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Genetics of Methyl-Accepting Chemotaxis Proteins in *Escherichia coli*: Null Phenotypes of the *tar* and *tap* Genes

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The *tar* and *tap* genes are located adjacent to one another in an operon of chemotaxis-related functions. They encode methyl-accepting chemotaxis proteins implicated in tactic responses to aspartate and maltose stimuli. The functional roles of these two gene products were investigated by isolating and characterizing nonpolar, single-gene deletion mutants at each locus. Deletions were obtained by selecting for loss or a defective Mu dl prophage inserted in either the *tar* or *tap* gene. The extent of the *tar* deletions was determined by genetic mapping against a set of deleted λ transducing phages. The extent of *tap* deletions was determined by physical mapping with Southern hybridization. Representative deletion mutants were surveyed for chemotactic responses on semisolid agar and by temporal stimulation in a tethered cell assay to assess flagellar rotational responses to chemoeffector compounds. The *tar* deletion strains exhibited complete loss of aspartate and maltose responses, whereas the *tap* deletion strains displayed a wild-type phenotype under all conditions tested. These findings indicate that the *tap* function is unable to promote chemotactic responses to aspartate and maltose, and its role in chemotaxis remains unclear.

Methyl-accepting chemotaxis proteins (MCPs) play a key role in regulating flagellar rotational behavior in *Escherichia coli* (39). These transmembrane proteins possess binding sites for monitoring the levels of chemoeffector compounds in the environment, either directly as in the case of serine and aspartate (6, 12, 45) or indirectly via periplasmic binding proteins as in the case of maltose, ribose, and galactose (17, 18, 33). The cytoplasmic portion of MCP molecules contains several glutamic acid residues that are capable of undergoing reversible carboxylmethylation, catalyzed by a methyltransferase encoded by the *cheR* gene and a methylesterase encoded by the *cheB* gene (4, 5, 8, 9, 16, 40, 41, 43). Changes in either chemoeffector concentration or methylation state are capable of modulating the flagellar signaling properties of MCP molecules. Chemical stimuli elicit MCP signals that alter the steady-state pattern of flagellar rotation, initiating a chemotactic response. Subsequent restoration of prestimulus swimming behavior (sensory adaptation) is accompanied by a net change in MCP methylation state, which effectively cancels the excitatory signals (38). Although the nature of these signals is not yet understood, the transducer properties of bacterial MCPs constitute an excellent system for investigating the mechanisms of stimulus detection and sensory adaptation at the molecular level.

Three MCP species have been extensively studied in *E. coli*; each handles a specific subset of sensory inputs. The Tsr protein mediates chemotactic responses to serine (12, 24) and is also involved in behavioral responses to temperature (22), weak acids (15, 32, 36), and various repellents (25, 42). The Trg protein mediates responses to ribose and galactose (11, 18). The Tar protein mediates responses to aspartate and maltose and is also involved in several repellent responses (31, 42). Unlike the *tsr* and *trg* loci, which are not located near any other chemotaxis-related genes, the *tar* locus lies at the promoter-proximal end of an operon containing several *che* genes whose products are required for all chemotactic responses (Fig. 1) (35). Most of the *tar* point mutations that have been used to define Tar function (31) are partially polar and cause general defects in chemotactic ability due to reduced expression of the downstream *che* functions (35). Thus some of the transducer functions that have been ascribed to the Tar protein may reflect polar effects rather than loss of *tar* activity per se.

Genetic and physical investigations of the *tar* region have

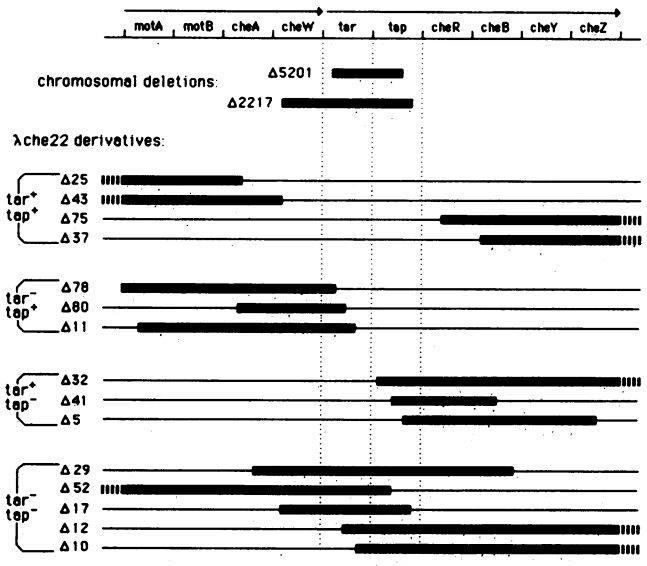

FIG. 1. Genetic map of the *tar-tap* region and deletions used in the reconstruction of null mutants. Strains containing the chromosomal deletions were lysogenized with the various transducing phages to reconstruct single-gene lesions as detailed in the text. The deletions were isolated and characterized in a previous study (30).

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TABLE 1. Bacterial strains

Strain	Relevant markers	Comments	Reference or source
RP437	*eda-50 thr-1*	Standard background for characterization of chemotaxis mutations	(30)
RP506	Δ(*lac*)*U169 eda-50*	Parent of Mu dl mutants	(35)
RP4324	Δ(*tar-tap*)*5201*	Host strain for null mutant reconstructions	(35)
RP2364	Δ(*tar-tap*)*5201* (λ *imm*^{434})	Host strain for null mutant reconstructions	This work
RP1078	Δ(*cheW-tap*)*2217*	Host strain for null mutant reconstructions	(30)
RP1362	*tar-362*::Mu dl	Mu dl insertion	(35)
RP1365	*tap-365*::Mu dl	Mu dl insertion	(35)
RP1367	*tap-367*::Mu dl	Mu dl insertion	(35)
RP1368	*tar-368*::Mu dl	Mu dl insertion	(35)
RP1384	*tap-384*::Mu dl	Mu dl insertion	(35)
RP1385	*tar-385*::Mu dl	Mu dl insertion	(35)
RP1386	*tar-386*::Mu dl	Mu dl insertion	(35)
RP2008	*tar-17*::Mu dl	Mu dl insertion	(35)
RP4368	*tsr-1 eda-50*	Recipient strain used to construct multiple mutants	(29)
RP5724	*tsr-501*::Tn*5*	Recipient strain used to construct multiple mutants	A. Callahan
RP5714	Carries pSP4	Preparation of plasmid DNA	(35)

also revealed a fourth MCP gene—designated *tap*—located immediately downstream from the *tar* locus (3, 35, 44). The *tap* gene shares considerable sequence homology with other MCP genes (3, 19) and makes a protein that undergoes *cheR*-dependent methylation (3, 35). Wang et al. (44) have suggested that *tap* is a functional duplicate of the *tar* gene and that either *tar* or *tap* function is sufficient for chemotactic responses to aspartate and maltose. This model predicts that loss of only one of the two genes should have little effect on chemotactic ability.

In an attempt to clarify the roles of Tar and Tap in chemotaxis, we have isolated nonpolar deletion mutants lacking either the *tar* or the *tap* gene. We found that *tar* deletion strains had wild-type swimming patterns in the unstimulated state, but were completely defective in carrying out chemotactic responses to aspartate and maltose stimuli. Other attractant responses in the *tar* deletion mutants were normal. By contrast, *tap* deletion mutants exhibited wild-type responses to all stimuli, including aspartate and maltose. These findings demonstrate that under physiological conditions Tar is the primary—perhaps only—transducer of aspartate and maltose signals in the cell. It is not clear what role, if any, the Tap protein plays in chemotaxis.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains were derivatives of *E. coli* K-12. Those employed as source strains in this work are summarized in Table 1. All phenotypic characterizations of chemotaxis mutants were done in derivatives of RP437 (30). Mutations at the *tar* and *tap* loci were transferred into RP437 by cotransduction with the *eda* locus (27); mutations at the *tsr* locus were transferred either by cotransduction with the *thr* locus (29) or by direct transduction of a *tsr*::Tn*5* insertion mutation (A. Callahan and J. S. Parkinson, manuscript in preparation). Lysogens containing specialized transducing phages were constructed as detailed below. The RP437 derivatives resulting from these manipulations are not listed individually in Table 1, but rather are discussed below by reference to the relevant chemotaxis mutations they contain.

Phage strains. λche22 is a nondefective, specialized transducing phage that carries the entire *tar* operon and the adjacent mocha operon (Fig. 1) (30). Deletion derivatives of λche22 used in null mutant reconstructions are shown in Fig. 1; those used for fine structure mapping of new *tar* deletion mutations are shown in Fig. 3.

Media. Tryptone broth, tryptone swarm agar, and minimal swarm agar have been described previously (27). Minimal swarm agar was supplemented with required amino acids (0.1 mM), the required vitamins (1.0 μg/ml), and the desired attractant (0.2 mM). In the case of aspartate and serine plates, glycerol (1.0 mM) was also added as a carbon source. Phage stocks were prepared in liquid NZ-amine medium as previously described (30).

Construction of λche22 lysogens. Lysogens of λche22 or any of its deletion derivatives were made by spotting about 10^8 phage particles onto a lawn of the recipient host strain on a tryptone plate. After overnight incubation at 30°C, bacteria from the center of phage growth were picked and tested for stable lysogeny by cross-streaking against λ *b2 c*. Because λche22 has no attachment system, the frequency of lysogens was typically less than 1%, and it was sometimes necessary to identify lysogens by streaking the cells on Tryptone plates seeded with about 10^9 particles of λ *b2 c* to select immune colonies. Individual colonies were then tested for sensitivity to λ *imm*^{434} *c* to eliminate resistant cells and retested for immunity to λ *b2 c* to confirm that they were stable lysogens. These lysogens are formed by homologous recombination of the prophage with the *tar* region of the host (30). No attempt was made to test for polylysogeny, but it is unlikely that lysogens formed under these conditions have more than one prophage.

Mapping methods. The general strategy for mapping *tar* mutations with deleted transducing phages has been described previously (35). Both Tar$^+$ and Tar$^-$ strains form large swarms on semisolid tryptone agar; to distinguish Tar$^+$ recombinants from Tar$^-$ parental cells in crosses, we routinely performed the crosses in a *tsr* genetic background, which in combination with a *tar* defect produces a generally nonchemotactic phenotype. Both low-resolution (37) and high-resolution (30) mapping methods were employed.

Isolation of deletions from Mu dl insertion mutants. Individual colonies of Mu dl insertion strains were inoculated into tryptone broth and grown overnight at 30°C. Cells from each culture were diluted 100-fold into separate flasks containing 10 ml of tryptone broth and grown at 42°C for 4 h. The cells were pelleted and suspended in 0.5 ml of Tryptone broth, and the entire sample was placed in a streak on either a tryptone plate or minimal maltose swarm plate and incubated overnight at 37°C to identify Che$^+$ survivors. Cells from Che$^+$ swarms were purified by single-colony isolation on a tryptone plate and then picked to MacConkey-lactose plates (Difco Laboratories) at 30°C to assess their Lac phenotype and to tryptone plates containing 25 μg of ampicillin per ml at 42°C to assess ampicillin sensitivity.

Only one Lac$^-$ Amp^s isolate was saved from each original culture to ensure independent deletion events.

Analysis of swimming and flagellar rotation patterns. Bacteria used in both swimming and tethering experiments were grown at 30°C in tryptone broth plus 0.5% maltose to a density of approximately 5×10^8 cells per ml. Swimming patterns were evaluated by light microscopy as previously described (27). For tethering studies, flagella were removed from cells by shearing in a Waring blender (14). The treated cells were washed twice in KEP motility buffer (0.1 mM EDTA, 10 mM K-PO_4, pH 7.0) (27) and then suspended in tethering buffer (KEP plus 10 mM sodium lactate, 75 mM sodium chloride, and 100 μg of chloramphenicol per ml) at a density of approximately 1.5×10^8 cells per ml. Cells were mixed with anti-flagellar serum as previously detailed (27) and spotted on cover slips. After 10 to 30 min of incubation at room temperature to allow the cells to become attached, the cover slips were inverted into a drop of tethering buffer on a microscope slide to which two additional cover slips had been affixed with epoxy, forming a channel approximately 1 mm deep and 20 mm wide; 15-s records of individual rotating cells were made with a phase contrast microscope attached to a video camera, recorder, and monitor. Subsequent analysis involved classifying the rotational behavior into one of five categories: exclusively counterclockwise (CCW) or clockwise (CW), reversing frequently with no obvious bias, or reversing less frequently with an obvious bias in the CCW or in the CW sense.

Quantitative analysis of stimulus responses. Chemical stimuli were presented to cells tethered in the manner described above by flushing the tethering chamber with buffer containing attractant or repellent solution. The behavior of the cells was recorded continuously before, during, and after application of the stimulus by using a video display timer to provide an accurate timing record for the subsequent analysis. Typically, positive stimulus responses were marked by a rapid shift of rotational behavior to a full CCW mode for periods of up to several minutes, depending on the strength of the stimulus. The duration of the response was defined as the time elapsed before the cell made a full turn in the CW direction.

DNA hybridization and Southern blot analysis. Chromosomal DNA was extracted from cells by the method of Berman et al. (2) and digested with restrictions enzymes obtained from New England Biolabs or Bethesda Research Laboratories, under the conditions recommended by the suppliers. Samples containing approximately 1 μg of digested DNA were electrophoresed on 1% agarose gels, and DNA fragments were transferred to pretreated nitrocellulose filter paper (BA85, 0.45 μm; Schleicher & Schuell Co.) as previously described (2) and vacuum dried for 3 h at 80°C. The filter pretreatment involved incubation at 65°C for 4 h in prehybridization buffer containing 12.5 μg of sonicated salmon sperm DNA per ml. Prehybridization buffer contained 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1× Denhardt solution (0.02% bovine serum albumin, 0.02% Ficoll [molecular weight 400,000], 0.02% polyvinylpyrrolidone-360), and 0.5% sodium dodecyl sulfate. Plasmid DNA for probe construction was extracted by the method of Ish-Horowitz and Burke (13) and labeled with [α-^{32}P]dCTP (Amersham Corp.) by nick translation as described by Maniatis et al. (23). Labeled probe was purified on a spin column as previously described (2) and was denatured before hybridization by the addition of 0.1 volume of 1.0 M NaOH. The probe was neutralized by the addition of 0.1 volume of 1.8 M Tris-hydrochloride plus 0.2 M Tris base and immediately added to hybridization buffer (prehybridization buffer plus 0.1 g of dextran sulfate [molecular weight, 500,000] per ml) at approximately 5×10^5 cpm/ml. Hybridization was carried out at 65°C for 12 to 20 h. Filters were washed in 0.2× SSC–0.1% sodium dodecyl sulfate and vacuum dried at 80°C for 3 h. Labeled fragments were visualized by autoradiography as previously described (7).

TABLE 2. Swarm phenotypes of *tar* and *tap* null mutants

Genotype[a]	Tryptone swarms	Ring formation on minimal swarm plates with:		
		Maltose	Aspartate	Serine
Reconstructed strains				
tar: *Δ5201* (λche22Δ11)	Tar$^-$	−	−	+
Δ5201 (λche22Δ11-Δ37)	Tar$^-$	−	−	+
Δ5201 (λche22Δ78)	Tar$^-$	−	−	+
Δ5201 (λche22Δ80)	Tar$^-$	−	−	+
tap: *Δ5201* (λche22Δ5)	Wild type[b]	+	+	+
Δ5201 (λche22Δ32)	Wild type[b]	+	+	+
Δ5201 (λche22Δ41)	Wild type[b]	+	+	+
Δ2217 (λche22Δ5)	Wild type[b]	+	+	+
Δ2217 (λche22Δ32)	Wild type[b]	+	+	+
Δ2217 (λche22Δ41)	Wild type[b]	+	+	+
Single-gene deletion mutants				
tar-386::Mu d1 ΔMu2[c]	Tar$^-$	−	−	+
tap-365::Mu d1 ΔMu4	Wild type[b]	+	+	+
Wild-type control strains				
Δ5201 (λche22)	Wild type	+	+	+
Δ5201 (λche22Δ75)	Wild type	+	+	+
Δ2217 (λche22)	Wild type	+	+	+
RP437	Wild type	+	+	+
RP506	Wild type	+	+	+

[a] The indicated mutations and prophages were transferred into RP437 or RP506 for these tests.

[b] The inner ring structure sometimes appeared less complex than that observed in wild-type swarms, particularly at 30°C incubation.

[c] This deletion is designated *Δtar-386-2*; other deletions are similarly named.

RESULTS

Phenotypes of reconstructed *tar* and *tap* null mutants. In previous studies we obtained and characterized a number of mutant strains that entirely lacked *tar* or *tap* function (30, 35). For various reasons, none of those strains is ideally suited for determining the null phenotypes of *tar* and *tap*. The available insertion mutations in *tar* and *tap* are polar on the downstream *che* genes in the operon and consequently exhibit a generally nonchemotactic phenotype (35). With only one exception (Δ5201, discussed below), the available deletions in the *tar-tap* region are either polar on, or extend into, neighboring *che* or *mot* genes, causing a nonchemotactic or nonmotile phenotype (30). As an initial means of investigating the null phenotypes of *tar* and *tap*, we reconstructed single-gene mutants from strains containing the Δ2217 or Δ5201 deletions (Fig. 1). These deletions remove both *tar* and *tap* coding sequences; in addition, Δ2217 extends into the *cheW* locus. By lysogenizing these strains with appropriate specialized λ transducing phages (Fig. 1), all but one of the deleted functions could be restored. For example, a strain carrying the Δ5201 deletion was lysogenized with λche22Δ11 (*tar tap*$^+$) to produce a strain lacking only *tar* function. A number of host mutant-phage combinations of this sort were constructed and tested

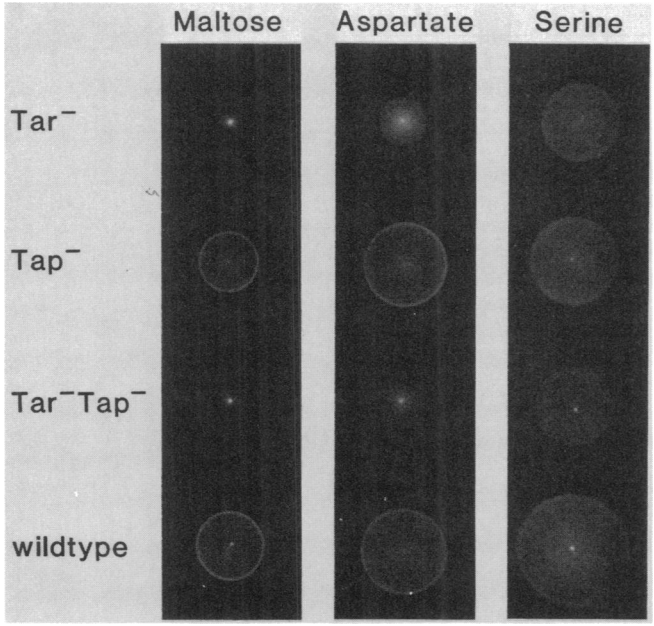

FIG. 2. Swarm phenotypes of *tar* and *tap* null mutants. Minimal swarm plates containing the indicated attractants were inoculated from colonies grown on tryptone plates and incubated at 35°C for 14 h. The strains were RP3841 [Δ*tar-386-2*] (Tar⁻), RP3525 [Δ*tap-365-4*] (Tap⁻), RP4324 [Δ(*tar-tap*)*5201*] (Tar⁻ Tap⁻), RP506 (wild type).

for chemotactic ability on various types of semisolid agar swarm plates (Table 2). Examples of Tar⁻ and Tap⁻ swarm phenotypes are shown in Fig. 2.

On tryptone swarm agar, the strains designed to lack only *tar* function formed large colonies, similar in diameter to wild-type swarms, but lacking the inner ring of cells associated with aspartate taxis. In contrast, the reconstructed *tap* strains formed large swarms that contained the inner aspartate taxis ring. Both types of mutants formed wild-type swarms on minimal serine swarm plates, but on minimal aspartate or maltose swarm plates, *tar* strains failed to swarm, whereas *tap* mutants swarmed as well as the wild type. In general, Tap⁻ swarms on all media were indistinguishable from wild-type swarms, although under some incubation conditions their inner ring on tryptone agar appeared to be less complex than that in wild-type swarms. (This effect was too subtle to photograph and difficult to reproduce reliably). These preliminary findings indicated that Tar function was essential for chemotactic responses to both aspartate and maltose, but not to serine, consistent with previous studies of *tar* point mutants (31). However, the Tap function was neither necessary nor sufficient for aspartate and maltose responses. It is also important to note that the swarm phenotype of strains lacking both *tar* and *tap* function (e.g., Δ5201) was identical to that of the reconstructed strains, which lacked only *tar* function. Thus loss of *tap* function does not seem to alter the chemotactic behavior of *tar* mutants.

It could be argued that the phenotypes exhibited by our reconstructed null mutants were tainted by alterations in the stoichiometry of the various chemotaxis gene products—due either to residual polarity effects, to changes in copy number, or to the expression of some genes from foreign promoters (e.g., Δ2211; Fig. 1). The importance of proper component stoichiometry in chemotaxis is not known, although it seemed unlikely that such effects alone could account for the major behavioral differences observed in *tar* and *tap* mutants. Nevertheless, it was conceivable that changes in the relative amounts of interacting gene products might have observable phenotypic consequences; perhaps the slight variation in inner ring structure occasionally seen in Tap⁻ tryptone swarms was due to such an effect. As a second means of investigating the null phenotypes of *tar* and *tap*, we isolated and characterized a series of nonpolar, single-gene deletions of the *tar* and *tap* loci. In designing schemes to obtain such deletions, we made the assumption that they would produce chemotaxis phenotypes similar—if not identical—to those of the reconstructed null mutants described above. This proved to be the case, as discussed below.

Isolation of *tar* deletion mutants. The *tar*::Mu dl insertion strains listed in Table 1 were generally nonchemotactic due to the polar block on *cheRBYZ* expression and unable to grow at 42°C due to the thermoinducibility of the Mu dl prophage. Mutants that had lost the Mu dl prophage by a prior spontaneous deletion event were selected by growth at high temperature. The survivors were placed on tryptone swarms plates to identify those that no longer had a polar block on downstream *che* gene expression in which the *che* genes had not been deleted. Finally, the chemotactic strains were tested for loss of the ampicillin-resistant and Lac⁺ properties conferred by the original Mu dl prophage. A total of 48 independent *tar* deletion mutants was obtained in this manner; all exhibited a characteristic Tar⁻ phenotype on tryptone swarm agar (i.e., large swarms lacking the inner ring).

Since the scheme for selecting *tar* deletion mutants required that they be chemotactic, none of these deletions should extend beyond the upstream end of the *tar* locus into the adjacent *cheW* gene or beyond the downstream end of the *tap* locus into the *cheR* gene (Fig. 1). However, it seemed likely that some of the deletions would prove to inactivate both *tar* and *tap* function, because, as discussed above, Tar⁻ Tap⁻ strains have the same swarm phenotype as Tar⁻ strains. To identify deletions that ended within the *tar* gene, the positions of the downstream deletion endpoints were determined by mapping tests with λ transducing phages that carried deletions entering the *tar* locus from the *tap* side. As expected, all of the deletions ended within the *tar-tap* region (Fig. 3). Some clearly ended within the *tar* gene because they were able to form *tar*⁺ recombinants with one or more of the λche22 deletions that removed the promoter-distal end of the *tar* gene. However, the majority of the strains failed to recombine with λche22Δ10, which had the most promoter-distal deletion endpoint in *tar*. Thus, many of the deletions could conceivably span the *tar-tap* border and extend into the *tap* coding region. Although the chemotaxis defect of these strains was corrected by complementation with *tar*⁺ *tap* phage, this does not necessarily mean that their deletions do not extend into the *tap* gene, because loss of *tap* function alone (as demonstrated in the reconstruction experiments above) does not result in a detectable change in swarm morphology. It was therefore not possible to map the downstream deletion endpoints in these latter strains by conventional genetic crosses, owing to the lack of a suitable phenotypic difference between *tap*⁺ and *tap* strains. Subsequent phenotypic characterizations were performed only with the deletions that clearly ended within the *tar* gene.

Isolation of *tap* deletion mutants. The basic approach for isolation of *tap* deletions was similar to that just described for *tar* deletions. Strains containing polar Mu dl insertions in

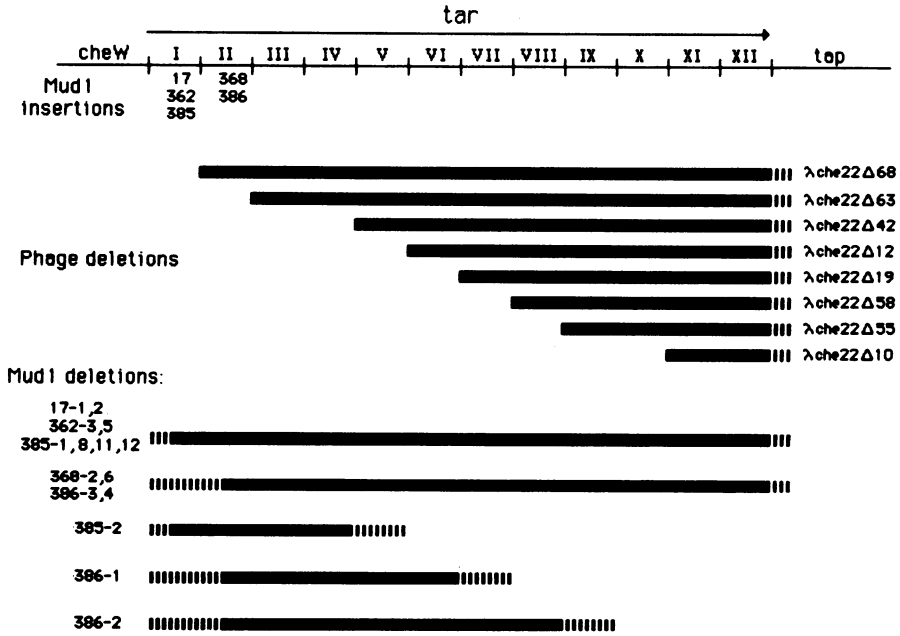

FIG. 3. Deletion map of the *tar* locus showing new deletions obtained from Mu dl insertions. The endpoints of these deletions were established by crosses against the transducing phage deletions shown at the top of the figure. The deletion segments within the *tar* gene were defined in a previous study (35).

the *tap* gene were grown at high temperature to select derivatives that had lost the Mu dl prophage. The survivors should include Tar$^-$ Tap$^-$ and Tar$^+$ Tap$^-$ deletion mutants, both of which were expected to form large swarms on semisolid tryptone agar. The distinctive behavior of Tar$^-$ strains suggested two different strategies for eliminating those mutants with *tar* defects. In the first method, high-temperature survivors were transferred to minimal maltose swarm plates to select chemotactic cells. Mutants with *tar* defects should not be able to swarm under these conditions, whereas mutants lacking only *tap* function should. In the second method, the starting *tap*::Mu dl insertion mutant also carried a *tsr* defect, and high-temperature survivors were transferred to tryptone swarm agar to select chemotactic cells. Strains lacking both *tar* and *tsr* function are generally nonchemotactic (10, 35), whereas reconstructed *tap tsr* strains still exhibited Tar-specific chemotactic responses under such conditions (see below). Note that both schemes demand that the selected strains retain *cheRBYZ* function, so none of the deletions obtained by either method should be polar or extend into the downstream *che* gene cluster.

Both methods yielded putative *tap* deletion strains with similar properties. In a *tsr*$^+$ genetic background, all of the putative *tap* deletion strains formed swarms with an inner ring on tryptone swarm agar, although the inner ring was occasionally less complex than that observed in wild-type cells. In a *tsr* genetic background, the *tap* deletion strains exhibited a typical Tsr$^-$ swarm phenotype on tryptone agar. Thus the deletions did not appear to enter the *tar* coding region, because the strains did not exhibit Tar-specific phenotypic defects. It was not possible to map the endpoints of these deletions by genetic means due to the lack of a phenotypic handle, so instead we mapped the deletions physically by Southern hybridization experiments to confirm that they did remove *tap* coding information.

DNA from each of 12 independent *tap* deletion strains was digested with *Pvu*II, *Sst*II, or *Ava*I restriction endonucleases, which have cleavage sites adjacent to or within the *tap* locus (Fig. 4). Chromosomal fragments containing sequences from the *tap* region were visualized by using labeled DNA from plasmid pSP4 (*cheA-cheY*) as a hybridization probe and compared with the corresponding fragments from wild-type cells. Examples of the restriction blots are shown in Fig. 5, with the findings summarized in Fig. 4.

In some strains, the size of the *Sst*II fragment spanning the site of the original Mu dl insertion was clearly decreased, consistent with a net deletion of material. The *Ava*I and *Pvu*II patterns of such strains revealed that the cleavage sites in the *tap* gene were no longer present, indicating that the deletions extended downstream from the original Mu dl insertion site. The upstream deletion endpoint is more difficult to locate precisely, but the phenotypic evidence discussed above argues that *tar* function is not altered in these strains. Since other deletions that remove only the carboxy-terminal coding segment of the *tar* gene cause loss of tar function (34; Slocum and Parkinson, manuscript in preparation), it seemed likely that these deletions ended within the *tap* gene.

In other putative *tap* deletion strains, the *Sst*II, *Ava*I, and *Pvu*II fragments spanning the original Mu dl insertion sites were larger than in the wild-type controls, and no alterations were evident in any of the flanking fragments. This result most likely is caused by the presence of some residual Mu dl material in the deleted strains. It is possible that in these mutants the deletion lies entirely within the Mu dl prophage; at best, it can only extend past one end of the prophage into *tap* sequences. However, since these strains were chemotactic, the remaining Mu dl sequence must have little or no polar effect on *cheRBYZ* expression, and we have regarded these mutants also as null for *tap* function.

Swimming patterns of *tar* and *tap* mutants. In the absence of chemotactic stimuli, wild-type cells swim about in a random walk pattern produced by alternating episodes of

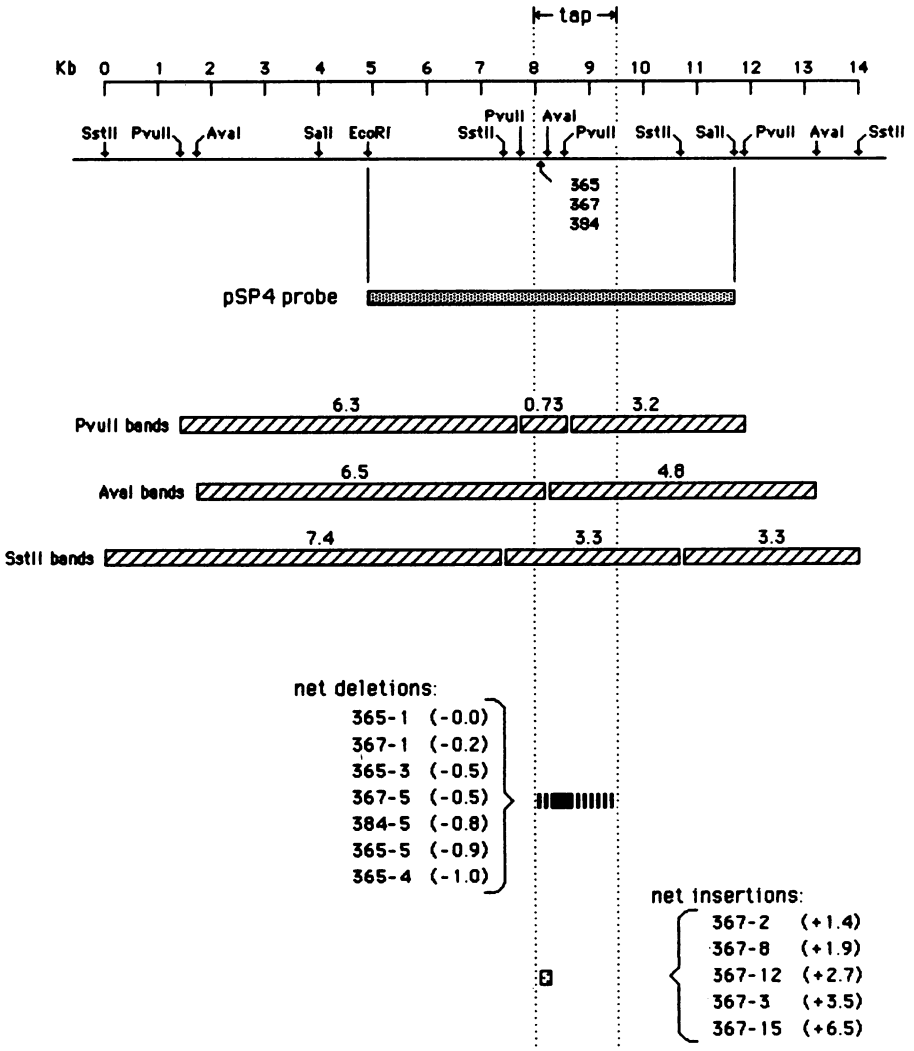

FIG. 4. Physical map of the *tap* region and new *tap* deletions. The restriction map at the top is based on previous work (35). Chromosomal DNA from putative *tap* deletion strains was digested with *Pvu*II, *Ava*I, or *Sst*II and subjected to Southern hybridization analysis as described in the text. The hybridization probe was plasmid pSP4, which carries the indicated portion of the *tap* region and vicinity. The restriction fragments (with approximate sizes indicated in kilobase pairs) detected in wild-type cells with this probe are indicated for each of the restriction enzymes used in the experiment. A number of the putative deletion strains exhibited alterations of the fragments spanning the *tap* locus (see Fig. 5). Some appeared to have undergone a net deletion of *tap* sequences; others probably retained a portion of the Mu d1 prophage.

CCW and CW flagellar rotation (1, 20, 21). We examined the unstimulated swimming pattern of the *tar* and *tap* deletion strains, but found no deviation from the wild-type behavior. A more quantitative analysis of flagellar rotation patterns in selected strains confirmed this finding (Table 3). Both *tar* and *tap* null mutants had a high frequency of reversing cells, similar to the wild-type control strains. Previous work suggested that loss of *tar* function resulted in a marked CCW bias in flagellar rotation pattern (31), but the present results indicate that this effect was probably due to the partially polar nature of the *tar* point mutations used in those studies.

Stimulus responses in *tar* and *tap* mutants. Because *tap* mutants showed slight deviations in the structure of the aspartate taxis ring on tryptone swarm plates, we investigated the possibility that *tap* plays a role in the detection of aspartate and possibly maltose stimuli by subjecting tethered cells to these compounds (Table 4). Strains carrying *tap* deletions showed response times and thresholds of detection similar to those of wild-type cells in all cases. Thus *tar* function alone appears to be sufficient for normal responses to aspartate and maltose. If *tap* function were also able to mediate these responses, even slightly, then *tar tap*$^+$ strains might behave differently than *tar tap* strains. We found, however, that neither *tar tap*$^+$ nor *tar tap* deletion strains were able to respond to aspartate and maltose stimuli, even at concentrations much higher than the threshold levels detected by wild-type cells.

Phenotypes of multiple MCP mutants. Mutant strains lacking various combinations of the *tsr*, *tar*, and *tap* functions were constructed and characterized in an attempt to find an observable phenotypic difference correlated with the presence or absence of *tap* function (Table 5). On swarm plates, strains lacking all three of these MCP functions exhibited a generally nonchemotactic phenotype, as has been found in previous work (10). Double mutants lacking *tar* and *tsr* function were also generally nonchemotactic and indistin-

TABLE 3. Swimming and flagellar rotation patterns of *tar* and *tap* null mutants

Genotype[a]	Swimming pattern	Flagellar rotation (% of cells with rotation pattern):				
		CCW	CCW-Reversing[b]	Reversing	CW-Reversing	CW
Reconstructed mutant[b]						
Δ*5201* (λche22Δ11) (Tar⁻)	Random	0	5	95	0	0
Single gene deletion mutants						
Δ*tar-386-2*	Random	0	3	91	6	0
Δ*tap-365-4*	Random	0	10	79	9	2
Wild-type control strains						
Δ*5201* (λche22Δ75)	Random	1	8	84	4	3
RP437	Random	0	2	88	10	2
RP506	Random	0	2	82	16	0

[a] These mutations and prophages were tested in the RP437 or RP506 genetic background.
[b] No Tap⁻ reconstructions were tested.

guishable from the triple mutant, indicating that the presence or absence of *tap* function in *tar tsr* strains had no influence on swarm behavior. Similarly, single *tsr* or *tar* mutants exhibited no change in their swarm appearance when *tap* function was absent. Thus, loss of *tap* function had no observable effect on swarm behavior in any of the mutant backgrounds. However, it appears that loss of *tap* function may have some effect on the unstimulated flagellar rotation patterns of *tar tsr* double mutants (Table 5). The *tar tsr* strain exhibited approximately 10% reversing cells, whereas the *tar tsr tap* strain had virtually no reversing cells. This result implies that *tap* function may play some role in regulating flagellar rotation.

DISCUSSION

Null phenotype of the *tar* locus. Studies of *tar* point mutants indicate that the Tar transducer probably mediates attractant responses to maltose and aspartate (and various aspartate analogs such as methionine, succinate, etc.) and repellent responses to divalent metal cations (24, 31). However, the *tar* strains used in those studies also exhibited a marked CCW bias in their unstimulated pattern of flagellar rotation and were partially defective in chemotaxis to other compounds, such as serine and ribose, whose signals are processed by other species of MCP molecules. We showed recently (35) that those *tar* point mutations had polar properties that reduced the expression of the *cheR*, *cheB*, *cheY*, and *cheZ* functions, which are required for all chemotactic responses (28). Thus, the CCW flagellar bias and partial chemotaxis defects observed in *tar* point mutants could be due to polarity effects rather than a specific requirement for *tar* function in those processes. The behavior of the single-gene *tar* deletion mutants isolated in the present study confirms this suspicion. The deletions removed only *tar* coding information and had no detectable polar effects on *che* gene expression. Strains containing the deletions responded normally to serine, but were completely defective in aspartate and maltose responses. (Responses to methionine and tricarboxylic acid cycle intermediates were not tested.) Moreover, the *tar* deletion strains had a wild-type pattern of flagellar rotation under unstimulated conditions, indicating that loss of *tar* function alone does not perturb the

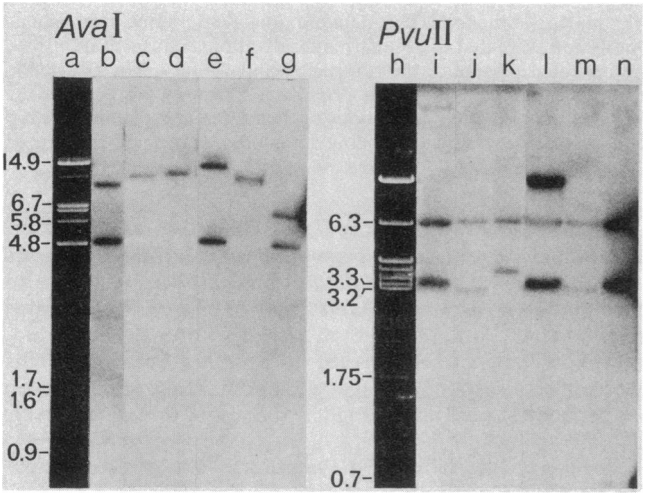

FIG. 5. Examples of Southern patterns of *tap* deletion strains. Details of the experiment are given in the legend to Fig. 4 and in the text. Lanes a through g are DNA samples cut with *Ava*I; lanes h through n are DNA samples cut with *Pvu*II. Lanes a and h contain digested λche22 DNA to serve as size markers. The other lanes contain digested DNA from the following deletion strains: Δ*367-12* (b and i); Δ*365-5* (c and j); Δ*365-3* (d and k); Δ*367-15* (e and l); Δ*365-4* (f and m); RP506 wild type (g and n).

TABLE 4. Stimulus responses of *tar* and *tap* null mutants

Mutation[a]	Attractant response[b]					
	Aspartate		Maltose		α-Amino-isobutyrate	
	Threshold (M)	Duration (S)	Threshold (M)	Duration (S)	Threshold (M)	Duration (S)
Δ*tar-386-2*	$>5 \times 10^{-2}$		$>5 \times 10^{-2}$		5×10^{-4}	60
Δ*tap-365-4*	5×10^{-6}	45	5×10^{-6}	85	5×10^{-4}	50
Wild type	5×10^{-6}	40	5×10^{-6}	75	5×10^{-4}	50

[a] The *tar* and *tap* deletions and the wild-type control were tested in the RP506 genetic background.
[b] Attractant compounds were applied to tethered cells as described in the text. These stimuli elicit CCW rotational responses whose duration is directly proportional to the magnitude of the stimulus. The values reported for duration of response are averages obtained from at least 10 rotating cells stimulated with the threshold concentration. The range in measured times for individual cells was about 10 s. The threshold concentration is defined as the lowest concentration that elicited readily observable responses.

TABLE 5. Phenotypes of multiple MCP mutants

MCP defects	Mutations[a]	Chemotaxis phenotype[b]	Swimming pattern	Flagellar rotation (% of cells with rotation pattern):				
				CCW	CCW-Reversing	Reversing	CW-Reversing	CW
tar tsr	Δ*tar-386-2 tsr-501*::Tn*5*	Che$^-$	Smooth	87	7	1	3	0
tar tap	Δ(*tar-tap*)*5201*	Tar$^-$	Random	0	14	83	3	0
tap tsr	Δ*tap-365-4 tsr-501*::Tn*5*	Tsr$^-$	Random	0	4	68	28	0
tar tsr tap	Δ(*tar-tap*)*5201 tsr-501*::Tn*5*	Che$^-$	Smooth	100	0	0	0	0

[a] MCP mutations were tested in the RP437 genetic background. All strains carried the wild-type allele at the *trg* locus.
[b] Phenotypes are based on swarm plate tests. Che$^-$ strains are generally nonchemotactic; the Tar$^-$ swarm pattern is described in Tabel 2; Tsr$^-$ strains form one-ringed swarms on tryptone plates, wild type swarms on minimal aspartate and maltose plates, and no swarm on minimal serine swarm plates.

cell's ability to control spontaneous tumbling episodes as it swims.

Null phenotype of the *tap* locus. The *tap* gene is located next to the *tar* gene and appears to specify a new species of MCP, since its protein product has the same general size and properties as other MCP molecules (3, 35, 44). In particular, the *tap* product is capable of being methylated by the MCP-specific methyltransferase (3, 35), but may also be subject to *cheB*-dependent demethylation and deamidation reactions. We attempted to determine the role of the tap gene product by isolating and characterizing mutants with null defects in *tap* function, but all such strains had essentially normal behavior in every respect examined. Their response thresholds for serine, aspartate, and maltose stimuli were identical to those of the wild type, and their unstimulated pattern of flagellar rotation was also the same as that of the wild type. Moreover, when combined with other MCP defects, *tap* mutations had no effect on the resultant swarming behavior.

***tap* and *tar* have different functions.** The null phenotypes of *tap* and *tar* mutants indicate that these two genes have distinctly different functions; they are not functional duplicates as Wang et al. have suggested (44). Our findings indicate that *tar* function is essential for responses to aspartate and maltose, whereas *tap* function is neither necessary nor sufficient. Mutants defective in *tar* activity were unable to detect and respond to aspartate and maltose regardless of whether they retained *tap* function. Conversely, deletion mutants lacking essentially all of the *tap* gene were able to respond normally to both types of stimuli. Comparison of the DNA sequences of the *tar* and *tap* genes also indicates it is unlikely that their products have similar chemoreceptor activities. The chemoreceptor domain of MCPs appears to reside in the amino-terminal half of the molecule, which probably protrudes through the cytoplasmic membrane into the periplasmic space (19). This portion of the *tar* and *tap* gene sequences is rather different, with less than 30% amino acid homology. In fact, the *tap* sequence is no more closely related to corresponding *tar* sequences than to corresponding *tsr* sequences.

The discrepancy between our results and those of Wang et al. could stem from a number of differences in our experimental systems. We attempted to preserve the normal stoichiometry of the chemotaxis gene products as much as possible in our *tap* and *tar* deletion mutants, whereas Wang et al. utilized a multicopy plasmid as their source of *tap* product. The *tap* gene carried on the plasmids was coupled to a foreign promoter and possibly expressed at higher than normal levels, although this was not quantitated. Differences in the level of expression could conceivably have been responsible for the weak stimulus responses Wang et al. attributed to *tap* function. It also possible that the responses they observed were not due to stimulation with aspartate or maltose per se, but rather to accompanying changes in oxygen concentration or temperature when the stimuli were applied.

Possible functions of the *tap* gene. Strains carrying the *tar-tap* deletion Δ5201 have been extensively studied in previous work and shown to have normal responses to other chemotactic stimuli such as oxygen, phosphotransferase transport system sugars, ribose, and galactose and to temperature, indicating that *tap* function is not required for any of these responses (10, 22, 26). These findings raise doubts that *tap* has a necessary chemotaxis-related function. Perhaps *tap* is merely the remnant of an MCP gene that is no longer functional in *E. coli*, even though it still makes a product with methyl-accepting activity. In this regard, it is interesting to note that *Salmonella typhimurium* does not seem to possess a *tap* gene—at least not one that is located in an analogous chromosomal position—and yet it exhibits essentially the same range of chemotactic responses as *E. coli* (A. Russo and D. Koshland, personal communication). However, it is still possible that *tap* has a chemotaxis-related function, but one that is too subtle or inappropriate to detect in laboratory situations. For example, Tap protein might serve as the transducer for some untested class of chemoeffectors such as peptides, vitamins, or nucleotides. Alternatively, Tap might function only under anaerobiosis or some other special set of growth conditions.

A possible clue to the role of Tap in chemotaxis is provided by the flagellar rotation patterns of multiple MCP mutants. Loss of any one MCP species has no effect on the unstimulated rotational behavior of the cell, implying that spontaneous flagellar reversals are not generated exclusively by one MCP type. However, double mutants lacking Tar and Tsr, the two major MCP species, have a pronounced CCW flagellar bias and are defective in adapting to stimuli that are processed by other MCP pathways (10). The basis for this effect is not understood, but it suggests that MCPs play a collective role in regulating the rate of spontaneous flagellar reversals. Perhaps when the available supply of methyl-accepting sites is too low (for example, in *tar tsr* strains), the methyltransferase and methylesterase reactions are poorly regulated and the cell is unable to adapt to background levels of chemoeffectors in the environment. The fact that *tap tar tsr* triple mutants are even more CCW biased than *tar tsr* double mutants indicates that Tap protein may serve as a reservoir of potential methylation sites and thereby buffer these effects to some extent.

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