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An *rpoN*-Like Gene of *Alcaligenes eutrophus* and *Pseudomonas facilis* Controls Expression of Diverse Metabolic Pathways, Including Hydrogen Oxidation

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Pleiotropic mutants of *Alcaligenes eutrophus* with the phenotype Hno$^-$ have been characterized previously. They are deficient in several diverse metabolic activities, including hydrogen oxidation, nitrate and urea assimilation, denitrification, and various substrate transport systems. Phenotypically similar mutants were identified among hydrogenase-deficient strains of *Pseudomonas facilis*. The Tn*5*-labeled *hno* gene was cloned from a genomic DNA library of *A. eutrophus* and used to identify the corresponding unimpaired wild-type DNA sequence. The recombinant plasmid pCH148 contained an insert of 12.3 kilobase pairs and was shown to restore the Hno$^+$ phenotype to mutants of *A. eutrophus* and *P. facilis*. A cosmid isolated from a DNA library of *P. facilis* also exhibited intergeneric Hno-complementing activity. The cloned *hno* loci from both organisms showed DNA homology by Southern blot hybridization. A subclone of pCH148 which contained a 6.5-kilobase-pair insert was constructed. The resulting hybrid, pCH170, not only was able to complement Hno$^-$ mutants but also relieved glutamine auxotrophy in NtrA$^-$ mutants of enteric bacteria. This suggests that the *hno* gene product from *A. eutrophus* is functionally similar to the NtrA protein, which has been identified as a novel sigma factor (σ^{54}) of RNA polymerase.

Alcaligenes eutrophus is capable of assimilating carbon for growth entirely as CO_2 via the reductive pentose phosphate cycle. The energy for this process can be derived either from formate through catalysis of formate dehydrogenase or from molecular hydrogen by a NAD-linked soluble hydrogenase and a membrane-bound electron transport-coupled hydrogenase. As a facultative chemolithoautotroph, *A. eutrophus* is able to grow heterotrophically with a variety of organic substrates or mixotrophically by using organic and inorganic compounds concomitantly. Although *A. eutrophus* is classified as a strict aerobic bacterium, it can be cultivated in the absence of oxygen provided nitrate or nitrite is present as a terminal electron acceptor. The latter two compounds can also be utilized as nitrogen sources in addition to urea, formamide, or other nitrogen-containing substrates (5). *Pseudomonas facilis*, another member of the facultative lithoautotrophic bacteria, resembles *A. eutrophus* physiologically. However, it contains only a single membrane-bound hydrogenase and fails to grow anaerobically with nitrate.

Hydrogen-oxidizing (10) and denitrifying (34) activities have been shown to be genetically linked in *A. eutrophus* H16 to a large plasmid of 450 kilobase pairs. A lithoautotrophic gene cluster has previously been identified spanning a DNA sequence of about 100 kilobase pairs (21). It consists of structural and regulatory hydrogenase (*hox*) genes (6) and a copy of *cfx* genes involved in autotrophic CO_2 fixation (19). In the course of mutant characterizations it was found that in one class of Hox$^-$ mutants, the mutation maps on the chromosome (15). However, in contrast to the plasmid-borne Hox$^-$ mutants, the chromosomal mutants, designated Hno$^-$, were extremely pleiotropic. They were impaired in at least eight metabolic functions, including hydrogen oxidation, autotrophic CO_2 fixation, denitrification, the utilization of nitrate and urea as nitrogen sources, and the uptake of nickel ions and C-4 dicarboxylic acids (35). Although the *hno* mutation restricts the metabolic versatility of the organism, it does not affect its normal mode of life. We have previously isolated Hox$^-$ mutants from *P. facilis* with an Hno$^-$-like pleiotropic phenotype (43).

In this paper, we report the cloning of an *hno*-bearing DNA sequence from a genomic DNA library of *A. eutrophus* and *P. facilis*. The cloned genes were successfully used in intergeneric complementation of Hno$^-$ mutants. Moreover, we present evidence that *hno* from *A. eutrophus* complements the auxotrophic phenotype of NtrA$^-$ mutants of enteric bacteria. These mutants are defective in the σ^{54} subunit of RNA polymerase, which determines promoter specificity (13, 16, 33).

MATERIALS AND METHODS

Organisms and plasmids. The strains and plasmids used are listed in Table 1.

Media and growth conditions. Cells of *A. eutrophus* and *P. facilis* were grown in mineral salts medium (37). The gas atmosphere for autotrophic growth contained a mixture of hydrogen, oxygen, and carbon dioxide in a ratio of 8:1:1 (vol/vol/vol). Organic carbon sources were added at a concentration of 0.4% (wt/vol) unless otherwise stated. The concentration of the nitrogen source was 0.2% (wt/vol). Anaerobic growth with nitrate as the electron acceptor was conducted in tubes sealed with screw caps. The concentration of potassium nitrate was 0.2% (wt/vol). Cells of *Escherichia coli* and *Klebsiella aerogenes* were propagated in M9 medium (27) supplemented with glutamine as indicated.

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Characteristics[a]	Source
A. eutrophus		
H16	Wild type, Hno	ATCC 17699, DSM 428
HF09	Hno$^-$	10
HF149	Hno$^-$ Km^r	15
K. aerogenes		
KC1043	Wild type, NtrA GlnA	32
KC1570	NtrA$^-$	2
MK9011	GlnA$^-$	41
E. coli		
ET8000	Wild type, NtrA	
ET8045	NtrA$^-$	25
P. facilis		
J	Wild type, Hno	ATCC 17695, DSM 550
JF88	Hno$^-$	This study
Plasmids		
pSUP202	Tc^r Cm^r Ap^r	39
pCH148	hno_A Tc^r Cm^s Ap^r	This study
pCH170	hno_A Tc^s Cm^r Ap^r	This study
pCH137	hno_A::Tn*5-mob*	This study
pVK101	Tc^r Km^r	20
pVK102	Tc^r Km^r	20
pGE45	hno_A Tc^r Km^r	This study
pGE57	hno_P Tc^r Km^s	This study
pMM17	$ntrA_K$	29
pTH700	$ntrA_E$	B. Magasanik, Cambridge, Mass.
pKR7649	$nifD'_B$-*lacAYZ'* $nifA$(Con)$_K$ Ap^r	H. M. Fischer and H. Hennecke, Zurich, Switzerland

[a] Subscripts indicate the origin of the gene (A, *A. eutrophus*; E, *E. coli*; K, *K. pneumoniae*; P, *P. facilis*; B, *Bradyrhizobium japonicum*). *nifA*(Con), Constitutive expression of the gene.

Complex media used were Luria broth for enteric bacteria and nutrient broth for hydrogen-oxidizing bacteria.

Standard recombinant DNA techniques. Total DNA was isolated from stationary-phase cells grown in mineral medium as described previously (28). Large-scale vector and recombinant plasmid DNAs from *E. coli* were prepared by ethidium bromide-cesium chloride gradient centrifugation after alkaline lysis of the cells (4). Rapid, small-scale DNA isolation for clonal analysis was done by the method of Birnboim and Doly (4). Restriction endonucleases and T4 DNA ligase were used as recommended by the manufacturer. *E. coli* S17-1 and HB101 were transformed with the ligated DNA by the method of Mandel and Higa (26). A genomic DNA library of *A. eutrophus* was constructed by using the vector pSUP202 (39). For construction of a gene library of *P. facilis* DNA, the cosmid vector pVK102 (20) was used and DNA was packaged in vitro with a DNA-packaging kit. Complementation studies with *A. eutrophus* DNA were conducted with plasmid pVK101 (20). The transfer of plasmids was achieved by mating the cells on solid nutrient broth medium as previously described (6). Selective media contained 15 μg of tetracycline per ml and 350 μg of kanamycin per ml for *A. eutrophus* transconjugants and 3 μg of tetracycline per ml for *P. facilis* transconjugants. Recombinant DNA-containing *E. coli* strains were selected in the presence of 15 μg of tetracycline, 30 μg of kanamycin, and 50 μg of chloramphenicol per ml. Agarose gel electrophoresis was performed as previously described (14). DNA-DNA hybridization was conducted by the method of Southern (40). For labeling and detection of DNA, biotin-11-dUTP, Nick Translation Kits, and BlueGene Kits (Bethesda Research Laboratories, Inc., Karlsruhe, Federal Republic of Germany) were employed as recommended by the manufacturer.

Cloning of the *hno* gene from *A. eutrophus*. For cloning of the *hno* gene of *A. eutrophus*, we proceeded as follows. The Tn*5*-labeled *hno* gene was cloned in *E. coli* from a genomic DNA library of mutant HF149 (Table 1). The resulting hybrid plasmid pCH137 was introduced into the *A. eutrophus* wild type and rescued by homologous recombination. A single crossover led to insertion of the whole hybrid pCH137 into the recipient chromosome and resulted in a Km^r Tc^r Hno$^+$ phenotype of heterogenote recombinants. The DNA of such a recombinant was partially digested with *Eco*RI, immediately ligated, and transformed into *E. coli*. Transformants were selected for a Tc^r Km^s Cm^s phenotype, which was indicative of a wild-type *hno* gene-containing clone.

Enzyme assays. Cells for hydrogenase assay were grown heterotrophically with a substrate mixture of fructose and glycerol at a concentration of 0.2% (wt/vol) each. Soluble hydrogenase activity was measured by hydrogen-dependent NAD^+ reduction with whole cells. Particulate hydrogenase activity was determined with membrane fractions and methylene blue as the electron acceptor (9). Cells for the ribulose 1,5-bisphosphate carboxylase assay were grown with formate as the carbon source, and enzymatic activity was measured as described previously (23). Urease activity was assayed by determining the quantity of ammonia formed within a given period of time (35). β-Galactosidase activity was measured with cells from the exponential phase of growth by the method of Miller (31). Protein was determined with bovine serum albumin as the standard (24).

Chemicals. Biotin-11-dUTP, Nick Translation Kits, and Blue Gene Kits were purchased from Bethesda Research Laboratories. Restriction endonucleases, T4 DNA ligase, and the lambda DNA-packaging kit were purchased from C. F. Boehringer & Soehne GmbH, Mannheim, Federal Republic of Germany. $NaH^{14}CO_3$ was purchased from The Radiochemical Centre, Amersham, United Kingdom. All other chemicals were purchased from E. Merck AG, Darmstadt, Federal Republic of Germany.

RESULTS

Analysis of *hno*-containing hybrid plasmids. *A. eutrophus* HF149 is an Hno-deficient mutant that was isolated by Tn*5* mutagenesis. Plasmid pSUP5011 (38) had been used to introduce Tn*5* into *A. eutrophus*. The vector contains an IncP1-specific *mob* sequence as part of the transposon. The total DNA was isolated from strain HF149, digested with restriction endonuclease *Eco*RI, and separated on a 0.8% agarose gel. Southern blots of these gels with radiolabeled Tn*5* DNA as a probe revealed a single Tn*5* insertion site on the HF149 chromosome. Two other independently isolated Hno$^-$ mutants exhibited the same insertion pattern (data not shown). Since there are no *Eco*RI sites in the 7.7-kilobase-pair Tn*5-mob* DNA sequence (38), the 20-kilobase-pair insert from the HF149 chromosome, which hybridized to Tn*5* DNA, contained 12.3 kilobase pairs of flanking chromosomal DNA. The 20-kilobase-pair fragment was cloned in *E. coli*

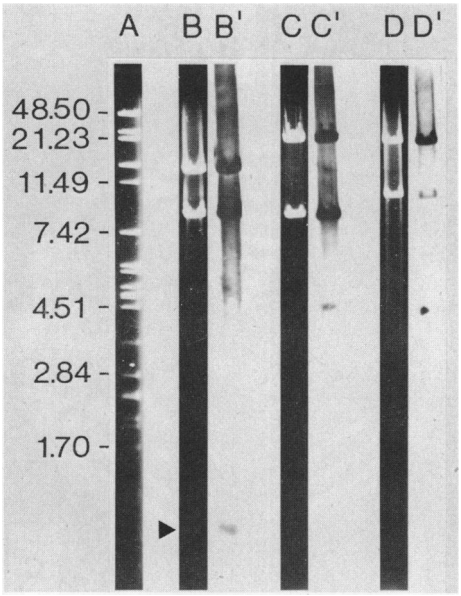

FIG. 1. Southern hybridization analysis of *hno*-containing DNA sequences. Biotin-11-dUTP-labeled DNA of plasmid pCH170 with a 6.5-kilobase insert of *A. eutrophus* DNA was hybridized to *Eco*RI-digested DNA of pCH148 (B'), the Tn*5*-containing hybrid pCH137 (C'), and the *P. facilis hno* hybrid pGE59 (D'). Lanes marked without primes contain the corresponding DNA fragments separated by agarose gel electrophoresis. Lane A contains size markers that are undigested and *Pst*I- and *Eco*RI-digested bacteriophage λ DNA. Sizes are indicated in kilobase pairs. Arrowhead indicates 0.5-kilobase-pair *Eco*RI fragment.

and used to identify and to clone the corresponding wild-type DNA sequence. This was achieved by the straightforward process that is described in Materials and Methods.

Southern blot analyses and restriction maps of the mutant and the wild-type DNA sequences are shown in Fig. 1 and 2. The Tn*5*-containing hybrid plasmid pCH137 was digested with *Eco*RI. It revealed the expected 20-kilobase-pair fragment (Fig. 1C) which exhibited homology to the corresponding wild-type fragment of 12.3 kilobase pairs of plasmid

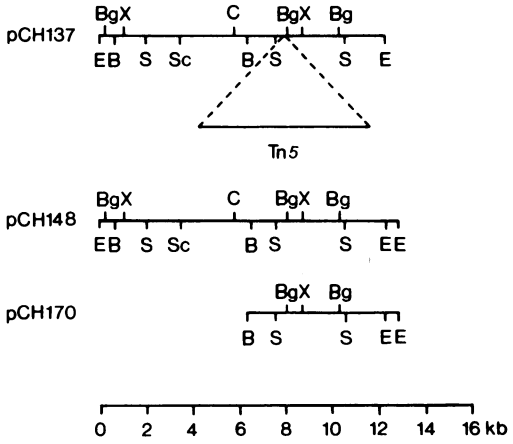

FIG. 2. Restriction endonuclease map and the site of Tn*5* insertion in *hno* DNA-containing hybrid plasmids constructed with *A. eutrophus* DNA. Restriction enzyme sites: E, *Eco*RI; S, *Sal*I; X, *Xho*I; Bg, *Bgl*II; B, *Bam*HI; Sc, *Sac*I; C, *Cla*I.

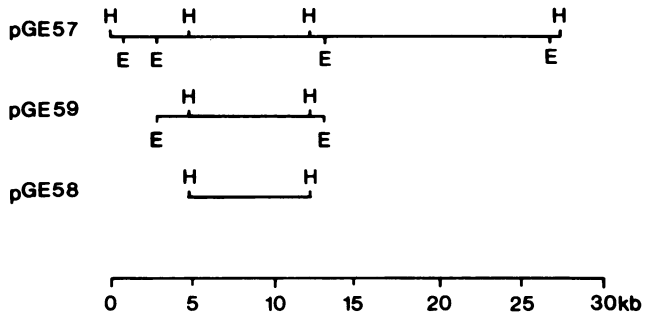

FIG. 3. Hybrid plasmids with *hno* DNA of *P. facilis*. Restriction enzyme sites: E, *Eco*RI; H, *Hin*dIII.

pCH148 (Fig. 1B). Bands occurring at a size of 8 kilobase pairs were due to hybridization to pSUP202 vector DNA. pCH148 contained an additional 0.5-kilobase-pair *Eco*RI fragment as a result of incompletely digested DNA (Fig. 1B, arrowhead). A plasmid with subcloned DNA was generated from pCH148 by restriction with the endonuclease *Bam*HI. The resulting hybrid pCH170 exhibited a *Bam*HI fragment of 6.5 kilobase pairs of *A. eutrophus* DNA and 4.5 kilobase pairs of pSUP202 vector DNA; the latter section is not shown (Fig. 2).

A homologous DNA fragment which hybridized to the *A. eutrophus* DNA insert of pCH170 was identified in a cosmid DNA library of total DNA from *P. facilis* (Fig. 3). This is demonstrated with subcloned DNA of plasmid pGE59, which contains a 10.2-kilobase-pair *Eco*RI insert in the broad host range vector pVK101 (Fig. 1D). The upper band in Fig. 1D is due to hybridization to *mob*-specific DNA sequences common to both vector systems used in this study.

Complementation of Hno⁻ mutants. The cloned *hno* DNA-containing sequences of both hydrogen bacteria included in this study were examined for the ability to complement independently isolated Hno⁻ mutants. For this purpose, the large 12.3-kilobase-pair *Eco*RI insert of pCH148 (Fig. 2) was cloned into the vector pVK101. The resulting hybrid, pGE45, was introduced into the point mutant HF09 as well as the Tn*5* insertion mutant HF149 by mating with the *E. coli* donor strain. Transconjugants were selected on tetracycline-containing fructose-mineral medium and subsequently tested for autotrophic growth with hydrogen, assimilation and dissimilation of nitrate, and utilization of fumarate. The experimental results are summarized in Table 2. They clearly show that pGE45 was capable of restoring the selected traits which were impaired in Hno⁻ mutants.

Enzymatic analysis revealed that pGE45-harboring transconjugants of HF09 had recovered almost the wild-type amount of soluble hydrogenase and urease activity, whereas

TABLE 2. Growth characteristics of complemented Hno⁻ *A. eutrophus* mutants

Strain	Doubling time (h) in:			
	H_2, CO_2, O_2	Nitrate + O_2	Nitrate − O_2	Fumarate
H16 (wild type)	4.1	3.9	7.8	2.1
HF09 (Hno⁻)	ng[a]	ng	ng	24
HF09(pGE45)	4.4	4.4	11.8	2.4
HF149 (Hno⁻)	ng	ng	ng	7.5
HF149(pGE45)	4.2	4.6	9.4	2.5

[a] ng, No growth.

TABLE 3. Restoration of enzymatic activities by cloned *hno* DNA from *A. eutrophus* and *P. facilis*

Strain	Enzymatic activity (U/mg of protein)[a]		
	Soluble hydrogenase	Particulate hydrogenase	Urease
A. eutrophus			
H16 (wild type)	1.75	1.17	0.128
HF09 (Hno$^-$)	0	0	0.006
HF09(pGE45)	1.00	0.22	0.054
HF09(pGE57)	1.50	0.19	0.080
P. facilis			
J (wild type)	np[b]	0.58	0.066
JF88 (Hno$^-$)	np	0.02	0.009
JF88(pGE45)	np	0.10	0.059
JF88(pGE57)	np	1.19	0.065

[a] Growth and assay conditions are described in Materials and Methods.
[b] np, Not present.

the membrane-bound hydrogenase activity was lower than that of the parent strain (Table 3). Attempts to complement the Hno$^-$ phenotype in mutants from *A. eutrophus* and *P. facilis* with cloned *hno* DNA sequences from either one of the two bacterial genera were successful. *P. facilis* DNA, present in pGE57, was as active as the equivalent *A. eutrophus* DNA of pGE45. Again, it was observed that the latter was less efficient in restoring membrane-bound hydrogenase activity in *P. facilis* JF88.

Hno of *A. eutrophus* has RpoN-like properties. The fact that Hno mutants were extremely pleiotropic and could be restored to the wild-type phenotype even by intergeneric complementation raised the possibility of Hno being a global control element whose existence was not restricted to lithoautotrophic bacteria. Thus we introduced the *hno*-containing DNA sequence into two classes of pleiotropic regulatory mutants of enteric bacteria. Fnr$^-$ mutants and RpoN$^-$ (NtrA$^-$) mutants were chosen as recipients. Fnr$^-$ mutants are known to be impaired in the expression of anaerobic metabolic traits (22), whereas the *rpoN (ntrA)* mutation was reported to predominantly affect functions involved in the nitrogen metabolism of the cell (13, 16). DNA of plasmid pCH148 which contained the *hno* gene from *A. eutrophus* was transferred into the *E. coli* Fnr$^-$ strain. The resulting transformants failed to grow anaerobically with nitrate or formate and behaved exactly like the mutant strain (data not shown). Unexpectedly, the *hno*-containing transformants derived from NtrA$^-$ mutants had recovered the ability to grow almost as rapidly as the wild type on minimal medium in the absence of glutamine. This is representatively demonstrated on an agar plate in Fig. 4. The NtrA$^-$ mutant of *E. coli*, ET8045, required glutamine for normal growth due to a very low level of glutamine synthetase (Fig. 4b). This deficiency could be complemented by transfer of the native *E. coli rpoN* gene (Fig. 4c) and by the subcloned *hno* gene of plasmid pCH170 (Fig. 4d). Inactivation of *hno* by transposon Tn*5* abolished complementing activity (Fig. 4e).

Table 4 summarizes the growth data and shows that *hno* from *A. eutrophus* also restored the NtrA$^-$ phenotype of *K. aerogenes*. Transfer of plasmid pCH170 into a *glnA* mutant which was a glutamine auxotroph as a result of a mutation in the glutamine synthetase structural gene did not restore prototrophy (data not shown). Thus, *hno* seems to be specifically related to *rpoN*. Attempts to complement NtrA$^-$ mutants with the *hno* gene from *P. facilis* were not successful. Moreover, there was no DNA homology by Southern

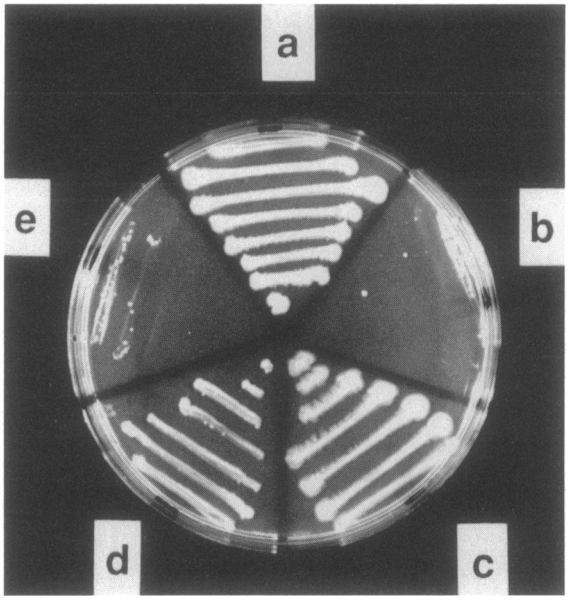

FIG. 4. Relief of *ntrA*-dependent glutamine auxotrophy by *hno* DNA. The following *E. coli* strains were streaked on a glucose-ammonium-mineral agar plate: ET8000 wild type (a), ET8045 NtrA$^-$ (b), ET8045(pTH700) complemented with *ntrA* (c), ET8045(pCH 170) complemented with *hno* (d), and ET8045(pCH137) complemented with *hno*::Tn*5* (e).

blot hybridization between *hno* and *rpoN* DNAs from *E. coli* and *K. pneumoniae*, respectively (data not shown). Although glutamine auxotrophy was complemented by pCH170 on the basis of growth, glutamine synthetase activity was only slightly enhanced. NtrA$^-$ strains of *E. coli* and *K. aerogenes* contained approximately 4% of the wild-type glutamine synthetase activity; this level was increased to 7% by introduction of *hno*. The strain-specific *rpoN* gene restored glutamine synthetase activity completely. A possible explanation for the discrepancy between growth and enzymatic activity is discussed below.

TABLE 4. Growth of NtrA$^-$ mutants and *hno*-complemented derivatives on glucose-minimal medium

Strain	Doubling time (min)	Origin of complementing plasmid
E. coli		
ET8000 (wild type)	85	nr[a]
ET8045 (NtrA$^-$)	180	nr
ET8045(pTH700)	127	*E. coli*
ET8045(pMM17)	105	*K. pneumoniae*
ET8045(pCH170)	115	*A. eutrophus*
ET8045(pCH137)	210	*A. eutrophus*
K. aerogenes		
KC1043 (wild type)	85	nr
KC1570 (NtrA$^-$)	275	nr
KC1570(pTH700)	nt[b]	*E. coli*
KC1570(pMM17)	140	*K. pneumoniae*
KC1570(pCH170)	120	*A. eutrophus*
KC1570(pCH137)	405	*A. eutrophus*

[a] nr, Not relevant.
[b] nt, Not tested.

TABLE 5. Activation of the *B. japonicum nifD* promoter by Hno of *A. eutrophus*

E. coli host strain	Plasmids[a]	β-Galactosidase activity[b] (mU/mg of protein):	
		A	B
ET8000 (wild type)	pKR7649	610	791
ET8045 (NtrA⁻)	pKR7649	4.4	8.6
ET8045 (NtrA⁻)	pKR7649 + pGE45	23.1	29.3

[a] Plasmids were introduced by transformation.
[b] Cells were grown in M9 medium with 0.2% (wt/vol) NH_4Cl and 0.04% (wt/vol) glutamine (A) or with 0.2% (wt/vol) glutamine as the sole nitrogen source (B).

In a second approach, we tested the activity of *hno* on *rpoN*-controlled *nifD* expression. Plasmid pKR7649, which contained a *nifD'-lacAYZ* fusion in addition to a constitutive *nifA* activator gene (1, 8), was transferred into the NtrA⁻ mutant *E. coli* ET8045. Subsequently the strain was transformed with DNA of the *hno* hybrid pGE45 (Table 1). Plasmid pCH170 could not be used in these experiments due to incompatibility problems. The results of the complementation (Table 5) demonstrated a clear increase (between three- and fivefold) of β-galactosidase activity caused by *hno* of *A. eutrophus*.

DISCUSSION

Multiple forms of sigma factors exist in various bacterial species. They regulate transcription by interacting with catalytically active core RNA polymerase and thus confer a distinct promoter specificity to the holoenzyme. The most prominent examples of metabolic functions that are controlled by alternative σ factors are vegetative and sporulation-specific genes in *Bacillus subtilis* (7), heat shock genes (12), and nitrogen-regulated genes (13, 16, 33) in enteric bacteria.

In this paper, we report that two chemolithoautotrophic organisms, *A. eutrophus* and *P. facilis*, contain an *rpoN*-like gene, designated *hno*, whose product is required for the following rather diverse physiological processes: oxidation of molecular hydrogen by hydrogenase, utilization of nitrate and urea as sole nitrogen sources, aerobic growth on fumarate, and anaerobic growth on nitrate. The range of metabolic activities affected by *hno* seems to be even more extensive. It has been shown previously that mutants impaired in *hno* are also defective in the metabolism of nickel ions, the expression of ribulosebisphosphate carboxylase activity, and growth on formamide, succinate, glutamate, and malate (35). An additional pleiotropic *hno* marker was detected in *A. eutrophus* recently, namely, growth on 1-butanol (D. Römermann and B. Friedrich, unpublished result).

The conclusion that Hno represents an RpoN-like sigma factor of RNA polymerase was drawn from complementation studies using RpoN⁻ (NtrA⁻) mutants of enteric bacteria as recipients. So far, this analogy is certainly based on indirect evidence, and to explore the putative *rpoN*-like gene further, DNA sequence analysis and the identification of the *hno* product and its target promoter sequences are necessary. Preliminary nucleotide sequence data indicate the existence of two tandemly arranged RpoN-specific promoter boxes upstream of the soluble hydrogenase structural genes, whereas a regular *E. coli* consensus promoter sequence is missing in this DNA region (A. Tran-Betcke and B. Friedrich, unpublished result).

The *rpoN* gene from *Azotobacter vinelandii* was shown to exhibit conserved nucleotide sequences to *rpoN* from *Klebsiella pneumoniae* and *Rhizobium meliloti* (30). Despite this fact, the *rpoN* promoter of the latter organism was not active in *E. coli* (36). Moreover, only weak complementation of glutamine synthetase in *E. coli* by the *Klebsiella rpoN* gene has been reported (3). Some of our data, namely, the failure to demonstrate a significant enhancement of glutamine synthetase activity by *hno* and the absence of complementing activity of the *hno* gene from *P. facilis*, may be due to weak expression of *hno* as a result of different codon usage of the G+C-rich DNAs of *A. eutrophus* (68 mol%) and *P. facilis* (63 mol%) and a low promoter activity in enteric bacteria. Nevertheless, *hno* of *A. eutrophus* restored glutamine auxotrophy, although glutamine synthetase activity was rather low. This discrepancy may be explained by a low threshold level to serve biosynthetic purposes. More convincing was the fourfold activation by *hno* of β-galactosidase with the *nifD-lacZ* fusion (1, 8). The latter system was less complex than glutamine synthetase, since the coactivator NifA, which is required in addition to σ^{54} and is analogous to the *glnA* coactivator, NtrC (13, 16), was expressed constitutively.

The observation that *hno* is implicated not only in nitrogen-regulated operons but also in pathways involved in energy metabolism and substrate transport was initially rather confusing. The only feature common to these metabolic activities is the fact that they represent alternative pathways in *A. eutrophus* and *P. facilis* which are not essential for aerobic heterotrophic growth and thus for the survival of the organisms. It seems likely that in other bacterial species, *rpoN* also has a broader physiological significance than was originally envisaged. In *R. meliloti*, the *rpoN* gene is required for diverse metabolic functions, such as C-4 dicarboxylate transport, nitrate assimilation, and symbiotic nitrogen fixation (36). RpoN-sensitive promoter sequences preceding genes for pilin formation (18) and the *xylABC* operon on Tol plasmids (17) have been detected in *Pseudomonas* species. The *rpoN* gene product was reported to be involved in anaerobic metabolism of *E. coli*. Two enzymatic activities, formate dehydrogenase and hydrogenase isoenzyme 3 (3), were shown to be affected by *rpoN*.

Provided *hno* codes for an RpoN-like sigma factor of RNA polymerase, we have presented two additional examples for RpoN-controlled hydrogenase genes. In a previous study we showed that structural and regulatory hydrogenase genes are clustered on megaplasmid pHG1 of *A. eutrophus* (21). One of the cloned DNA sequences contains a regulatory gene locus, *hoxC*, which is essential for a coordinate expression of both the soluble and the particulate hydrogenases whose structural genes were identified in two separate operons (6). One of the products of *hoxC* acts as a positive regulator and confers temperature sensitivity to *hox* structural gene expression (11). Moreover, it responds to an as-yet-unknown intracellular mediator that signals energy deficiency to the cell, a condition that is required for activation of *hox* gene transcription in *A. eutrophus* (6; G. Eberz and B. Friedrich, unpublished result). In conclusion, hydrogenase synthesis strictly depends on the chromosomal *hno* gene, whose product acts in concert with at least one other transcriptional activator, encoded by *hoxC* on the megaplasmid DNA. These observations are consistent with those

made for NtrA-controlled systems, which imply transcriptional coactivators such as NtrC, NifA, DctD, XylR (42).

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