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CheA protein, a central regulator of bacterial chemotaxis, belongs to a family of proteins that control gene expression in response to changing environmental conditions

(*Salmonella typhimurium*/*Escherichia coli*/receptors/sensory transduction)

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ABSTRACT **During bacterial chemotaxis, the binding of stimulatory ligands to chemoreceptors at the cell periphery leads to a response at the flagellar motor. Three proteins appear to be required for receptor-mediated control of swimming behavior, the products of the *cheA*, *cheW*, and *cheY* genes. Here we present the complete nucleotide sequence of the *Salmonella typhimurium cheA* gene together with the purification and characterization of its protein product. The protein is a 73,000 M_r cytoplasmic constituent. Amino acid-sequence comparisons indicate that it belongs to a family of bacterial regulatory proteins including the products of the *cpxA*, *dctB*, *envZ*, *ntrB*, *phoR*, *phoM*, and *virA* genes. Each member of this family has a conserved domain of ≈200 residues within its C terminus. We have previously shown that another chemotaxis protein, CheY, represents a domain of protein structure that has been conserved within a second large family of bacterial regulatory proteins. Each protein of the CheA family seems to function as a regulator of a different CheY homologue. Although each pair of proteins appears to produce a specialized response to a distinct type of stimulus, the relationships in primary structure suggest that a similar molecular mechanism may be involved.**

During bacterial chemotaxis, receptor proteins at the cell surface modulate motor behavior in response to binding of extracellular ligands (for reviews, see refs. 1–3). The receptors communicate indirectly with the flagellar motor via a signal transduction system within the cytosol. Genetic studies have shown that the products of three genes, *cheA*, *cheW*, and *cheY*, are required for this process. CheY protein appears to interact directly with proteins at the motor, whereas CheA and CheW appear to be necessary for receptor-mediated control of CheY activity (4–8).

The amino acid sequence of CheY indicates that this component is homologous to a family of bacterial regulatory proteins, each of which functions to generate adaptive responses to changing environmental conditions (3, 9, 10). These include proteins such as OmpR, which controls porin expression (11), NtrC, which controls responses to nitrogen depletion (12), PhoB, which controls phosphate assimilation (13), and SpoOA, which controls sporulation in response to nutrient deprivation (14). Most members of this family appear to be regulated by a second component that functions as a sensor for environmental change (10). Each sensory component contains a similar domain of ≈200 residues within its carboxyl terminus. Thus, in bacteria, two primordial domains of protein structure have apparently evolved to provide a core processor for the transduction of sensory information into appropriate responses.

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In this communication we report the nucleotide sequence of the *Salmonella typhimurium cheA* gene.* The predicted product of *cheA* is a 73,000 M_r protein that is similar to the family of sensory proteins identified in other bacterial regulatory systems. We have also purified and characterized the CheA protein. Unlike most members of the sensor family, CheA does not appear to be membrane associated. During chemotaxis CheA probably receives information from a variety of different membrane chemoreceptor proteins and transduces it via CheY into a signal that controls motor behavior. Thus, proteins CheA and CheY may provide the central sensory processing mechanism for signal transduction in chemotaxis.

MATERIALS AND METHODS

Strains and Plasmids. Part of the *cheA* gene used for sequencing was from pME1 (15), an *S. typhimurium*–pUC12 hybrid containing an 8.4-kilobase (kb) *Pst* I genomic DNA fragment from *S. typhimurium* strain ST1 spanning a region between *cheA* and *flaM* (Fig. 1). M13 phage subclones, M13mo1A and M13mo1B, were created by inserting a 3.1-kb *Pst* I–*Sma* I fragment of pME1 modified with *Bam*HI linkers in both orientations into the *Bam*HI site of M13mp10 (16). The remainder of *cheA* was obtained from an *S. typhimurium*–pUC12 hybrid plasmid, designated pMO1. This plasmid was generated by ligating 8-kb *Eco*RI–*Sst* I genomic DNA fragments from *S. typhimurium* PS1 into pUC12, transforming into *Escherichia coli* HB101 (17), and screening by the colony hybridization procedure of Grunstein and Hogness (18). A 0.9-kb *Sst* I–*Pst* I fragment of pME1 labeled with ^{32}P by nick-translation (17) was used as a probe. M13 phage subclones, M13mo2A and M13mo2B, were created by inserting a 2.7-kb *Hin*dIII fragment of pMO1 in both orientations into the *Hin*dIII site of M13mp10. The *E. coli recA* strain, JM109 (obtained from J. Messing, Rutgers), was used as host for all sequencing procedures. The expression vector used to produce CheA for purification was constructed in two steps. A *Nae* I–*Pst* I pMO1 fragment spanning the last 40 bp of *motB* and first 184 bp of *cheA* was first ligated into the *Hin*cII–*Pst* I sites of pUC12 to create a plasmid, designated pMO3, with an in-frame fusion of the pUC12 *lacZ'* gene and the 3' end of *motB*; then the 1.9-kb *Pst* I–*Eco*RI pME1 fragment encoding the remainder of *cheA* was ligated into the *Pst* I–*Hin*dIII sites of pMO3 to yield the complete expression vector, pMO4.

Determination of the Nucleotide Sequence of the *S. typhimurium cheA* Gene. Processive deletions through the inserts in M13mo1A, M13mo1B, M13mo2A, and M13mo2B were

*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03611).

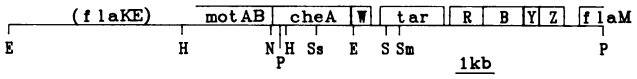

FIG. 1. Organization of the *che* genes in *S. typhimurium*. The region shown spans ≈16 kb at ≈40 min on the *S. typhimurium* genome. Restriction sites used in cloning and subcloning are indicated: E, *Eco*RI; H, *Hin*dIII; N, *Nae* I; P, *Pst* I; S, *Sal* I; Sm, *Sma* I; Ss, *Sst* I.

generated using exonuclease III (New England Biolabs) digestion as described elsewhere (19). The complete nucleotide sequence of *cheA* was determined on both strands by the dideoxynucleotide chain-termination procedure of Sanger *et al.* (20) using [α-^{32}P]dATP, DNA polymerase I Klenow fragment (Bethesda Research Laboratories), an M13 universal primer (New England Biolabs), and the deletion phage plus strands as templates. The FASTP and RDF programs of Lipman and Pearson (21) were used to search for homologies and help align sequences.

Purification of CheA. *E. coli* HB101 containing the CheA expression vector pMO4 was grown at 37°C in L broth (17) supplemented with ampicillin at 40 μg/ml to a density of 10^9 cells per ml, harvested by centrifugation, resuspended in 0.1 M sodium phosphate, pH 7.0 (3 ml/g of cells, wet weight), and lysed with a Heat Systems/Ultrasonics (Plainview, NY) W-225 sonicator. The resulting extract was centrifuged at 100,000 × *g* for 90 min to remove membranes, and ammonium sulfate was added to 40% saturation. The pellet was collected by centrifugation, resuspended in 20 mM Tris·HCl, pH 7.5, containing 1 mM dithiothreitol, and dialyzed against the same buffer. This fraction was then subjected to high-performance liquid chromatography using a 5 × 50 mm Mono Q HR 5/5 column (Pharmacia) and eluting with a 30-ml linear gradient of 0.25–0.75 M NaCl in 20 mM Tris·HCl, pH 7.5, at a flow rate of 1 ml/min. CheA, eluting at 0.54 M NaCl, was collected and subjected to high-performance liquid chromatography using a 7.1 × 300 mm GF-200 column (Sota Chromatography, Crompond, NY) equilibrated in 0.1 M sodium phosphate, pH 7.0. All steps except chromatography were performed at 0–4°C. CheA from the Mono Q column was subjected to automated Edman degradation after blotting onto polyvinylidene difluoride membranes according to the method of Matsudaira (22).

RESULTS

Nucleotide Sequencing of the *cheA* Gene. The complete nucleotide sequence of the *cheA* gene and flanking regions, together with the deduced amino acid sequence of the CheA protein, is shown in Fig. 2. The presumed site of CheA initiation is a GTG codon just distal to the TGA termination codon of the *motB* gene (23). The GTG is preceded by a ribosomal binding site (24, 25) comprised of 8 bases with perfect complementarity to a sequence at the 3′ end of 16s rRNA. This initiation site is followed by an open reading frame extending 2013 bp to a TGA termination codon just 20 bp proximal to the ATG initiation codon of the *cheW* gene (15). The predicted product of *cheA* would have a M_r of 73,000. This corresponds well with the estimated M_r of 76,000 obtained from NaDodSO$_4$/polyacrylamide gel electrophoresis of the ^{35}S-labeled product of *cheA* (26, 27). A second *cheA* product with an apparent M_r of 68,000 has also been observed. It has been proposed that this protein is produced from a secondary site of translational initiation at the 5′ end of the gene (28). We can detect only one possible candidate for such a site, an ATG initiation codon preceded by a Shine–Dalgarno ribosomal binding consensus. The predicted product initiated from this site would have a M_r of 64,000.

The nucleotide sequence of a third of the *E. coli cheA* gene encoding the C terminus of the protein has previously been reported (29). The region corresponds to the *S. typhimurium* sequence extending from Ile-447 to Ala-671. The *E. coli* and *S. typhimurium* amino acid sequences exhibit only about a 15% difference, with most changes resulting in conservative substitutions. Similar results have been obtained in comparing other chemotaxis genes in the two species (9, 15, 29, 30, 31). There is one anomalous region, however. Between Gly-564 and Lys-583 the *E. coli* and *S. typhimurium* amino acid sequences appear completely different. An analysis of codon usage within the corresponding nucleotide sequences indicates that this apparent divergence is due to a shift in reading frame. A base is missing in the *E. coli* sequence at the position of G-1693 in the *S. typhimurium* gene, and the *S. typhimurium* reading frame is restored in the reported *E. coli* sequence by the introduction of a guanine just after the base corresponding to A-1752 in the *S. typhimurium* sequence.

Relationship Between CheA and a Family of Bacterial Sensory Transduction Proteins. The predicted amino acid sequence of CheA was compared to the collection of 4791 protein sequences in the National Biomedical Research Foundation Protein Sequence Database† as well as to a set of over 7000 protein sequences translated from GenBank.‡ The results clearly indicate that a sequence of 150 residues in CheA extending roughly from Ser-382 to Leu-529 is related to a corresponding region in the family of bacterial regulatory proteins that include the products of the *cpxA, dctB, envZ, ntrB, phoM, phoR*, and *virA* genes from a wide range of different bacterial species (Fig. 3). Each of these proteins functions in the regulation of a protein that is homologous to CheY of the chemotaxis system: EnvZ regulates OmpR (11), PhoR regulates PhoB (13), PhoM may regulate ORF2 (34), CpxA regulates SfrA (37), VirA may regulate VirG (38), NtrB regulates NtrC (39), and CheA is required for the regulation of CheY (8), as well as another homologous protein, CheB (40). It seems likely that the conserved domain shared by all the CheA homologues corresponds to a structure that interacts with the corresponding CheY homologue.

There appear to be four highly conserved sequences shared by all members of the CheA family (Fig. 4). These correspond to a sequence in CheA around His-393; the sequence beginning at Asn-415, N X X X N; the sequence beginning at Asp-437, D X G X G; and the sequence beginning at Gly-488, G X G X G. The proteins tend to be relatively variable between these sequences with several insertions and deletions. It seems likely that at least some of the highly conserved residues participate directly in the interaction between CheA and CheY.

Most members of the CheA family appear to be membrane proteins. This has been directly demonstrated for EnvZ (41), VirA (36), and CpxA (personal communication, P. Silverman), and inferred from hydropathy analyses of the sequences of PhoM (34), PhoR (35), and DctB (personal communication, C. Ronson). Predicted membrane spanning sequences are indicated in Fig. 3. NtrB is an exception in that it is clearly a cytoplasmic constituent (42). A hydropathy analysis of the CheA protein indicates that it, like NtrB, has no extended hydrophobic sequences that could span the cytoplasmic membrane.

Identification and Purification of the CheA Protein. The CheA protein was tentatively identified with a band that appeared on Coomassie blue-stained NaDodSO$_4$/poly-

†Protein Identification Resource (1987) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 12.0.

‡EMBL/GenBank Genetic Sequence (1987) GenBank (Bolt, Beranek, and Newman Laboratories, Cambridge, MA), Tape Release 48.0.

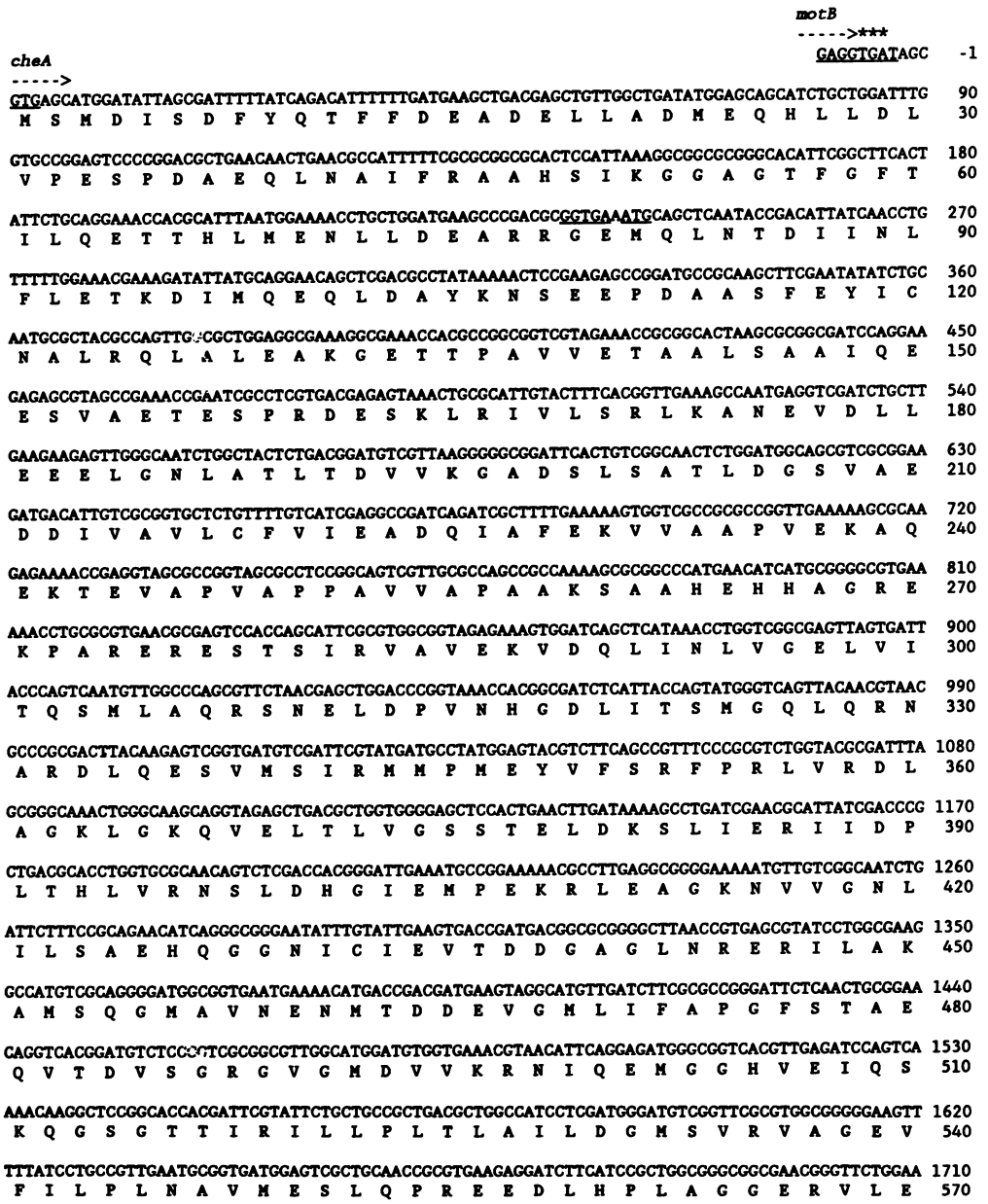

FIG. 2. Nucleotide sequence of *cheA* and the predicted amino acid sequence of its protein product. The nucleotide sequence is numbered relative to the first base of the *cheA*-coding region. The amino acid sequence is numbered with respect to the initiator methionine. The TGA termination codon of *motB* is indicated, and critical bases involved in the initiation of *cheA* and *cheW* are underlined. A possible secondary site of *cheA* initiation is also underlined.

acrylamide gels of *E. coli* cells transformed with a *cheA* expression vector, pMO4, but the protein was missing in cells transformed with the parent plasmid, pUC12 (Fig. 5). This material was purified by ammonium sulfate fractionation, ion exchange, and molecular sieve chromatography. Amino terminal-sequence analysis confirmed its identity with the predicted product of the *cheA* gene. Two overlapping sequences were detected in approximately 1:1 stoichiometry, Ser-Met-Asp-Ile-Ser-Asp-Phe-Tyr-Gln-Phe and Met-Asp-Ile-Ser-Asp-Phe-Tyr-Gln-Phe-Phe. The first sequence confirms the GTG initiation codon indicated in Fig. 2. The second could result from proteolytic cleavage of the N-terminal serine during the purification procedure, or from ambiguity in translational initiation. The purified protein

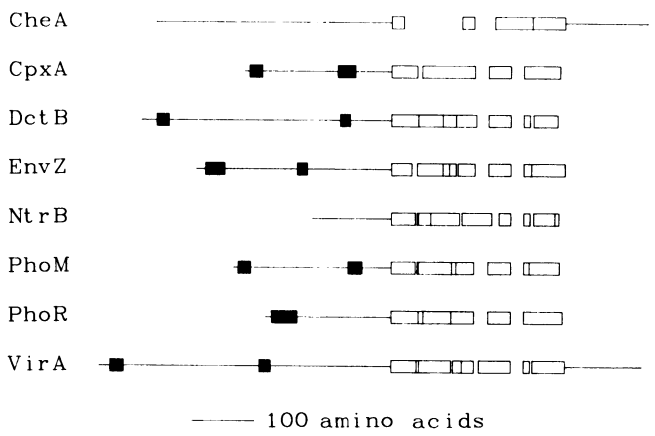

FIG. 3. Regions of similarity between CheA and a family of bacterial regulatory proteins. Homologous regions are indicated by white boxes with similar sequences aligned as indicated in Fig. 4. In these regions the number of identical residues at corresponding positions in CheA, and each similar protein ranges from 16 to 25%. Black boxes indicate predicted membrane-spanning sequences. Sequences were obtained from the following sources: CpxA (10, 49); DctB (50); EnvZ (32); NtrB (33); PhoM (34); PhoR (35); and VirA (36).

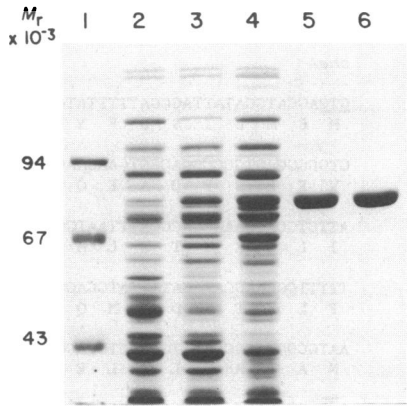

FIG. 5. $NaDodSO_4$/polyacrylamide gel electrophoresis of fractions from the CheA purification. Lanes: 1, M_r standards; 2, 50 μg of 100,000 × *g* supernatant from *E. coli* HB101 containing pUC12; 3, 50 μg of 100,000 × *g* supernatant from *E. coli* HB101 containing the *cheA* expression vector, pMO4; 4, 50 μg of the 40% ammonium sulfate precipitation fraction; 5, 10 μg of the 0.54 M NaCl peak from the Mono Q HR 5/5 column; 6, 10 μg of 240,000 M_r peak after molecular sieve chromatography. Samples were electrophoresed in 7% polyacrylamide according to the method of Laemmli (48), and the gel was stained with Coomassie blue.

exhibited an apparent native M_r of ≈240,000 during molecular sieve chromatography. It seems likely, therefore, that under these conditions CheA is a trimer or tetramer.

DISCUSSION

In *S. typhimurium* and *E. coli* only six genes exhibit a Che$^-$ null phenotype: *cheA, cheB, cheR, cheW, cheY*, and *cheZ*. All have now been sequenced, and their protein products have been purified and characterized (9, 15, 29–31, 43). Two of these components, CheR and CheB, appear to serve a peripheral role as modulators of receptor activity (44). CheZ also seems to be peripheral because mutants totally lacking this protein still respond to attractant stimuli (45). Studies of the effects of plasmids that produce varying levels of CheZ

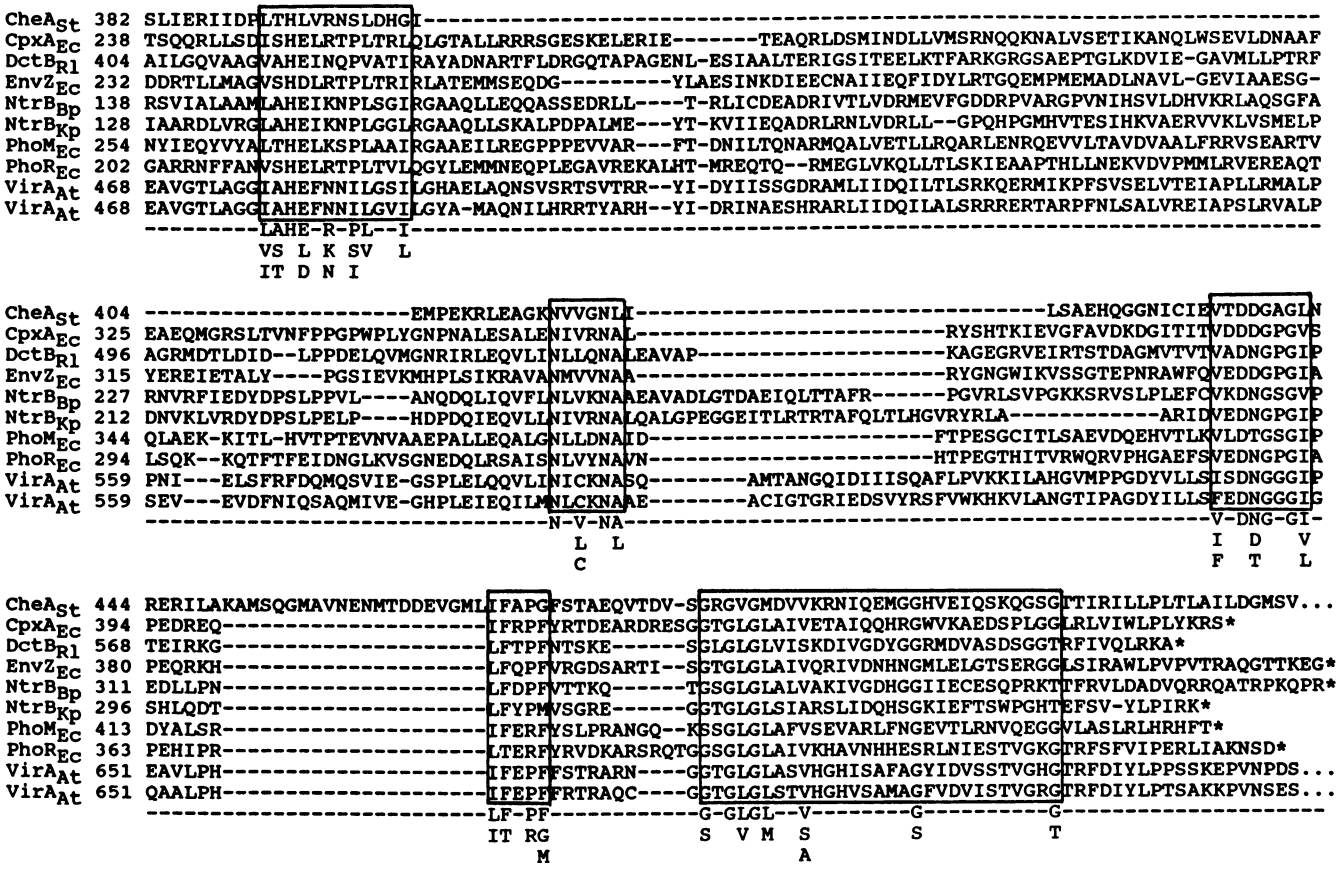

FIG. 4. Amino acid sequence similarities between family of related bacterial regulatory proteins. The most highly conserved regions are boxed with conserved residues indicated beneath the boxes. Sequences are from references cited in Fig. 3.

protein (8, 46) indicate that CheZ acts to antagonize the effects of CheY. Thus, the only components that appear to be absolutely essential for signal transduction are proteins CheA, CheW, and CheY. Both CheY and CheW are small compared to CheA with M_r values of 14,000 and 18,000, respectively (9, 15). Several lines of evidence suggest that CheY interacts directly with the flagellar motor and that CheA acts to modulate this activity (4–9). CheW may serve as a link between CheA and the membrane chemoreceptor proteins (5, 8, 15). As a regulator of CheA, the function of CheW would be analogous to that of the protein, designated P_{II}, that interacts with proteins NtrB and NtrC to regulate the expression of glutamine synthetase and the nitrogen fixation system (47). In comparing the amino acid sequences of the Che signaling proteins to components of cognate regulatory systems it is apparent that only the CheY domain and a portion of CheA have been conserved through evolution. These structures apparently provide an essential sensory transduction function. In all the systems except chemotaxis, the output involves positive transcriptional regulation (10). This finding suggests a fundamental relationship between sensory–motor regulation and control of gene expression. With the availability of pure component proteins it should be possible to begin to unravel the molecular mechanisms that underlie this relationship.

Note Added in Proof. Recent results have established a common mechanism of action between the CheA and CheY proteins and the corresponding homologues of the nitrogen regulation system, the NtrB and NtrC proteins. Previous studies showing that NtrB is a protein kinase that phosphorylates NtrC (39) have been extended to the chemotaxis system. We have shown that the purified CheA protein is a CheY kinase (51).

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