coli* is stimulated by activator bound to sites far from the promoter. Cell **45**:785–792.
104. **Romeo, J. M., and D. R. Zusman.** 1987. Cloning of the gene for myxobacterial hemagglutinin and isolation and analysis of structural gene mutations. J. Bacteriol. **169**:3801–3808.
105. **Romermann, D., M. Lohmeyer, C. G. Friedrich, and B. Friedrich.** 1988. Pleiotropic mutants from *Alcaligenes eutrophus* defective in the metabolism of hydrogen, nitrate, urea, and fumarate. Arch. Microbiol. **149**:471–475.
106. **Romermann, D., J. Warrelmann, R. A. Bender, and B. Friedrich.** 1989. An *rpoN*-like gene of *Alcaligenes eutrophus* and *Pseudomonas facilis* controls expression of diverse metabolic pathways. J. Bacteriol. **171**:1093–1099.
107. **Ronson, C. W., and P. M. Astwood.** 1985. Genes involved in the carbon metabolism of bacteroids, p. 201–207. *In* H. J. Evans, P. J. Bottomley, and W. E. Newton (ed.), Nitrogen fixation research progress. Martinus Nijhoff, Dordrecht, The Netherlands.
108. **Ronson, C. W., P. M. Astwood, and J. A. Downie.** 1984. Molecular cloning and genetic organization of C4-dicarboxylate transport genes from *Rhizobium leguminosarum*. J. Bacteriol. **160**:903–909.
109. **Ronson, C. W., P. M. Astwood, B. T. Nixon, and F. M. Ausubel.** 1987. Deduced products of C4-dicarboxylate transport regulatory genes of *Rhizobium leguminosarum* are homologous to nitrogen regulatory gene products. Nucleic Acids Res. **15**:7921–7934.
110. **Ronson, C. W., P. Lyttleton, and J. G. Robertson.** 1981. C4-dicarboxylate transport mutants of *Rhizobium trifolii* form ineffective nodules on *Trifolium repens*. Proc. Natl. Acad. Sci. USA **78**:4284–4288.
111. **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**:2424–2430.
112. **Ronson, C. W., B. T. Nixon, and F. M. Ausubel.** 1987. Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. Cell **49**:579–581.
113. **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.
114. **Santero, E., F. Luque, J. R. Medina, and M. Tortolero.** 1986. Isolation of *ntrA*-like mutants of *Azotobacter vinelandii*. J. Bacteriol. **166**:541–544.
115. **Santero, E., A. Toukdarian, R. Humphrey, and C. Kennedy.** 1988. Identification and characterization of two nitrogen fixation regulatory regions, *nifA* and *nfrX* in *Azotobacter vinelandii* and *Azotobacter chroococcum*. Mol. Microbiol. **2**:303–314.
116. **Sasse-Dwight, S., and J. D. Gralla.** 1988. Probing the *Escherichia coli glnALG* upstream activation mechanism in vivo. Proc. Natl. Acad. Sci. USA **85**:8934–8938.
117. **Schmitz, G., K. Nikaido, and G. F.-L. Ames.** 1988. Regulation of a transport operon promoter in *Salmonella typhimurium*: identification of sites essential for nitrogen regulation. Mol. Gen. Genet. **215**:107–117.
118. **Spooner, R. A., K. Lindsay, and F. C. H. Franklin.** 1986. Genetic, functional and sequence analysis of the *xylR* and *xylS* regulatory genes of the TOL plasmid pWWO. J. Gen. Microbiol. **132**:1347–1358.
119. **Stewart, V.** 1988. Nitrate respiration in relation to facultative metabolism in enterobacteria. Microbiol. Rev. **52**:190–232.
120. **Stragier, P., C. Parsot, and J. Bouvier.** 1985. Two functional domains conserved in major and alternate bacterial sigma factors. FEBS Lett. **187**:11–15.

121. **Swanson, J.** 1973. Pili: their role in attachment of gonococci to tissue culture cells. J. Exp. Med. **137**:571–589.
122. **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 activation of nitrogen fixation genes in free-living and symbiotic cells. J. Bacteriol. **169**:1423–1432.
123. **Szeto, W. W., J. L. Zimmerman, V. Sundaresan, and F. M. Ausubel.** 1984. A *Rhizobium meliloti* symbiotic regulatory gene. Cell **36**:1035–1043.
124. **Thony, B., H.-M. Fischer, D. Anthamatten, T. Bruderer, and J. Hennecke.** 1987. The symbiotic nitrogen fixation regulatory operon (*fixR nifA*) of *Bradyrhizobium japonicum* is expressed aerobically and is subject to a novel, *nifA*-independent type of activation. Nucleic Acids Res. **15**:8479–8499.
125. **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.
126. **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.
127. **Warrelmann, J., and B. Friedrich.** 1986. Mutants of *Pseudomonas facilis* defective in lithoautotrophy. J. Gen. Microbiol. **132**:91–96.
128. **Watt, P. J., and M. E. Ward.** 1981. Adherence of *Neisseria gonorrhoeae* and other *Neisseria* strains to mammalian cells, p. 255–288. *In* E. Beachey (ed.), Bacterial adherence. Chapman & Hall, Ltd., New York.
129. **Wei, G. R., and S. Kustu.** 1981. Glutamine auxotrophs with mutations in a nitrogen regulatory gene, *ntrC*, that is near *glnA*. Mol. Gen. Genet. **183**:392–399.
130. **Wong, P.-K., D. Popham, J. Keener, and S. Kustu.** 1987. In vitro transcription of the nitrogen fixation regulatory operon *nifLA* of *Klebsiella pneumoniae*. J. Bacteriol. **169**:2876–2880.
131. **Woods, D. E., D. C. Straus, W. G. Johanson, Jr., V. K. Berry, and J. A. Bass.** 1980. Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. Infect. Immun. **29**:1146–1151.
132. **Worsey, M. J., F. C. H. Franklin, and P. A. Williams.** 1978. Regulation of the degradative pathway enzymes coded for by the TOL plasmid (pWWO) from *Pseudomonas putida* mt-2. J. Bacteriol. **134**:757–764.
133. **Zimmerman, J. L., W. W. Szeto, and F. M. Ausubel.** 1983. Molecular characterization of Tn5 induced, symbiotic (Fix^-) mutants of *Rhizobium meliloti*. J. Bacteriol. **156**:1025–1034.