

# Identification of the *tip*-Encoded Receptor in Bacterial Sensing

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**A chemotaxis gene encoding a protein with receptorlike properties has been identified in *Salmonella typhimurium* and termed *tip* for taxis-involved protein. Based on the stringency of DNA hybridization, the *tip* gene has about 75% homology with a region of the *tar* gene encoding the cytoplasmic domain of the aspartate receptor. Introduction of the *tip* gene into a smooth-swimming *Escherichia coli* receptor mutant (*tar tsr tap*) restored both chemotaxis ability on soft-agar-tryptone plates and a wild-type swimming phenotype. We have shown, by overexpressing the CheY protein, that shifting of the mutant swimming bias in the absence of receptors is insufficient to restore chemotaxis ability. This suggests that in addition to resetting the swimming bias, the *tip* gene product functions as a receptor. By functional criteria, we found that Tip is not a duplicate aspartate (Tar) or serine (Tsr) receptor gene. Based on behavioral properties, the *S. typhimurium* Tip receptor provides functional features similar to those of the *E. coli* Tap receptor.**

Bacteria detect and respond to a variety of chemicals in their surroundings (4, 16, 27). The receptor genes for several of these chemoeffectors have been identified and cloned from *Escherichia coli* and *Salmonella typhimurium* strains. The membrane-bound primary receptors for aspartate and serine are encoded by the *tar* and *tsr* genes, respectively (25, 26, 33). The *trg* gene encodes a receptor which recognizes the ligand-occupied forms of the ribose- and galactose-binding proteins (12). An additional gene with receptorlike properties, *tap* (taxis-associated protein) has been identified tandem to the *E. coli* aspartate receptor gene (3, 25, 34). The specificity of the *tap* receptor is not known, but it is not a duplicate aspartate or serine receptor gene (25).

Recently, the sequences for the *tar*, *tsr*, *tap*, and *trg* receptor genes were determined (1, 2, 18, 24). The receptor sequences show a striking degree of structural homology (1, 18). Although the different receptors from both *E. coli* and *S. typhimurium* are homologous, this identity is more pronounced in certain regions of the sequence. In the carboxy-terminal domains there is greater than 75% identity between the aspartate and serine receptors, yet in the amino-terminal halves the sequences diverge to less than 25% identity. This divergence probably reflects differences in substrate specificity. Therefore, for DNA hybridization a probe covering the carboxy-terminal region would be most likely to detect receptorlike sequences. Such an approach has been used to identify several potential receptor genes in the *Salmonella* chromosome (D. Clegg and D. E. Koshland, Jr., unpublished observations).

In the initial cloning of chemotaxis genes from *S. typhimurium*, DeFranco and Koshland (9) isolated an *EcoRI* restriction fragment which complemented the chemotactic migration of a triple-receptor mutant (RP4372) on soft-agar plates containing tryptone (A. DeFranco, Ph.D. thesis, University of California, Berkeley, 1979). This mutant lacks the aspartate (Tar), serine (Tsr), and Tap (unknown specificity) receptors and has a smooth-swimming phenotype, in contrast to the random swimming behavior of cells containing one or more of these receptors (11, 23, 25). Because RP4372 has a receptor deficiency it is unable to migrate to

chemotactic stimuli within the tryptone soft-agar plates. Tryptone is a rich undefined medium consisting of a pancreatic digest of casein, and has particularly high levels (5 mM) of aspartate and serine, which are recognized by the *tar* and *tsr* gene products respectively. It should be noted that although RP4372 contains the *trg* gene (taxis to ribose and galactose), the contribution from Trg receptors is relatively minor because Trg is expressed at about 10-fold-lower levels than the other receptors (12) and tryptone contains primarily amino acids (Difco Laboratories).

On the basis of this complementation and studies described below, we have identified a receptorlike gene. This gene has been named *tip*, for taxis-involved protein, following the example set in naming the *tap* gene (taxis-associated protein), for which ligand specificity is also not known (3). In this report we describe the structural and functional properties of Tip receptors and compare Tip receptors with the possibly analogous Tap receptors in *E. coli* cells. For simplicity in this discussion we shall use the abbreviation Tip to refer to the protein encoded by the *tip* gene. Similarly, Tap, Tar, etc., will be used to refer to the proteins encoded by the *tap*, *tar*, etc., genes.

## MATERIALS AND METHODS

**Bacterial strains and media.** *E. coli* chemotaxis strains were obtained from J. S. Parkinson, University of Utah. The strains used were RP437 (21), RP4324  $\Delta$ (*tar-tap*)5201 (25), RP4368 *tsr-518* (22), RP4372  $\Delta$ (*tar-tap*)5201 *tsr-518* (25), and RP4315 *cheY217* (21). The properties of strain NO1406 have been described previously (9). Lambda bacteriophages and plasmids used were  $\lambda$  *tip* ( $\lambda$ cheS3), pTip (pRK49),  $\lambda$  *tar-meche* ( $\lambda$ cheS1) (9),  $\lambda$  *tar* ( $\lambda$ cheS1 $\Delta$ 4) (9), pTar (pWK34) (33), p $\Delta$ CheY (pCK65) (8), pCheY (pCK63) (8), and pDK1 (9). The cloning of recombinant lambda-containing *tip* DNA was performed as described previously for  $\lambda$ cheS1 (9; DeFranco, Ph.D. thesis). Stable lysogens containing the *tip* gene were constructed by spotting lambda-Tip phage on *E. coli* grown in L broth with 0.4% maltose at 30°C, essentially as described previously (9, 20). Lambda-resistant colonies were streaked several times to remove residual phage and tested for temperature sensitivity (42°C) conferred by the temperature-sensitive *cI* repressor. Cells were grown in L broth (1% tryptone [Difco], 0.5% yeast extract [Difco], 1% NaCl) (20), tryptone (1% tryptone, 1% NaCl), or VBC (32)

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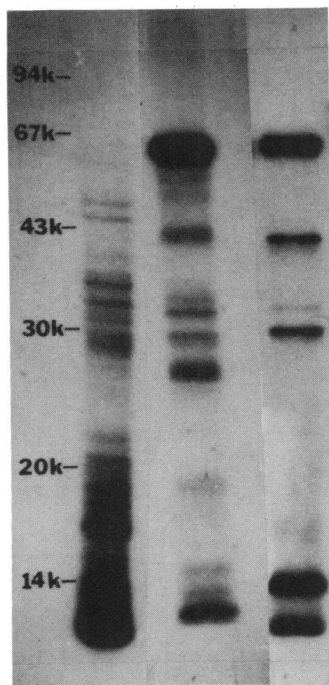


FIG. 1. Polypeptides synthesized from hybrid  $\lambda$  phages. Strain NO1406 was irradiated with UV light, and then hybrid  $\lambda$  phage and [ $^{35}$ S]methionine were added to label phage-coded proteins. The samples were electrophoresed in a 12.5% SDS-polyacrylamide gel, which was then autoradiographed. Phages used were  $\lambda$  gt4 (left),  $\lambda$  Tip (center), and  $\lambda$ cheS1 (right). The positions of marker proteins phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and  $\alpha$ -lactalbumin are indicated. Identities of  $\lambda$ cheS1-encoded proteins are shown based on results from DeFranco and Koshland (9).

minimal medium plus either glycerol (1%) or glucose (0.4%) and required supplements. Nutritional supplements were 50  $\mu$ g/ml for methionine, histidine, thiamine, threonine, and leucine. Plasmids were maintained in 150  $\mu$ g of ampicillin per ml or 12.5  $\mu$ g of tetracycline per ml as applicable. In VBC media, tetracycline was used at 8  $\mu$ g/ml.

**Behavior assays.** For tethering *E. coli* strains, the flagellum copy number was reduced as described previously (24). Cells were grown in VBC minimal medium containing glycerol (1%), glucose (0.4%), lactate (10 mM) plus supplements. At an optical density at 650 nm or 0.5, the cells were shifted to 30°C and treated with chloramphenicol (100  $\mu$ g/ml) to stop protein synthesis during the experiment. After 20 minutes, cells were tethered to cover slips coated with anti-flagellin sera. Data were videotaped from 20 to 30 individual rotating cells during the 15-min prestimulus period and then during addition of stimulus. Free-swimming behavior was measured with cells grown in VBC-1% glycerol with supplements at 30°C to mid-exponential phase (optical density at 650 nm, 0.5). The response time for free-swimming as well as tethered cells was defined as the time at which half the cells had returned to the prestimulus behavior. In all cases when cells were analyzed by tethering and free-swimming assays, the behavior and response times were the same. Migration rates on soft-agar plates were determined from 3 to 6 measurements of the colony diameter at 30°C for 15 to 30 h. Plates contained either 0.35% agar with L broth or 0.4% agar with VBC-glycerol medium and either 0.5 mM L-aspartate or 0.5

mM L-serine. As a control, cells transformed with the plasmid vector pACYC184 had the same behavior as untransformed cells.

**DNA manipulations.** Lambda DNA was extracted from purified phage by treatment with formamide and plasmid DNA isolated as described previously (20). Restriction enzymes were obtained from New England BioLabs and Bethesda Research Laboratories, and digestions were performed as described previously (20). For Southern hybridization of receptor genes, DNA restriction fragments were electrophoresed on a 0.7% agarose gel and transferred to nitrocellulose as described previously (20). The nitrocellulose bands containing  $^{32}$ P-labeled probe were excised and incubated in 5 $\times$  SSPE-50% formamide (20) initially at 37°C and then at temperatures increasing in 5°C increments to 82°C, for 20 min at each temperature. The fragments were *Pvu*II-*Eco*RI and *Ava*I-*Eco*RI digests of pWK51 (34), the *Sa*II-*Eco*RI digest of  $\lambda$  Tip, and the *Cl*aI digest of pDK1 (9).

**Other assays.** Receptor methylation was determined under *in vivo* labeling conditions as described previously (17). Lambda-hybrid phage protein labeling was performed as described previously (9).

## RESULTS

**Identification of the Tip receptor.** The *tip* gene was originally cloned from *S. typhimurium* on an *Eco*RI fragment inserted into lambda gt4 (DeFranco, Ph.D. thesis). The recombinant phage containing *tip* DNA was selected by its ability to complement the chemotaxis defect of *E. coli* receptor mutant RP4372 on tryptone soft-agar plates. The polypeptides synthesized by this hybrid phage were resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis after UV irradiation of the host cell. The major product encoded by  $\lambda$  Tip was a 63-kilodalton protein, which is in agreement with the sizes of other chemotaxis receptors (Fig. 1). For comparison, the proteins from a hybrid phage encoding the 63-kilodalton *S. typhimurium* aspartate receptor and meche operon proteins ( $\lambda$ cheS1) (9) is shown (Fig. 1). The identities of the other peptides apparently encoded by  $\lambda$  Tip are unknown, although the labeling pattern indicates that  $\lambda$  Tip does not encode duplicate meche or moche chemotaxis operons (9).

To study the effects of Tip overproduction on behavior, we cloned the *tip* gene onto a multicopy plasmid. The 10.1-kilobase (kb) fragment from the original lambda clone was inserted into the *Eco*RI site of plasmid pACYC184. The recombinant plasmid pTip produced a 63-kilodalton protein detected on SDS-polyacrylamide gels, in agreement with the size of the major product synthesized from hybrid-lambda phages. This protein was detected in whole-cell lysates of the receptor mutant RP4372 transformed with the Tip plasmid, but not receptor mutant transformed with vector alone. On the basis of Coomassie blue staining, the amount of expression was comparable to that seen with the *Salmonella* aspartate receptor plasmid (pWK34) expressed under parallel conditions.

Methylation of Tip protein was shown under *in vivo* labeling conditions. The receptor mutant with *tip* plasmid [RP4372(pTip)] was grown in [methyl- $^3$ H]methionine, and the proteins were electrophoresed on SDS-polyacrylamide gels. The 63-kilodalton band was excised from the gel, and base-labile methylation was measured by a simple diffusion assay (6). The methylation level (3 pmol/10<sup>9</sup> cells) was significantly above the RP4372 background level (0.5 pmol/10<sup>9</sup> cells) and was comparable to that seen with overproduced aspartate receptor in the absence of stimulus (5

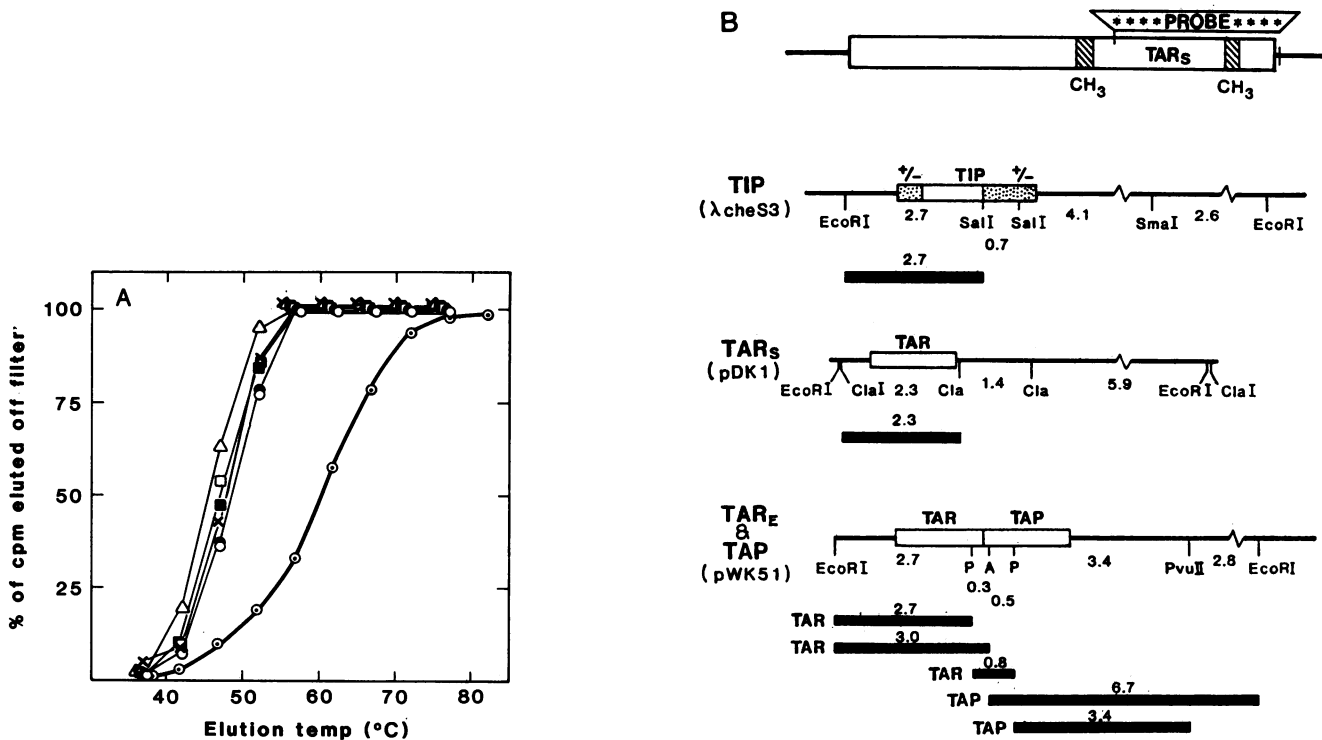


FIG. 2. Relative homology of the receptor genes. (A) The amount of  $^{32}\text{P}$ -labeled *S. typhimurium* aspartate receptor DNA (*tar<sub>S</sub>*) eluted from nitrocellulose filters containing receptor DNA fragments was counted to generate the melting curve shown. The radioactivity in counts per minute (cpm) initially bound to each restriction fragment was as follows: *tar<sub>E</sub>*, *Ava*I (3.0 kb), 6,818 cpm, *Pvu*II (2.7 kb), 4,951 cpm; *tap*, *Ava*I (6.7 kb), 1,584 cpm, *Pvu*II (3.4 kb), 1,774 cpm; *tar<sub>S</sub>*, *Cla*I (2.3 kb), 4,986 cpm; *tip*, *Sal*I-*Eco*RI (2.7 kb), 1,110 cpm; *tar<sub>E</sub>* carboxy-terminal 240 nucleotides (0.8 kb), 253 cpm. In separate experiments the *tar<sub>S</sub>* self-hybrid had twice the counts per minute bound as the *tar<sub>E</sub>* gene. The low amount in this experiment was due to poor transfer and excision of the self-hybrid DNA band. Symbols:  $\odot$ , self-hybrid *tar<sub>S</sub>*, 2.3 kb;  $\square$ , *tar<sub>E</sub>*, 3.0 kb;  $\blacksquare$ , *tar<sub>E</sub>*, 2.9 kb;  $\triangle$ , *tar<sub>E</sub>* 0.8 kb;  $\circ$ , *tap*, 6.7 kb;  $\bullet$ , *tap*, 3.4 kb;  $\times$ , *tip*, 2.7 kb. (B) Receptor gene fragments which were identified by hybridization with the *tar<sub>S</sub>* probe are shown as closed boxes beneath the genes. Restriction fragment sizes are given in kilobases (P, *Pvu*II; A, *Ava*I). Plasmid pDK1 was used to obtain *Salmonella tar*, plasmid pWK51 was used for *E. coli tar* and *tap*, and lambda cheS3 was used for *Salmonella tip*. The 0.65-kb *Sal*I-*Cla*I fragment of *Salmonella tar* was used as the hybridization probe. The methylation sites of *tar<sub>S</sub>* are shown as cross-hatched regions for orientation. The scale for the probe is five times larger than the target genes. The locations of the *tar<sub>S</sub>*, *tar<sub>E</sub>*, and *tap* genes were determined by their sequences. The location of *tip* was approximated on the basis of hybridization and its gene product size, so that the gene may extend from the open area into the stippled portions.

pmol/ $10^9$  cells). Because Trg receptor expression is so low (12) and Trg methylation is especially low in RP4372 (12), we were not able to resolve Tip from Trg for the methylation analysis. However, because of the low expression of Trg, it is unlikely that Trg can account for all the methylation seen in RP4372 containing overproduced Tip receptors. Furthermore, all the detectable methylation from either RP4372 pTip or RP4372 pTar appeared to be in a single band visible on SDS-polyacrylamide gels, whereas Trg has been reported to electrophorese differently from Tar (12).

To establish homology between *tip* and other chemotaxis receptor genes, hybridization to aspartate receptor DNA was determined. The *Salmonella* aspartate receptor gene (*tar<sub>S</sub>*) was hybridized to the *tip* gene, and as controls, to *E. coli* aspartate receptor (*tar<sub>E</sub>*) and *tap* genes for which the sequences are known. The portion of *tar<sub>S</sub>* encoding the carboxy-terminal domain of the receptor was chosen as the hybridization probe, since this region has the greatest degree of homology among the sequenced receptor genes (18, 24).

The homology of the *tip* and aspartate receptor DNA was quantitated by incubating the nitrocellulose filters containing the hybridized DNA at increasing temperature to generate a melting curve (Fig. 2). Mismatching of 1 to 3% decreases the  $T_m$  (temperature of 50% melting) of the duplex DNA by

about  $1^\circ\text{C}$ , based on model DNA polymers and sequenced genes (5, 14). The *tip-tar<sub>S</sub>* hybrid melted  $12.5^\circ\text{C}$  lower than the *tar<sub>S</sub>* self-hybrid, corresponding to about 75% sequence homology. A comparable degree of homology is predicted for the other receptor genes on the basis of the melting curves (Fig. 2). Homology between *tar<sub>S</sub>*, *tar<sub>E</sub>*, and *tap* genes was confirmed by sequence analysis, which shows 74 to 84% identity over 80% of the regions used for hybridization analysis. Therefore, on the basis of the differences in DNA duplex melting temperatures, about 75% identity is expected between the regions encoding the C-terminal halves of *tip* and other chemotaxis receptor genes.

**Behavioral properties of cells containing Tip receptors.** The behavior of cells containing the Tip receptor was determined by two assays. First, the prestimulus steady-state behavior and ability of various stimuli to change the motility of the cells were determined by either free-swimming or tethered-cell analysis methods. Second, the migration rates of cells inoculated onto soft-agar plates were determined to measure chemotaxis to various stimuli.

Introduction of either a low or high gene dosage of Tip into the receptor mutant RP4372 changed prestimulus behavior from smooth-swimming to frequent reversals of rotation and random swimming. This shift from smooth to random swim-

TABLE 1. Signaling in cells with Tip receptors

Strain	Response time (min) <sup>a</sup> for			
	aspartate	serine	indole	phenol
RP437 wild type	2.3	2.5	1.3	1.8
RP4372 <i>tar tap tsr</i>	— <sup>b</sup>	—	2.3	1.5
RP4372 $\lambda$ Tip	0	0	1.0	1.8
RP4372 p Tip	0	0	1.8	2.0
RP4324 <i>tar tap</i>	0	2.0		
RP4324 $\lambda$ Tip	0	1.1		
RP4368 <i>tsr</i>	3.0	0		
RP4368 $\lambda$ Tip	2.0	0		

<sup>a</sup> The free-swimming prestimulus behavior for all the cells was random, except for RP4372, which was smooth swimming. Responses to attractants (1 mM aspartate or 1 mM serine) were smooth swimming, and responses to repellents (0.2 mM indole or 1 mM phenol) were tumbling. The response times are averaged from at least four independent determinations, with a standard error of the mean of less than 20%.

<sup>b</sup> Because RP4372 prestimulus behavior was smooth swimming, a smooth-swimming response to aspartate or serine would not be measurable.

ming was also seen when the *S. typhimurium* aspartate receptor was expressed at either high or low levels (24). Consequently, there appears to be a general threshold of receptor number required for random motility, and this requirement can be met by Tip receptors.

Since Tip allowed a receptor mutant lacking the aspartate, serine, and Tap receptors to migrate on L broth plates, these receptors were considered likely candidates for correspondence to Tip. Although the aspartate and serine receptor genes in *S. typhimurium* have been functionally defined by ligand-binding and by mutations (7; S. Panasenko and D. E. Koshland, Jr., unpublished observations), only the aspartate receptor gene has been cloned (9). Therefore, the possibility that *tip* was actually a serine or duplicate aspartate receptor gene was tested. Unfortunately, the specificity of Tap is not known, and so complementation of Tap could not be directly tested.

Introduction of the *tip* gene into receptor mutants did not restore aspartate or serine signaling activity (Table 1). This result was confirmed by tethered-cell analysis under conditions for which the shortest detectable response was about 15 s. A response shorter than 15 s would not be expected if Tip recognized aspartate, since wild-type cells respond for 2 to 3 min and cells with overproduced aspartate receptors respond to aspartate for 45 min (17, 24). The same logic applies for serine recognition. Therefore, Tip is not a duplicate aspartate receptor, nor is it a serine receptor.

The chemotaxis ability of cells containing Tip receptors was measured on soft-agar plates. The receptor mutant RP4372 could not migrate on L broth or tryptone, whereas the presence of only aspartate or serine receptor genes allowed migration at the wild-type rate (Table 2). Tip receptors expressed in RP4372 receptor mutant restored migration on tryptone or L broth plates, although at only 15 to 20% the wild-type rate (Fig. 3; Table 2).

An intriguing observation was that overproduction of Tip slightly increased the migration rate on L broth plates relative to the rate with a low gene dosage of Tip (Table 2). Overproduction of Tip from a multicopy plasmid (high gene dosage) was confirmed by SDS-polyacrylamide gel electrophoresis, which indicated about 10-fold-higher levels than from stable lysogens expressing Tip (low gene dosage). This is in sharp contrast to overproduction of the aspartate receptor, which reduces migration on L broth to 30% of the low gene dosage rate (24) (Table 2). For the overproduced

aspartate receptor, the decreased migration is probably due to the 10-fold-longer response time to aspartate stimulation. The possibility that whatever Tip recognizes in tryptone is not saturating, and consequently that there are relatively short response times even with overproduced Tip receptors, is discussed below.

The inability of Tip to recognize aspartate or serine shown by the free-swimming and tethered cell analysis was confirmed by measuring chemotaxis ability on minimal soft-agar plates containing aspartate or serine. Expression of Tip in receptor mutants RP4324  $\Delta$ (*tar-tap*)5201 RP4368 (*tsr-518*) or RP4372 [ $\Delta$ (*tar-tap*)5201 *tsr-518*] did not allow migration on the appropriate minimal plates (Fig. 3; Table 2). As controls, cells lacking the aspartate (RP4324) or serine (RP4368) receptors could not migrate well toward those attractants on minimal soft-agar plates (Table 2). These results are consistent with the direct behavioral assay, demonstrating that Tip does not recognize aspartate or serine.

Since it has previously been noted that metabolic waste products are potent repellents (31), the possibility that Tip is a repellent receptor was addressed. The poor migration of cells containing Tip on minimal medium plates indicates that Tip is not a receptor for repellents produced as metabolic byproducts during cell growth. However, we cannot completely exclude repellent recognition by Tip, since a repellent could be produced when cells are grown on tryptone but not on minimal media. Two relatively potent repellents, indole and phenol, were tested to see whether these chemicals might be focused through Tip. However, Tip does not appear to be an indole or phenol receptor, since comparable responses were seen in the receptor mutant RP4372 with or without Tip receptors (Table 1).

**Separation of swimming bias and chemotactic ability.** It could be argued that Tip expression simply shifted the smooth-swimming bias to random, which then allowed the cells to respond to stimuli detected by other receptors still present in RP4372 (e.g., sugars or oxygen). This possibility was addressed by expressing the CheY protein from a multicopy plasmid (8) to allow the receptor mutant RP4372 to reverse swimming directions in the absence of Tip receptors. This plasmid contains *cheY* behind the *tac* promoter, which is regulatable by isopropylthiogalactopyranoside (IPTG). Expression of the *cheY* gene, even in the absence of inducer IPTG, was sufficient to shift the receptor mutant prestimulus behavior from counter-clockwise to clockwise flagellar rotations as described by Clegg and Koshland (8).

TABLE 2. Chemotaxis ability of cells containing Tip

Strain	Migration rate (mm/h) <sup>a</sup> in:		
	L-Broth	Aspartate	Serine
RP437 wild type	4.0	1.12	1.34
RP4372 <i>tar tap tsr</i>	0.15	0.05	0.05
RP4372 $\lambda$ Tip	0.56	0.06	0.06
RP4372 p Tip	0.76	0.04	0.04
RP4372 $\lambda$ Tar	3.9	ND	ND
RP4372 p Tar	0.66	0.10	0.02
RP4324 <i>tar tap</i>	3.8	0.54	1.98
RP4324 $\lambda$ Tip	ND <sup>b</sup>	0.50	1.66
RP4368 <i>tsr</i>	4.0	1.12	0.46
RP4368 $\lambda$ Tip	ND	0.86	0.42

<sup>a</sup> Migration rates were measured on soft-agar plates as the increase in colony diameter in millimeters per hour. The average of three experiments is given, from which the standard error of the mean was less than 20%.

<sup>b</sup> ND, Not determined.

As controls, CheY overexpression shifted the tethered-cell behavior of wild-type (random) and the *cheY* mutant (counter-clockwise) to clockwise flagellar rotations similar to those of the receptor mutant.

Since clockwise rotation is ordinarily a nonchemotactic phenotype, it was critical to demonstrate first that wild-type cells containing high levels of CheY protein could migrate on the soft-agar plates, and second that these levels of CheY could complement the smooth-swimming and nonchemotactic phenotype of *cheY* mutants. It seemed reasonable that cells with excess CheY could still migrate on soft-agar plates, since wild-type cells with CheY plasmid can still respond to serine (8), which was a major component of the plates. In addition, there would be a selection for cells with less clockwise bias, for example, cells with a lower plasmid copy number, that could migrate on the soft-agar plates. When migration was measured, we found that wild-type cells

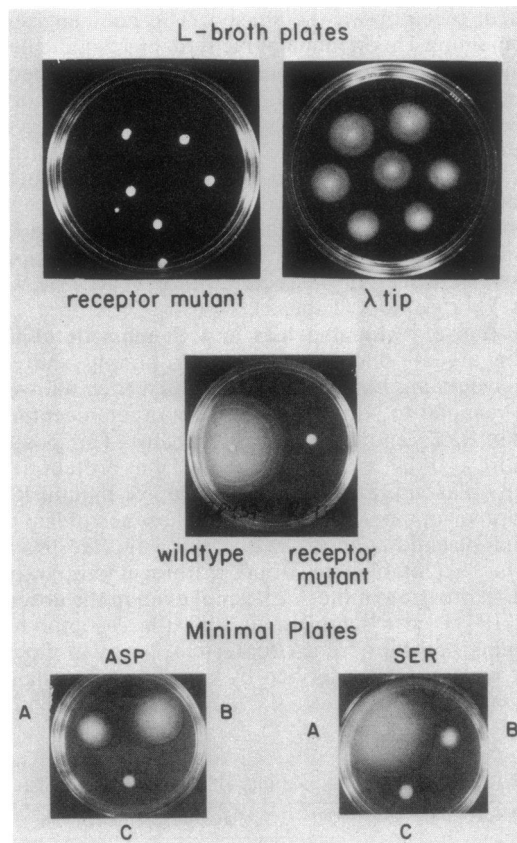


FIG. 3. Effect of Tip receptor on chemotaxis. Top, single colonies of receptor mutant RP4372 and RP4372  $\lambda$  Tip incubated on L broth soft-agar plates for 24 h. Middle, wild-type RP437 and receptor mutant RP4372 for comparison at 12 h. Bottom, cells incubated for 42 h on VBC-glycerol soft-agar plates containing either L-aspartate (left) or L-serine (right). The cells are as follows: (A) the *tar tap* receptor mutant RP4324, which did not migrate well on aspartate plates; (B) the *tsr* receptor mutant RP4368, which did not migrate on serine plates; (C) the *tar tap tsr* receptor mutant RP4372 lysogenized with a lambda phage containing the *tip* gene, which did not restore migration ability on either aspartate or serine plates. As controls, RP437 wild-type migrated at the same rate on both plates, RP4372 did not migrate on either plate, and *tip* expressed in RP4324 or RP4368 did not complement their migration defects (Table 2).

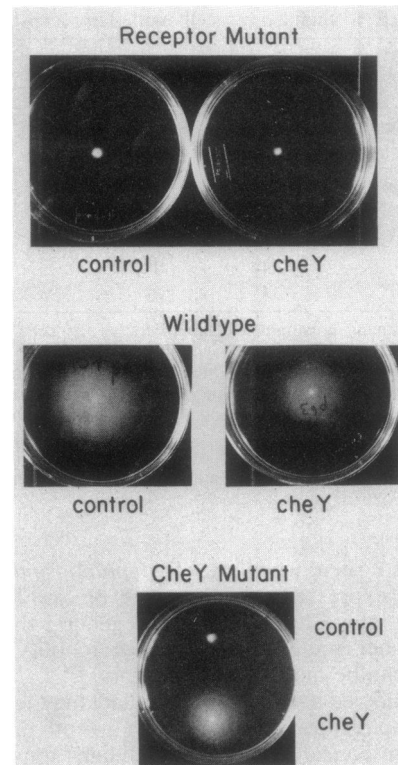


FIG. 4. Chemotaxis ability of receptor mutant containing over-produced CheY. Receptor mutant RP4372 was transformed with CheY plasmid pCheY or control plasmid p $\Delta$ CheY, and transformants were incubated on L broth-soft-agar plates for 20 h. As controls, CheY and control plasmids were expressed in wild-type RP437 cells and *cheY* mutant RP4315 cells. The migration rates were as follows: RP437p $\Delta$ CheY, 3.7 mm/h; pCheY, 3.2 mm/h; RP4315 p $\Delta$ CheY, 0.4 mm/h; pCheY, 3.2 mm/h; RP4372 p $\Delta$ CheY; 0.3 mm/h; pCheY, 0.3 mm/h measured as increase in colony diameter, CheY expression was confirmed by measuring the rotational bias of tethered cells. The cells with CheY plasmid had almost 100% clockwise rotation, and the controls had the expected behavior (RP4372, counter-clockwise; RP437, reversing; RP4315, counter-clockwise).

with plasmid-encoded CheY could still migrate despite the clockwise rotation bias, although at a slower rate than cells with control plasmid (Fig. 4). In addition the *cheY* mutant with plasmid-encoded CheY could now migrate on chemotaxis plates, whereas without CheY plasmid it was unable to do so (Fig. 4).

The CheY plasmid was then introduced into the receptor mutant RP4372, and the cells were tested for migration on L broth-soft-agar plates. We found that the receptor mutant could not migrate even when its smooth swimming had been corrected by CheY (Fig. 4). These results indicate that Tip does not simply act by correcting the defective smooth-swimming motility of receptor mutant RP4372. Presumably, Tip allows migration on tryptone plates by recognizing one or more chemotactic stimulus in the medium. Since tryptone is a pancreatic digest of casein, it seems likely that the stimuli are amino acids or derivatives of amino acids, such as phosphoserine.

## DISCUSSION

We have described an *S. typhimurium* gene which encodes a receptorlike protein. This gene, termed *tip* for taxis-

involved protein, has considerable homology with a family of receptor genes identified with bacterial chemotaxis. Within this family of receptors encoded by the *tar*, *tsr*, *tap*, and *trg* genes from *E. coli* and *S. typhimurium*, there is a striking degree of structural and functional homology. The primary sequences of these receptors predict that all share a similar structural motif (1, 18, 24). There are two potential membrane-spanning regions which define a variable extracellular amino-terminal domain and a constant cytoplasmic carboxy-terminal domain (24). Functional homology is demonstrated from cross-species complementation (9, 10) and because these receptors are a common substrate for the CheR methylase and CheB demethylase (28, 30). The Tip receptor also appears to share this sequence homology and methyl-accepting capacity.

For the homologous chemotaxis receptors, circumstantial evidence leads to a comparison between *S. typhimurium* Tip and *E. coli* Tap as possible corresponding receptors. The potential for correspondence is supported by common behavioral properties discussed below. However, one argument that these genes differ is that *tip* is not located tandem to the *S. typhimurium* aspartate receptor gene (D. Clegg and D. E. Koshland, Jr., manuscript in preparation), whereas *tap* is tandem to the *E. coli* aspartate receptor gene (3, 34). This is in sharp contrast to the similar chromosomal locations seen so far among the chemotaxis genes from *E. coli* and *S. typhimurium* (9, 10). Although it is possible that *tip* and *tap* were originally in the same relative location and have since rearranged, the different chromosomal locations suggest that these genes arose after *E. coli* and *S. typhimurium* species diverged. In this regard, several chemoeffectors have been identified which are species specific; for example, citrate, which is recognized as a chemoeffector and utilized as a carbon source by *Salmonella* spp. but not by *E. coli* (13, 15). Therefore, it is a reasonable possibility that Tip and Tap are species-specific receptors which recognize different chemicals in tryptone.

Although positive correspondence of Tip and Tap must await identification of their ligand specificity, these proteins do share some common functional features on the basis of behavioral properties reported here for Tip and by Clegg and Koshland (manuscript in preparation) for Tap. First, introduction of either Tip or Tap into the triple-receptor mutant allows a migration rate on the soft-agar plates that is only 20% that of the wild type. In contrast, comparable expression of the aspartate receptor in this mutant completely restored chemotactic behavior. One explanation for the partial complementation seen with Tip and Tap is that the unknown chemoeffectors recognized by these receptors are not at an optimal concentration in the tryptone plates. Reduced migration ability of cells containing aspartate receptors can be observed when the amount of aspartate in minimal soft-agar plates is decreased. Second, overproduction of Tip or Tap slightly increased the migration rate on soft-agar L broth plates relative to that produced by a low gene dosage. For Tap, there was increased migration on soft-agar plates when RP4372 pTap cells were treated with 1 mM IPTG to induce the TAC promoter controlling *tap* expression (0.2 to 0.7 mm/h) (Clegg and Koshland, Jr., manuscript in preparation). This is in sharp contrast to the threefold-decreased migration rate observed when the aspartate receptor is overproduced. This decreased migration is attributable to the 10-fold-increased adaptation time to saturating levels of aspartate found in tryptone medium (4.8 mM; Difco) (24). Consequently, there exists a rough correlation between adaptation time and migration rate, as

well as between adaptation time and receptor level (17) and chemoeffector concentration (19). With these relationships, one explanation for why migration rates were not decreased with Tip and Tap overproduction would be that their chemoeffectors were at subsaturating concentrations in tryptone. Taken together, these results suggest that the Tip receptor directly recognizes an effector in tryptone, possibly similar to the effector recognized by Tap receptors, and that this effector is at subsaturating levels.

An alternative explanation is that Tip (and Tap) partially complement RP4372 by an indirect mechanism. In this case, Tip would allow an improvement in taxis to stimuli not directly recognized by Tip, such as oxygen, phosphotransferase system sugars, or Trg sugars. The possibility that Tip allowed taxis to other stimuli by simply resetting the basal swimming bias was ruled out by the CheY experiments. However, although both Tip and CheY reset the bias, they most likely do so at different steps in the information processing from receptors to flagella (8). In this regard, it has been reported that, for unknown reasons, RP4372 has defective adaptation to Trg sugars and PTS sugars, and has low methylation of Trg receptors (11). Consequently, expression of Tip might conceivably restore taxis to other stimuli by resetting the adaptation machinery or possibly the signal processing system. Elucidation of the ligand specificity of Tip should resolve the role of Tip in bacterial sensing as well as the relation between the *S. typhimurium* Tip and *E. coli* Tap receptors.

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