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Cloning and Sequencing of the *Escherichia coli chlEN* Operon Involved in Molybdopterin Biosynthesis

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The nucleotide sequence of a *Hin*PI-*Hpa*II restriction nuclease fragment which complemented a Δ*chlE* strain of *Escherichia coli* was determined. Two open reading frames were deduced to be the structural genes for ChlE and ChlN proteins, which have molecular weights of 44,067 and 26,719, respectively. Both proteins were required for complementing a chromosomal deletion of the *chlE* locus. The *chlE* and *chlN* genes were transcribed from a common promoter, *chlEp*, located upstream of *chlE*. Transcriptional and translational signal sequences were recognized in this region.

All molybdoenzymes except nitrogenase share a common cofactor, which is a complex of molybdopterin with a Mo atom (9, 12, 13). The best-known mutations affecting the molybdoenzymes are in the chlorate-resistant loci (*chl*) of *Escherichia coli*, designated *chlA* through *chlE* and *chlG* (1). With the exception of *chlC*, which is subdivided into the *narCHI* operon, coding for α- and β-subunits of nitrate reductase and cytochrome b_{NR} (4, 7, 30), these *chl* mutants are pleiotropic; they lack nitrate reductase as well as other molybdoenzyme activities. The *chlA* (resolved into *chlA* and *chlM*) and *chlE* (resolved into *chlE* and *chlN*) gene products are thought to be involved in the biosynthesis of molybdopterin (14, 15, 28, 36), while the *chlB*, *chlD*, and *chlG* gene products seem to be required for the transport or processing of Mo (11, 18, 22, 34). These *chl* gene products need to be identified for a better understanding of the biosynthetic pathway of Mo cofactor.

The *chlE* gene was suggested to be involved in the regulation of the *narCHI* operon (28, 29) in addition to the *narK* and *narL* genes (10, 36) and the *fnr* (*nirR*) gene (5, 17, 32). The *chlE* locus has two complementation groups (37) and was recently subdivided into *chlE* and *chlN* (15). It would therefore be interesting to know how these genes are involved in the biosynthesis of molybdopterin and in the expression of the *nar* operon.

The *chlE* gene was cloned during the course of our studies on the *glnHPQ* operon (26, 27), which is located between *chlA* and *chlE* (1). We have determined the DNA sequence of a fragment from the wild-type *E. coli* chromosome that complements *E. coli* Δ*chlE* mutants. Two open reading frames were identified as the *chlE* and *chlN* structural genes on the basis of the results of complementation tests and gene product analysis.

MATERIALS AND METHODS

Bacteria and plasmid vectors. All strains used in this study were derivatives of *E. coli* K-12. BK9MDG and TNK23 are *glnP-chlE* deletion strains used as hosts for cloning (25, 26). JRG97 (*chlE5*; CGSC 4459) and PSM180 (*chlE180*) were used as hosts for the *chlE* complementation test (20). XL1-Blue (*lacI*^{q}*Z*Δ*M15*), obtained from Stratagene, was used for α-complementation tests of LacZ activity as well as for propagating M13 phage.

Plasmid pTN1028 was constructed from pBR322 by replacing the *bla* marker gene with the *kan* gene from pUC4-KIXX (Pharmacia P-L Biochemicals). For analysis of promoter activity, plasmid pTN1051, which was derived from pTN257 and used previously for assaying *glnHp* (26), was used. The *glnHp1* and *glnHp2* promoters were deleted from pTN257, and the *Eco*RI linker was inserted instead to obtain pTN1051. Plasmid pTN1051 is LacZ$^-$ in XL1-Blue, though it carries the ribosome-binding site and initiation codon of the *glnH* gene fused in frame to *lacZ'* as in pTN118 (25).

Media. Nutrient broth medium contained 8 g of nutrient broth (Difco Laboratories) and 5 g of NaCl per liter and was used routinely for growth and maintenance of bacteria. Solid media for plates contained 1.5% agar. When required, 50 μg of ampicillin, 25 μg of kanamycin, and 12.5 μg of tetracycline were added per ml. Selection of Chl$^+$ transformants was performed on the lactate-nitrate medium (38) by incubating them in anaerobic chambers (Becton Dickinson and Co.). Chlorate resistance (Chl$^-$) was determined on the nutrient broth medium containing 0.2% $KClO_3$ and 0.2% glucose by anaerobic incubation. LacZ activity was assayed on the medium containing 50 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

DNA manipulation. Plasmid DNA was prepared as described by Davis et al. (6). The standard methods for the analysis of DNA, such as restriction enzyme digestion, polishing with T4 DNA polymerase, ligation with T4 DNA ligase, transformation with plasmid and phage DNAs, agarose gel electrophoresis, isolation of DNA fragments from agarose gels, etc., were adopted from Maniatis et al. (19).

DNA sequence determination. DNA sequencing was performed by the dideoxy-chain termination method of Sanger et al. (31) with [α-^{35}S]thio-dATP and buffer gradient gels (3). Single-stranded DNA templates were derivatives of the phage vector M13mp19 carrying restriction fragments from the *chl* region of plasmids pTN402 and pTN405. The DNA inserts in pTN402 and pTN405 were isolated after digestion with *Eco*RI and *Hin*dIII, which recognized sites just outside of the *Cla*I cloning site of pTN1028 vector, made blunt ends with T4 DNA polymerase, and ligated at the *Hin*cII site of M13mp19. Nested deletions extending into cloned DNA fragments from the end proximal to the universal M13 primer-binding site on the vector were generated by exonuclease III and mung bean nuclease treatment after *Sac*I-*Bam*HI double digestion. To improve gel readings of areas of high G+C content, 7-deaza-2'-deoxyguanosine-5'-triphosphate was used in place of dGTP (2, 23).

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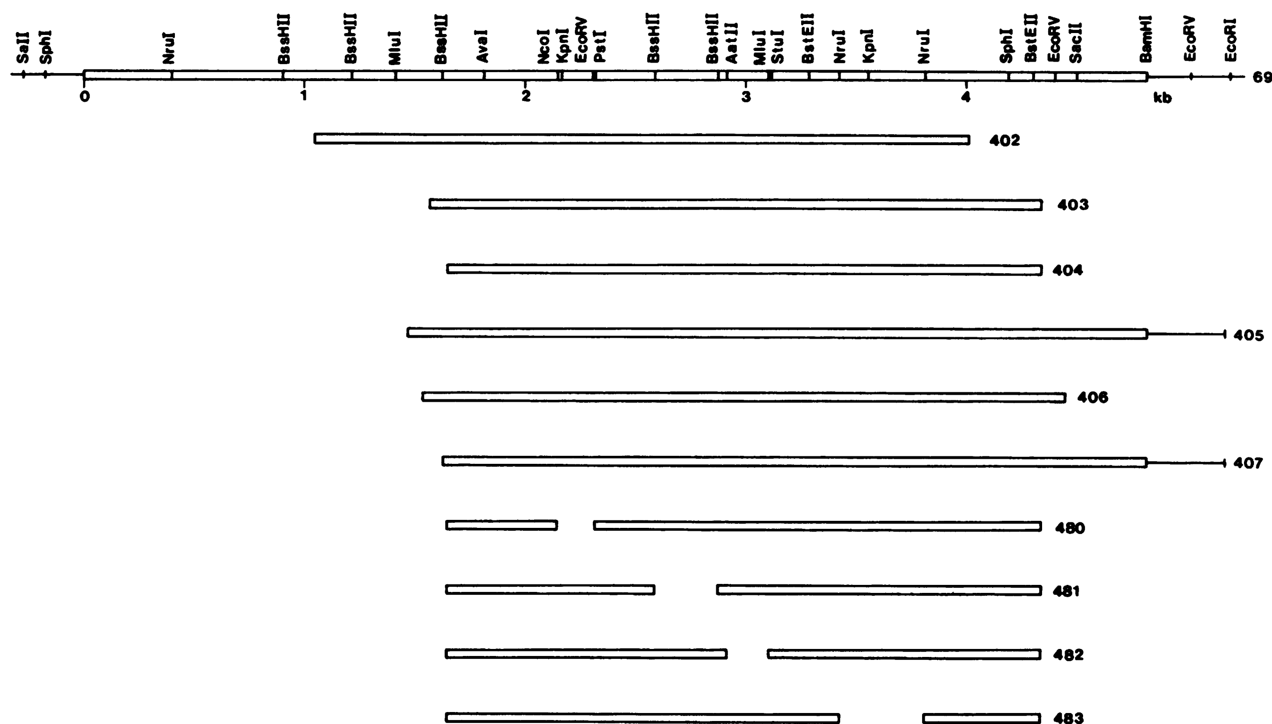

FIG. 1. Restriction map of pTN69 and its subclones. DNA derived from the *chlE* locus of the wild-type strain is shown by the open bar. Plasmids pTN402 to pTN407 (402 to 407, below restriction map) carry portions of DNA from pTN69 in a pTN1028 vector; the vector DNA is not presented here. Plasmids pTN480 to pTN483 (480 to 483, below restriction map) are the deletion subclones of pTN404.

Analysis of total cellular proteins. Analyses of proteins accumulated by plasmid-bearing strains on sodium dodecyl sulfate (SDS)-polyacrylamide gels were carried out after dissolving cells directly in SDS sample buffer as described previously (16). Proteins were stained with Coomassie brilliant blue R-250.

RESULTS

Subcloning of the *chlE* region. Construction of plasmid pTN69 (Fig. 1), which carries a 4.8-kilobase (kb) *Sau*3AI fragment that complements a Δ*chlE* strain, has been described previously (27). Southern blot hybridization of the chromosomal DNAs from several Δ*chlE* strains demonstrated that the DNA inserted at the *Bam*HI site of pBR322 in pTN69 was derived from the *chlE* locus of the wild-type strain (27). The pTN69 DNA was partially digested with either *Hpa*II or *Hin*PI followed by complete digestion with *Cla*I and *Sal*I and ligated with pTN1028 at the *Cla*I site. A Δ*glnP-chlE* strain, BK9MDG, was transformed with the DNA and selected on lactate-nitrate medium containing kanamycin by incubating anaerobically for 2 days at 37°C. Large colonies were picked up and streaked on chlorate medium to see whether they were sensitive to chlorate. Plasmid DNAs were prepared from the Chl^+ (chlorate-sensitive) cells and designated pTN402 to pTN407 after restriction site mapping (Fig. 1). All of the plasmids carried at least the DNA from the *Ava*I site at 1.81 kb to the *Nru*I site at 3.81 kb shown in Fig. 1. We constructed plasmids pTN480, pTN481, pTN482, and pTN483 from pTN404 by deleting *Nco*I (2.14 kb)-*Pst*I (2.31 kb), *Bss*HII (2.58 kb)-*Bss*HII (2.87 kb), *Aat*II (2.91 kb)-*Mlu*I (3.10 kb), and *Nru*I (3.42 kb)-*Nru*I (3.81 kb) fragments (Fig. 1).

Complementation tests were performed with these plasmids in two representative *chlE* strains, JRG97 and PSM180. The results are summarized in Table 1. Since some of the cells bearing the plasmid showed intermediate growth on lactate-nitrate medium as well as on chlorate medium, both media were used for the assay. The *chlE* mutations in JRG97 and PSM180 were equally complemented only by pTN483 and not by pTN480 to pTN482. The plasmid pTN483 itself was, however, incapable of conferring a Chl^+ phenotype on a Δ*chlE* strain such as BK9MDG. These results suggest that at least one more gene, which appears to be identical to the

TABLE 1. Complementation of *chlE* mutations by pTN404-derived plasmids

Strain (mutation)	Chlorate sensitivity[a]/growth on lactate-nitrate medium[b] with plasmid:					
	pTN1028	pTN404	pTN480	pTN481	pTN482	pTN483
BK9MDG (Δ*chlE*)	−/−	+/+	−/−	−/−	−/−	−/−
JRG97 (*chlE5*)	−/−	+/+	−/−	−/−	±/−	±/+
PSM180 (*chlE180*)	−/−	+/+	−/±	−/−	±/−	+/+

[a] +, Sensitive; ±, partially resistant; −, resistant.
[b] +, Rapid; ±, slow; −, no growth.

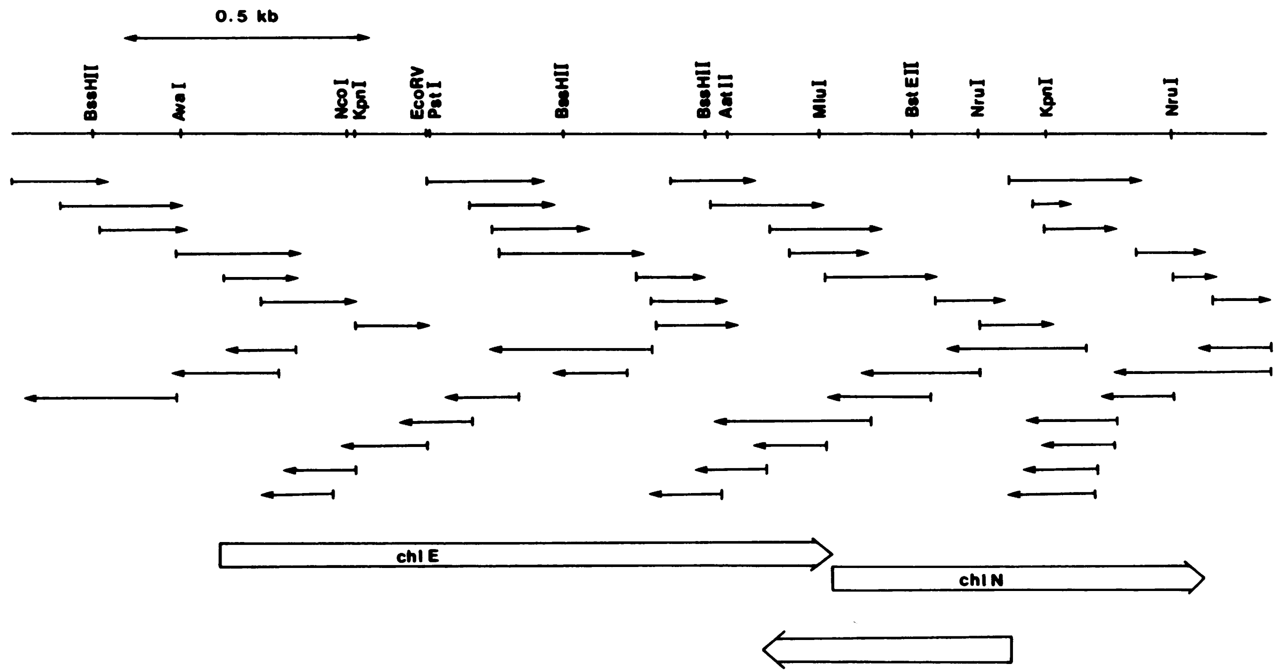

FIG. 2. Sequencing strategy of the *chlEN* operon. The top line presents the location of restriction sites in the region shared by pTN402 to pTN405. Arrows in the middle section give the length and direction of sequencing. At the bottom are presented the locations of open reading frames and their assignments, as described in the text.

chlN gene proposed by Johnson and Rajagopalan (15), is present near the *chlE* gene. We were unable to obtain their *chlN* strain and therefore were unable to confirm this hypothesis. The *chlE* and *chlN* genes are localized in the *Nco*I (2.14 kb)-*Mlu*I (3.10 kb) and the *Nru*I (3.42 kb)-*Nru*I (3.81 kb) regions, respectively, as will be shown below.

Nucleotide sequence of *chlE* and *chlN*. For nucleotide sequence analysis of the two genes, the DNA inserts in pTN402 and pTN405 were subcloned into M13mp19, and nested deletions were generated as described in Materials and Methods. The nucleotide sequence in the region common to pTN402 and pTN405 was determined on both strands by means of the sequencing strategy shown in Fig. 2. Three translational reading frames of significant length were deduced from the nucleotide sequence (Fig. 3). Among them, *chlE* and *chlN* structural genes were assigned as shown in Fig. 2 by complementation tests and gene product analysis (described below).

The ChlE protein could be detected in the total cellular protein extract prepared from a Δ*chlE* host, TNK23, which carries pTN404 and pTN483, but not in extract prepared from TNK23, which carries pTN480 and pTN482 (Fig. 4A). The size of the unique protein band on the SDS-polyacrylamide gel (Fig. 4A) was in good agreement with the calculated size of the ChlE protein (molecular weight, 44,067). On the gel shown in Fig. 4A, it is difficult to see the band corresponding to the ChlN protein with the calculated molecular weight of 26,719. To detect the *chlN* gene product encoded on the plasmid pTN402, an *Ava*I (1.81 kb)-*Hin*dIII (4.02 kb) fragment of pTN402 was ligated at *Eco*RI-*Hin*dIII sites of pUC18 after polishing of *Ava*I and *Eco*RI ends with DNA polymerase so that the *chlE* and *chlN* genes would be transcribed under the control of the *lac* promoter. The resultant plasmid, pTN525, was used to transform a *lacI*^{q} strain, XL1-Blue. The total proteins, which accumulated after induction with isopropyl-β-D-thiogalactopyranoside, were examined on an SDS-polyacrylamide gel (Fig. 4B). Two prominent protein bands with molecular weights that correspond to those expected for the ChlE and ChlN proteins (44,000 and 27,000, respectively) were observed.

Promoter for *chlEN* operon. Presence of a promoter in the 5′-flanking region of the *chlE* gene was demonstrated by construction of *lacZ* transcription fusions. The *Mlu*I (1.41 kb)-*Kpn*I (2.16 kb) fragment of pTN402 was inserted at the unique *Eco*RI site of pTN1051 to obtain plasmid pTN518, which conferred LacZ$^+$ activity on XL1-Blue (Fig. 5). The upstream and downstream regions of the insert relative to the *Eco*47III site were deleted to obtain pTN519 and pTN532, respectively. The results indicated in Fig. 5 implicate that a promoter for the *chlEN* operon (*chlEp*) should be located in the sequence up to the *Eco*47III site at nucleotide −83 from the initiation codon of *chlE* (Fig. 3). Furthermore, ChlE protein could be demonstrated in TNK23, which carries a pTN404-derived plasmid devoid of the sequence upstream of the *Ava*I site (1.81 kb) (data not shown). The promoter sequence is, therefore, tentatively assigned as shown in Fig. 3.

DISCUSSION

We have determined the nucleotide sequence of the DNA derived from the *chlE* locus of wild-type chromosome. Two structural genes, *chlE* and *chlN*, were identified by gene product analysis as well as by complementation tests with several deletion subclones. The *chlE* and *chlN* genes are located in this order to form an operon and are preceded by a promoter, *chlEp* (Fig. 3 and 5). The *chlE* reading frame extends from nucleotide 1 to nucleotide 1233, encoding a protein of molecular weight 44,067. The *chlE* coding region ends with the sequence TAATGG at nucleotide 1234. The ATG in this sequence is at the beginning of *chlN* reading

```
      -351      .         .         .         .       -301      .         .         .         .  BssHII .         .
ccacatccagcgcactttcgcccgcttccagcatttctgcccggtttcaacaatggcagacaacgcctcgatgtagcgtaattcctgttgcagactcatctgcgcgcggctaattgcac

          .         .         .       -201      .         .         .         .       -151      .         .         .
ctgcgccaccatgaattgcaatgactgctttgcccatagtattcgtccattaagggtggttcgccacttttgctataaatatcagaatttttatgaattactcattcaatttttgaatat

          .       -101      .    Eco47III AvaI .         .        -51   ("-35")      .        ("-10")   (RBS)      -1
agaaagatggatatggcatgtaaaggcaggacccagcgctacgcccgaggtggcatacagtctgtggcgtttttCTGACAtaataggcaaattcgattTTGCCTccgcAGGAGtgttttc

chlE -->.         .         .         .         50        .         .         .         .         .        100        .         .
atggaatttaccaccggattgatgtcgctcgacaccgcgcttaatgagatgctttctcgcgtcaccccactgaccgcccaggaaacgctgccactggtacagtgttttggtcgtattctg
 M  E  F  T  T  G  L  M  S  L  D  T  A  L  N  E  M  L  S  R  V  T  P  L  T  A  Q  E  T  L  P  L  V  Q  C  F  G  R  I  L

          .         .        150        .         .         .         .         .        200        .         .         .         .         .
gcgagcgatgtcgtttcgccgcttgatgttccggggtttgataactccgcaatggacggctacgcggtgcgtttagccgatattgcctccggcaaccgctgcccgttgccggtaaatcc
 A  S  D  V  V  S  P  L  D  V  P  G  F  D  N  S  A  M  D  G  Y  A  V  R  L  A  D  I  A  S  G  Q  P  L  P  V  A  G  K  S

       250     NcoI       .     KpnI      .        300        .         .         .         .         .        350        .
tttgccggtcagccataccatggtgaatggcctgcgggtacctgcattcgtattatgaccggtgcgccggtgccggaaggctgcgaagcggtggtgatgcaggagcagactgaacaaatg
 F  A  G  Q  P  Y  H  G  E  W  P  A  G  T  C  I  R  I  M  T  G  A  P  V  P  E  G  C  E  A  V  V  M  Q  E  Q  T  E  Q  M

          .         .         .        400        .         .    EcoRV .PstI      .        450        .         .         .         .
gacaatggcgtgcgttttactgctgaagtgcgtagcgggcaaaatattcgccgtcgcggtgaagatatctctgcaggtgcggttgttttcccggcgggaactcgcctgactaccgcagag
 D  N  G  V  R  F  T  A  E  V  R  S  G  Q  N  I  R  R  R  G  E  D  I  S  A  G  A  V  V  F  P  A  G  T  R  L  T  T  A  E

          .        500        .         .         .         .    Eco47III550        .         .         .         .         .        600
ctgccagtaattgcttcactggggattgccgaagttccggtgattcgtaaagtgcgtgtagcgctttttctaccggtgatgaactccagttgcccggtcagccgctgggcgacggccaa
 L  P  V  I  A  S  L  G  I  A  E  V  P  V  I  R  K  V  R  V  A  L  F  S  T  G  D  E  L  Q  L  P  G  Q  P  L  G  D  G  Q

          .         .         .         .         .        650        .         .         .         .         .   BssHII        .         .
atctacgataccaaccgtctcgccgtacacctgatgttagaacagttgggatgcgaggtaattaacttagggattatccgcgacgatccccatgccctgcgcgccgcatttattgaagcc
 I  Y  D  T  N  R  L  A  V  H  L  M  L  E  Q  L  G  C  E  V  I  N  L  G  I  I  R  D  D  P  H  A  L  R  A  A  F  I  E  A

          .         .        750        .         .         .         .         .        800        .         .         .         .         .
gacagccaggcggatgtggtgatcagttccggcggtgtttcagtgggtgaggcggattacaccaaaacgattcttgaagagctgggggagatcgccttctggaagctggcgattaaacca
 D  S  Q  A  D  V  V  I  S  S  G  G  V  S  V  G  E  A  D  Y  T  K  T  I  L  E  E  L  G  E  I  A  F  W  K  L  A  I  K  P

       850        .         .         .         .         .        900        .         .         .         .         .        950        .
ggtaaaccgttcgcgttcggtaaactcagcaatagctggttctgcggcctgccgggcaacccggtttcagcgacgctgaccttctatcaactggtacagcctttgctggcaaaactaagc
 G  K  P  F  A  F  G  K  L  S  N  S  W  F  C  G  L  P  G  N  P  V  S  A  T  L  T  F  Y  Q  L  V  Q  P  L  L  A  K  L  S

          .         .    BssHII     1000        .         .      .AatII     .       1050        .         .         .
ggtaacaccgccagcggcctgcccgcgcgccagcgcgtacgcacagcgtcccgcctgaaaaaaacgccaggacgtcttgatttccagcgcggcgtgctgcaacgcaacgccgatggcgaa
 G  N  T  A  S  G  L  P  A  R  Q  R  V  R  T  A  S  R  L  K  K  T  P  G  R  L  D  F  Q  R  G  V  L  Q  R  N  A  D  G  E

          .       1100        .         .         .         .         .       1150        .         .         .         .       1200
ctggaagtgacgaccaccggacatcagggttcacatatatttagctcctttagcctcggcaactgctttatcgtgctggaacgcgatcgcggcaatgtggaagtgggcgaatgggtggaa
 L  E  V  T  T  T  G  H  Q  G  S  H  I  F  S  S  F  S  L  G  N  C  F  I  V  L  E  R  D  R  G  N  V  E  V  G  E  W  V  E

          .   MluI  .    (RBS).     chlN -->  1250        .         .         .         .       1300        .         .
gtagaaccgtttaacgcgttgttcGGAGGcctgtaatggcggaactcagcgatcaggagatgctgcgctacaaccggcaaatcattctgcgcggctttgattttgacggccaggaggcgc
 V  E  P  F  N  A  L  F  G  G  L  * M  A  E  L  S  D  Q  E  M  L  R  Y  N  R  Q  I  I  L  R  G  F  D  F  D  G  Q  E  A

          .         .       1350        .         .         .         .         .       1400        .         .         .         .         .
tgaaagattctcgcgtgctgatagtgggcctgggcggcctcggctgtgcagcctcgcagtatctggcaagcgccggtgtcggtaacctgacgctgctcgacttcgacacggtttcgctct
 L  K  D  S  R  V  L  I  V  G  L  G  G  L  G  C  A  A  S  Q  Y  L  A  S  A  G  V  G  N  L  T  L  L  D  F  D  T  V  S  L

      1450        .         .         .         .         .       1500        .         .         .     NruI      1550        .
cgaatctgcaacgccagacactgcacagtgatgccacggtcgggcaaccgaaggtggaatccgcccgtgacgccctgacgcggatcaacccacatatcgcgattacgccagtcaatgcac
 S  N  L  Q  R  Q  T  L  H  S  D  A  T  V  G  Q  P  K  V  E  S  A  R  D  A  L  T  R  I  N  P  H  I  A  I  T  P  V  N  A

          .         .         .       1600        .         .         .         .         .       1650        .        . KpnI  .
tgctggatgacgcagaacttgcagcattgattgctgaacacgatctggtgctcgactgtacggataacgttgcggtacgtaatcaactgaacgcaggctgttttgccgcgaaggtaccgc
 L  L  D  D  A  E  L  A  A  L  I  A  E  H  D  L  V  L  D  C  T  D  N  V  A  V  R  N  Q  L  N  A  G  C  F  A  A  K  V  P

          .       1700        .         .         .         .         .       1750        .         .         .         .       1800
tggtttccggcgcggcaattcgtatggaaggtcaaatcaccgtctttacttatcaggacggtgaaccgtgctatcgctgccttagccgtttgtttggtgaaaatgcattaacctgcgtgg
 L  V  S  G  A  A  I  R  M  E  G  Q  I  T  V  F  T  Y  Q  D  G  E  P  C  Y  R  C  L  S  R  L  F  G  E  N  A  L  T  C  V

          .         .         .         .         .       1850        .         .         .         .         .       1900        .         .
aagcaggcgtaatggcaccgttgatcggcgtaattggttcgttgcaggcaatggaagcgatcaaaatgctggcaggttatggcaaaccggccagcgggaaaatcgtcatgtacgatgcga
 E  A  G  V  M  A  P  L  I  G  V  I  G  S  L  Q  A  M  E  A  I  K  M  L  A  G  Y  G  K  P  A  S  G  K  I  V  M  Y  D  A

          .   NruI  .       1950        .         .         .         .         .       2000        . (REP)   .         .         .
tgacctgtcagtttcgcgaaatgaaactgatgcgtaatccggggtgtgaggtgtgtgggcagtaattgccacctccAGGCCTGATAAGACGCGGCACTCCGCCACGTCATCAGCaattta
 M  T  C  Q  F  R  E  M  K  L  M  R  N  P  G  C  E  V  C  G  Q  *

      2050        .         .         .         .       2100        .         .         .
tgaggatgcagaacgtccggttaaatcgacgttctgccaaacgctccctgccagtcctgctcaaacttcgccacagcggcatcaaccgccgg
```

FIG. 3. Nucleotide sequence of the *chlEN* operon. The sequence is numbered from the first base of the *chlE* gene. The predicted amino acid sequences are indicated in one-letter code below the nucleotide sequence. Possible transcriptional and translational signal sequences (capital letters in nucleotide sequence), promoter sequences ("−35" and "−10"), ribosome-binding site (RBS), and repetitive extragenic palindromic sequence (REP) are indicated. Several restriction sites are also shown above the sequence.

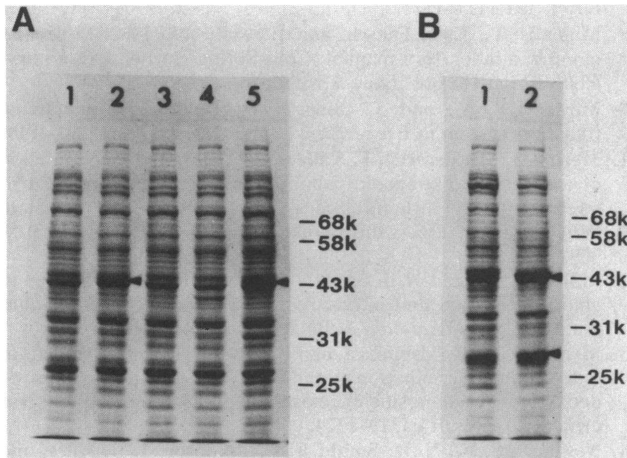

FIG. 4. SDS-polyacrylamide gel electrophoresis of total cellular proteins. Positions of size markers are indicated on the right. (A) The total proteins were prepared from a Δ*chlE* strain, TNK23, harboring plasmids pTN1028 (lane 1, control), pTN404 (lane 2), pTN480 (lane 3), pTN481 (lane 4), or pTN483 (lane 5). Cells were cultured in medium containing kanamycin until $A_{660} \simeq 0.6$. The ChlE protein is indicated by an arrowhead in lanes 2 and 5. (B) The total proteins accumulated in a *lacI*^{q} strain, XL1-Blue, harboring plasmids pUC18 (lane 1, control) and pTN525 (lane 2), after 2 h induction with 2 mM isopropyl-β-D-thiogalactopyranoside were prepared from cells that had been cultured in the medium containing ampicillin until $A_{660} \simeq 0.2$. The ChlE and ChlN proteins are indicated by arrowheads in lane 2.

frames between nucleotides 1236 and 1982, encoding a protein of molecular weight 26,719. Both *chlE* and *chlN* initiation codons are preceded by potential ribosome-binding-site sequences (33), AGGAG and GGAGG, at appropriate distances (Fig. 3). Therefore, the translation of *chlN* may be coupled with that of *chlE*. However, the *chlN* gene was more weakly expressed than *chlE*, as indicated by the relative amounts of ChlN and ChlE proteins (Fig. 4). This is reflected in the difference in codon usage preference (8): the *chlN* gene contains fewer codons preferred by highly expressed genes than does the *chlE* gene.

Although there is no typical rho-independent terminator sequence downstream of the *chlN*-coding region up to nucleotide 2132, one copy of a repetitive extragenic palindromic sequence (35) is observed in the 3'-flanking region of *chlN* (Fig. 3). This sequence seems to serve as a stabilizer for *chlEN* mRNA by forming a secondary structure (21, 24).

The −35 and −10 sequences are recognized in the *chlEp* region (Fig. 3), although there is no direct evidence for the exact location of the promoter sequence. The −10 sequence has poor homology to the canonical *E. coli* promoter sequence.

Both ChlE and ChlN proteins are required to complement the defect in Δ*glnP-chlE* mutants. These proteins are thought to be involved in the biosynthesis of a demolybdo cofactor, molybdopterin (15), which is necessary for the activities of nitrate reductase and other molybdoenzymes by forming a complex with a Mo atom. The ChlE protein appears to be identical to one of the biosynthetic enzymes (termed molybdopterin-converting factor) having a molecular weight of 45,000 (14), since the factor has been ascribed to either *chlE*, *chlM*, or *chlN*. The deduced amino acid sequence of the ChlN protein indicates an enrichment of Cys residues, especially in the carboxy-terminal half. There are two copies of Cys-X-X-Cys sequence which may bind to a metal, such as Mo. Therefore, the ChlN protein is considered to play a role in Mo insertion into molybdopterin to form active Mo cofactor.

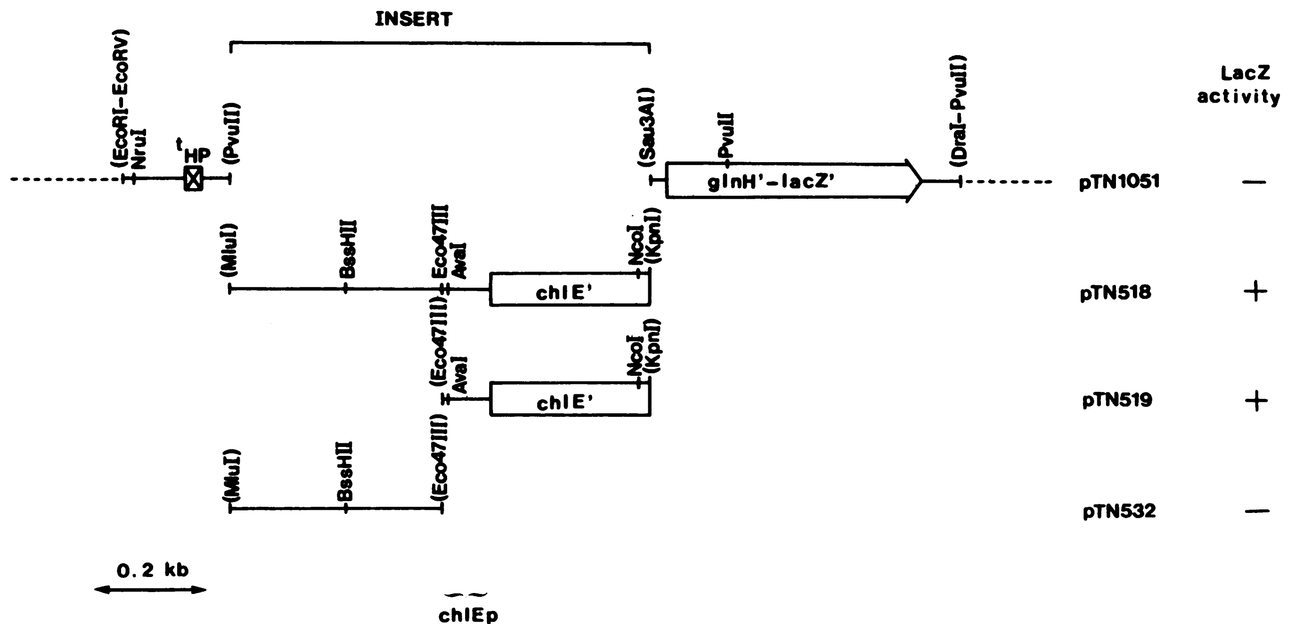

FIG. 5. Promoter activity in the upstream region of the *chlEN* operon. DNA fragments from the *chlEN* operon were inserted into pTN1051 as indicated in the figure. There is an in-frame stop codon with the *glnH'* gene between the cloning site and the initiation codon (26) which prevents reading through from the *chlE'* gene in pTN518 and pTN519. The vector pTN1051 contains a terminator sequence, t_{HP}, upstream of the cloning site. Broken line denotes DNA from pBR322. Restriction sites lost during construction are in parentheses. LacZ activity assayed in XL1-Blue is indicated on the right.

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