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Chimeric chemosensory transducers of *Escherichia coli*

(chemotaxis/methyl-accepting chemotaxis proteins/gene family/gene fusion)

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ABSTRACT **The *tar* and *tsr* genes of *Escherichia coli* encode homologous transducer proteins that mediate distinct chemotactic responses. We report here the construction of two *tasr* chimeric genes in which the 5′ coding region of the *tar* gene is fused to the 3′ coding region of the *tsr* gene at either of two conserved restriction sites. Both chimeric genes code for chemotactically functional proteins. Results of analyses of behavior and methylation in cells carrying the chimeric genes support existing models for the disposition of transducer domains across the cell membrane and reveal that the receptors for internal pH map in a specific region of the COOH-terminal (cytoplasmic) domain.**

The chemosensory system of *Escherichia coli* regulates motility by controlling the direction of flagellar rotation. Signals for a number of attractants and repellents are processed by a structurally related set of transmembrane proteins, known as transducers or methyl-accepting chemotaxis proteins (MCPs), that receive information from the environment and transduce that information into signals that converge on the flagellar motors. The transducers, in turn, are covalently but reversibly modified in a process that changes their signaling capacity and allows for adaptation (reviewed in refs. 1 and 2).

Four transducer genes (*tsr, tar, tap*, and *trg*) and their products (proteins of *ca.* 60 kDa) have been identified in *E. coli* (reviewed in refs. 2–4). The Tsr (MCP I) and Tar (MCP II) proteins have been studied most extensively. The Tsr protein binds the attractant serine; is required for chemotaxis to the repellents leucine, indole, and weak acids; and mediates pH taxis and thermotaxis. The Tar protein binds the attractant aspartate and the maltose-binding protein and is required for chemotaxis to the repellents nickel and cobalt (reviewed in ref. 2). Adaptation to a positive stimulus (addition of attractant or removal of repellent) is accompanied by methyl esterification of specific glutamic acid residues on the corresponding transducer, while adaptation to a negative stimulus is accompanied by hydrolysis of these esters (reviewed in ref. 5).

Comparisons of nucleic acid sequence homologies between *tsr, tar*, and *tap* suggested the constitution of a gene family with products comprised of discrete structurally and functionally differentiated domains (6). Determination of complete nucleic acid sequences for the four transducer genes in *E. coli* (7–9) and for the *tar* gene in *Salmonella* (10) has expanded our understanding of the structure of these domains. Each transducer protein contains two stretches of hydrophobic amino acids thought to be transmembrane sequences. One is at the NH_2 terminus of the protein and resembles a signal sequence. The other is near the middle of the protein and resembles a membrane-spanning region that divides the protein into an NH_2-terminal periplasmic domain and a COOH-terminal cytoplasmic domain. The putative cytoplasmic domains are highly conserved, while the periplasmic domains are divergent. The sites of methylation are clustered in two cytoplasmic regions that have been isolated as tryptic peptides (11, 12). Finally, a highly conserved region of 48 contiguous amino acid residues is found to be identical in the Tar and Tsr proteins; this region is flanked on either side by the methylation sites (Fig. 1).

The functions of these domains have been revealed through specific changes in structure. For example, certain mutations that map in regions specifying the NH_2-terminal part of Trg have been shown to affect ligand binding activity (9), and *tsr* mutant strains that no longer respond to Tsr attractants but still respond to repellents have been isolated (13). Truncated genes have been shown to encode proteins with residual ligand binding activity that are defective in signaling or adaptation (10, 14). While these experiments indicate that one can selectively interfere with different transducer functions, they do not always allow one to define clearly the portion of the protein that is involved in each activity. If the notion of discrete domains is correct, these domains ought to be interchangeable. In particular, it should be possible to build functional chimeric proteins comprised of different parts of Tar and Tsr. The construction and characterization of two such chimeras are described here.

MATERIALS AND METHODS

Chemicals. Restriction enzymes were purchased from New England Biolabs, T4 DNA ligase was from Boehringer Mannheim, *Eco*RI linkers were from Collaborative Research, and T4 polymerase was from Bethesda Research Laboratories. Synthetic amino acids were used for chemotaxis and methylation assays: L-aspartic acid, sodium salt, was purchased from ICN, and L-serine and L-leucine were purchased from K & K. [^{35}S]Methionine was purchased from Amersham, and L[*methyl*-^{3}H]methionine was from ICN. Other chemicals were reagent grade.

Bacterial Strains. RP437 (F-*thi, thr, leu, his, met, eda, rpsL*) is an *E. coli* K-12 derivative that is wild-type for motility and chemotaxis. RP4372, a derivative of RP437, carries a *tar–tap* deletion (*52Δ1*) and the *tsr-1* allele, and RP5698 carries the *tsrΔ1-28* allele. These strains were given to us by J. S. Parkinson, University of Utah. AB1200 is a derivative of RP437 carrying the *52Δ1* and *tsrΔ1-28* alleles. *recA* derivatives of RP437 and RP4372 were constructed as described by Boyd *et al.* (6).

Plasmid and Phage Constructions. The plasmid pAK106 contains a 6.1-kilobase (kb) *Eco*RI–*Hin*dIII fragment cloned into the vector pBR322 (6). This fragment carries several chemotaxis genes, including the *tar* gene. A 4.3-kb fragment extending from the *Ava* I site within the insert to the *Ava* I

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Abbreviations: MCP, methyl-accepting chemotaxis protein; kb, kilobase(s); CW, clockwise; CCW, counterclockwise.

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site in pBR322 was deleted from pAK106 to generate the plasmid pAK101, which contains the *tar* coding region but lacks the downstream chemotaxis genes (see Fig. 1). A 2.7-kb *Eco*RI–*Cla* I fragment derived from pAK106 was cloned into the vector pBR322 to give the plasmid pAB153. The insert contains the coding region of the *tar* gene up to nucleotide 1407 (numbered as in ref. 8). The plasmid pAB157 (see Fig. 1) was constructed by replacing the 0.35-kb *Cla* I–*Bam*HI fragment of pAB153 with the 0.9-kb *Cla* I–*Bgl* II fragment from the *tsr* plasmid pAB100 (6). The plasmid pAB160 was constructed by ligating the 5′ region of the *tar* gene, contained on a 2.0-kb *Eco*RI–*Nde* I restriction fragment from pAK101, and the 3′ region of the *tsr* gene, contained on a 1.6-kb *Nde* I–*Hin*dIII fragment from pAB100, into the *Eco*RI–*Hin*dIII sites of pBR322.

Insert DNA from these plasmids was introduced into the single *Eco*RI restriction endonuclease site of the vector λgt4 (15). The *tsr*-containing *Eco*RI fragment of pAB100 was cloned into the *Eco*RI site of the phage vector to generate the hybrid λgt4-*tsr*. The other plasmids were first digested with the following restriction enzymes: pAK101, *Ava* I; pAB153, *Hin*dIII; pAB157, *Sal* I; and pAB160, *Sal* I. The ends of the fragment containing the transducer gene were filled in by using T4 polymerase, and *Eco*RI linkers were ligated to the blunt ends (16). The resulting inserts flanked by *Eco*RI sites were cloned into the λgt4 vector to generate the corresponding λ transducing phage, which were then used to prepare lysogens of RP4372(*recA*).

Tethered Cell Assays. Cells were grown in tryptone broth at 30°C and harvested at midexponential phase. They were washed and tethered as described in Block *et al.* (17) except that the buffer was 10 mM potassium phosphate, pH 7/67 mM NaCl/0.1 mM Na_2 EDTA/0.001 mM methionine/10 mM sodium lactate. Tethered cells were placed in a flow chamber (18) and observed in phase contrast. Their behavior was recorded on videotape before and after shifts (via 15-s flows) to media containing attractants or repellents.

Methionine Labeling. Polypeptides were labeled with [^{35}S]methionine by using λgt4-directed protein synthesis in UV-irradiated cells as described (19).

[^{3}H]Methyl Labeling. Methylated proteins were assayed as described by Slonczewski *et al.* (20). Plasmid-bearing strains and lysogens were grown at 30°C in tryptone broth to early exponential phase. Cells were washed twice in 10 mM potassium phosphate/0.1 mM EDTA at pH 7.3 or at pH 5.5 and resuspended to a final concentration of $\approx 10^8$ cells per ml. A carbon source (10 mM glycerol) and chloramphenicol (150 μg/ml) were added prior to a 10-min incubation at 30°C; 10 mM sodium citrate (pH 5.5) was added to the medium in experiments requiring pH 5.5. L[*methyl*-^{3}H]Methionine (10 Ci/mM; 1 Ci = 37 GBq) was added to a final concentration of 5 μM, and cells were incubated for another 50 min prior to CCl_3COOH precipitation. Attractants and repellents were added at specific times before CCl_3COOH precipitation: 10 mM serine or 10 mM aspartate for 20 min, 0.5 mM nickel sulfate for 30 s, and sodium acetate for 10 min.

Electrophoresis. Cell pellets were resuspended in NaDod-SO_4 sample buffer, boiled for 2 min, and electrophoresed as described (21). Gels were assayed by fluorography (22).

RESULTS

Polypeptide Products of Chimeric Genes. Two restriction endonuclease sites, *Nde* I and *Cla* I, are found in the DNA sequence encoding the conserved COOH-terminal region of both Tsr and Tar (7, 8). These sites enabled us to construct the chimeric genes *tasr* (*Cla* I) and *tasr* (*Nde* I) shown in Fig. 1. The *tasr* (*Cla* I) gene has almost all of the *tar* coding region, including the sequences encoding one block of methylation sites (the K1 peptide; ref. 23). However, the nucleotides coding for the last 84 amino acids of *tar* are replaced by a fragment derived from *tsr* coding for 65 amino acids. The fusion leaves the coding region in phase, and the new gene should encode a complete chimeric protein. The *tasr* (*Nde* I) construct contains the 5′ coding region of *tar* fused to a substantial section of the 3′ coding region of *tsr*, a section that specifies 275 amino acids, including all of the methylation sites. Finally, the truncated gene *tar* (*Cla* I)

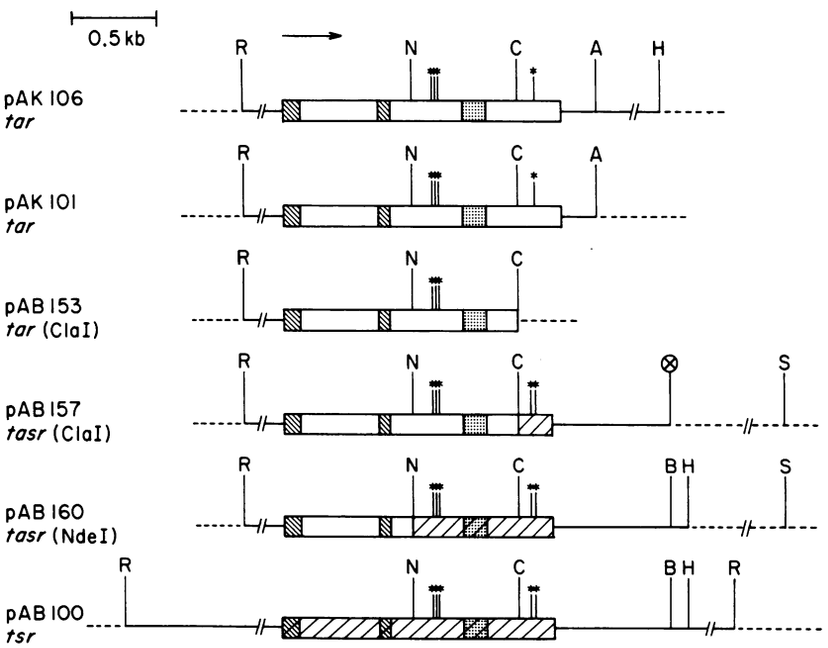

FIG. 1. Physical maps of plasmids described in *Materials and Methods*. ---, Vector DNA; —, insert DNA; boxed regions, DNA-encoding transducer genes; arrow, direction of transcription; □, regions of the *tar* gene; ▨, regions of the *tsr* gene; ▧, regions within each gene of DNA-encoding putative signal sequences and membrane spanning regions; ▩, nucleotide sequences coding for a 48-amino-acid region of identity between the Tar and Tsr proteins; *, sites of methylation for the respective transducer protein. Symbols: A, *Ava* I; B, *Bam*HI; C, *Cla* I; H, *Hin*dIII; N, *Nde* I; R, *Eco*RI; S, *Sal* I; ⊗, *Bam*HI/*Bgl* II fusion.

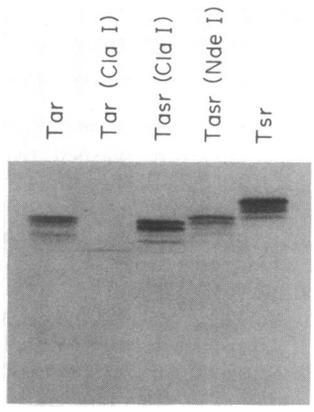

FIG. 2. Fluorogram of gel of transducer polypeptides encoded by λgt4 phage labeled with [^{35}S]methionine in UV-irradiated cells. Electrophoresis was from top to bottom on polyacrylamide in the presence of $NaDodSO_4$.

contains most of the coding region of *tar* but lacks sequences that specify the last 84 amino acids; the 3′ truncated end is juxtaposed to sequences in the pBR322 plasmid that encode lysine and leucine and a stop codon. Therefore, the *tar* (*Cla* I) gene should encode a truncated polypeptide in which 84 amino acids at the COOH-terminal end are replaced by 2 amino acids, lysine and leucine.

Insert DNA from pAB153, pAB157, pAB160, pAK101, and pAB100 was introduced into the vector λgt4. Polypeptides labeled with [^{35}S]methionine were identified after electrophoresis of material obtained from UV-irradiated cells that had been infected with the appropriate recombinant phage (Fig. 2). Note that both Tasr (*Cla* I) and Tasr (*Nde* I) proteins migrated with about the same electrophoretic mobility as Tar, and that both chimeras showed the banding pattern characteristic of multiple levels of transducer methylation (21, 24–26). Thus, the chimeric genes encode products that are structurally similar to the wild-type transducers. Tar (*Cla* I) migrated slightly faster than the other transducer proteins and failed to show a multiple banding pattern (Fig. 2), suggesting that the truncated Tar polypeptide could not be methylated. This was confirmed by an experiment in which [*methyl*-^{3}H]methionine was used to label proteins in a strain carrying the *tar* (*Cla* I) plasmid: no methylated species corresponding to the truncated polypeptide could be found. A multi-copy plasmid was used for this experiment because the truncated polypeptide appears to be subject to proteolysis: the Tar (*Cla* I) polypeptide was found in substantially smaller amounts than Tar (Fig. 2), even though it is transcribed from the same promoter.

Rotational Bias of Cells Carrying Chimeric Genes. Strain RP437(*recA*) showed both clockwise (CW) and counterclockwise (CCW) rotation, as expected for the wild type. The *tsr, tar, tap*-defective strain RP4372(*recA*) showed only CCW rotation. When RP4372(*recA*) was lysogenized with phage carrying *tsr* or *tar* or one of the chimeric genes, normal rotational bias was restored, and responses to both attractants and repellents could be elicited (see below). But when this strain was lysogenized with phage carrying the truncated *tar* (*Cla* I) gene, the cells continued to rotate CCW and did not respond to any of the attractants or repellents tested. However, when the truncated gene was introduced into either wild-type (RP437) or *tsr, tar, tap*-defective cells (AB1200) on the multicopy plasmid pAB153, the cells showed a strong CW bias and failed to respond to attractants. Wild-type cells carrying the plasmid vector pBR322 behaved normally. Thus, the truncated transducer can both shift the rotational bias CW and inhibit wild-type transducer functions.

Response to Attractants. Lysogens carrying the wild-type genes on λgt4 showed the normal spectrum of responses to attractants. Thus RP4372(λgt4-*tar*) responded to aspartate by prolonged CCW rotation but failed to respond to serine, whereas RP4372(λgt4-*tsr*) failed to respond to aspartate but showed prolonged CCW rotation when exposed to serine. When lysogens carrying the chimeric genes were tested, their responses were similar to the λgt-*tar*-bearing strain, with recovery times that were even longer. These results are summarized in the first two columns of Table 1.

The specificities of the receptors were tested further by examining transducer methylation patterns. In the absence of a chemical stimulus, Tar, Tsr, and the chimeric proteins all showed a basal level of methylation (Fig. 3, lanes 0). The addition of aspartate resulted in an increased level of methylation of Tar, Tasr (*Cla* I), and Tasr (*Nde* I) proteins, as evidenced by a set of more intense, faster-migrating bands (Fig. 3, lanes Asp). Tsr failed to respond. The addition of serine resulted in changes in the banding pattern of only Tsr (Fig. 3, lanes Ser). Thus, in methylation as well as in behavioral experiments, the chimeric proteins showed a response dictated by the NH_2-terminal region of the protein.

Response to Repellents. Addition of nickel elicits a repellent response mediated by the Tar protein (28). Removal of nickel elicits an attractant response that is more easily measured. This response was found in all of the lysogens except that carrying the truncated gene, as indicated in the third column of Table 1. However, the response of the strain that carried the *tsr* gene was relatively short. The addition of nickel resulted in specific demethylation of Tar, Tasr (*Cla* I), and Tasr (*Nde* I) but not of Tsr (Fig. 3, lanes Ni^{2+}). Addition of leucine elicits a repellent response mediated by the Tsr protein (28). Responses to the removal of leucine are shown in the fourth column of Table 1. There was a strong CCW response to the removal of leucine in the wild-type and *tsr* strains, but the other strains showed only a weak CCW

Table 1. Chemotactic responses of tethered cells

Strain	L-Aspartate 0 → 2.5 μM Resp./duration	L-Serine 0 → 1.25 μM Resp./duration	$NiCl_2$ 1 → 0 mM Resp./duration	L-Leucine 10 → 0 mM Resp./duration	pH 7 → pH 6 Resp./duration	NaOAc* 0 → 10 mM Resp./duration
RP437(*recA*)	CCW/132 ± 7	CCW/119 ± 7	CCW/147 ± 13	CCW/157 ± 13	CW†/—	CW/64 ± 6
RP4372(λgt4-*tar*)	CCW/148 ± 4	NR/—	CCW/342 ± 18	CCW/23 ± 3	CCW/70 ± 3	CCW/81 ± 5
RP4372[λgt4-*tasr* (*Cla* I)]	CCW/316 ± 13	NR/—	CCW/>480	CCW/29 ± 3	CCW/68 ± 5	CCW/73 ± 5
RP4372[λgt4-*tasr* (*Nde* I)]	CCW/318 ± 27	NR/—	CCW/>480	CCW/25 ± 1	CCW/93 ± 9	CW/46 ±3
RP4372[λgt4-*tar*(*Cla* I)]	NR/—	CCW/94 ± 5	CCW/78 ± 7	CCW/101 ± 9	CW†/—	CW/105 ± 14

Data are means ± SEM of duration of response (resp.) in seconds (time from onset of flow to first reversal) for 8–10 cells adapted to one medium (at pH 7 unless otherwise specified) and then shifted to a second medium as indicated. CW is a tumble response, CCW is a run response, and NR is no response. Strain RP4372[λgt4-*tar* (*Cla* I)] failed to respond to any of these stimuli (not shown).

*At pH 6, with cells adapted at this pH before exposure to acetate.

†Clearly CW, but too brief to measure.

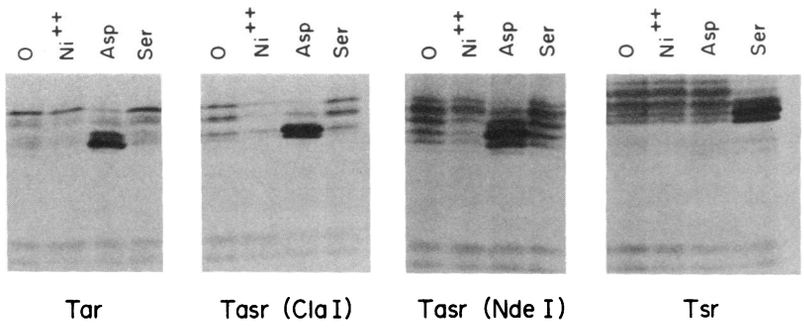

FIG. 3. Gel patterns of [^{3}H]methyl-labeled transducers encoded by λgt4 lysogens obtained after addition (at pH 7.3) of [*methyl*-^{3}H]methionine (lanes 0) and exposure to the repellent nickel (lanes Ni^{2+}) or to the attractants aspartate (lanes Asp) or serine (lanes Ser). The upper sets of bands are transducers, and the lower sets are the EF-Tu protein (27). Electrophoresis was as in Fig. 2.

response. These results suggest that specificity for the repellents nickel and leucine also is determined by the NH_2-terminal portion of the transducers.

Mapping the Response to pH. Wild-type cells respond to changes in either external or internal pH (20). A rapid decrease in external pH (e.g., from pH 7 to pH 6) elicits a repellent response. This was observed for the wild-type and *tsr* strains; however, the *tar* and *tasr* strains showed an inverted response (Table 1, column 5). Changes in internal pH are elicited by addition of weak acids (29, 30). This gives a repellent response in wild-type strains (31) but an inverted (attractant) response in *tsr*$^-$ *tar*$^+$ mutant strains (32). Responses to such treatment are shown in the last column of Table 1. The wild-type strain and the strains carrying *tsr* or *tasr* (*Nde* I) showed a repellent response, while the strains carrying *tar* or *tasr* (*Cla* I) showed the inverted response. Similar results were obtained in methylation studies. When cells adapted to pH 5.5 were given acetate (10 mM), Tsr and Tsr (*Nde* I) showed marked demethylation compared to Tasr (*Cla* I) and Tar (Fig. 4). These results suggest that detection of changes in cytoplasmic pH is specified by a region of the *tsr* and *tar* genes that maps between the *Nde* I and *Cla* I restriction sites.

DISCUSSION

Sequence analysis of the *tsr*, *tar*, and *tap* genes of *E. coli* allowed the definition of putative structural and functional transducer domains and a model for their disposition in the cell membrane (7, 8). A similar model was proposed for the closely related Tar transducer of *Salmonella typhimurium* (10). Recently, the more distantly related *E. coli trg* gene was sequenced, and its product was found to follow a similar design (9). Evidence that the ligand binding sites of this transducer lie in the NH_2-terminal domain was obtained by using specific *trg* mutations to construct *trg* recombinants (9). Our present results both support and extend the model of

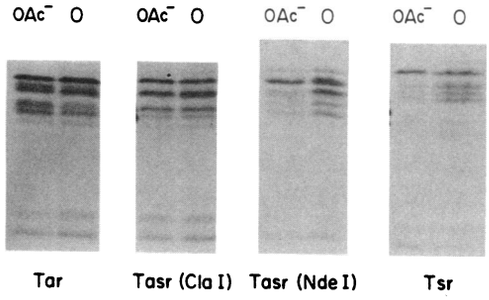

FIG. 4. Gel patterns of [^{3}H]methyl-labeled transducers encoded by λgt4 lysogens obtained after addition (at pH 5.5) of [*methyl*-^{3}H]methionine (lanes 0) and exposure to the repellent sodium acetate (lanes OAc^-).

Krikos *et al.* (8). Homologous domains of the Tar and Tsr proteins are interchangeable, yielding chimeras that are chemically stable and chemotactically competent. Specific receptor functions can be assigned to each of these domains.

These functions and the disposition of the transducers across the cytoplasmic membrane are shown schematically in Fig. 5. The behavior of the chimeric transducers can be predicted from linear superposition of the component parts. The specificity of response to the attractants aspartate and serine and the repellents nickel and leucine segregates with the NH_2-terminal (periplasmic) domain (Table 1 and Fig. 3). Such specificity for nickel and leucine is clear, even though binding sites for these repellents have not been identified (see ref. 2). The specificity for external hydrogen ion (H^+), which acts as an attractant for Tar and a repellent for Tsr (Table 1), segregates in the same way. The specificity for internal hydrogen ion, that also acts as an attractant for Tar and a repellent for Tsr, segregates with the region of the COOH-terminal (cytoplasmic) domain encoded by DNA between the *Nde* I and *Cla* I restriction sites (Fig. 1). Thus, the Tasr (*Cla* I) chimera showed CCW responses on addition of aspartate, removal of nickel, or depression of external or internal pH (Table 1, row 3), whereas the Tasr (*Nde* I) chimera showed CCW responses on addition of aspartate, removal of nickel, or depression of external pH, but a CW response on depression of internal pH (Table 1, row 4). We note that nickel elicited a smaller but significant repellent response in the *tsr* strain (as judged on its removal; Table 1); however, it did not generate significant shifts in methylation (Fig. 3). Since nickel is known to inhibit motility at substantially smaller concentrations than that used in our studies (33), this probably is a nonspecific effect. We note, also, that leucine had a small repellent effect in the *tar* and *tasr* strains (Table 1); the reason for this is not known.

It is possible that histidine residues, with pKs of about 6, are involved in detection of changes in internal pH (20). Within the pH-sensitive region, the Tar protein contains three histidine residues, at positions 267, 337, and 350, while the Tsr protein contains one at position 328. The latter is an obvious candidate for a functional group that could be changed by site-specific mutagenesis. Slonczewski *et al.* (20) suggested that pH-sensitive sites are found in Tsr in both periplasmic and cytoplasmic parts of the molecule. Our results confirm that prediction and show, in addition, that analogous sites (specifying responses of opposite sign) are found in Tar. Note that the Tasr (*Nde* I) chimera gave responses of one sign for changes in external pH and of the other sign for changes in internal pH (Table 1); therefore, responses to changes in external pH are not mediated by changes in internal pH. The external and internal sensitivities segregate independently (Fig. 5).

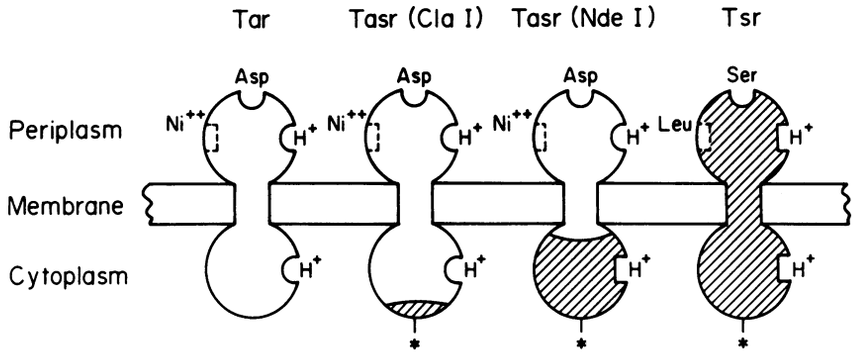

FIG. 5. Schematic representation of wild-type and chimeric transducers. Round sites give CCW responses on the addition of the ligand indicated, whereas square sites give CW responses. Inverse responses are obtained on removal of the ligand. Sites for Ni^{2+} and Leu are shown dashed because it is not known whether these repellents interact with the transducers directly. The asterisk represents an extra methylation site.

One other difference between the chimeras and Tar, evident in Fig. 5, is the addition of a methylation site (shown by an asterisk). Since methylation is required for adaptation (5), one might expect the chimeras to show differences in adaptation times. This could explain why the responses to aspartate and nickel were substantially longer in the *tasr* strains than in the *tar* strain (Table 1). However, this was not seen in responses to changes in external pH. Note that the responses to aspartate in the *tar* strain and to serine in the *tsr* strain were similar to the corresponding responses in the wild type (Table 1). We did not see the enhanced responses in tar^- or tsr^- mutants noted by Springer *et al.* (34). The difference probably is due to our use of chemically synthesized amino acids (35).

It is more difficult to assess the functional defects of the truncated transducer Tar (*Cla* I). This transducer failed to relay signals for aspartate, even when overproduced [as observed for the truncated transducers Δ59 or Δ60 by Koshland *et al.* (14)]. However, Tar (*Cla* I) shifted the rotational bias CW and interfered with the chemotactic functions of wild-type transducers, suggesting that it retained some activity. In addition, it appeared to be subject to proteolysis. This illustrates the difficulty in interpreting experiments with proteins that could have grossly perturbed three-dimensional structures.

Modification of genes within a family by exchange of homologous nucleic acid, coupled with detailed analysis of behavior of organisms expressing the chimeric gene products, provides a powerful means for dissection of mechanisms of chemosensory signal transduction.

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