The *narX* and *narL* genes encoding the nitrate-sensing regulators of *Escherichia coli* are homologous to a family of prokaryotic two-component regulatory genes

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ABSTRACT

The nucleotide sequence of a 4.4-kilobase SacII-SspI fragment encoding the narXL operon and a part of the narK gene of Escherichia coli has been determined. The narX and narL genes encode proteins of molecular weight 67,275 and 23,927, respectively, and are transcribed from a common promoter, narXp, locating within 429 bases upstream of narX. Transcription from narXp is not significantly induced by nitrate under anaerobiosis, whereas transcription from narK promoter, which overlaps narXp region and is transcribed divergently, is fully induced by nitrate. The N-terminal two-thirds of the NarL protein has extensive homology with those of a diverse set of prokaryotic regulatory proteins, including OmpR, PhoB, SfrA, UhpA, CheY, CheB, NtrC, DctD, FixJ, VirG, Spo0F, and Spo0A. A segment locating in the C-terminal half of the NarL protein seems to have potential most likely to form the helix-turn-helix structure characteristic of a class of DNA-binding protein. The protein is considered to play a role as a transcriptional activator of the nitrate reductase operon, narCHJI, and the narK gene. The C-terminal region of the NarX protein also has homology with other regulatory proteins known as counterparts of two-component regulatory systems, such as EnvZ, PhoR, PhoM, CpxA, NtrB, DctB, FixL, and VirA. Presence of two copies of hydrophobic segments in the N-terminal half of the NarX protein suggests the role as a transmembrane receptor sensing nitrate.

INTRODUCTION

Escherichia coli can exploit a variety of exogenous compounds as a terminal electron acceptor for respiratory electron transfer, including, in order of decreasing redox potential, oxygen, nitrate, trimethylamine N-oxide (Me₃NO), and fumarate. Use of these compounds is hierarchical, thereby maximizing buildup of proton motive force across the plasma membrane. This is effected by a complex regulatory network of gene expression that enables *E. coli* cells to take advantage of the available electron acceptor with the highest redox potential (reviewed in refs. 1 and 2).

The enzyme nitrate reductase consists of three membrane-bound subunits and contains molybdenum cofactor and heme (2). All three of the subunit polypeptides are encoded by the *narCHJI* operon located at 27 min on the *E. coli* chromosome (3, 4). The operon is positively controlled by an activator protein encoded by the closely linked *narL* gene in response to nitrate as the inducer (5), as well as by a pleiotropic transcriptional activator, the *fnr* gene product, which mediates anaerobic induction of several respiratory enzymes (6). In addition, the *narL* gene product has been shown to act as a repressor in the presence of nitrate on the operons *tor* and *frd* that encode Me₃NO and fumarate reductases, respectively (7, 8). Thus the NarL protein plays an important role as a genetic switch in the differential expression of these operons.

By cloning and operon fusion studies, regulatory region of the nar operon including

narL has been characterized (9). The narL gene is transcribed divergently with respect to the *narCHJI* operon accompanying the proximal *narX* gene. The *narX* and *narL* genes encoding proteins with estimated molecular weights of 66,000 and 28,000, respectively, may constitute an operon, and their expression is relatively insensitive to Fnr and nitrate. Therefore, one hypothesis to explain the hierarchical control of the reductase operons by nitrate is that the NarX and/or NarL proteins are transcriptional regulators of nar, tor, and *frd* operons eventually sensing the nitrate availability.

In the studies reported here, we have sequenced entire region of the *narX* and *narL* genes, which were subcloned from the insert of lambda 13H6 bearing upstream region of the *narCHJI* operon (10). The sequence revealed that NarX and NarL have homology in the deduced amino acid sequence with other prokaryotic regulatory proteins which belong to a two-component regulatory system and transduce environmental signals to transcriptional apparatus (11).

MATERIALS AND METHODS

Bacterial strains and media

E. coli K-12 strains used in this study were XL1-Blue (recA1 Δlac endA1 gyrA96 thi hsdR17 supE44 relA1 { F' proAB lacl lacZ Δ M15 Tn10}, obtained from Stratagene), NM522 (supE thi Δ (lac-proAB) hsdR5 { F' proAB lacI⁴ lacZ Δ MI5}) (12), and TNK50 (NM522 with $\Delta narXL$::kan). The narXL deletion strains were constructed by the gene replacement method (13) utilizing homologous recombination of plasmid-encoded Δ narXL::kan genes in pNR66 (see below) with the chromosomal narXL alleles in polA12 strain MM383 (14). Subsequent P1 vir transductions were performed by the method of Miller (15) to obtain TNK50. Defined and complex media used for routine culture were as described previously (16). MacConkey nitrate agar was used to score Nar phenotype as described previously (17). When necessary, ampicillin (50 μ g/ml), tetracycline (12.5 μ g/ml), kanamycin (25 μ g/ml), chloramphenicol (10 μ g/ml), isopropyl- β -D-thio-galactopyranoside (IPTG, 24 μ g/ml), or 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal, 40 μ g/ml) was added. Induction by nitrate was accomplished in the medium containing 1% KNO₃.

Plasmids and DNA sequencing

The *narX* and *narL* genes sequenced in this study was from pNR17, which was constructed by subcloning an 8.4-kilobase (kb) SalI-EcoRI fragment from lambda 13H6 into pBR322 (10). A 6.4-kb BalI fragment of pNR17 bearing the complete coding region of the narK, narX, and narL genes was further subcloned in two orientation into SmaI site of pTN1058 to obtain pNR24 and pNR30 (Fig. 1). Derivatives of pNR24 and pNR30 were constructed by deleting various restriction fragments as shown in Fig. 1. The same 6.4-kb Ball fragment of pNR17 was subcloned into EcoRI-DraI sites of pBR322 after filling an end with T4 DNA polymerase. From the resultant plasmid, pNR57, most of the narXL coding sequence from XhoI site at 3.8 kb to Bg/II site at 1.9 kb (Fig. 1) was replaced with kan gene from pUC4K (18) to obtain pNR66.

DNA sequencing was done by the dideoxy-chain termination method (19) with $[\alpha^{-35}S]$ thio-dATP and modified T7 DNA polymerase (Sequenase, United States Biochemical Corp.). Single-stranded templates were prepared from derivatives of M13 phage (20) carrying various restriction endonuclease fragments from pNR24. Both strands were sequenced with minimum overlapping of 30 bases (strategies not shown). The DNA sequencing information was processed using the GENETYX software package (Software Development Co., Ltd., Tokyo).

Promoter analysis

Plasmid pTN1058 used for assaying promoter activity was constructed by replacing unique EcoRI site of pTN1051 (21) with the polylinker sequence of pUC18 from EcoRI site to SalI site. The plasmid pTN1058 contains a terminator sequence within 120 bases upstream of the cloning sites which effectively prevents transcriptional reading through from the P4 and P5 promoters present in pBR322 (22, 23). There is an in-frame stop codon with the downstream lacZ' gene between the cloning sites and the initiation codon which prevents translational fusion from an inserted gene. Promoter activity in the *narK-narX-narL* region was determined by subcloning various restriction fragments into the multiple cloning sites of pTN1058. The activity was estimated by the α -complementation of the LacZ activity in XL1-Blue host on X-gal plate containing IPTG with or without nitrate under aerobic or anaerobic condition.

Gene product analysis

The NarX and NarL proteins were overexpressed in XL1-Blue harboring plasmid pNR71, pNR73, or pNR74 (Fig. 1) constructed in pHSG398 (24). The plasmids pNR71, pNR73, and pNR74 contained 2.9-kb DraI-AvaI fragment, 2.0-kb DraI-BgII fragment, and 1.2-kb *NsiI* fragment, respectively, from pNR24 inserted into the multiple cloning sites of pHSG398 such that the *narX* and *narL* genes would be transcribed from the *lac* promoter.



Figure 1. Genetic and physical map of the *narK-narX-narL* region. The top line represents restriction sites determined by the enzymatic digestion. In the middle portion show the locations of *narK*, *narX*, and *narL* coding sequences. Arrows in the bottom section give the length and orientation of the inserts shared by the plasmids pNR24 to pNR52 and pNR63 (24 to 52 and 63 in the figure) constructed in pTN1058 to detect promoter activity: -, no; +, constitutive; *, nitrate-inducible promoter activities determined by the LacZ assay. The promoter region of each *nar* operon was indicated as *narCp*, *narKp*, *narXp*, and *narLp*.

← narK	
gttgaccgcgttgttgccagaacgcaggatcttccggtcgccaatctgtaatgacagctccagtagccctttcgggggggg	100
\overline{ctct} gatactcgtttcgatgtctgccaccttagtgtctgtagctaaaggcaatttgatgtaaatcaaacgataagataactttatcattgatattt	200
atcattacccatagtgagtacagtgacttcataaaaattatgagatttttcacggtgctgtaaaatccctacccttaccgatgtaaagcgactaaccaca	300
cggcaaataaggagtaactctttccgggtatgggtatacttcagccaatagccgagaatactgccattccagaatgtatcgtcacattcatt	400
$t_{geteatttaaageetgaaggaaggtttaeatgettaaaegttgtetetete$	500
narX→MLKRCLSPLTLVNQ <u>VALIVLLST</u>	(23)
tgctattggactggcagggatggcggtttctggctggctg	600
<u>AIGLAGMAVSGWLV</u> QGVQAAPMRSTKRDALRMQ	(56)
agttaccgtctgttggcggcagtgccattaagcgagaaagacaagccttaattaa	/00
SYRLLAAVPLSEKDKPLIKEMEQIAFSAELIKA	(89)
	(123)
	900
S A D V S O F V A G L D O L V S G F D R T T E M R I E T V V L V H	(156)
cgggtaatggcggtatttatggcacttttactggtgttcactattatctggttgcgggcgcgactgctacaaccgtggcggcaactgctggcaatggCga	1000
R V M A V F M A L L L V F T I I W L R A R L L Q P W R Q L L A M A	(189)
gtgccgtcagtcatcgcgattttacccaacgcgcaaacatcagcgggcgcaacgaaatggcgatgcttggaactgcgttgaacaatatgtctgcagaact	1100
ŜĂVŜHRDFTQRĂNIŜĜŔNEMĂMLĠTĂLNNMSAEL	(223)
ggccgaaagttatgccgtacttgagcagcgggttcaggagaaaaccgccgggctggagcataaaaatcagatcctctcttttttatggcaggctaaccgc	1200
A E S Y A V L E Q R V Q E K T A G L E H K N Q I L S F L W Q A N R	(256)
cgtttgcattcccgcgccccgctgtgtgaacgcctgtcacctgtactcaacggcttacagaatttaaccctgctacgtgatatcgaattgcgggtgtatg	1300
R L H S R A P L C E R L S P V L N G L Q N L T L L R D I E L R V Y	(289)
acactgatgatgatgatgaagagaatcatcaggagtttacctgccagcca	(222)
	1500
C D D C T T T V U D I A D S H T O V C I I I A T I D O C D H I S H	(356)
at car as a start of to start contrast car	1600
D O O O L V D T L V E O L T A T L A L D R H O E R O O O L I V M E	(389)
agcgtgccaccattgcgcgcgaactgcatgattctattgcccaatctctctttgcatgaagatgcaggtgagttgtttacagatgcagggcgatgcgct	1700
ĔŘATIĂŘELHĎSIĂQSLSČMKMŎVŠČLŎMŎŢŎĀL	(423)
gccagaaagcagccgcgaactgttaagtcagatccgtaacgaactgaatgcatcctgggcgcagttgcgtgaattgctcaccacattccgcttgcagctgcagctgcagttgcagttgcagctgcagttgcag	1800
PESSRELLS QIR NEL NASWA QLRELLTTFRL QL	(456)
accgagctggattacgtccggcgctggaggcgagttgcgaagagtacagcgccaaatttggcttcccggtgaagctggattatcaattgccgcctcgcc	1900
T E P G L R P A L E A S C E E Y S A K F G F P V K L D Y Q L P P R	(489)
tggtgccttcgcatcaggcaatccacttgttgcaaattgcccgtaggcaattagtaacgccctcaaacattcgcaagcgagtgaagtcgtggtgacggt	(523)
	2100
A O N D N O V K L T V O D N G C G V P F N A T R S N H Y G M T T M	(556)
greatestescaaagtitaceaegegattgccgccgtcgtcgtgaatcaggtggcaccgaagtggtggtcaccttattcccgaaaaaactttca	2200
R D R A Q S L R G D C R V R R R E S G G T E V V V T F I P E K T F	(589)
cagacgtccaaggagatacccatgagtaatcaggaaccggctactatcctgctgattgacgatcacccgatgctgcgaactggcgtaaaacagcttatca	2300
T D V Q G D T H E *	(598)
narL→M S N Q E P A T I L L I D D H P M L R T G V K Q L I	(26)
gtatggcaccagatatcaccgtggttggcgaagcgagtaatggcgaaccagggtattgaactggcggagtctcttgatcccgatctgatcctgttagatct	2400
S M A P D I T V V G E A S N G E Q G I E L A E S L D P D L I L L D L	(60)
caatatgcccggcatgaacggtctggaaacgctggataactgcgcgaaaagtcctttcagggcgcattgtggtattcagcgtttttagcgtctttaaccatgaagaa	2500
N M P G M N G L E I L D K L K E K S L S G K I V V F S V S N H E E	2600
gatgiggicactgeactgeactgeactggeggatggeiattggatggatggatggatggattggat	(126)
aatof at taag coast taac oct of to to concare the core taac of to coact act taag co cast taac cast taac coact act coact act taac coact act act taac coact act taac coact act taac coact act act act act act act act act act	2700
E M V L S E A L T P V L A A S L R A N R A T T E R D V N O L T P R E	(160)
gcgcgatattctcaagctgattgcccagggtttgccgaacaagatgattgcccgccgcctggatatcaccgaaagcacagtaaaagtgcacgtcaagcac	2800
<u> </u>	(193)
atgetgaagaaaatgaagetcaagtetegegtggaageageggtatgggtgeteegggggegeattttetgattaeggtteeeagegeagttegtegttttetgattaeggtteeeagegeagttegtegttttetgattaeggtteeeagegeagttegtegtttttetgattaeggtteeeagegeagttegtegtttttetgattaeggtteeeagegeagttegtegtttttetgattaeggtteeeagegeagttegtegttegt	2900
M L K K M K L K S R V E A A V W V H Q E R I F *	(216)
gacaatgcatcgaacggttcgacaagcgttagctgatctcattggaggagacacgctgcccctggttatcttccaccaccacgacaatcgccagtgatt	3000
gctgccccttccccgtctgccggcgtcggcgctgtcggcgctgtggctggcgccggggtcaaacttaatatctgcgtatcg	3100
ccctgccaaatcagttgccgaataccgtaacgaCtgcggatttgtaatttcagcggcactgtttcgccaggttttagatcccacggcggtgtcgccagaa	3200
acaccyttaac	3211

Figure 2. Nucleotide sequence of the *narXL* operon and deduced amino acid sequence of their products. Possible ribosome binding sites and ATG initiation codons are overlined. Two regions of sufficient hydrophobicity to span the membrane in NarX and one region of helix-turn-helix structure to be concerned with DNA binding in NarL are underlined.

Total cellular proteins accumulated after IPTG induction were dissolved in sodium dodecyl sulfate (SDS) sample buffer, and analyzed by SDS-polyacrylamide gel electrophoresis as described previously (25).

Enzyme assay

Aerobic culture were grown on a vigorously shaking platform at 37°C in midexponential phase. Anaerobic culture were grown in filled 20-ml syringes standing undisturbed at 37°C. β -Galactosidase activity was measured in toluene-permeabilized cells by monitoring the hydrolysis of *o*-nitrophenyl- β -D-galactopyranoside. Nitrate reductase activity was determined as previously described (26).

RESULTS

Subcloning of narX and narL genes

The recombinant plasmids pNR24 and pNR30 contain a 6.4-kb *Ball* restriction fragment which, in addition to the *narX* and *narL* genes encoding the nitrate regulators, bears the *narK* gene and a part of the *narC* operon (9, 27). A detailed restriction map of this region was determined using the enzymes shown in Fig. 1, and is in good agreement with that reported previously (9, 28).

Previous studies have located the *narX* and *narL* genes in the region from 1.5 kb to 4.1 kb on the scale in Fig. 1 (9). Functionality of this region was verified using the *narXL* deletion strain TNK50 by analyzing nitrate induction of the reductase activity under anaerobic condition. Defect in the nitrate induction in TNK50 was restored by transforming with pNR71 as well as pNR24. The *narK* gene with unknown function is also mapped in the 4.3–6.0-kb region. Transcriptional control region of the *narC* operon was already sequenced (27), and shown to correspond to the 5.7-6.4-kb region. However, it was not clear whether the *narX* and *narL* genes constitute an operon. We determined the promoter activity in this region using pTN1058.

Promoter for the narX and narL genes

Anaerobically inducible and nitrate-inducible promoters could be detected in XL1-Blue host with plasmids pNR31, pNR42, pNR43, pNR44, pNR45, and pNR46 but not with pNR34, pNR35, and pNR47 (Fig. 1). Plasmid pNR31 contains a promoter of the *narC* operon (27, 29) and confers on the aerobically growing host cells characteristic blue color on X-gal medium as the center of the colony turned deeper blue with white periphery due to the development of anaerobic central area (4). The similar color reaction was also observed with plasmids pNR44, pNR45, and pNR46 bearing promoter region of *narK*.

The promoters in the *narX-narL* region were complicated. Plasmids pNR26, pNR27, pNR38, pNR39, pNR40, pNR48, pNR49, and pNR52 showed constitutive promoter activity that was essentially independent of anaerobiosis and the presence of nitrate. No promoter activity was detected with plasmids pNR36, pNR50, pNR51, and pNR63. The results suggest that there are at least two promoters operated separately at *XhoI* site in the *narX* coding region. The absence of promoter in the immediate upstream region of *narL* covered by pNR51 implicates that the *narX* and *narL* genes form an operon, which is transcribed from a common promoter located in the upstream region of *narX*. This is confirmed by the analysis of the nucleotide sequence of this region (see Fig. 2). The promoter activity detected with pNR52 seems to imply an additional promoter within the *narX* coding region, which may in part be responsible for the increased expression of *narL* relative to *narX* in the absence of added nitrate (9).

Nucleotide sequence of the narXL operon

The physical map of the *narXL* region (Fig. 1) was used as a basis for nucleotide sequencing of a 4.4-kb SacII-SspI fragment from pNR24. The sequence data (Fig. 2) revealed two major open reading frames (ORFs) with a high coding probability and a leftward polarity, as supported from the findings on the promoter activity. No significant ORF with a high coding probability could be identified with the opposite orientation in the region shown in Fig. 2. We designated the first ORF of 1,794 nucleotides as *narX* and the second ORF of 648 nucleotides as *narL* from the following evidence. Both ORFs were preceded by a strong ribosome binding site sequence at appropriate distance from the first initiation codon ATG. The *narX* and *narL* genes, as mapped in this region with the correct orientation (9), encode proteins with apparent molecular weights of 62,000-66,000 and

	1 10	20	30	40	50	60
Narl (Ec)	MSNOEPATILIT		ISMAPDIT	VVGEASNGEO		
	MTEVNIVII		IDEEDTEE		ADIVENVUE	
$U_{U_{2}}(22k) (E_{2})$	CILVINI VIII			VVCCASCCCD		
	MITUAL		LEDIKGIK	VVGEASCGED	AVKWURINAV	OVVLMUMSMPGI
UNDA (EC)	MIIVALI	DUHLIVRSGFAQL	LGLEPDLQ	VVAEFGSGRE	ALAGLPGRGV	QVCICDISMPDI
FixJ (Rm)	MIDYTVHIV	DDEEPVRKSLAFM	ILTMNGF.A	VKMHQ.SAEA	FLAFAPDVRN	IGVLVTDLRMPDM
OmpR (Ec)	MQENYKNLVV	DDDMRLRALLERY	'LTEQGF.Q	VRSVA.NAEQI	MDRLLTRESF	HLMVLDLMLPGE
PhoB (Ec)	MARRILVV	EDEAPIREMVCFV	LEQNGF.Q	PVEAE.DYDS	AVNQLNEPWF	PDLILLDWMLPGG
PhoM26k (Ec)	MQRETVWLV	EDEQGIADTLVYM	ILQQEGF.A	VEVFE.RGLP	VLDKARKQVF	PDVMILDVGLPDI
SfrA (Ec)	MOTPHILIV	EDELVYRNTLKSI	FEAEGY.D	VFEAT.DGAE	MHOILSEYDI	NUVIMDINUPGG
VirG (At) 22	LKGEPLKHVLLV	DDDVAMRHITTEY	TTHAFK	VTAVA DSTO	FTRVI SSATV	DVVVVDL NI VRF
CheY (Ec)	MADKELKELVV	DESTMERIVEN	I KELGENN	VEFAE DOVD	AL NKL DAGGY	GEVISDWNMPNM
SpollF (Bs)	MNEKTTITV		ENKEGY O	TEDAA NCLO		
N+rC (Kr)	MODELANIN			CTTEE SCNE		
$D_{0+}D_{1}$				VEAVD CAVA		
			LELAGE.S	VSATU.GAKA	ALAULPAUF	AGPVVIDIRMPEI
PGTA (St)	MENDEUSILLI	DUDVDVLDATIP	ILEUAGY.R	VRGFI.HPFE	AKEWVKAUWE	GIVESDVCMPGC
Cher (FC)	MSKIRVLSV	DDSALMRQIMIEI	INSHSDME	MVATAPDPLV	ARDLIKKENE	PDVLTLDVEMPRM
SpoOA (Bs)	MEKIKVSVA	DDMRELVSLLSEY	IEGQEDME	VIGVAYNGQE	CLSLFKEKDF	PDVLVLDIIMPHL
	****	•• •• • •	·	• •	•	**** ** ** *
	70	80 90) 1	00 1	10 1	130
NarL (Ec)	NGLETLDKLREKSL	SGRIVVFSVSM	HEEDVVTA	LKRGADGYLL	KDMEPEDLLK	ALHOAAAGEMVL
DeaU (Bs)	NGVEATKOLVELYP	ESKVIILSIHE	DENYVTHA	LKTGARGYLL	KEMDADTI LE	AVKVVAFGGSYL
UvrC23k (Ec)	GGI FATRKIARSTA	DVKTTMLTVH1	ENPI PAKV	MOAGAAGYLS	KGAAPOEVVS	ATRSVYSGORYT
UhpA (Fc)	SGLELL SOL PKGMA	TIMISVH	SPAL VENA	INACAPCELS		AVHTVATCCCVI
Fiv.1 (Pm)	SOVELLONICOLKI	NIDSIVITCH		MYACANDETE		
				I CICADDVID	KFFEDIVIIE	
	DOLOTIKU KOCON		SEE VURIVG	LEIGADDYIP	KPENPRELLA	ARIKAVLKRUANE
PHOB (EC)	SGIUFIKHLKKESM	INDIPVVMLTAR	ELEDRAKG	LEIGADDYII	KPFSPKELVA	ARIKAVMERISPM
PROMZEK (EC)	SGFELCRULLALHP	ALPVLFLTARS	DEEVDRLLG	LEIGADDYVA	KPFSPREVCA	ARVRTLLRRVKKF
SfrA (Ec)	NGLLLARELREQAN	VALMFLTGRE	DNEVDKILG	LEIGADDYIT	KPFNPRELTI	(RARNLLSRTMNL
VirG (At)	DGLEIVRNLAAKSD	IPIIIISGDRL	.EETDKVVA	LELGASDFIA	KPFSIREFL <i>F</i>	ARIRVALRVRPNV
CheY (Ec)	DGLELLKTIRADGA	MSALPVLMVTAEA	KKENIIAA	AQAGASGYVV	KPFTAATLEE	EKLNKIFEKLGM*
SpoOF (Bs)	DGIEILKRMKVIDE	NIRVIIMTAY	SELDMIQES	KELGALTHFA	KPFDIDEIRD	DAVKKVLPLKSN*
NtrC (Kp)	DGLALLKOIKORHP	MLPVIIMTAHS	SDLDAAVSA	YOOGAFDYLP	KPFDIDEAVA	ALVDRAISHYOEO
DctD (R1)	DGLOLFATLOGMDV	DL PVILMTGHO	DIPMAVOA	TODGAYDETA	KPFAADRI VO	SVRRASEKRRI V
PatA (St)	SGIDI MTI FHODDD	OL PTLL TTGH	DVPMAVDA	VKKGAWDELO	KPSTRAKLL	I TEDAL RORRSV
CheB (Ec)	DGI DELEKI MRI RPI	MPVVMVSSI TCK	SEVT I PA	I FI GATDEVT	KPOL GIPECN	AL AVNENTAEKVP
Spo(A (Bs)	DGI AVI EDI DESDI					
Shooy (P2)	DOLATELKERESDE			VDLUASTFIL	KFF DHENLY	
	140		150	100	170	100
No. 1 (F)	140		150	160	1/0	180
Narl (EC)	SEALIPVLAA		NRATIERU	VNULIPRERD	ILKLIAQGL	NKMIARREDITE
DegU (Bs)	HPKVTHNLVNEFRR	LATSGVSAHPQHE	EVYPEIRRP	LHILTRRECE	VLQMLADGKS	SNRGIGESLFISE
UvrC23k (Ec)	ASDIAQQMAL	S.Q1	EPEKTESP	FASLSERELQ	IMLMITKGQH	(VNEISEQLNLSP
UhpA (Ec)	TP	DIA1	KLASG.RQ	.DPLTKRERQ	VAEKLAQGMA	AVKEIAAELGLSP
FixJ (Rm)	AAEA	DVDI	ANDIRAR.	LOTLSERERO	VLSAVVAGLT	PNKSIAYDLDISP
	**		•			
	190 2	00 210	216			
NarL (Ec)	STVKVHVKHMI KKM	KI KSRVFAAVWV	IOFRIF*			
Deall (Bs)	KTVKNHVSNTLOKM	NVNDRTOAVVVA	KNGWVFMD	*		
llvr(23k (Fc)	KTVNSVDVDMESKI			*02217		
				12334		
	DTVEVUDANUMAUM		ACCECTCAC+			
FIXJ (KIII)	RIVEVHKANVMAKM	NANSLPHLVRMAL	AUGLEPS*			

Figure 3. Sequence alignment of NarL with E. coli UvrC ORF2, UhpA, OmpR, PhoB, PhoM ORF2, SfrA, CheY, and CheB, Rhizobium meliloti FixJ, Agrobacterium tumefaciens VirG, Bacillus subtilis DegU, Spo0F, and Spo0A, K. pneumoniae NtrC, R. leguminosarum DctD, and Salmonella typhimurium PgtA. Conserved residues are marked below the sequence, defined as more than 70% of amino acids belonging to one of the groups P A G S T; H K R; Q N E D; I L V M F Y W; C. Possible helix-turn-helix DNA-binding motifs of NarL, UhpA, and FixJ are underlined.

25,000–28,000, respectively, in reasonable agreement with the calculated values of 67,275 and 23,927. Gene product analysis using pHSG398 expression vector located the *narX* and *narL* coding sequences between *Dra*I site at 407 and *Bgl*II site at 2395 and between *NsiI* site at 1748 and *NsiI* site at 2905, respectively (data not shown).

Structure of the NarX and NarL proteins

The deduced amino acid sequences of the NarX and NarL proteins were compared with sequences from National Biomedical Research Foundation data base. NarL was found to have extensive similarity over the first 120 amino acids to the N termini of the OmpR protein from *E. coli* (30, 31) and the NtrC protein from *Klebsiella pneumoniae* (32, 33).

		390	400	410	420	430	440
NarrY	(E_{C})	388MEEDATI				ESCOLISOI	
DogS		179	SPETHDODAO			AEDCECETENI	
Dey3		222 DDDTIIN					
			AGVAUETNOD	LIKIRLAN		TLAESI	NKUIEEUNAIIEUF
DCTR	(RI)	404AILGUVA	AGVAHEINUP	VATIRATA	DNARTFLURGU	TAPAGENLESI	AALTERIGSTIEEL
PgtB	(St)	3/1AVVGQIM	TILAHEIDQP	LINAL SMYL	FTAGRATEQGQ	SGQARNTLRKA	EGLINRIDAIIRSL
СрхА	(Ec)	238TSQQRLL	SDISHELRTP	.LTRLQLGT/	ALLRRRSGE	SKELERI	ETEAQRLDSMINDL
PhoR	(Ec)	202GARRNFF	ANVSHELRTP	LTVLQGYLI	EMMNEQPLEGA	VREKALHTM	IREQTORMEGLVKQL
PhoM	(Ec)	254NYIEQYV	YALTHELKSP	.LAAIRGAAI	EILREGPPPEV	VARFTDNI	LTQNARMQALVETL
NtrB	(Kp)	128IAARDLV	RGLAHEIKNP	LGGLRGAA	QLLSKALPDPA	LMEYTKVI	IEQADRLRNLVDRL
VirA	(At)	468EAVGTLA	GGIAHEFNNI	LGSILGHA	ELAQNSVSRTS	VRRYIDYI	ISSGDRAMLIIDQI
FixL	(Rm)	229NEMGEMA	STLAHELNOP	LSAIANYS	IGCTRLLRDMD	.DAVATRIREA	LEEVASOSLRAGOI
		••	`-				• • • • •
		450 460	470	480)	490	500
NarX	(Ec)	LTTFR.LOLTEPG	LRPALEASCE	YSAKEGEP	KLDYOL	PPRI VPS	HOATHLLOTARFAL
DeaS	(Bs)	I YOL RPMAL DDL G	I TPTI RKYLY	TEEYNG K	KTHEOC	IGETEDORI AP	OFFVAL FRI AOFAV
EnvZ	(Ec)	IDYL RTGOEMPME	MADI NAVI GE	TAAFSGYF	REIF	TALYPGSTEVK	MHPI STKRAVANMV
DetB	(RI)	KTFARKGRGSAFR	TGI KOVIEGA	MII DTDEA			
Dat B) c+(VOVDADOTEV				
CovA			CETTKANOLU				
Срхя			SETTKANULW	SEVEDNAAFI	ALU.MGKSLT	VNFPPGPWPLT	GNPNALESALENIV
PHOR	(EC)	LILSKIEAAPIHL	LNEKVUVPMMI	RVVEREAU	LSQ. KKUIF	TFEIDNGLKVS	GNEDQLKSAISNLV
Phom	(EC)	LRQARLENRQEVV	LTAVDVAALFI	RVSEARTV	LAEKKIT	LHVTPTEVNVA	AEPALLEQALGNLL
NtrB	(Kp)	LGPQHPGMHVTES	IHKVAERVVKI	VSMELPDN	/KLVI	RDYDPSLPELP	HDPDQIEQVLLNIV
VirA	(At)	LTLSRKQERMIKP	FSVSELVTEI/	VPLLRMALP	PNIELS	FRFDQMQSVIE	GSPLELQQVLINIC
FixL	(Rm)	IKHLREFVTKGET	EKAPEDIRKL	/EESAALAL\	/GSREQGVRTV	FEYLPGAEMVL	VDRIQVQQVLINLM
		• •	•			• • •	• • • • • • • •
		510		520	530	540	550
NarX	(Ec)	510 SNALKHSQASE		520	530 AQNDNQVKLTV	540 DNGCGV.PEN	550 AIRSNHYGMIIMRD
NarX DegS	(Ec) (Bs)	510 SNALKHSQASE SNALKHSESEE		520 VVVTV/	530 AQNDNQVKLTVO EITKDFVILMII	540 QDNGCGV.PEN KDNGKGFDLKE	550 AIRSNHYGMIIMRD AKEKKNKSFGLL
NarX DegS EnvZ	(Ec) (Bs) (Ec)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW		520 VVVTV/ ITVKVE IKVSS(530 AQNDNQVKLTV EITKDFVILMII GTEPNRAWFOVI	540 QDNGCGV.PEN KDNGKGFDLKE EDDGPGIAPEO	550 AIRSNHYGMIIMRD AKEKKNKSFGLL .RKHLFOPFV
NarX DegS EnvZ DctB	(Ec) (Bs) (Ec) (R1)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW ONALFAVAPKAG.		520 VVVTV/ ITVKVE IKVSSO IKVSSO	530 AQNDNQVKLTV EITKDFVILMII STEPNRAWFQVI STDAGMVTVTV	540 QDNGCGV.PEN KDNGKGFDLKE EDDGPGIAPEQ ADNGPGI.PTE	550 AIRSNHYGMIIMRD AKEKKNKSFGLL .RKHLFQPFV IRKGLFTPEN
NarX DegS EnvZ DctB PatB	(Ec) (Bs) (Ec) (R1) (St)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW QNALEAVAPKAG. ANALDACSIDA		520 IVVVTV/ ITVKVE IKVSSO .EGRVEIRTS	530 AQNDNQVKLTV(ITKDFVILMI) STEPNRAWFQVI STDAGMVTVTV/ DTOGFALFVYI/	540 QDNGCGV.PEN KDNGKGFDLKE EDDGPGIAPEQ ADNGPGI.PTE ADNGPGW PVA	550 AIRSNHYGMIIMRD AKEKKNKSFGLL .RKHLFQPFV IRKGLFTPFN I PSII KPFT
NarX DegS EnvZ DctB PgtB CnvA	(Ec) (Bs) (Ec) (R1) (St)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW QNALEAVAPKAG. ANALDACSIDA PNALDASIDA.		520 VVVTV/ ITVKVE IKVSSO VIAVTWO VIAVTWO	530 AQNDNQVKLTVG ITKDFVILMI STEPNRAWFQVI TDAGMVTVTV/ TQGEALEVYI/ VNPCPGITITVI	540 QDNGCGV.PEN KDNGKGFDLKE EDDGPGIAPEQ ADNGPGI.PTE ADNGPGW.PVA	550 AIRSNHYGMIIMRD AKEKKNKSFGLL .RKHLFQPFV IRKGLFTPFN LLPSLLKPFT BEDIEDBEY
NarX DegS EnvZ DctB PgtB CpxA PboP	(Ec) (Bs) (Ec) (R1) (St) (Ec)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW QNALEAVAPKAG. ANALDACSIDA RNALRYSHTK VNAVMUTBECTU		520 VVVTVA ITVKVE IKVSSO VIAVTWO IEVGFVO	530 AQNDNQVKLTVO ITKDFVILMII STEPNRAWFQVI STDAGMVTVTV/ AVGEALEVYI/ AVDKDGITITVI	540 QDNGCGV.PEN KDNGKGFDLKE EDDGPGIAPEQ ADNGPGI.PTE ADNGPGW.PVA DDDGPGVSPED	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQFFV IRKGLFTPFN LLPSLLKPFT REQIFRPFY
NarX DegS EnvZ DctB PgtB CpxA PhoR	(Ec) (Bs) (Ec) (R1) (St) (Ec) (Ec)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW QNALEAVAPKAG. ANALDACSIDA RNALRYSHTK YNAVNHTPEGTH. DNAIDETDESCO		520 VVVTVA ITVKVE IKVSSO VIAVTWO IEVGFA	530 AQNDNQVKLTVO EITKDFVILMII STEPNRAWFQVI STDAGMVTVTV/ AVDKDGITITVI RVPHGAEFSVI VNDCFUTEVI	540 QDNGCGV.PEN KDNGKGFDLKE EDDGPGIAPEQ ADNGPGI.PTE ADNGPGW.PVA DDDGPGVSPED EDNGPGIAPEH	550 AIRSNHYGMIIMRD AKEKKNKSFGLL .RKHLFQPFV IRKGLFTPFN LLPSLLKPFT .IPRLTERFY IPRLTERFY
NarX DegS EnvZ DctB PgtB CpxA PhoR PhoM	(Ec) (Bs) (Ec) (R1) (St) (Ec) (Ec) (Ec)	510 SNALKHSQASE SMALKHSESEE VNAARYGNGW QNALEAVAPKAG. ANALDACSIDA RMALRYSHK VNAVNHTPEGTH. DNAIDFTPESGC.		520 VVVTV/ ITVKVE IKVSS(IKVSS(IKVSS(IKVRV IEVGF/ ITVRV(ITLSAE	530 AQNDNQVKLTVI EITKDFVILMII STEPNRAWFQVI STDAGMVTVV/ QTQGEALEVYI/ VDKDGITITVI RVPHGAEFSVI VDQEHVTLKVI	540 QDNGCGV.PEN KDNGKGFDLKE EDDGPGIAPEQ ADNGPGU.PTE ADNGPGW.PVA DDDGPGVSPED EDNGPGIAPEH LDTGSGI.PDV	550 AIRSNHYGMIIMRD AKEKKNKSFGLL .RKHLFQPFV IRGGFTPFN LLPSLLKPFT .IPRLTERFY ALSRIFERFY ALSRIFERFY
NarX DegS EnvZ DctB PgtB CpxA PhoR NtrB	(Ec) (Bs) (Ec) (R1) (Ec) (Ec) (Ec) (Kp)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW QNALEAVAPKAG. ANALDACIDA YNAVNHTPEGTH. DNAIDFTPESGC. RNALQALGPEGG	EITLRTR	520 VVVTV/ ITVKVE ITVKVE VIAVTWC IEVGF/ IEVGF/ ITVRWC ITLSAE AFQLTLHGG	530 AQNDNQVKLTVU ITKDFVILMII STEPNRAWFQVI STDAGMVTVTV/ AVDKDGITITVI RVPHGAEFSVI VDQEHVTLKVI (AVRLAARIDV)	540 QDNGCGV.PEN KDNGKGFDLKE EDDGPGIAPEQ ADNGPGI.PTE ADNGPGW.PVA DDDGPGVSPED EDNGPGI.PDY EDNGPGI.PDY	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV IRKGLFTPFN LPSLLKPFT IPRLTERFY LSRIFERFY LQDTLFYPMY
NarX DegS EnvZ DctB PgtB CpxA PhoR NtrB VirA	(Ec) (Bs) (Ec) (R1) (Ec) (Ec) (Ec) (At)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW QNALEAVAPKAG ANALDACSIDA YNAVNHTPEGTH VNAVNHTPEGTH NALGHEPEGG. KNASQAMTANGQI	EITLRTR DIIISQAFLP1	520 VVVTV/ IVVSC IKVSSC VIAVTWC IVGF/ IVRWC ITVRWC ITVRWC ITVRWC ITLSAE VAFQLTLHGV /KKILAHGV	530 AQNDNQVKLTVI EITKDFVILMII STEPRRAWFQVI STDAGMVTVTV/ QTQGEALEVYI/ VDDKDGITITVI QRVPHGAEFSVI VDDQEHVTLKVI (RYRLAARIDVI MPPGDYVLLSI:	540 QDNGCGV.PEN EDDGPGIAPEQ ADNGPGI.PTE ADNGPGW.PVA DDGPGVSPED EDNGPGIAPEH DTGSGI.PDY EDNGPGI.PSH EDNGGGI.PEA	550 AIRSNHYGMIIMRD AKEKKNKSFGLL. .RKHLFQPFV IRKGLFTPFN LPSLLKPFT .IPRLTERFY ALSRIFERFY LQDTLFYPMV VLPHIFEPFF
NarX DegS EnvZ DctB PgtB CpxA PhoR PhoM NtrB VirA FixL	(Ec) (Bs) (Ec) (R1) (St) (Ec) (Ec) (Ec) (At) (Rm)	510 SMALKHSQASE SNALKHSESEE VNAARYGNGW QMALEAVAPKAG. ANALDACSIDA RNALRYSHTK JNAVHNTPEGTH. DNAIDFTPESGC. RNALQALPEGG KNASQAMTANGQI RNAIEAMRHVDR.	EITLRTR1 DIIISQAFLP1	520 VVVTV/ ITVKVE IKVSSG GRVEIRTS IEVGF/ ITVRWG ITVRWG ITLSAE AFQLTLHGG /KKILAHGVM .RELTIRTMF	530 AQNDNQVKLTVG ITKOFVILMII STEPNRAWFQVI STDAGMVTVTV/ AVDKDGITITVI RVPHGAEFSVI VDQEHVTLKVI VRVRLAARIDVI PADPGEVAVVLSI ADPGEVAVVV	540 QDNGCGV.PEN CDNGKGFDLKE EDDGPGIAPEQ ADNGPGI.PTE ADNGPGI.PTE EDNGPGIAPEH DTGSGI.PDY EDNGGGI.PEA EDTGGGI.PEA	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV IRKGLFPPFN LLPSLLKPFT IPRLTERFY ALSRIFERFY LQDTLFYPMV VAGQLFKPFY
NarX DegS EnvZ DctB PgtB CpxA PhoR PhoR NtrB VirA FixL	(Ec) (Bs) (R1) (St) (Ec) (Ec) (Kp) (At) (Rm)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW QNALEAVAPKAG. ANALDACSIDA RNALRYSHTK NAVNHTPEGTH. DNAIDFTPESGC. RNALQALGPEGG. KNASQAMTANGQI RNAIEAMRHVDR.	EITLRTR DIIISQAFLP	520 VVVTV/ IVKVE IKVSSC .EGRVEIRTS VIAVTWC ITVGF/ ITVRWC ITLSAE GAFQLTLHGV KKILAHGVM RELTIRTM	530 AQNDNQVKLTVI EITKDFVILMII STEPRAWFQVI TQGEALEVVI/ VDCBGIIITVI RVPHGAEFSVI VDQEHVTLKVI RVPLAARIDVI RYRLAARIDVI RYRLAARIDVI ADDGEVAVVI	540 QDNGCGV.PEN KONGKGFDLKE EDDGPGIAPEQ ADNGPGI.PTE ADNGPGV.PVA DDDGPGVSPED EDNGPGIAPEH DTGSGI.PEY EDNGGGI.PEA DTGGGI.PEA	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV IRKGLFTPFN LPSLLKPFT ALSRIFERFY LQDTLFYPMV VLPHIFEPFF VAGQLFKPV
NarX DegS EnvZ DctB PgtB CpxA PhoR PhoM NtrB VirA FixL	(Ec) (Bs) (R1) (St) (Ec) (Ec) (Kp) (At) (Rm)	510 SNALKHSQASE SNALKHSESEE YNAARYGNEW QNALEAVAPKAG. ANALDACSIDA RNALRYSHTK YNAYNHTPEGTH. DNAIDFTPESGC. RNALQAPEGG. KNASQAMTANGQI RNAIEAMRHVDR. 560 570	EITLRTR DIIISQAFLP1 580	520 VVVTV/ ITVKVE IKVSS(EGRVEIRTS VIAVTMO ITVRM ITVRM ITVRM ITVRM ITLSAE AFQLTLHG /KKILAHGV 	530 AQNDNQVKLTVA ITKDFVILMII STEPRRAFQVI STDAGMVTVTV VDKDGITITVI VDKDGITITVI VDCHVTLKVI VDQEHVTLKVI PADPGDVVLLSI PADPGEVAVVVI 598	540 DNGCGV.PEN CONGKGFDLKE EDDGPGIAPEQ ADNGPGW.PVA DDDGPGVSPED EDNGPGIAPEH LDTGSGI.PSH DDNGGGI.PEE EDTGGGI.PEE	550 AIRSNHYGMIIMRD AKEKKNKSFGLL. .RKHLFQPFV IRKGLFTPFN .REQIFRPFY ALSRIFERFY UQTLFYPMV VLPHIFEPFF VAGQLFKPFV
NarX DegS EnvZ DctB PgtB CpxA PhoR PhoM NtrB VirA FixL	(Ec) (Bs) (Ec) (St) (Ec) (Ec) (Kp) (At) (Rm) (Ec)	510 SMALKHSQASE SMALKHSESEE VMAARYGNGW QMALEAVAPKAG. ANALDACSIDA RMALRYSHTK DNAIDFTPEGTH. DNAIDFTPEGTH. DNAIDFTPEGTGC. RMAIQALGPEGG. KMASQAMTANGQI RMAIEAMRHVDR. 560 570 RAQSLRGDCRYRR	EITLRTR DIIISQAFLPY 580 RESGGTEV.VI	520 VVVTV/ ITVKVE ITVKVE IKVSSG VIAVTWG IEVGF/ ITVRWG ITVRWG ITVRWG ITVRWG ITVRWG ITVRWG ITVRWG ITVRWG 	530 AQNDNQVKLTVI EITKDFVILMI TTEPRAMFQVI TOGEALEVYI/ VDCROETITVI VDCROETITVI VDQEHVTLKVI VRVPHGAEFSVI VDQEHVTLKVI VRVPLAARIDV PADPGEVAVVVI 9598 TDVQGDTHE*	540 DNGCGV.PEN KDNGKGFDLKE DDGPGIAPEQ ADNGPGI.PTE ADNGPGW.PVA DDGPGVSPED DNGPGIAPEH DTGSGI.PEY DDNGGGI.PEA DDTGGGI.PEE	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV IRKGLFPFFN LLPSLLKPFT IRKGLFRFY ALSRIFRFY ALSRIFERFY VLPHIFEPFF VAGQLFKPFY
NarX DegS EnvZ DctB PgtB CpxA PhoR NtrB VirA FixL NarX DegS	(Ec) (Bs) (Ec) (Ec) (Ec) (Ec) (At) (Ec) (Ec) (Ec) (Bs)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW QNALEAVAPKAG. ANALDACSIDA RNALRYSHTK YNAVNHTPEGTH. DNAIDFTPESGC. RNALQALGPEGG. KNASQAMTANGQI RMAIEAMRHVDR. 560 570 RAQSLRGDCRVRR GMKERVDLLEGT	EITLRTR DIIISQAFLP 580 RESGGTEV.VI .MTIDSKIGL(520 VVTVV ITVKVE ITVSS EGRVEIRTS EGRVEIRTS ITVRWG ITVRWG AFQLTLHGN KKILAHGW RELTIRTMF 590 /TFIPEKTFI TFIMIKVPL	530 AQNDNQVKLTVI TITKDFVILMII STEPRRAWFQVI TIQGEALEVVI VDCBGIIITVI RVPHGAEFSVI VADCHVTLKVI (RYRLAARIDVI (RYRL	540 DDNGCGV.PEN CDNGKGFDLKE EDDGPGIAPEQ ADNGPGW.PVA DDDGPGVSPED EDNGPGIAPEH DTGSGI.PDY EDNGPGI.PSH SDNGGGI.PEE EDTGGGI.PEE	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV IRKGLFTPFN LPSLLKPFT REQIFRPFY LSRIFERFY LQDTLFYPMV VLPHIFEPFF VAGQ_FKPFV
NarX DegS EnvZ DctB PgtB CpxA PhoR PhoM NtrB VirA FixL NarX DegS EnvZ	(Ec) (Bs) (Ec) (Ec) (Ec) (Ec) (At) (Rm) (Ec) (Bs) (Ec)	510 SMALKHSQASE SMALKHSESEE VWAARYGNGW QMALEAVAPKAG. ANALDACSIDA RNALRYSHTK DMAIDFTPESGTH. DMAIDFTPESGTH. DMAIDFTPESGTH. CRASQSLRGDCRYRR .GMKERVDLLEGT RGDSA	EITLRTR DIIISQAFLP S80 RESGGTEV.VI .MTIDSKIGLGL/	520 VVVTV/ ITVKYE IKVSS(EGRVEIRTS VIAVTWG ITUSAE ITUSAE AFQLTLHG (KKILAHGVM, RELTIRTMF 590 /TFIPEKIKPI IVQRI.VD/	530 AQNDNQVKLTV(ITKDFVILMI) TTEPRAMFQVI TOGEALEYYI YUQEALEYYI YUQEALEYYI YUQEALEYYI YUQEALEYYI YADPGOYVLSI ADPGE YAYYVI 598 TOYQGDTHE* SL*	540 DNGCGV.PEN KDNGKGFDLKE DDGPGIAPEQ DNGPGI.PTE ADNGPGW.PYA DDDGPGVSPED DNGGGI.PEA EDNGGGI.PEA EDTGGGI.PEA EDTGGGI.PEA	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV LLPSLLKPFT IRKQLFTPFY JPRITERFY ALSRIFERFY VAGQLFKPFV VAGQLFKPFV
NarX DegS EnvZ DctB PgtB PhoR PhoR NtrB VirA FixL NarX DegS EnvZ DctB	(Ec) (Bs) (Ec) (Ec) (Ec) (Ec) (At) (Rm) (Ec) (Bs) (Ec) (R1)	510 SMALKHSQASE SMALKHSESEE VMAARYGNGW QMALEAVAPKAG. ANALDACSIDA RMALRYSHTK YMAVMHTPEGTH. DNAIDFTPESGC. RMALQALGPEGG. KMASQAMTANGQI RMAIEAMRHVDR. 560 570 RAQSLRGDCRVRR .GMKERVDLLEGT RGDSA	EITLRTR DIIISQAFLP 580 RESGGTEV.VI .MTIDSKIGLQ RTISGTGLGL SKESGLGLGL	520 VVVTV/ ITVKY5 IKVSS VIAVTWC ITVRW ITVRW ITVRW ITVRW ITVR ITVRW ITVR 	530 AQNDNQVKLTVI EITKDFVILMII TTEPRAWFQVI TOGEALEVYI/ VDCROGIIITVI VDCROGIIITVI VDCROGIITVI VDQEHVTLKVI (RYRLAARIDVI HPGDYVLLSII ADPGEVAVVVI 598 TDVQGDTHE* .SL* IHNGMLELGTSI	540 DNGCGV.PEN KDNGKGFDLKE DDGPGIAPEQ DNGPGI.PTE ADNGPGV.PVA DDGPGVSPED DTGSGI.PDY DTGSGI.PES DTGGGI.PEE DTGGGI.PEE CTGGGLSIRAWL GGTRFIVQLR	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV ILPSLLKPFT ILPSLLKPFT ALSRIFERFY ALSRIFERFY ALSRIFERFY VLPHIFEPFF VAGQLFKPFV PVPVTRAQGTTKEG* KA*
NarX DegS EnvZ DctB PgtB CpxA PhoM NtrB VirA FixL NarX DegS EnvZ DctB PgtB	(Ec) (Bs) (Ec) (St) (Ec) (Ec) (Ec) (At) (Ec) (At) (Ec) (Ec) (At) (Ec) (St)	510 SNALKHSQASE SNALKHSESEE VNAARYGNGW QNALEAVAPKAG. ANALDACSIDA RNALRYSHTK YNAVNHTPEGTH. DNAIDFTPESGC. RNALQALGPEGG. KNASQAMTANQQI RMAIEAMRHVDR. 560 570 RAQSLRGDCRVRR RGDSA 	EITLRTR 580 RESGGTEV.VI .MTIDSKIGL(RTISGTGLGL/ SKESGLGLGL SKAVGLGIGLS	520 VVVTVJ ITVKVE IKVSS .EGRVEIRTS IEVGFJ ITUSAG AFQLTLHGV KKILAHGVN .RELTIRTMF 590 /TFIPEKTFI STFIMIKVPI LIVQRI.VDP (ISKDI.VDF) [ISVSL.MA	530 QNDNQVKLTVI ITKDFVILMII STEPRRAWFQVI TTQGFALEVYI/ VDKDGIIITVI RVPHGAEFSVI VDQEHVTLKVI (RYRLAARIDVI (RYRLAARIDVI (RYRLAARIDVI PPGDVVLLSI SADPGEVAVVVI 0 598 DVQGDTHE* SL* HHNGHLELGTSI YYGGRMDVASD SYGGRMDVASD	540 DDNGCGV.PEN CDNGKGFDLKE EDDGPGIAPEQ ADNGPGW.PVA DDNGPGW.PVA DDDGPGVSPED EDNGPGI.PDY EDNGPGI.PSH EDTGGGI.PEA ETGGGLSIRAWL SGGTRFIVQLR TRNACVVLOF	550 AIRSNHYGMIINRD AKEKKNKSFGLL RKHLFQPFV IRKGLFTPFN LPSLLKPFT LQTLFRPFY LQDTLFYPMV VLPHIFEPFF VAGQLFKPFV PVPVTRAQGTTKEG* KA* SVTDVDDVE*
NarX DegS EnvZ DctB CpxA PhoR PhoM NtrB VirA FixL NarX DegS EnvZ DctB CpxA	(Ec) (Bs) (Ec) (St) (Ec) (Kp) (Ec) (Kt) (Ec) (Ec) (Ec) (Ec) (Ec) (Ec) (Ec) (Ec	510 SMALKHSQASE SNALKHSESEE VNAARYGNGW QMALEAVAPKAG. ANALDACSIDA RALRYSHTK VMAVNHTPEGTH. DNAIDFTPESGC. RNALQALGPEGG. KNASQAMTANGQI RMAIEAMRHVDR. 	580 EITLRTR DIIISQAFLPY MTIDSKIGLGJ/ SKESGLGLGL SKAVGLGIGL SKAVGLGIGL	520 VVVTV/ ITVKY5 IKVS5 ITVRW5 ITVRW6 ITVRW6 ITVRW6 ITVRW7 ITVRW7 ITVRW7 ITVRW7 S90 	530 AQNDNQVKLTV(ITKDFVILMI) ITEPRAMFQVI ITGEALEVYI/ VDKDGIIITV) VDCKDGIIITV) VDQEHVTLKVI VDQEHVTLKVI VADPGEVAVVVI) 598 TDVQGCTHE* SL* HNGMLELGTSI VGGRMDVASD MKGQLRLASTI	540 DNGCGV.PEN KDNGKGFDLKE DDGPGIAPEQ DDNGPGI.PTE ADNGPGI.PTE DDNGPGI.PEL DTGSGI.PDY DDNGGGI.PEA EDTGGGI.PEE ERGGLSIRAWL SGGTRFIVQLR TRNACVVLQF LGGLRLVIWL	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV ILPSLLKPFT ILPSLLKPFT ALSRIFERFY ALSRIFERFY VAGQLFKPFV VAGQLFKPFV PVPVTRAQGTTKEG* KA* SVTDVDDVE* PLYKRS*
NarX DegyZ EnvZB PgtB CpxA PhoR NtrA Fix NargS EnvZ DctB PgtA PgtA PhoR	(Ec) (Ec) ((R1) ((Ec) ((Ec) ((Rm) ((Ec) ((Rm) ((Ec) ((Rm) ((Ec) ((Ec)) ((Ec)) ((Ec)) ((Ec))	510 SMALKHSQASE SNALKHSESEE VWAARYGNGW QNALEAVAPKAG. ANALDACSIDA RNALRYSHTK YNAVNHTPEGTH. DNAIDFTPESGC. RNALQALGPEGG. KNASQAMTANGQI S60 570 RAQSLRGDCRVRR .GMKERVDLLEGT RGDSA T TTDRDB.AR	580 RESGGTEV.VI MTIDSKIGLO RTISGTGLGL SKESGLGLGL SKAVGLGIGLS RESGGTGLGL/	520 VVVTV/ ITVKY5 ITVKY5 ITVSA IT	530 AQNDNQVKLTVI EITKDFVILMII STEPRAWFQVI TTQGEALEVYI/ VDCBOGIIITVI RVPHGAEFSVI RVPHGAEFSVI VDDEHVTLKVI (RYRLAARIDVI HPGDYVLLSI: SL* DVQGDTHE* SL* HNGMLELGTSI)MKGDLRLASTI HRGMLVKAEDSI MKGDLRLASTI HRGMLVKAEDSI	540 DNGCGV.PEN KDNGKGFDLKE DDGPGIAPEQ DNGPGI.PTE DNGPGVSPED DTGSGI.PDY DTGSGI.PDY DTGSGI.PES DTGGGI.PEE DTGGGI.PEE CGGTRFIVQLR TRNACVYLQF LGGLRVIWL	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV IPSLLKPFT IPSLLKPFT IPRLTERFY ALSRIFERFY UQDTLFYPMV VLPHIFEPFF VAGQLFKPFV PVPVTRAQGTTKEG* KA* SVTDVDDVE* PLYKRS* PERLIAKNSD*
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NarX DegS EnvZ PgtB Phot NtrB NtrB NtrB NtrA DegS DctB Phot Phot Phot NtrB NtrB	(Ec) (Bs) (Ec) ((Ec) ((Ec) ((Ec)) ((Ec)) ((Ec)) ((Ec)) ((Ec)) ((Ec)) ((Ec)) ((Ec)) ((Ec))	510 SMALKHSQASE SMALKHSESEE VWAARYGNGW QMALEAVAPKAG. ANALDACSIDA RRALRYSHTK JMAVHHTPEGTH. DNAIDFTPESGC. RRALQALGPEGG. KMASQAMTANGQI RNAIEAMRHVDR. 560 570 RAQSLRGDCRVRR .GMKERVDLLEGT RGDSA T RTDE.ARD RDE.ARD SLPRA	580 RESGGTEV.VI MTIDSKIGLG KESGLGLGL/ SKESGLGLGL/ SKAVGLGIGLS SKAVGLGIGLS RQTGGSGLGL/ NGQKSSGLGL/	520 VVVTV/ ITVKV5 IKVSS GRVEITS VIAVTWC ITVRWM ITVRWM ITVRWM ITVRW ITVRW ITVRW ITVRW ISKDI.VG SSUI.VG	530 AQNDAQVKLTVI EITKDFVILMII STEPRAMFQVI STDAGMVTVTVI YDGEALEVYI/ VDDKDGIIITVI VDQEHVTLKVI VADPGEVAVVVI PADPGVVLSI PADPGEVAVVVI SS8 FDVQGDTHE* SL* HNGMLELGTSI MKGGURASDI HNGMLELGTSI HESRLNIESTI - FNGEVTLRVI - FNGEVTLRVI	540 DNGCGV.PEN KDNGKGFDLKE DDGPGIAPEQ DNGPGI.PTE ADNGPGI.PTE DNGPGI.PTE DNGPGI.PEA DTGSGI.PEA EDTGGI.PEA EDTGGI.PEA ETGGI.PEA E	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFV ILPSLLKPFT ILPSLLKPFT ALSRIFERFY ALSRIFERFY VAGQLFKPFY VAGQLFKPFY VAGQLFKPFY PVPVTRAQGTTKEG* KA* SVTDVDDVE* PERLIAKNSD* HRHFT* T0K*
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NarX DegS DrtB PgtA PhoR Phor NtrB NarX DegtA PhoM NtrA DegtB DctB DctB PgtB NtrB PhoM NtrB Fixl	(Ec) (Bs) (R1) (Ec) (Ec) (Ec) (Ec) (Ec) (Bs) (C1) (Ec) (Ec) (Ec) (C1) (Ec) (Ec) (C1) (Ec) (Ec) (C1) (Ec) (C1) (Ec) (C1) (Ec) (C1) (Ec) (C1) (Ec) (C1) (Ec) (C1) (Ec) (C1) (Ec) (C1) (Ec) (C1) (Ec) (C1) (Ec) (C1) (C1) (C1) (C1) (C1) (C1) (C1) (C1	510 SMALKHSQASE SMALKHSESEE VWAARYGNGW QMALEAVAPKAG. ANALDACSIDA RNALRYSHTK DMAIDFTPEGTH. DMAIDFTPEGTH. DMAIDFTPEGT. CRASQLRGDCRVRR CMKERVDLLEGT CMKERVDLEGT CMKERVD CMKERVDLEGT CMKERVD CMKERVD CMKERVD CMKERVD CMKERVD CMKERVD CMKE	580 RESGGTEV.VI MTIDSKIGLGL/ SKESGLGLGL/ SKESGLGLGL/ SKAVGLGIGLS/ RQTGGSGLGL/ NGQKSSGLGL/ ARNGGSGLGLGL	520 VVVTV/ ITVKY5 ITVKY5 ITVKY5 ITVRW6 ITVRW6 ITVRW6 ITVRW6 ITVRW7 ITVRW7 ITVRW7 ITVRW7 ITVRW7 ITVRW7 	530 AQNDAQVKLTV(ITKDFVILMI) ITEPRAMFQVI ITGEALEVYI/ VDCKDGIIITV) VDCKDGIIITV) VDQEHVTLKVI VADPGEVVLSI: PADPGEVVLSI: PADPGEVVLSI: ADPGE VAVVULSI: ADPGE VAVVU ADGE TETSWI FAGVIDYSCHI FAGVIDYSCHI ADGE TSWI FAGVIDYSCHI	540 DNGCGV.PEN KDNGKGFDLKE DDGPGIAPEQ DDNGPGI.PTE ADNGPGI.PTE DDNGPGI.PTE DDNGGGI.PTA DTGSGI.PDY DDNGGGI.PEA EDTGGGI.PEA EDTGGGI.PEA ETGGGI.SIRAWL SGGTRFSVQLP 2.GGLRLVIWL QGKTEFSVVLP GHTEFSVVLP GGHTEFDYVLP	550 AIRSNHYGMIIMRD AKEKKNKSFGLL RKHLFQPFY LLPSLLKPFT IRKGLFTPFY JPRITERFY ALSRIFERFY UQDTLFYPMY VAGQLFKPFY YAGQLFKPFY PVPVTRAQGTTKEG* KA* SYTDVDDVE* PLYKRS* PERLIAKNSD* HRHFT* IRK* PPSSKEPVNPDSFF-

Figure 4. Sequence alignment of NarX with *E. coli* EnvZ, CpxA, PhoR, and PhoM, *B. subtilis* DegS, *R. leguminosarum* DctB, *S. typhimurium* PgtB, *K. pneumoniae* NtrB, *A. tumefaciens* VirA, and *R. meliloti* FixL. Conserved residues are marked as in Figure 3.

OmpR is involved in osmotic regulation of the synthesis of outer membrane proteins, and NtrC is involved in nitrogen control of several nitrogen assimilatory genes. It has been reported that OmpR and NtrC share homologous N-terminal domain with other bacterial regulatory proteins such as PhoB (34), PhoM ORF2 (35), SfrA (36), VirG (37, 38), DctD (39), PgtA (40), CheY (41, 42), SpoOF (43), CheB (41, 42), and SpoOA (44). This family also includes UhpA (45), FixJ (46), DegU (47, 48), and UvrC ORF2 (49), which, in addition to the homologous N-terminal domain, shared extensive similarity to NarL over their entire lengths (Fig. 3). Therefore, the most proteins of this family are subdivided into four different groups according to their C-terminal domain: (i) NarL, DegU, UvrC ORF2, UhpA, and FixJ; (ii) OmpR, PhoB, PhoM ORF2, SfrA, and VirG; (iii) NtrC, DctD, and PgtA; and (iv) CheY and SpoOF. The UhpA and FixJ proteins of the first group



Figure 5. Model for nar gene activation in E. coli. See text for details.

were shown to have helix-turn-helix DNA-binding motif in the C-terminal domain (45, 46). By predicting the secondary structure of the NarL protein with a GENETYX program, we found the motif in the conserved C-terminal domain as a potential candidate (Fig. 2). It is also possible to assign the motif in the highly homologous region with FixJ and UhpA between Leu 171 and Lys 192, although this region is most likely to form the helix-beta-helix structure (Fig. 3). In any case, the bihelical region seems to confer NarL to interact with the control region of the nitrate-responsive *narC* and *narK* genes, consistent with genetically characterized phenotypes of *narL* (2, 5).

The regulatory proteins shown in Fig. 3 are known to regulate gene expression in combination with a second class of regulatory protein (11). Members of this second family, including EnvZ (30), DctB (39), PgtB (50), CpxA (51), PhoR (52), PhoM (35), NtrB (53), VirA (54), and FixL (46), share similarity to each other over their C-terminal 250 amino acids. NarX and DegS (47, 48) also shared this similarity to some extent (Fig. 4). Four or more conserved regions were recognized, some of which had extensively conserved residues with NarX. However, these two proteins are truncated and have poor homology in the very C terminus to a region which is highly conserved in the rest of the family. Most members of the EnvZ family except NtrB and DegS are transmembrane proteins and contain two regions of sufficient hydrophobicity outside of the conserved domain. We therefore screened the NarX sequence for potential transmembrane helices and found two such sequences, one at residues 15 to 37 and the other at residues 152 to 174 (Fig. 2). Thus, NarX is predicted to be a transmembrane protein, which acts as a sensor for nitrate and transduces signal of nitrate availability to the *narK* and *narC* operons via NarL.

DISCUSSION

The nucleotide sequence of the nitrate regulator operon, which is adjacent to the *narK* and *narC* operons at 27 min on the *E. coli* chromosome (3, 10), was determined. The *narXL* operon is transcribed essentially independent of anaerobiosis and nitrate availability. Two ORFs of the operon were designated *narX* and *narL* to indicate their order relative to the promoter on the basis of coding polarity and the size of the gene product. There is five-nucleotide overlap between the coding region of *narX* and *narL* which implies translational coupling of the genes. Under anaerobic condition, however, the *narL* gene

was expressed differently from the narX gene (9). An additional promoter present within the narX coding region may be responsible for the elevated expression of narL relative to narX in the absence of nitrate.

The *narL* gene encodes the pleiotropic transcriptional regulator which acts as an activator on the *nar* operon and a repressor on the *frd* and *tor* operons (5, 7, 8). Analysis of the deduced amino acid sequence of NarL revealed characteristic helix-turn-helix motif in the C-terminal region which is responsible for binding to the regulatory region of a target gene (Fig. 2). Extensive similarity of N-terminal amino acid sequence of NarL to other prokaryotic regulatory proteins such as OmpR and PhoB (Fig. 3) strongly suggests that NarL acts directly on the regulatory region of the *narK* and *narC* operons after some modification to an active form in the presence of nitrate. This modification is likely to be effected by the product of the proximal *narX* gene, since NarX has a homologous Cterminal domain with other family of regulatory proteins such as EnvZ and PhoR (Fig. 4), which are known as a counterpart of two-component regulatory systems (11). Thus, the NarX/NarL system belongs to sensor/regulator systems widely observed in prokaryotic gene regulation responding to a variety of environmental stimuli.

Most members of the EnvZ family are anchored in the inner membrane, and recognize physical or chemical signals through a periplasmic domain. The signal recognition domain has been assigned in non-conserved N terminus of each sensor class protein. According to the model (11), this domain transduces the stimulus to the conserved C-terminal cytoplasmic domain, and the activated C-terminal domain of the sensor protein then interacts with and modifies the conserved N-terminal domain of the regulator protein. The modification plays as a switch between inactive and active forms through a conformational change in the C terminus of the regulator. Structural similarity of the NarX/NarL system allows us to suppose the following mechanism operative in the positive control of the nar operon with nitrate (Fig. 5): NarX and NarL are constitutively produced independent of anaerobiosis and nitrate availability. Binding of nitrate to the periplasmic face of NarX triggers an allosteric change in the cytoplasmic portion of NarX, causing activation of NarX. Activated NarX then interacts with the N-terminal region of NarL, resulting in an allosteric or covalent modification of NarL to an active form. Activated NarL in conjunction with Fnr then activates transcription of the narK and narC operons, but represses transcription of other operons such as frd and tor.

It has been demonstrated that NtrB is a kinase/phosphatase capable of converting NtrC to a phosphorylated active form (55, 56) and that CheA is autophosphorylated at a His residue and able to transfer the phosphoryl group to CheB and CheY (57, 58). Similar observation was also made in the EnvZ/OmpR system (Mizuno, T. and Mizushima, S., 11th Annual Meeting of the Molecular Biology Society of Japan, 1988) and in the PhoR/PhoB system (Makino, K. *et al.*, *op. cit.*). These findings suggest the phosphorylation/dephosphorylation as a common mechanism for all members of sensor class to modulate their partners. In the NtrB/NtrC system, a His residue in NtrB and an Asp residue in NtrC were identified as the phosphorylated amino acids (59). It was also shown that PhoR was autophosphorylated at His 213, and transferred phosphoryl group to PhoB at Asp 53 to activate it (Makino, K. *et al.*, *op. cit.*). These His and Asp residues were conserved in all members of the sensor/regulator systems including NarX/NarL (Figs. 3 and 4). It is possible that the NarX protein activates the NarL protein through the similar mechanism.

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