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Hybrid *Escherichia coli* Sensory Transducers with Altered Stimulus Detection and Signaling Properties

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The *tar* and *tap* loci of *Escherichia coli* encode methyl-accepting inner membrane proteins that mediate chemotactic responses to aspartate and maltose or to dipeptides. These genes lie adjacent to each other in the same orientation on the chromosome and have extensive sequence homology throughout the C-terminal portions of their coding regions. Many spontaneous deletions in the *tar-tap* region appear to be generated by recombination between these regions of homology, leading to gene fusions that produce hybrid transducer molecules in which the N terminus of Tar is joined to the C terminus of Tap. The properties of two such hybrids are described in this report. Although Tar and Tap molecules have homologous domain structures, these Tar-Tap hybrids exhibited defects in stimulus detection and flagellar signaling. Both hybrid transducers retained Tar receptor specificity, but had reduced detection sensitivity. This defect was correlated with the presence of the C-terminal methyl-accepting segment of Tap, which may have more methylation sites than its Tar counterpart, leading to elevated steady-state methylation levels in the hybrid molecules. One of the hybrids, which carried a more extensive segment from Tap, appeared to generate constitutive signals that locked the flagellar motors in a counterclockwise rotational mode. Changes in the methylation state of this transducer were ineffective in canceling this aberrant signal. These findings implicate the conserved C-terminal domain of bacterial transducers in the generation or regulation of flagellar signals.

Many of the chemotactic responses exhibited by motile *Escherichia coli* are mediated by a class of inner membrane proteins known as methyl-accepting chemotaxis proteins (MCPs) (33). These proteins function as sensory transducers, analogous to those in higher organisms, and are excellent models for investigating the molecular details of stimulus detection and transmembrane signaling. MCP molecules have two distinct functions: they serve as chemoreceptors for monitoring attractant or repellent concentrations in the environment, and they generate or regulate signals that control the rotational behavior of the flagella, the locomotor organelles (reviewed in reference 23). As the organism moves about, spatial gradients of chemoeffectors are sensed as temporal changes in MCP receptor occupancy. These stimuli in turn modulate MCP signaling activity to produce an appropriate chemotactic response. Subsequent changes in the methylation state of the transducer molecules bring about sensory adaptation by cancelling these signals and restoring the unstimulated swimming pattern.

Four MCP species are known in *E. coli*, each of which processes a different set of stimuli: Tar (aspartate, maltose, and several repellents), Trg (ribose and galactose), Tsr (serine and various repellents), and Tap (dipeptides). Sequence analyses of MCP genes in *E. coli* (2, 3, 15) and *Salmonella typhimurium* (27) have shown that MCP molecules are composed of discrete structural and functional domains (Fig. 1). The N-terminal half of the molecules contains two hydrophobic segments (designated S and M), which serve to anchor the transducer in the cytoplasmic membrane, and an intervening region of variable sequence that comprises a periplasmic chemoreceptor domain (4, 20, 37). The C-terminal half of the molecules constitutes a cytoplasmic domain, characterized by a highly conserved region flanked by segments (designated K and R) that contain the methylation sites (10, 13, 36). The function(s) of the conserved cytoplasmic domain is not yet known, but presumably involves activities common to all transducer species. Since the flagellar signals produced by different transducers are probably identical (1, 34), this region is thought to be involved in flagellar signaling.

Krikos et al. (14) tested the MCP domain model by constructing gene fusions that produced hybrid transducer molecules. They found that transducers containing the periplasmic domain of Tar and the cytoplasmic domain of Tsr mediated responses to Tar-specific attractant stimuli, demonstrating that those chemoreceptor functions segregate with the periplasmic domain. However, the hybrid molecules exhibited normal signaling properties and could not provide information about the location of the transducer signaling domain. In this report we describe the properties of two hybrid transducers in which portions of the cytoplasmic domain of Tar were replaced with the corresponding segments from Tap. Both of these Tar-Tap hybrids retained Tar receptor specificity but had reduced detection sensitivities that might be caused by changes in the number and distribution of methylation sites. In addition, one of the hybrid transducers exhibited defects in signaling behavior which indicate that the cytoplasmic domain of MCP molecules plays an important role in generating or regulating the signals that control flagellar rotation.

MATERIALS AND METHODS

Strains. All bacterial strains used in this work were derivatives of *E. coli* K-12 from our laboratory collection. RP4324 [Δ(*tar-tap*)*5201*; see Fig. 2)] (31) was used as the null control for *tar* and *tap* function. RP3098 (32) was used for growth of bacteriophage λ stocks and phage crosses. RP5762

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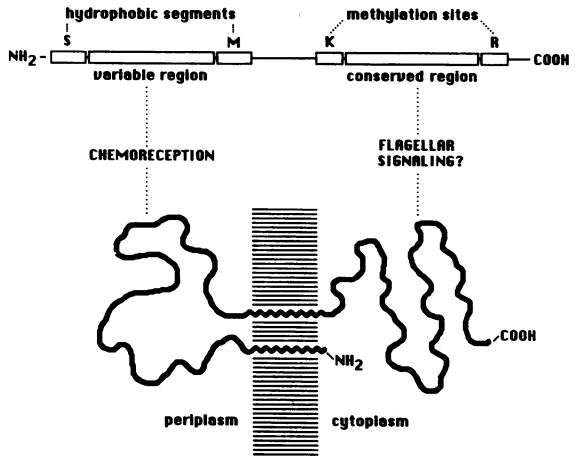

FIG. 1. Structural domains of MCP molecules. Common structural features of bacterial transducers are shown in the upper part of the figure. S, Signal sequence required for membrane insertion; M, membrane-spanning segment; K, lysine-containing tryptic peptide; R, arginine-containing tryptic peptide. The transmembrane organization of transducer molecules is shown in the lower part of the figure. The variable region in the N-terminal half of the molecule comprises a periplasmic domain with chemoreceptor function. The conserved C-terminal cytoplasmic domain contains the sites of methylation and may be responsible for flagellar signaling.

(*che*$^+$), RP5763 [Δ(*cheA-cheR*)*2216*], and RP5764 [Δ(*tar-cheB*)*2234*] were used as host strains in UV light programming experiments (6). Strains constructed in the course of this work were derived from RP437 (24), which is wild type for chemotaxis. Deletion derivatives of λche22 (see Fig. 2), a specialized transducing phage that carries the entire *tar* operon (24), were used for mapping and complementation tests, following standard methods (22, 30, 32).

Chemicals. Restriction enzymes were purchased from Bethesda Research Laboratories, Boehringer Mannheim, or New England Biolabs. DNA ligase was from Collaborative Research, and DNA polymerase (Klenow fragment) was from Boehringer Mannheim. [^{35}S]methionine and [^{32}P]dATP were purchased from Amersham and New England Nuclear Corp., respectively. Dideoxy nucleotides were from P-L Biochemicals, and "universal primer" for DNA sequencing reactions was purchased from Bethesda Research Laboratories.

Sequence analysis of deletion endpoints. Restriction fragments carrying the deletions in λche22Δ30 and λche22Δ38 were excised from phage DNA, purified on DEAE-cellulose (7), and inserted into M13mp11 (18) replicative form (RF) DNA. Sequencing reactions were done by the dideoxy method (28) with commercial primer and a modified gel procedure (8).

Introduction of Δ*2275* into fusion phages. The *cheR* and *cheB* genes carried by λche22Δ30 and λche22Δ38 were eliminated by crossing in the deletion from λche22Δ75 (see Fig. 2). To facilitate detection of the desired double-deletion recombinants, a transposon Tn*5* insertion located between the *Ava*I and *Pvu*II sites in *tap* (see Fig. 2) was first crossed into λche22Δ75 to provide a counterselectable marker. This derivative was then crossed to λche22Δ30 and λche22Δ38, and the progeny were fractionated on equilibrium bouyant density gradients. Phage particles from the predicted density position of double-deletion recombinants were screened for absence of the Tn*5* (kanamycin resistance) marker and for inability to complement a *cheY* tester mutant. Phage that met these criteria were subsequently examined by restriction mapping to confirm the presence of both deletions.

Radiolabeling and PAGE analysis of fusion proteins. Cells were grown in modified M9 medium (50 mM Tris base, 25 mM Na_2HPO_4, 1 mM Na_2SO_4, 20 mM NH_4Cl, 3 mM $MgCl_2$, 3 μM $FeCl_3$, pH 7.0) supplemented with 1% glycerol, 0.5% maltose, 1 μg of thiamine hydrochloride per ml, and required amino acids at 1 mM. UV irradiation, infection, and labeling of phage-encoded proteins with [^{35}S]methionine were performed as previously described (32). Samples were electrophoresed on discontinuous polyacrylamide gels (16) (11% polyacrylamide–0.075% bisacrylamide gels, 0.8% sodium dodecyl sulfate [SDS], pH 8.3) and analyzed by autoradiography after polyacrylamide gel electrophoresis (PAGE).

Construction of chromosomal *tar-tap* fusion strains. The deletions present in λche22Δ30 and λche22Δ38 were transferred to the RP437 chromosome by an integration-excision cycle as described previously (24). Putative deletion-bearing strains were tested for production of *tar*$^+$ recombinants in crosses to λche22 strains with deletion endpoints in *tar* (30, 31) (see Fig. 2) to confirm that they carried the appropriate fusion.

Behavioral analysis of fusion strains. Cells were grown, tethered with antiflagellar antibodies, and analyzed as described previously (31).

RESULTS

Isolation of *tar-tap* fusions. The *tar* and *tap* loci map next to one another at the promoter-proximal end of an operon containing the *cheR*, *cheB*, *cheY*, and *cheZ* genes, which are required for all chemotactic responses (30) (Fig. 2). Since *tar* and *tap* have the same orientation and exhibit sequence homology over much of their C-terminal coding regions (5, 15), *tar-tap* deletions could conceivably be produced by homologous recombination events, giving rise to gene fusions that make hybrid transducer molecules. In a previous study, we isolated a series of spontaneous, independent deletion derivatives of λche22, a specialized transducing phage that carries the entire *tar* operon (24). Preliminary genetic characterization indicated that three of those phage strains (λche22Δ30, λche22Δ35, and λche22Δ38) might have

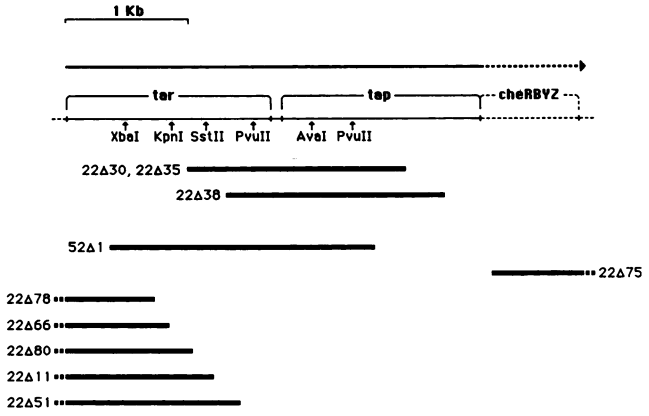

FIG. 2. Physical map of the *tar-tap* region and λ transducing phage deletions used in this study. The relative sizes and positions of the genes are based on DNA sequence information (15). The direction and extent of transcription are indicated by the arrow above the genes. Deletion positions were determined from genetic properties and the presence or absence of the indicated restriction sites.

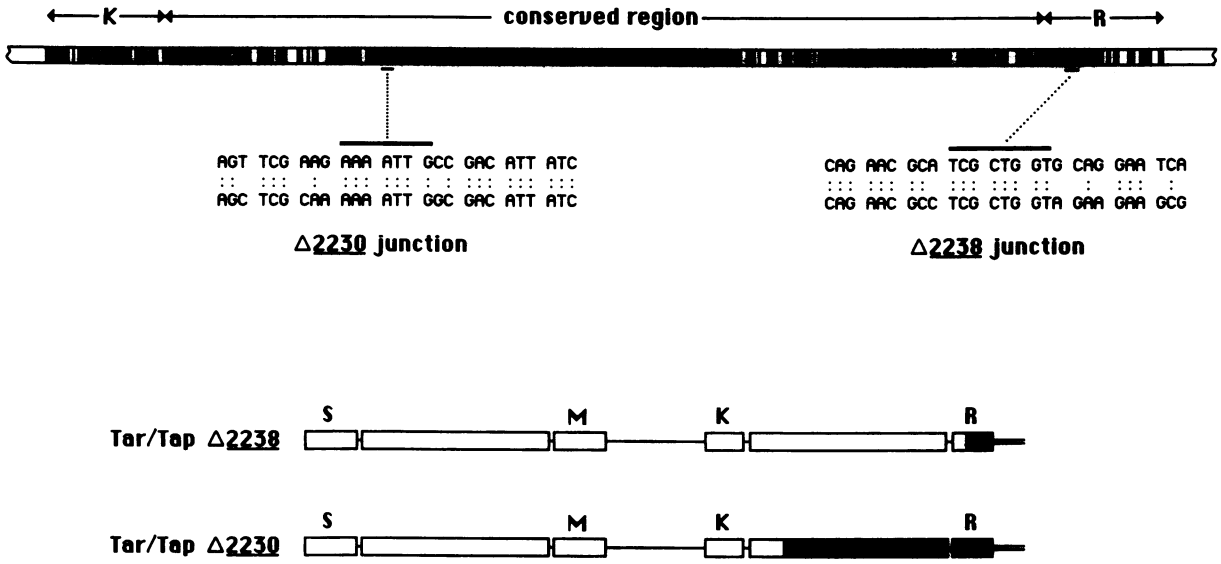

FIG. 3. Structures of *tar-tap* gene fusions and their products. The nucleotide sequence homology between the C-terminal portions of the *tar* and *tap* coding regions is shown in the upper part of the figure (15). Solid bars represent bases that are identical in the two genes. The positions of the *Δ2230* and *Δ2238* fusion junctions are shown in the expanded view: the upper sequence is from *tar*, the lower is from *tap*. The inferred structures of the *Δ2230* and *Δ2238* fusions proteins are shown at the bottom of the figure: unshaded segments are derived from Tar, shaded segments from Tap.

in-frame fusions of the *tar* and *tap* genes: both deletion endpoints fell within the *tar-tap* region, and expression of downstream *che* genes was not reduced by polarity effects. The deletions carried by these phage strains have been designated *Δ2230*, *Δ2235*, and *Δ2238*. Their physical position and extent were determined by restriction mapping of phage DNA (Fig. 2). All three deletions removed about 1.7 kilobases (kb) of material and spanned restriction sites in both the *tar* and *tap* genes. They appeared to fuse the C-terminal coding regions of *tar* and *tap*, indicating that they may have been formed by recombination between homologous segments. Only two of these deletions, *Δ2230* and *Δ2238*, are described in this report, as the structure and functional properties of *Δ2235* proved to be similar to those of *Δ2230* (Fig. 2).

Sequence analysis of *tar-tap* fusion junctions. The DNA sequences at the fusion junctions of *Δ2230* and *Δ2238* support the idea that they were formed by a recombinational mechanism (Fig. 3). Both deletions joined homologous regions of the *tar* and *tap* genes, creating in-frame fusions of the *tar* and *tap* coding sequences. The *Δ2238* fusion should make a hybrid protein in which the C-terminus of Tar, including the R peptide segment, has been replaced with the corresponding region from Tap. The *Δ2230* fusion should replace a larger segment of the Tar molecule with Tap sequences, including most of the conserved cytoplasmic region as well as the remainder of the C terminus.

Modification patterns of Tar-Tap fusion proteins. MCP molecules undergo several posttransitional modifications catalyzed by the *cheR* and *cheB* gene products. The CheR methyltransferase attaches methyl groups to glutamic acid residues in the K and R segments; the CheB methylesterase hydrolyzes the methylated sites to re-form glutamic acid residues. Steady-state MCP methylation levels reflect the relative activities of these two enzymes. In addition, some of the methyl-accepting groups are initially synthesized as glutamine residues and subsequently converted to glutamic acids (9). CheB catalyzes these irreversible MCP deamidation reactions as well. Both methylation state and amidation state affect the electrophoretic mobility of transducer molecules and can be assessed by SDS-PAGE analyses.

To examine the modification properties of Tar-Tap fusion proteins, derivatives of λche22Δ30 and λche22Δ38 lacking the *cheR* and *cheB* genes were used to program protein synthesis in host strains with different combinations of *cheR* and *cheB* defects. The fusion proteins were analyzed by SDS-PAGE to assess their ability to serve as substrates for deamidation and methylation reactions (Fig. 4). In the absence of *cheR* and *cheB* activity, both phage strains produced proteins that migrated as single bands with mobilities roughly comparable to those of Tar and Tap molecules (Fig. 4, first and fourth lanes). That these bands represented Tar-Tap fusion proteins was indicated by the fact that they shifted positions in the presence of MCP-specific methyltransferase or methylesterase activity. CheB function alone

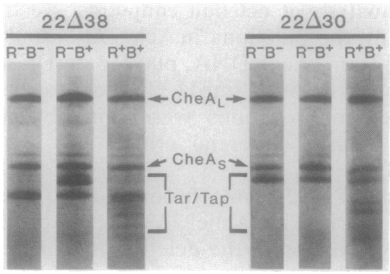

FIG. 4. SDS-PAGE gel of Tar-Tap fusion proteins. Proteins were labeled by UV programming with derivatives of λche22Δ30 and λche22Δ38 that carried the *Δ2275* deletion to eliminate phage expression of the *cheRBYZ* genes. The host strains were RP5764 (*cheR cheB*) (first and fourth lanes), RP5763 (*cheR cheB*$^+$) (second and fifth lanes), and RP5762 (*cheR*$^+$ *cheB*$^+$) (third and sixth lanes). Phage-encoded proteins in the 60 to 70-kDa size range are shown. The fusion proteins migrated just below the small CheA (CheA$_s$) protein (M_r, 64,000).

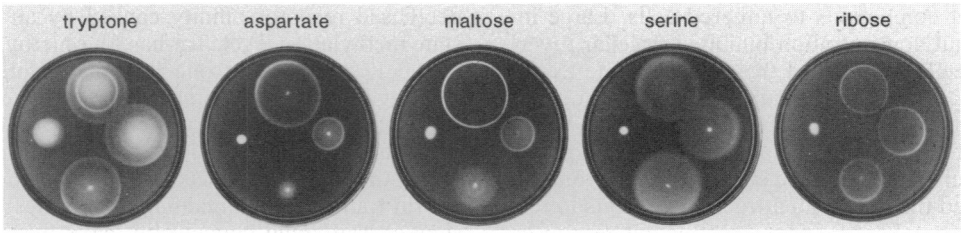

FIG. 5. Swarm phenotypes of strains containing Tar-Tap hybrid transducers. Cells were transferred to tryptone and minimal swarm plates, and colonies were photographed after 12 and 24 h of incubation, respectively, at 35°C. Aspartate and maltose responses are mediated by the Tar transducer, serine responses by the Tsr transducer, and ribose responses by the Trg transducer. Reading clockwise from the top, the strains used were RP437 (wild type), RP2924 (*Δ2238*), RP4324 (*Δ5201*), and RP2918 (*Δ2230*).

caused an upward shift characteristic of deamidated MCP molecules (Fig. 4, second and fifth lanes); CheR function produced a downward shift of the deamidated bands characteristic of methylated MCP molecules (Fig. 4, third and sixth lanes). Thus, *Δ2230* and *Δ2238* produced apparently functional fusion proteins capable of undergoing both deamidation and methylation reactions. Although the predicted sizes of the *Δ2230* and *Δ2238* fusion proteins were nearly identical, their band positions differed significantly, presumably due to differences in primary sequence. Single amino acid substitutions in the Tsr transducer are known to cause equally dramatic changes in band mobility (6).

Two deamidated forms of the *Δ2238* transducer were observed in addition to some unmodified molecules (Fig. 4, second lane). By analogy to previous studies of Tar and Tsr molecules (26, 29), we infer that the lower of the two deamidated bands represents an intermediate reaction product, implying that this fusion protein has at least two deamidation sites. The methylation pattern of the *Δ2238* protein (Fig. 4, third lane) was more complex than that of wild-type Tar and Tap molecules labeled under the same conditions (not shown) and could reflect differences in the number or reactivity of the methylation sites in the hybrid molecule.

It was difficult to assess the deamidation pattern of the *Δ2230* transducer because one of the deamidated forms comigrated with the small CheA protein. Nevertheless, it appears that this hybrid was deamidated less readily than the *Δ2238* protein, and the paucity of methylated species (Fig. 4, sixth lane) was consistent with this conclusion. The *Δ2230* transducer may be a relatively poor substrate for deamidation, or CheB deamidation activity may be globally depressed in cells containing this transducer. Both of these effects have been observed with other transducer mutants (11; D. I. Sherris, Ph.D. thesis, University of Utah, Salt Lake City, 1984).

Functional properties of Tar-Tap fusion proteins. The *Δ2230* and *Δ2238* mutations were transferred from the corresponding phage strains into the bacterial chromosome to assess their effects on chemotactic behavior. Although at the outset we could not predict the phenotypes of the fusion strains, we found that a high proportion of the suspected recombinants produced non-wild-type swarms on semisolid tryptone agar. Mapping crosses to other λche22 deletion phages confirmed that these strains carried deletions with the expected *tar* endpoint. The swarm phenotypes of the strains are shown in Fig. 5. On tryptone swarm agar, colonies of *Δ2238* strains exhibited a faint inner ring, indicative of a partial defect in aspartate taxis. On minimal agar, these strains exhibited normal swarm responses to serine and ribose but decreased responses to maltose and aspartate. In contrast, strains containing the *Δ2230* fusion failed to make swarms on any of these attractants. The partial chemotaxis defect of *Δ2238* strains could be corrected by complementation with λ transducing phages that carried a wild-type *tar* gene, whereas the generally nonchemotactic phenotype of *Δ2230* strains could not (data not shown). The failure to complement the *Δ2230* defect cannot be due to polar effects on *cheRBYZ* expression, because phages that supply those functions also failed to complement (data not shown). The dominant nature of the *Δ2230* mutation indicates that the hybrid transducer itself plays an active role in inhibiting chemotactic responses mediated by other sensory pathways.

Mutants with null defects in the Tar and Tap transducers exhibited wild-type swimming behavior, and their unstimulated flagellar rotation pattern was punctuated by frequent reversals (Table 1). Strains containing the *Δ2238* transducer had a similar phenotype, whereas *Δ2230* strains exhibited predominantly counterclockwise (CCW) flagellar rotation, with a very low rate of spontaneous reversals (Table 1). This rotational pattern caused the cells to swim in smooth trajectories, with few of the tumbling episodes that characterize wild-type motility. Many classes of *che* mutants are also smooth swimmers, and the aberrant swimming patterns of *Δ2230* strains probably account for their generally nonchemotactic behavior.

We investigated the response thresholds and adaptation times in strains containing hybrid Tar-Tap transducers by

TABLE 1. Behavioral properties of Tar-Tap fusions

Strain (mutation)	Swimming pattern	Rotation pattern[a] (%)					Stimulus threshold[b] (M)		
		CCW	CCW^R	REV	CW^R	CW	Aspartate	Maltose	α-Amino-isobutyrate
RP2924 (*Δ2238*)	Wild type	0	11	61	28	0	5×10^{-5}	5×10^{-5}	1×10^{-4}
RP2918 (*Δ2230*)	Smooth	98	2	0	0	0	5×10^{-5}	5×10^{-5}	1×10^{-4}
RP4324 (*Δ5201*)	Wild type	0	37	63	0	0	$>5 \times 10^{-4}$	$>5 \times 10^{-4}$	1×10^{-4}
RP437 (Wild type)	Wild type	0	2	88	10	0	5×10^{-6}	5×10^{-6}	1×10^{-4}

[a] At least 100 rotating cells were each observed for 15 s and classified as follows: exclusively CCW; predominantly CCW, but reversing (CCW^R); frequently reversing, with no apparent bias (REV); predominantly CW, but reversing (CW^R); and exclusively CW. The percentage of cells in each class is given.

[b] The concentration of attractant required to produce a measurable CCW response (see text).

applying attractant compounds to tethered cells. Large increases in attractant concentration inhibited flagellar reversals in wild-type cells and caused the cells to rotate exclusively in the CCW direction until adaptation was complete. Because *Δ2230* strains exhibited predominantly CCW flagellar rotation, we first subjected them to a repellent stimulus (25 mM sodium benzoate) to induce clockwise (CW) rotation and then challenged them with an attractant stimulus to test for reversal of the repellent response. Attractant responses in the other strains were tested in similar fashion, but because they were not CCW biased, only 10 mM benzoate was required to induce a comparable repellent response. Under these conditions, both hybrid transducers mediated attractant responses to Tar-specific compounds (Table 1). Their response thresholds to α-amino-isobutyrate, a Tsr-specific stimulus, were unaltered, whereas their aspartate and maltose thresholds were approximately 10-fold higher than that of the wild type. Adaptation times were essentially normal in all cases (data not shown). These results demonstrate that the *Δ2230* and *Δ2238* transducers detect Tar-specific stimuli, but with reduced sensitivity. Although the *Δ2230* transducer distorted the normal swimming pattern and precluded chemotaxis on swarm plates, it was still able to produce appropriate changes in signal output and undergo sensory adaptation in these more sensitive tests.

DISCUSSION

MCP molecules comprise a family of transducer proteins with homologous structures and functions. Hybrid proteins containing domains from different transducer species are therefore useful for exploring structure-function relationships in MCP molecules. The hybrid transducers described in this report contain the N-terminal periplasmic domain of Tar joined to the C-terminal cytoplasmic domain of Tap. These Tar-Tap hybrids exhibited defects in stimulus detection and flagellar signaling that shed new light on the functional architecture of bacterial transducers.

The *Δ2238* fusion replaced the R peptide and C terminus of Tar with the corresponding segments from Tap (Fig. 3). Cells containing this transducer exhibited wild-type swimming patterns in the absence of stimuli and responded in normal fashion to attractants detected by the Tsr or Trg transducers. They also responded to aspartate and maltose stimuli, but with elevated thresholds, indicating that the hybrid transducer retained Tar receptor specificity but had reduced sensitivity. Tap itself is known to be a functional transducer (17), so this behavior cannot be due to substitution of a defective segment from Tap, but rather to functional incompatibility of the Tap and Tar components of the hybrid molecule. Since Tar-Tsr hybrids function normally, even though their C termini distal to the R peptide differ greatly in sequence (14), the aberrant properties of the *Δ2238* transducer may be caused by the Tap R peptide rather than the remainder of the Tap C terminus. Moreover, extensive mutational studies of the Tsr and Tar molecules have revealed very few C-terminal point mutations (19, 25; M. K. Slocum, Ph.D. thesis, University of Utah, Salt Lake City, 1984), suggesting that this portion of the molecule may not be critical for transducer function. Although deletions of the Tar C terminus can cause transducer defects (14, 27), they may simply increase the susceptibility of the molecule to proteolytic degradation.

The ligand affinity of MCP receptors is influenced by the methylation and amidation states of the molecules (38). The impaired sensitivity of the *Δ2238* transducer may reflect decreased receptor affinity caused by an elevated steady-state methylation level. Tar has four methylation sites, three in the K peptide and one in the R peptide (36) (Fig. 6). Although the methylation sites of Tap have not been explicitly identified, comparison of the Tap and Tar sequences suggests that Tap may also contain four methylation sites, two in the K peptide and two in the R peptide (Fig. 6). Thus, hybrid transducers containing the K sites of Tar and the R sites of Tap could conceivably have five methylation sites and consequently higher steady-state methylation levels than either of the parental MCP molecules. Moreover, the R peptide of Tap may be intrinsically more effective than its Tar counterpart in modulating receptor affinity. The putative Tap R peptide methylation sites conform closely, in both sequence and relative spacing, to the consensus motif of K peptide sites present in all MCP species. The Tap sites may therefore be particularly efficient substrates for deamidation and methylation reactions. In contrast, the single R peptide site of Tar is the only known example of a Gln-Glu pair and may be less reactive than its Tap counterpart(s).

The receptor specificity and detection sensitivity of the *Δ2230* transducer, which contained most of the conserved region as well as the R peptide and C terminus from Tap (Fig. 3), resembled those of the *Δ2238* transducer. However, cells containing the *Δ2230* transducer exhibited very low tumbling rates while swimming and were generally nonchemotactic. The dominant nature of this defect implies that the *Δ2230* transducer may generate a constitutive flagellar signal that locks the rotational machinery in the CCW mode. Such a signal might also inhibit CheB activity, which is thought to be globally regulated by the flagellar signaling system (12). This could account for the inefficient deamidation observed with this transducer. Alternatively, the hybrid molecules might have altered substrate properties for the CheB enzyme. In either case, the *Δ2230* transducer still accepted methyl groups and underwent sensory adaptation following stimulation. Evidently, changes in methylation state cannot fully compensate for the altered signaling properties of this transducer, because they failed to restore flagellar rotation to the normal steady-state pattern.

The aberrant signaling behavior of the *Δ2230* hybrid suggests that the conserved domain located between the K and R peptide methylation sites plays an important role in generating or regulating transducer signals. The conserved regions of Tar and Tap differ at a number of residues (Fig. 6), and it could be that the Tar and Tap segments juxtaposed by the *Δ2230* fusion cannot interact properly. Alternatively, the Tap signaling domain might be designed to optimize signal output or communication with its own receptor domain. Since *tap* is expressed at a much lower level than *tar* (5, 30), most likely due to an inefficient translational start site (15), it may need to make a transducer with a particularly loud voice in order to be heard in the presence of other transducer species. When coupled to the Tar receptor and expressed at Tar levels, the Tap signaling domain may generate an abnormally strong output that dominates the steady-state pattern of flagellar rotation. Point mutations in the *tsr*, *tar*, and *trg* genes can cause similar behavioral defects, provided that the mutant transducer comprises a substantial proportion of the MCP molecules in the cell (6, 19, 21).

We have shown that functional gene fusions can be formed by homologous recombination between the highly conserved C-terminal portions of the *tar* and *tap* coding regions. In fact, such events appear to account for many of the spontaneous deletions that span the *tar-tap* gene border (N. F. Halden, M.S. thesis, University of Utah, Salt Lake City, 1985). The

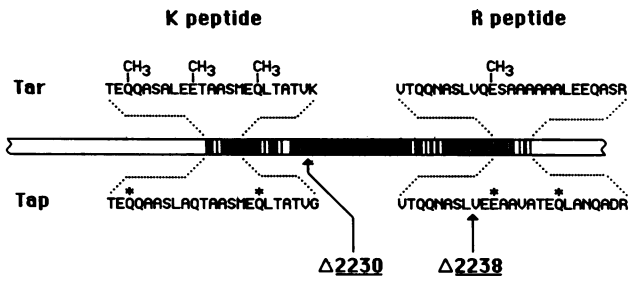

FIG. 6. Comparison of the cytoplasmic domains of Tar and Tap. The amino acid sequence homology between the C-terminal portions of Tar and Tap is indicated by the striped line, with solid bars denoting identical residues in the two transducers. The sequences of the K and R peptides are expanded to show sites of methylation. The Tar sites are from recent work (36), although it should be noted that the position of the first K peptide site is inconsistent with that found in a previous study (35). Tap methylation sites (shown as asterisks) are inferred by analogy to the Tar sites. The positions of the substitution join points in the *Δ2230* and *Δ2238* hybrid molecules are indicated by arrows.

MCP genes of *E. coli* probably arose from a common ancestral gene. Although they have since diverged in sequence, they appear to retain sufficient sequence homology to permit construction of a variety of transducer hybrids by forcing or selecting appropriate recombinational exchanges. We would predict, for example, that fusion of the C terminus of Tap to the Tsr or Trg receptor would lead to domain incompatibilities similar to those observed in the present study. Since it is unlikely that the functional properties of such transducers could be mimicked by single point mutations, it might be particularly instructive to examine partial revertants in which the behavioral defect of the hybrid transducer is compensated for by other structural changes. In this way it should be possible to identify functional interactions both within and between transducer domains and perhaps gain insight on the mechanism of transmembrane signaling.

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