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Bacillus subtilis CheN, a Homolog of CheA, the Central Regulator of Chemotaxis in *Escherichia coli*

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The *Bacillus subtilis cheN* gene was isolated, sequenced, and expressed. It encodes a large negatively charged protein with a molecular weight of approximately 74,000. The predicted protein sequence has 33 to 34% identity with the *Escherichia coli* and *Salmonella typhimurium* CheA and *Myxococcus xanthus* FrzE sequences. These proteins are found to autophosphorylate and are members of the same histidine kinase signal modulating family. CheN has several conserved regions (including the histidine that is phosphorylated in CheA) that coincide with other autophosphorylated signal transducers. A null mutant is defective in attractant-induced methanol formation and shows no behavioral response to chemoeffectors. These results imply that in *B. subtilis* the mechanism of chemotaxis involves phosphoryl transfer similar to that in *E. coli*. However, the CheN null mutant mostly tumbles, whereas CheA mutants swim smoothly, and only in *B. subtilis* does excitation lead to methyl transfer and methanol formation. Thus, the overall mechanism of chemotaxis is different in the two organisms.

Cells of the gram-positive bacterium *Bacillus subtilis* have a unique chemotactic mechanism for sensing the environment and migrating to more-favorable conditions (20, 21, 31, 32, 34). Methyl groups are apparently transferred from methyl-accepting chemotactic proteins (MCPs) to an intermediate carrier or regulator (31–34) throughout the adaptation period. In contrast, in *Escherichia coli* no methyl transfer beyond the MCPs is known to occur upon the addition of attractant, and methanol is believed to evolve directly from the MCPs (9, 30). Chemotactic behavior in *E. coli* is controlled by a balance of autophosphorylation, phosphotransfer, and phosphatase activity (8). Phosphorylation has not yet been shown to be involved in *B. subtilis* chemotaxis.

To understand the chemotaxis mechanism in *B. subtilis*, a collection of *che* mutants was generated by treatment of cells with ethyl methanesulfonate (EMS), which normally causes point mutations (20). Many of the mutations were mapped and characterized (21). Most mutations were localized to a large operon between *pyrD* and *spcB* (38). One mutation, *cheN1088*, was found to lie in a 1.6-kb *Pst*I fragment in the distal part of this operon (38). Five other randomly generated mutations were also found to lie in *cheN* by complementation (21).

CheN$^-$ mutants have a complex phenotype. Of the EMS-produced CheN$^-$ mutants, four mostly tumble, one moves randomly, and one swims smoothly (20, 21). Tumbling is uncoordinated motion, without forward progress. Smooth swimming is uninterrupted straight or gently curved motion. Random motion is a mixture of smooth swimming and tumbling and has the appearance of erratic swimming. One of these mutants, OI1088, shows no behavioral response to the removal of attractants or repellents in tethering experiments in which a flagellum is immobilized and the resulting cellular rotation is monitored (21). This implies that one function of CheN may be to transmit the response signal for removal of attractants and repellents (21). Furthermore, OI1088 does not adapt to the addition of repellents (21), suggesting that CheN helps bring about adaptation.

In view of the unusual phenotype of OI1088 and of the considerable differences in chemotaxis-associated methylation between *B. subtilis* and *E. coli*, we did not anticipate that a phosphoryl transfer cascade might underlie chemotaxis in *B. subtilis* as it does in *E. coli*. In this article, we report the sequence of *cheN* and the characterization of a null mutation in that gene.

MATERIALS AND METHODS

Plasmids and strains. A 10.9-kb *Eco*RI fragment containing *cheN* was previously subcloned into pUC18 (35) to create pGO104 (38) (Fig. 1). The DNA from the middle *Eco*RV site to the distal *Sal*I site in pGO104 (Fig. 1) was subcloned in several steps into the *B. subtilis* expression vector pSI-1 (36) to create pDF103; an 0.8-kb *Pst*I-*Sal*I fragment was subsequently deleted to create pDF106, which expresses *cheN* only, under the control of the *spac* promoter (36). The segment from the middle *Eco*RV site in pGO104 to the *Kpn*I site was put into pT7-6, which expresses proteins under the control of T7 RNA polymerase (28), to generate pDF102.

All strains used are described in Table 1. Transformations for subcloning and plasmid preparations were done with strain TG-1 (Amersham Corp., Arlington Heights, Ill.).

DNA sequencing. The Sanger dideoxy chain-termination method (23) for plasmid DNA was used. Deletions were generated in both directions by using exonuclease III (Bethesda Research Laboratories) (7). Additional constructs were made by using appropriate restriction and modifying enzymes (Bethesda Research Laboratories). The Promega (Madison, Wis.) and Sequenase (U.S. Biochemicals) kits and protocols were used with [^{35}S]dATP from Amersham. DNAStar (Madison, Wis.) was used to correlate and analyze sequence information, such as aligning CheA to CheN (14).

Complementation. Transformations involving plasmids were performed as previously described for *B. subtilis* (1) or *E. coli* (6). The chemotaxis phenotype of transformants was tested on mannitol and tryptone swarm plates by the method of Ordal et al. (20). Expression was induced with 1 mM

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TABLE 1. Bacterial strains

Strain	Genotype	Source of reference
B. subtilis		
OI1085	*trpF7 hisH2 metC che*$^+$	1
OI1179	*trpF7 hisH2 metC che*$^+$ *recE4*	1
OI1088	*trpF7 hisH2 metC cheN1088*	5
OIA839	*trpF7 hisH2 metC cheN1088 recE4*	This work
OIA841	*trpF7 hisH2 metC cheN1088 recE4* (pDF106)	This work
OIA842	*trpF7 hisH2 metC cheN1088 recE4* (pSI-1)	This work
OIA840	*trpF7 hisH2 metC cheN::cat*	This work
OIA843	*trpF7 hisH2 metC cheN::cat recE4*	This work
OIA844	*trpF7 hisH2 metC cheN::cat recE4* (pDF106)	This work
E. coli		
TG-1	Δ(*lac-proAB*) *supE thi hsdΔ5* (r_K^- m_K^-)/F′ *fraD36 proAB lacI*$_q$	Amersham Corp
D404	F$^-$ *thi thr leu his met*(Am) *strA* Δ*cheA*	46
KO685	Δ*recA* Δ*cheA*	8
OIA964	D404(pGP1-2)	This work

isopropyl-β-D-thiogalactopyranoside (IPTG) where appropriate.

Expression using T7 polymerase. Strain D404 carrying pGP1-2, which encodes T7 RNA polymerase, was transformed with pDF102 (pT7-6::*cheN*). pT7-6 was used as a control. The promoter was induced and expressed proteins were radiolabelled by the method of Tabor and Richardson (28). The labeled proteins were fractionated on a sodium dodecyl sulfate-polyacrylamide gel (13) and autoradiographed with Kodak XAR-5 film.

Characterization. A continuous-flow assay to monitor the effect of adding and removing attractant on methanol production was carried out as previously described (33). Expression of *cheN* in the pSI-1 background was varied by using IPTG induction as described previously (2). The behavior of late-exponential-phase cells was recorded with a Panasonic AG-1960 video cassette recorder connected to a Zeiss RA microscope via a Panasonic BL200 camera. Linear advances were regarded as swimming and stationary rotation were regarded as tumbling.

Nucleotide sequence accession number. The sequence reported in this paper has been deposited in the EMBL and GenBank databases under accession no. M51894. In future articles, the designations *cheA* and CheA will replace *cheN* and CheN. The original *cheA* locus (20) is being renamed *orf219* and, after characterization of its null mutant phenotype, will be renamed.

RESULTS

Sequence of *cheN*. The complete nucleotide sequence of *cheN* is presented in Fig. 2. A large open reading frame (ORF) found in this DNA encodes a 671-amino-acid polypeptide, which corresponds to a mass of 73 kDa. The direction of transcription agrees with that for the operon as a whole (38), and there is a putative ribosome binding site (AAAGAGAGG [5]) with a ΔG of −16.0 kcal/mol (ca. −66.9 kJ/mol) (calculated by the method of Tinoco et al. [29]) upstream of the AUG start. There does not appear to be a secondary translational start site further down in the sequence generating a smaller protein as is found in *E. coli* (11). A hydropathy plot (data not shown) suggests that CheN has two regions at the C-terminal end with the potential to be transmembrane regions by the algorithm of Kyte and Doolittle (12). It is not known whether CheN is actually membrane bound.

Evidence to identify the CheN ORF is as follows. First, all six EMS mutations in *cheN* (20) were repaired by recombination, using the 1.6-kb *Pst*I fragment (Fig. 1). In this fragment there are two ORFs. The smaller one encodes only three amino acids. It is very probable that all the mutations are in the large ORF and not in the small ORF. Second, the 1.6-kb *Pst*I fragment alone will not complement the mutant phenotype. Therefore, CheN must overlap the promoter-proximal *Pst*I site. Third, an expression plasmid (pDF106) containing the large ORF, including upstream regions, complemented *cheN1088* in a *recE4* background on tryptone (data not shown) and mannitol swarm plates (data not shown). Fourth, a null mutant was constructed by inserting a promoterless *cat* gene into an *Eco*RV site internal to the ORF in question but upstream of the 1.6-kb *Pst*I fragment (Fig. 1). The behavior and swarm plate morphology of this mutant were similar to those of the majority of EMS mutants. The null mutation (in strain OIA843) and *cheN1088* (in strain OIA839) were both complemented by pDF106, which expresses *cheN* (data not shown), and thus lie in the same gene and are not polar on downstream genes. Cured strains returned to the original mutant phenotype (data not shown).

Expression of CheN in *E. coli*. *cheN* in pT7-6 (pDF102) was introduced into OIA964, a *cheA*-deleted strain of *E. coli* that also carried pGP1-2, which encodes the bacteriophage T7 RNA polymerase. Following heat induction to destroy the repressor and allow synthesis of this polymerase, [^{35}S]methionine and rifampin were added. Under these conditions, only genes under control of the T7 promoter are expressed and labelled, as rifampin blocks cellular RNA polymerase. A radiolabelled band was observed at 74 kDa, which was not present in the control strain having pT7-6 without an insert (data not shown). This value agrees with that predicted from the nucleotide sequence.

Homology between *B. subtilis* CheN and other proteins. To determine whether CheN was similar to any proteins, the NBRF data base was searched. CheN had high homology to *E. coli* (11) and *Salmonella typhimurium* CheA (24) and to *Myxococcus xanthus* FrzE (16), with 33, 34, and 34% amino acid identities, respectively. The regions believed to be involved in autophosphorylation are conserved in all species pictured (Fig. 3 and 4). However, the highly conserved region at the C terminus of CheA seems to be specific to chemotaxis in *B. subtilis* and *E. coli* (Fig. 3).

CheA and FrzE are members of a family of autophosphorylating sensory transduction proteins, and CheN bears many of the hallmarks of this family (10, 26) (Fig. 3). In particular, there are seven residues that were found to be invariant for all members of this group (26) (see the residues in boldface type in Fig. 3). All seven are also conserved in CheN, except for CheA Asn-419, which is Lys-433 in CheN. His-46 of CheN, which corresponds to His-48 of CheA, the autophosphorylated residue, is also conserved.

Attempted complementation of CheA by CheN. In view of the strong conservation of certain amino acids and the potential parallel nature of CheN and CheA, an attempt was made to complement a *cheA* mutant of *E. coli*. Plasmids pDF103 (which encodes CheN and a CheW homolog [4a]) and pDF106 (which encodes only CheN) were introduced

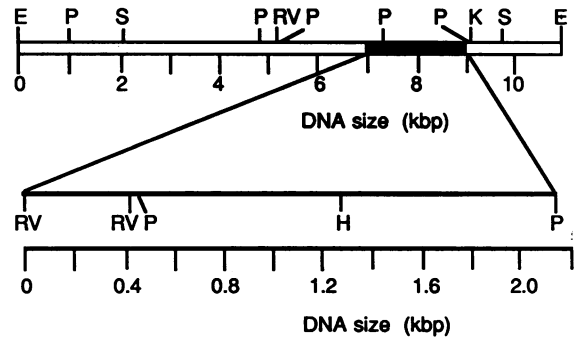

FIG. 1. Restriction map of the 10.9-kb *Eco*RI fragment (20) from pGO104 (38). The ORF of *cheN* is shown by the dark bar. Restriction site abbreviations: E, *Eco*RI; P, *Pst*I; S, *Sal*I; RV, *Eco*RV; B, *Bal*I; K, *Kpn*I; H, *Hin*dIII.

into *E. coli* KO685, a *cheA* deletion mutant. No complementation for chemotaxis occurred (data not shown). However, a plasmid which encodes *E. coli* CheA and CheW did complement the KO685 strain (data not shown). Therefore, CheN is not sufficiently similar to CheA to substitute for it in vivo.

Characterization. The swimming behavior of the null mutant was compared with that of the wild type, strain OI1085. It was found that the null mutant tumbled 88% of the time on average under the conditions tested (see Materials and Methods) as opposed to 40% for the wild type in *B. subtilis*. In the wild type, all stimuli, both positive and negative, cause production of methanol, probably to bring about adaptation (33). One particularly sensitive method of measuring this methanol is a continuous-flow assay in which methyl-labelled cells are placed on a membrane and buffer, with or without attractant, is flowed by the cells and collected in a fraction collector (33). The radiolabelled methanol is subsequently quantitated. In this assay, the null mutant produced only a baseline response to addition and removal of attractant (Fig. 5). In addition, the null mutant showed no

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     EcoRV                                                                M D M N Q Y L D V F I D E S K E
1    GATATCGCAGCATCCATCACAAGCTGTGTGAAAAAAGAGAGGGTGTGATTCAAATGGATATGAATCAGTATTTAGATGTCTTTATTGATGAGAGTAAAGA
                                  ***
      H L Q T C N E K L L L L E K D P T D L Q L V H D I F R A A H T L K
101  ACATTTACAAACATGTAATGAAAAGCTTCTTCTTTTAGAGAAAGACCCGACTGATCTTCAGCTCGTACATGATATATTCAGGGCTGCCCATACATTAAAA
      G M S A T M G Y T D L A H L T H L L E N V L D A I R N G D M E V T S
201  GGAATGAGCGCAACGATGGGCTACACGGATTTAGCACATCTTACCCATCTGCTTGAAAACGTGCTGGATGCAATCCGAAACGGAGACATGGAAGTCACCT
      D W L D I L F E A L D H L E T M V Q S I I D G G D G K R D I S E V
301  CAGACTGGCTGGATATTTTGTTTGAAGCTCTGGATCACCTGGAAACAATGGTTCAGTCTATTATTGATGGCGGGGATGGTAAAAGAGATATCTCAGAAGT
      S A K L D V N G A H A E S A A S A E P A E A Q S S A S D W E Y D E
401  GAGTGCCAAGCTTGATGTGAATGGTGCGCACGCTGAATCCGCTGCCTCCGCTGAACCTGCAGAAGCACAGAGTTCAGCATCTGATTGGGAATATGATGAA
      F E R T V I Q E A E E Q G F K R Y E I K I S L N E N C M L K A V R V
501  TTTGAACGCACAGTTATACAAAAGCGGAGGAGCAAGGCTTCAAACGATATGAAATCAAGATTTCTCTGAACGAAAACTGTATGTTAAAAGCAGTTCGTGT
      Y M V F E K L N E V G E V A K T I P S A E V L E T E D F G T D F Q
601  CTATATGGTGTTTGAAAAACTAAATGAAGTCGGAGAAGTAGCCAAAACAATTCCAAGTGCTGAAGTGCTTGAAACAGAAGATTTTGGAACTGACTTTCAA
      V C F L T H Q S A E D I E Q L I N G V S E I E H V E V I Q G A P L T
701  GTTTGTTTCTTAACACATCAATCAGCGGAAGACATTGAACAATTAATCAATGGCGTGTCGGAAATTGAGCATGTTGAAGTCATTCAAGGGGCTCCTTTAA
      S A E K P E E S K Q E D S P A A A V P A N E E K Q K Q P A K N D E
801  CATCAGCTGAAAAGCCTGAAGAATCTAAGCAAGAAGATTCACCAGCAGCAGCTGTACCTGCGAACGAGGAGAAACAAAAACAGCCTGCTAAAAACGATGA
      Q A K H S A G G S K T I R V N I D R L D S L M N L F E E L V I D R
901  ACAGGCGAAGCATTCAGCCGGCGGATCTAAAACAATTCGTGTCAACATTGACAGGCTTGATTCTTTAATGAACTTATTTGAAGAGCTTGTCATTGACCGC
      G R L E Q I A K E L E H N E L T E T V E R M T R I S G D L Q S I I L
1001 GGGCGTCTCGAGCAGATTGCGAAAGAGCTTGAGCATAATGAACTGACTGAAACGGTTGAACGAATGACGAGAATTTCCGGAGATTTGCAATCGATTATTC
      N M R M V P V E T V F N R F P R M I R Q L Q K E L N K K I E L S I
1101 TGAATATGAGAATGGTCCCGGTTGAAACTGTTTTTAACAGATTCCCGAGAATGATTCGCCAGCTTCAGAAAGAGCTGAATAAAAAAATTGAACTCTCGAT
      I G A E T E L D R T V I D E I G D P L V H L I R N S I D H G I E A
1201 CATCGGTGCGGAAACTGAACTGGATCGTACAGTAATTGATGAAATCGGAGATCCACTCGTTCACTTGATCAGAAACAGTATTGACCATGGTATCGAGGCG
      P E T R L Q K G K P E S G K V V L K A Y H S G N H V F I E V E D D G
1301 CCGGAAACACGTTTGCAAAAGGGAAAACCGGAATCAGGAAAAGTTGTGCTTAAAGCTTATCACAGCGGCAACCATGTCTTTATCGAAGTAGAGGATGACG
      A G L N R K K I L E K P L E R V I T E K E A E T L E D N Q I Y E L
1401 GCGCAGGCCTTAATCGAAAAAAAATTCTGGAAAAACCGCTTGAGCGGGTCATAACGGAAAAAGAAGCTGAAACCTTAGAAGACAACCAAATTTACGAATT
      I F A P G F S T A D Q I S D I S G R G V G L D V V K N K L E S L G
1501 GATTTTTGCTCCGGGGTTCTCAACTGCTGACCAAATTTCTGATATTTCCGGCCGCGGTGTAGGACTTGACGTTGTAAAAAACAAACTGGAGTCTCTTGGC
      G S V S V K S A E G Q G S L F S I Q L P L T L S I I S V L L I K L E
1601 GGTTCAGTCAGTGTGAAATCAGCCGAGGGTCAAGGCTCTCTATTCAGCATCCAGCTGCCGCTTACCTTGTCTATTATTTCAGTTCTGCTGATTAAGTTGG
      E E T F A I P I S S I I E T A V I D R K D I L Q T H D R E V I D F
1701 AAGAAGAGACATTTGCCATTCCGATTTCTTCAATCATTGAGACAGCAGTTATTGACAGAAAAGACATTTTGCAAACGCATGACCGTGAAGTGATTGACTT
      R G H I V P V V Y L K E E F K I E D T R K D A E Q L H I I V V K K
1801 TAGAGGGCATATTGTCCCTGTCGTTTATTTAAAAGAAGAGTTTAAAATAGAAGATACAAGAAAAGATGCGGAACAGCTCCATATCATTGTCGTGAAAAAA
      G D K P T A F V V D S F I G Q Q E V V L K S L G D Y L T N V F A I S
1901 GGCGACAAACCTACTGCATTTGTGGTGGACTCCTTTATTGGCCAGCAGGAAGTTGTGCTGAAATCACTCGGAGATTATTTAACAAACGTCTTTGCAATTT
      G A T I L G D G E V A L I I D C N A L I I                    PstI
2001 CCGGAGCTACTATTTTAGGAGACGGAGAAGTAGCGCTCATTATTGATTGTAATGCACTGATTATTTAACCATTCGAGGAGGTGTCTCACATGACTGCAG
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FIG. 2. Nucleotide sequence and predicted amino acid sequence of CheN. Putative ribosome binding sites are underlined. Stop codons are indicated by three asterisks beneath the codon. Internal *Eco*RV and *Pst*I sites are not indicated.

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                                                *
CheN  MDMNQYLDVFIDESKEHLQTCNEKLLLLEKDPTDLQLVHDIFRAAHTLKGMSATMGYTDLAHLTHLLENVLDAIRNGDMEVTSDLDILFE  90
CheA  MD:::: :.F:DE:.E L.. ::.LL L  ::.D : ::.IFRAAH::KG:::T.G:T L.: THL:EN:LD. R.G:M::.:D:::::E  92
FrzE                                                                          M:..:  .:L .  12

CheN  ALDHLETMVQSIIDGGDGKRDISEVSAKLDVNGAHAESAASAEPAEAQSSASDWEYDEFERTVIQEAEEQGFKRYEIKISLNENCMLKAV 180
CheA  : D ::. ::: ::.: . .  E  : .:.:  E: :.:.PA .:::A . . :E  . :.E:E..  R E K:.:  : .LKA  176
FrzE    :  ...:Q.I  G  :  . :.  A  DV. .H .  :.A  . .: :  : .E  . :E:. ..  :: :. :  ... .  103

CheN  RVYMVFEKLNEVGEVAKTIPSAEVLETEDFGTDFQVCFLTHQSAEDIEQLINGVSEIEHVEVIQGAPLTSAEKPEESKQEDSPAAAVPAN 270
CheA   V :: E.L.:::.::..: :A: L    ::.:: . ::::.  .:  :: .. :I  .V: :AP:..A: .E  . .:PA...PA. 254
FrzE   .  . .  .  E:.: ::...  .. .::.. .V  . .::. :.. : . :.:.  .  AP:::: ...  : ::.AAA.::: 187

CheN  EEKQKQPAKNDEQAKHSAGGSKTIRVNIDRLDSLMNLFEELVIDRGRLEQIAKELEHNELTETVERMTRISGDLQSIILNMRMVPVETVF 360
CheA  .. ..:.  . E:: :. ::: :IRV.::::D L:NL .ELVI.::.L.Q ::EL H.::..::.:: R : DLQ. ::::RM:P:E VF 349
FrzE   . .. ...:.  A .SA :.::IRVN:: LD:L  L .:L:::RGRL  ..E  : ::  :R. R::.:::  .R:VP:.TVF 285

CheN  NRFPRMIRQLQKELNKKIELSIIGAETELDRTVIDEIGDPLVHLIRNSIDHGIEAPETRLQKGKPESGKVVLKAYHSGNHVFIEVEDDGA 450
CheA  :RFPR::R:L. .L.K::EL:::G:.TELD:::I: I DPL.HL:RNS:DHGIE PE.RL: GK  GN::L.A H G.:: IEV.DDGA 440
FrzE  : FPR :R:: :. .K.::L I .A:..:DR:::::: D:LVHL:RNS:DHG:E:P:TR Q GKP :GN: ::.  .G: : IEVEDDG  420

CheN  GLNRKKILEKPLERVITEKEAETLEDNQIYELIFAPGFSTADQISDISGRGVGLDVVKNKLESLGGSVSVKSAEGQGSLFSIQLPLTLSI 540
CheA  GLNR.::IL.K::.: :: :E  .:.D:::  LIFAPGFSTA:Q::D:SGRGVG:DVVK.::::.:GG V.::S :G G: : I.LPLTL:I 528
FrzE  G::..:: : ::.R:I.. :A.:L.:.:  ELIF PGFST DQ:S::SGRGVG:DVVK.K:E:LGGSV:V.S  G:GS :: :LP :L:: 511

CheN  ISVLLIKLEEETFAIPISSIIETAVIDRKDILQTHDREVIDFRGHIVPVVYLKEEFKIEDTRKDAEQLHIIVVKKGDKPTAFVVDSFIGQ 630
CheA  :. : ::::..E.F :P:::::E:     .D L.. :R V:: RG: :P:V L . F.::::::.:A.Q  :::::.:::. A::VD :IGQ 619
FrzE  :.VLL::L.::.:::P :.: ..  :...D L:. :  .:  RG. .::V L  : :::..:  :: . :VV:.G:..:A:VVD:F::: 600

CheN  QEVVLKSLGDYLTNVFAISGATILGDGEVALIIDCNALII
CheA  ::VV:K:L..   :V :IS:ATILGDG.VALI:D :AL
FrzE  :EV.:K: G::L...  I:G:: L.DG :A::::  .::
```

FIG. 3. Comparison of sequences of *B. subtilis* CheN, *E. coli* CheA, and *M. xanthus* FrzE proteins. Only residues identical to CheN are shown. The phosphorylated residue of CheA (His-48) is indicated by an asterisk. Highly conserved regions of the autophosphorylating sensory transduction family are underlined and invariant residues are in boldface type (26). A colon, period, or a blank between amino acids represents a positive, zero, or negative correlation, respectively.

behavioral response to the attractant (aspartate) or repellent (butyrate).

The effects of variation of expression of CheN were tested with pDF106, in which *cheN* was placed under the control of a *spac* promoter (36) whose expression may be controlled by added IPTG. It is apparent from Fig. 6 that even in the absence of IPTG, the null mutant showed excellent complementation, probably as a result of background expression of *cheN* from the *spac* promoter. However, IPTG-induced expression of CheN produced larger swarms (Fig. 6), suggesting that the background level of CheN expressed from the *spac* promoter is suboptimal for chemotaxis on a tryptone swarm plate. It should be noted that all strains carried the *recE4* mutation to avoid recombination between plas-

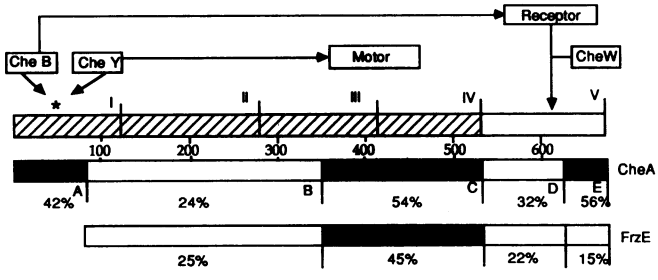

FIG. 4. Diagram showing the relationship between the predicted functional map of CheA (19) and regions of homology to CheN and FrzE. The region of CheA thought to be involved in autophosphorylation (19) (▨) and regions highly homologous to CheN (▨) are shown. Regions I to V are CheA regions defined by restriction enzymes (19). Region I of CheA is thought to be involved in phosphoryl transfer both to CheB and to CheY (19). The asterisk shows the site of phosphorylation on CheA (19). Regions of homology A to E were distinguished by a dot plot between CheA and CheN on the basis of a protein similarity search with a cutoff of 50% and a window of 20.

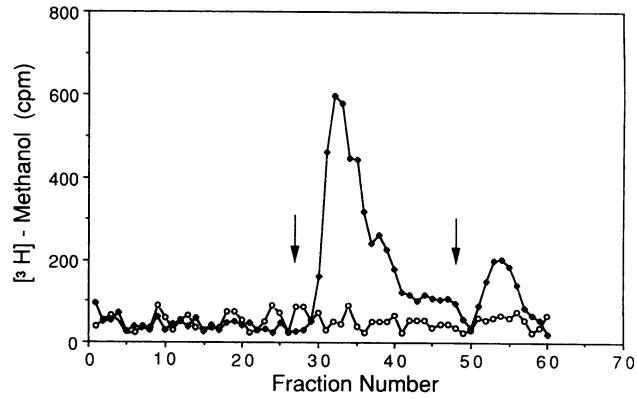

FIG. 5. Flow assay showing effects of addition and removal of attractant. Arrows represent (left to right) the addition and removal of attractant. Symbols: ○, OI1085; ●, OIA840 (null mutant).

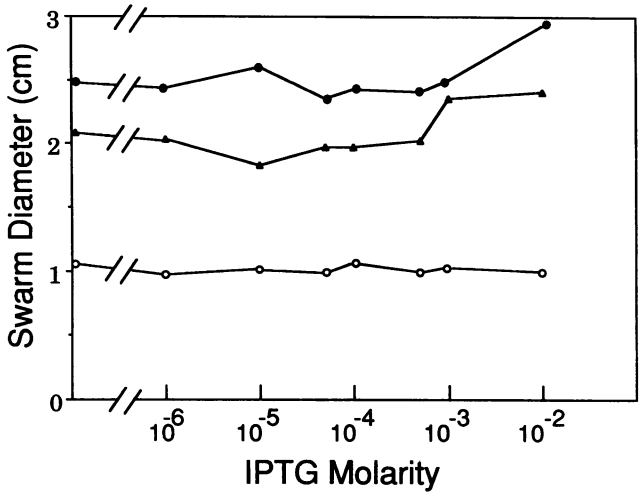

FIG. 6. Effect of variable induction of CheN. All points shown are the mean values for three experiments. Symbols: ●, OIA844 (*cheN*::*cat* with pDF106, a complementing plasmid); ▲, OI1179 (wild type); ○, OIA843 (*cheN*::*cat*).

mids and chromosome; the *recE4* allele makes the strains somewhat slow growing.

DISCUSSION

The major conclusion of this paper, based on homology of the deduced protein sequences, is that *B. subtilis* contains a chemotactic autophosphorylating kinase (CheN). Accordingly we speculate that a phosphotransfer signal transduction mechanism plays a significant role in *B. subtilis* chemotaxis. This hypothesis is supported by other similarities between *E. coli* and *B. subtilis* chemotaxis. Recently, evidence for a *B. subtilis* CheY has been found (2). In *E. coli*, CheY receives phosphoryl groups from CheA so that clockwise rotation of the flagella will occur (8). *B. subtilis* CheY shares the canonical sequences (26) of the phosphorylated regulator family with *E. coli* CheY, including the phosphorylated aspartate residue (2). In *E. coli*, the methyltransferase (27) and methylesterase (25) act on the MCPs (25, 27), and CheA and CheW both interact with the MCPs (17). The methylesterase is subject to activation through CheA-mediated phosphorylation (15). *B. subtilis* has both a methyltransferase and a methylesterase and they are sufficiently similar to those of *E. coli* that they work on both *B. subtilis* and *E. coli* MCPs (4, 18). A *B. subtilis* CheW has also been inferred by deduction from sequencing *B. subtilis* DNA (4a). Finally, a phosphorylation cascade as hypothesized for CheA also occurs for the onset of sporulation in *B. subtilis* (3, 22). Thus, it is reasonable to suppose that as in *E. coli*, CheN is an autophosphorylating kinase might transfer phosphoryl groups to CheY and the methylesterase in response to signals from the MCPs.

E. coli CheA is the excitatory enzyme for chemotaxis in that binding of attractant or repellent causes decreased or increased autophosphorylation of CheA and, hence, decreased or increased phosphorylation of CheY and CheB (methylesterase), respectively (8). Inactivation of *cheN* makes the bacterium refractory to any behavioral effect of attractant or repellent and to any stimulation of methanol production (Fig. 5). Methanol is produced as an adaptation reaction to stimulus and in the absence of stimulus, only background amounts are produced (Fig. 5). In *B. subtilis*, the actual excitatory reaction is believed to involve methyl transfer to a regulator (32, 33) not simply phosphoryl transfer, and it is reasonable to suppose that CheN governs the enzymes required for this transfer.

The behavioral phenotype of CheN$^-$ mutants also suggests that the chemotactic mechanism in *B. subtilis* is not the same as that in *E. coli*. The null mutant and four point mutants mostly tumble. In *E. coli*, CheA$^-$ mutants all exhibit smooth swimming, since they result in a deficiency of phosphorylated CheY (19). Interestingly, one of the point mutants with a mutation in *cheN* exhibits smooth swimming. Another (OI1088) is random in its behavior and shows a complex phenotype, not presently understood (see the introduction). Thus, the phenotypes of *B. subtilis* CheN mutants are not simply the opposite of that of *E. coli* CheA mutants and the characteristics of the EMS mutants should in time provide important clues for understanding the chemotactic mechanism in *B. subtilis*.

On the basis of homology, it is likely that CheN phosphorylates CheY in *B. subtilis* and that CheY interacts with the flagellar switch to affect behavior. However, the theme that *B. subtilis* chemotaxis works differently is reiterated on closer analysis of *B. subtilis* CheY mutants. The null mutants tumble but the point mutants exhibit smooth swimming (2). Overexpression, however, produces a tumbling phenotype as it does in *E. coli* (2). The flagellar switch, which controls whether a bacterium swims smoothly or tumbles, is also not the same. In *E. coli*, the switch has three components, FliM, FliG, and FliN, and in *B. subtilis*, it lacks FliN but has CheD and Orf219 in addition to FliM and FliG (2, 37). If we assume that excitation occurs, in part, because the methylated regulator (see above) binds to the switch, these differences in the switch components may reflect the requirement for binding this regulator in addition to CheY in its phosphorylated or unphosphorylated form.

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