infection with *L. major* may derive from the loss of the ability to generate IL-12–induced T_H1 responses rather than from an IL-4–induced T_H2 response (*23*). Here we propose that susceptibility to murine leishmaniasis, and possibly to other infectious diseases, is also determined by the ability of the infected host to mount a strong T_H2-polarized response against a restricted set of antigens. Thus, immune interventions aimed at modifying the T cell repertoire may be used to alter the course of an infection, as has been shown in the experimental treatment of autoimmune diseases (*24*). Tolerization protocols such as oral, intranasal, or intravenous administration of antigens (*25*) may be considered for down-regulation of deleterious immune responses and for conferring long-term protection against infectious diseases.

REFERENCES AND NOTES

1. T. R. Mosmann and S. Sad, *Immunol. Today* **17**, 138 (1996).
2. M. D. Sadick, F. P. Heinzel, V. M. Shigekane, W. L. Fisher, R. M. Locksley, *J. Immunol.* **139**, 1303 (1987); R. P. Heinzel, M. D. Sadick, B. J. Holaday, R. L. Coffman, R. M. Locksley, *J. Exp. Med.* **169**, 59 (1989); F. P. Heinzel, M. D. Sadick, S. S. Mutha, R. M. Locksley, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7011 (1991); L. Morris, T. Aebisher, E. Handman, A. Kelso, *Int. Immunol.* **5**, 761 (1993).
3. M. D. Sadick *et al.*, *J. Exp. Med.* **171**, 115 (1990).
4. R. A. Seder, W. E. Paul, M. M. Davis, B. Fazekas de St. Groth, *ibid.* **176**, 1091 (1992); R. A. Seder and W. E. Paul, *Annu. Rev. Immunol.* **12**, 635 (1994).
5. S. L. Reiner, S. Zheng, Z.-E. Wang, L. Stowring, R. M. Locksley, *J. Exp. Med.* **179**, 447 (1994).
6. A. Bendelac, N. Killeen, D. R. Littman, R. H. Schwartz, *Science* **263**, 1774 (1994); A. Bendelac *et al.*, *ibid.* **268**, 863 (1995).
7. S. Reiner, personal communication.
8. E. Mougneau *et al.*, *Science* **268**, 563 (1995).
9. S. L. Reiner, Z.-E. Wang, F. Hatam, P. Scott, R. Locksley, *ibid.* **259**, 1457 (1993).
10. Parasite-specific T cell clones were purified after incubation with SLA (50 μg/ml) and IL-2 (20 U/ml) as described (*26*). Reactivity to LACK was assessed by measurement of the amounts of IFN-γ and IL-4 released by each clone after incubation of 5×10^4 T cells with 2×10^6 mitomycin C–treated syngeneic splenocytes and SLA (50 μg/ml) or 30 μM LACK synthetic peptide (amino acids 158 through 173).
11. For the ELISPOT assay, 5×10^6 purified $CD4^+$ lymph node cells were activated in vitro with 5×10^6 mitomycin C–treated syngeneic splenocytes and SLA (50 μg/ml) or with an optimum concentration of LACK (amino acids 158 through 173) (FSPSLEHPIV-VSGSWD) (*27*), GP63 (amino acids 364 through 378) (GSCTQRASEAHASLL), or LDP23 (amino acids 120 through 137) (RAHWARKVFDEKDAKTPV) synthetic peptides in 1 ml of Dulbecco's minimum essential medium. SLA was prepared as previously described (*28*). After culture for 72 hours, live cells were recovered by passage through Ficoll-Hypaque, washed, and transferred by serial dilution (from 10^5 to 5×10^3 cells per well) in triplicate to 96-well microtiter plates (Millipore, Bedford, MA) that had been coated with the capturing monoclonal antibodies (mAbs) (2 μg/ml), antibody to IFN-γ, antibody to IL-4, or antibody to IL-5. After 24 hours, cells were removed and spot-forming cells were visualized with biotinylated detecting mAbs and avidin D peroxidase in conjunction with 3-amino-9-ethylcarbazole (Sigma) substrate. Spots were counted under a dissecting microscope, and the frequency of antigen-specific cells was determined from the difference between the number of spots seen with and without antigen.
12. L. R. B. Soarez, E. E. Sercarz, A. Miller, *Int. Immunol.* **6**, 785 (1994).
13. A. Campos-Neto *et al.*, *J. Exp. Med.* **182**, 1423 (1995).
14. IE-LACK transgenic mice were generated with a LACK cDNA subcloned into the pDOI transgene expression vector [W. Van Ewijk *et al.*, *Cell* **53**, 357 (1988)]. These mice were produced by microinjection of fertilized B6/D2 F_1 mouse embryos and implantation into pseudopregnant foster mothers. The IE-LACK trangenic mice studied were backcrossed eight generations to BALB/c mice and were maintained under specific pathogen-free conditions.
15. Mice were immunized with 50 μg of recombinant LACK antigen in IFA. Ten days later, popliteal lymph node cells were prepared, and 2×10^6 cells were incubated in triplicate for 72 hours in a 96-well flat-bottomed plate with 30 μM LACK peptide, concanavalin A (2 μg/ml), or medium alone. Cells were pulsed with 1 μCi of [^{3}H]thymidine during the last 18 hours of culture. Plates were harvested and counted on a Betaplate Reader (Pharmacia, LKB). Parallel cultures were set up to measure amounts of cytokines in the supernatants by enzyme-linked immunosorbent assay, with the use of specific mAbs to IL-4, IL-5, or IFN-γ (PharMingen, San Diego, CA).
16. Because the IE-LACK transgenic mice were initially made on a B6/D2 F_1 background, we cannot completely rule out the possibility that the transgene integrated near and segregated with a resistant gene despite eight generations of backcrossing to BALB/c mice. This is, however, very unlikely to happen.
17. S. Constant, C. Pfeiffer, A. Woodard, T. Pasqualini, K. Bottomly, *J. Exp. Med.* **182**, 1591 (1995); N. A. Hosken, K. Shibuya, A. W. Heath, K. M. Murphy, A. O'Garra, *ibid.*, p. 1579.
18. V. Kumar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9510 (1995).
19. T. F. Gajewski, M. Pinnas, T. Wong, F. W. Fitch, *J. Immunol.* **146**, 1750 (1991); T.-L. Chang *et al.*, *ibid.* **145**, 2803 (1990).
20. L. Carrera *et al.*, *J. Exp. Med.* **183**, 515 (1996).
21. M. Roberts, B. A. Mock, J. M. Blackwell, *Eur. J. Immunogenet.* **20**, 349 (1993).
22. A. H. Shankar and R. G. Titus, *J. Exp. Med.* **181**, 845 (1995).
23. M. L. Güler *et al.*, *Science* **271**, 984 (1996).
24. J. L. Urban *et al.*, *Cell* **54**, 577 (1988); H. Acha-Orbea *et al.*, *ibid.*, p. 263.
25. A. M. Mowat, *Immunol. Today* **8**, 93 (1987); K. L. Weiner *et al.*, *Annu. Rev. Immunol.* **12**, 809 (1994); R. S. Liblau *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3031 (1996).
26. L. Morris, A. B. Troutt, E. Handman, A. Kelso, *J. Immunol.* **149**, 2715 (1992).
27. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
28. P. Scott, E. Pearce, P. Natovitz, A. Sher, *J. Immunol.* **139**, 3118 (1987).

29. We thank K. Marcu, S. McSorley, and E. Sercarz for reviewing this manuscript; D. Mathis, C. Benoist, and C. Gabaglia for reagents; and F. Altare for preparing the IE-LACK transgene. Supported by grants from the Conseil Régional de la Région PACA, the Special Program for Research and Training in Tropical Diseases (United Nations Development Programme–World Bank–World Health Organization), and the Ministère de l'Education Nationale, de la Recherche et de l'Enseignement Supérieur (France).

31 May 1996; accepted 6 August 1996

Signaling by the *Escherichia coli* Aspartate Chemoreceptor Tar with a Single Cytoplasmic Domain per Dimer

Ichiro Tatsuno, Michio Homma, Kenji Oosawa, Ikuro Kawagishi*

Many transmembrane receptors are oligomeric proteins. Binding of a ligand may alter the oligomeric state of the receptor, induce structural changes within the oligomer, or both. The bacterial aspartate chemoreceptor Tar forms a homodimer in the presence or absence of ligands. Tar mediates attractant and repellent responses by modulating the activity of the cytoplasmic kinase CheA. In vivo intersubunit suppression was used to show that certain combinations of full-length and truncated mutant Tar proteins complemented each other to restore attractant responses to aspartate. These results suggest that heterodimers with only one intact cytoplasmic domain are functional. The signaling mechanism may require interactions between dimers or conformational changes within a single cytoplasmic domain.

The aspartate chemoreceptor Tar of enteric bacteria forms a homodimer (*1*), each subunit of which has two transmembrane segments (TM1 and TM2). The Tar dimer forms a stable complex with a homodimer of the cytoplasmic histidine protein kinase CheA and two molecules of the cytoplasmic protein CheW (*2*). Binding of a repellent or an attractant activates or inactivates, respectively, CheA autophosphorylation and phosphotransfer to the cytoplasmic protein CheY. Phospho-CheY switches the direction of flagellar rotation to clockwise (*3*).

Aspartate binds to one of two sites at the subunit interface of the Tar homodimer (*4*, *5*). Because artificially disulfide–cross-linked dimers still function (*1*), signaling appears not to be mediated by a monomer-dimer transition. The TM1/TM1′ pair seems to be static (*4*, *6*, *7*), but TM2 has been proposed to move vertically, tilt, or rotate in response to aspartate binding (*6*, *8*). This movement could

I. Tatsuno, M. Homma, I. Kawagishi, Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan.
K. Oosawa, Graduate School of Polymathematics, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan.

*To whom correspondence should be addressed.

change intradimer or interdimer interactions in the cytoplasmic domains. Oligomerization of cytoplasmic fragments of Tar occurs only in fragments derived from "smooth swimming" (CheA kinase–inhibiting) mutants (9). On the other hand, dimerization of a cytoplasmic fragment of Tar fused to a leucine-zipper domain activates CheA kinase (*10*).

We tested the in vivo signaling function of mutant heterodimers that have a single cytoplasmic domain. The Ala^{19}→Lys (A19K) substitution in TM1 is suppressible by the Ala^{198}→Glu (A198E) mutation in TM2 (Fig. 1A) (*11*). When the Thr^{154}→Pro (T154P) substitution, which causes a severe defect in aspartate sensing (*12*), was introduced into Tar-A198E, the Tar-A198E/T154P receptor did not mediate an aspartate response (Fig. 1B) (*13*). However, when Tar-A19K and Tar-A198E/T154P were coexpressed, the cells responded to aspartate, indicating that the heterodimer is functional (Fig. 1B) (*13*).

Seven deletion derivatives of *tar-A198E* were constructed (Fig. 1C); all encoded receptors lacking regions important for signal production (*14*). When expressed in strain RP4372*recA* (*15*), which itself lacks Tar, the truncated receptors, like Tar-A19K, did not allow the cells to respond to aspartate (*16*).

The truncated receptors were then coexpressed with Tar-A19K (*17*), and their aspartate responses were examined in the temporal stimulation assay (Fig. 2). When the cells, which originally swam smoothly (*16*), were treated with the repellent 1 M glycerol (*18*), the smooth swimming fraction fell below 5% (Fig. 2, A to F) (*19*). Subsequent addition of aspartate caused smooth swimming responses in cells expressing Tar-A19K together with each of the six deletion derivatives of Tar-A198E (Fig. 2, A to F) (*20*). The concentration of aspartate required to produce smooth swimming in 50% of these cells (about 3 μM) was only slightly greater than that for cells expressing wild-type Tar (about 0.3 μM) (Fig. 2H). These results suggest that one of the two cytoplasmic signaling domains in a dimer is dispensable for generating an attractant response to aspartate (*21*).

Cells coexpressing Tar-A19K and Tar-A198EΔNru showed only a weak response to aspartate (Fig. 2G). Tar-A198EΔNru lacks not only the signaling domain but also a large portion of the "linker" region, suggesting that intersubunit interactions in this region may be required for signaling.

Bacterial chemoreceptors are reversibly methylated at specific glutamate residues (*3*). Attractant and repellent stimuli cause net increases and decreases in the methylation level of the receptor, respectively, resulting in adaptation. Methylation in vitro of a disulfide–cross-linked Tar heterodimer consisting of one full-length subunit and one subunit lacking the cytoplasmic domain was shown to be regulated by aspartate (*22*). Similarly, in our experiments, an aspartate response was accompanied by increased methylation of the full-length Tar-A19K in the heterodimer (*16*). These results reinforce the conclusion that the one cytoplasmic domain attains an active conformation.

Our results raise the possibility that signaling by Tar may be mediated by interaction between dimers, conformational changes within a single cytoplasmic domain, or both. Although there is no direct evidence for interactions between dimers, receptors cluster with CheA and CheW at the poles of *Escherichia coli* cells (*23*), and methyltransferase bound to one receptor dimer is predicted to catalyze methylation of another receptor dimer (*24*). It has also been reported that oligomerization of the cytoplasmic domain affects signaling state (*9*, *10*). Therefore, direct or indirect (*25*) communication between Tar dimers might be important for signaling. On the other hand, receptor-mediated control of a CheA heterodimer with only one COOH-terminal regulatory domain has been reported (*26*). Therefore, a single cytoplasmic domain of a receptor might be able to regulate CheA activity at least under some conditions.

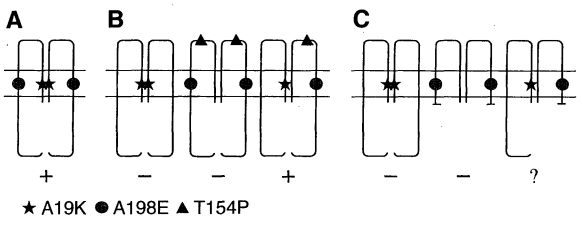

Fig. 1. Intersubunit suppression of the TM1 mutation A19K by the TM2 mutation A198E. (**A**) Tar-A19K/A198E homodimer. The A19K substitution in TM1 is suppressed by the A198E substitution in TM2 (*11*). (**B**) Intersubunit suppression between A19K and A198E (*13*). When Tar-A19K and Tar-A198E/T154P are coexpressed in a receptorless strain, three types of Tar dimers should be formed. (+) Capable of mediating an aspartate response; (−) incapable of mediating an aspartate response. (**C**) In vivo functional assay of Tar dimer lacking one cytoplasmic signaling domain.

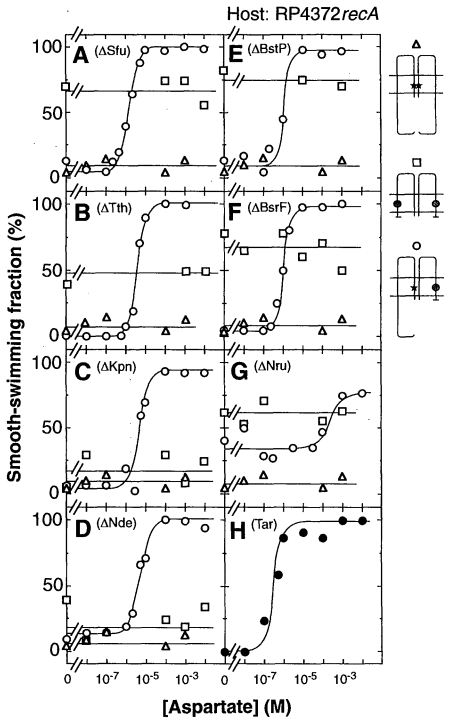

Fig. 2. Aspartate-sensing abilities of heterodimers with only one signaling domain. Chemotactic responses to aspartate of RP4372*recA* cells expressing Tar-A19K and a deletion derivative of Tar-A198E were measured with the temporal stimulation assay (*27*). Exponential cultures in tryptone-glycerol broth growing at 30°C were washed twice and resuspended in motility medium at 25°C. These cells were treated with 1 M glycerol to reduce the smooth swimming fraction and then stimulated with various concentrations of L-aspartate. After 30 s, the percentage of smooth swimming cells was measured. Triangles, Tar-A19K alone; squares, a deleted version of Tar-A198E alone; circles, Tar-A19K and a deleted version of Tar-A198E. The deleted versions are (**A**) Tar-A198EΔSfu, (**B**) Tar-A198EΔTth, (**C**) Tar-A198EΔKpn, (**D**) Tar-A198EΔNde, (**E**) Tar-A198EΔBstP, (**F**) Tar-A198EΔBsrF, and (**G**) Tar-A198EΔNru. As a control, responses of cells expressing wild-type Tar are shown in (**H**).

REFERENCES AND NOTES

1. D. L. Milligan and D. E. Koshland Jr., *J. Biol. Chem.* **263**, 6268 (1988).
2. J. A. Gegner, D. R. Graham, A. F. Roth, F. W. Dahlquist, *Cell* **70**, 975 (1992); S. C. Schuster, R. V. Swanson, L. A. Alex, R. B. Bourret, M. I. Simon, *Nature* **365**, 343 (1993).
3. For reviews, see R. B. Bourret, K. A. Borkovich, M. I. Simon, *Annu. Rev. Biochem.* **60**, 401 (1991); J. B. Stock, G. S. Lukat, A. M. Stock, *Annu. Rev. Biophys. Biophys. Chem.* **20**, 109 (1991); J. S. Parkinson and E. C. Kofoid, *Annu. Rev. Genet.* **26**, 71 (1992); M. D. Manson, *Adv. Microbial Physiol.* **33**, 277 (1992); J. S. Parkinson, *Cell* **73**, 857 (1993).
4. M. V. Milburn *et al.*, *Science* **254**, 1342 (1991); W. G. Scott *et al.*, *J. Mol. Biol.* **232**, 555 (1993); J. I. Yeh, H.-P. Biemann, J. Pandit, D. E. Koshland Jr., S.-H. Kim, *J. Biol. Chem.* **268**, 9787 (1993).
5. H.-P. Biemann and D. E. Koshland Jr., *Biochemistry* **33**, 629 (1994).
6. S.-H. Kim, G. G. Prive, J. Yeh, W. G. Scott, M. V. Mil-

burn, *Cold Spring Harbor Symp. Quant. Biol.* **57**, 17 (1992).
7. S. A. Chervitz and J. J. Falke, *Biochemistry* **34**, 9722 (1995); G. F. Lee, M. R. Lebert, A. A. Lilly, G. L. Hazelbauer, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3391 (1995).
8. I. N. Maruyama, Y. G. Mikawa, H. I. Maruyama, *J. Mol. Biol.* **253**, 530 (1995); S. A. Chervitz and J. J. Falke, *J. Biol. Chem.* **270**, 24043 (1995); *Proc. Natl. Acad. Sci. U.S.A.* **93**, 2545 (1996).
9. D. G. Long and R. M. Weis, *Biochemistry* **31**, 9904 (1992).
10. A. G. Cochran and P. S. Kim, *Science* **271**, 1113 (1996).
11. K. Oosawa and M. I. Simon, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6930 (1986).
12. L. Lee and Y. Imae, *J. Bacteriol.* **172**, 377 (1990).
13. I. Kawagishi, I. Tatsuno, M. Shibazaki, K. Oosawa, M. Homma, in preparation.
14. Tar-A198EΔSfu lacks three-quarters of the signaling region but retains the first three methylation sites (K1 region). Tar-A198EΔKpn completely lacks the signaling region. Tar-A198EΔTth lacks the first two-thirds of the cytoplasmic signaling domain and retains the COOH-terminal 160 residues, including the fourth methylation site (R1 region). Tar-A198EΔNde, Tar-A198EΔBstP, Tar-A198EΔBsrF, and Tar-A198EΔNru lack part of the linker region and all of the signaling domain. When expressed in strain RP4372*recA*, none of these mutant receptors mediated an aspartate response (Fig. 2).
15. This strain produces only the ribose-galactose chemoreceptor Trg [F⁻ *thi thr leu met eda rpsL* Δ(*tar-tap*)*5201 tsr-1*; A. Boyd, A. Krikos, M. I. Simon, *Cell* **26**, 333 (1981)].
16. I. Tatsuno, M. Homma, K. Oosawa, I. Kawagishi, unpublished results.
17. We also examined swarming abilities of the cells in tryptone semisolid agar (0.25%). Many of them swarmed, whereas cells expressing Tar-A198EΔTth, Tar-A198EΔBstP, Tar-A198EΔBsrF, and Tar-A198EΔNru did not. However, even those cells that did not swarm responded to aspartate (Fig. 2).
18. K. Oosawa and Y. Imae, *J. Bacteriol.* **154**, 104 (1983); *ibid.* **157**, 576 (1984).
19. Cells expressing only Tar-A19K also responded to 1 M glycerol (Fig. 2, A to G). However, when strain HCB339 [A. J. Wolfe, M. P. Conley, T. J. Kramer, H. C. Berg, *J. Bacteriol.* **169**, 1878 (1987)], which lacks all four chemoreceptors, was used as a host, cells coexpressing Tar-A19K and one of the deleted versions of Tar responded to glycerol, whereas cells expressing only Tar-A19K did not respond. Therefore, the Tar-A19K homodimer seems to lack the ability to mediate a glycerol response.
20. Similar results were obtained with strain HCB339 as the host, suggesting that Trg is not responsible for the aspartate responses of RP4372*recA* cells expressing the heterodimers.
21. These heterodimers were functional when the residue Thr^{154} in the truncated subunit was changed to Pro, but not functional when the residue in the full-length subunit was mutated, suggesting that the cytoplasmic domain of the subunit in which residue Thr^{154} is in contact with aspartate is responsible for signal production (*16*).
22. D. L. Milligan and D. E. Koshland Jr., *Science* **254**, 1651 (1991).
23. J. R. Maddock and L. Shapiro, *ibid.* **259**, 1717 (1993).
24. J. Wu, J. Li, G. Li, D. G. Long, R. M. Weis, *Biochemistry* **35**, 4984 (1996); H. Okumura, M. Homma, I. Kawagishi, unpublished results.
25. P. J. Gardina and M. D. Manson [*Science* **274**, 425 (1996)] discuss possible mechanisms for such indirect interaction.
26. A. J. Wolfe, B. P. McNamara, R. C. Stewart, *J. Bacteriol.* **176**, 4483 (1994).
27. I. Tatsuno, L. Lee, I. Kawagishi, M. Homma, Y. Imae, *Mol. Microbiol.* **14**, 755 (1994).
28. We thank M. D. Manson and P. J. Gardina for communicating unpublished results and for helpful discussions. Supported in part by grants-in-aid for scientific research to I.K. from the Ministry of Education, Science, and Culture of Japan.

16 April 1996; accepted 21 August 1996

Attractant Signaling by an Aspartate Chemoreceptor Dimer with a Single Cytoplasmic Domain

Paul J. Gardina and Michael D. Manson*

Signal transduction across cell membranes often involves interactions among identical receptor subunits, but the contribution of individual subunits is not well understood. The chemoreceptors of enteric bacteria mediate attractant responses by interrupting a phosphotransfer circuit initiated at receptor complexes with the protein kinase CheA. The aspartate receptor (Tar) is a homodimer, and oligomerized cytoplasmic domains stimulate CheA activity much more than monomers do in vitro. Intragenic complementation was used to show in *Escherichia coli* that heterodimers containing one full-length and one truncated Tar subunit mediated responses to aspartate in the presence of full-length Tar homodimers that could not bind aspartate. Thus, a Tar dimer containing only one cytoplasmic domain can initiate an attractant (inhibitory) signal, although it may not be able to stimulate kinase activity of CheA.

Proposed mechanisms for signaling by multimeric transmembrane receptors range from ligand-induced oligomerization to conformational changes within individual receptor subunits. For many eukaryotic receptors, alternative signaling states correspond to altered interactions among cytoplasmic domains within multimers (*1*).

The aspartate chemoreceptor Tar of *E. coli* mediates an attractant response to L-aspartate. Aspartate binds across the interface of the periplasmic domains of a Tar homodimer at one of two rotationally symmetric binding sites (*2*). Each periplasmic domain is anchored in the cytoplasmic membrane by an NH_2-terminal transmembrane segment (TM1) and a second membrane segment (TM2) that connects to the cytoplasmic (signaling) domain (*3*). Ligand-free Tar enhances autophosphorylation of a CheA dimer, which is the initial component of a signaling circuit. Phosphate is then transferred to the CheY protein, which regulates the direction of flagellar rotation. Aspartate-bound Tar inhibits its CheA activity (*4*).

For Tar, ligand-induced dimerization is not the signaling mechanism; Tar is a dimer in the presence and absence of aspartate (*5*), and disulfide–cross-linked dimers mediate normal responses to aspartate (*6*). Furthermore, aspartate increases adaptive methylation in vitro of Tar dimers containing one full-length and one cytoplasmically truncated subunit (*7*). Thus, ligands appear to induce conformational changes within a single subunit. On the other hand, oligomerization of Tar cytoplasmic domains through leucine zippers strongly stimulates CheA in vitro (*8*), indicating that interdomain contacts are important in generating kinase activity.

We used a complementation system in which two defective Tar subunits must form heterodimers to bind aspartate. Two mutant *tar* genes, encoding Tar-R73K (*9*) and Tar-T154I (*10*), were engineered onto compatible plasmids, pMK113 and pRBB16 (*11*), and introduced into *E. coli* strain VB12 (*12*). The residues Arg^{73} and Thr^{154} are located in opposite halves of the aspartate-binding site (Fig. 1A). Cells expressing either Tar-R73K or Tar-T154I from both plasmids did not respond to aspartate (Fig. 1B) (*13*). Coexpression of the proteins, which form heterodimers with one intact binding site, did restore aspartate taxis (Fig. 1C).

This system was extended to test whether a heterodimer containing one full-length and one cytoplasmically truncated subunit mediates responses to aspartate (Fig. 1D). Plasmids pMK113-229_{oc} and pMK113-258_{oc} each contain a UAA (ochre) nonsense codon that terminates Tar 17 or 46 amino acids after TM2. The resulting proteins lack 95 and 85%, respectively, of the cytoplasmic domain, are of the predicted size, and fail to support any aspartate chemotaxis (*14*).

Plasmid pMK113-258_{oc} complemented pRBB16-R73K to produce normal chemotactic swarms in aspartate soft agar. A strain containing these plasmids also responded well in an assay in which cells migrated up a gradient into capillaries containing aspartate (Fig. 2). These results indicate that a heterodimer containing only one cytoplasmic domain can mediate an attractant signal.

Plasmid pMK113-258_{oc} did not restore aspartate taxis in combination with pRBB16-T154I, although the Tar-T154I/Tar-258_{oc} heterodimer should bind aspartate as well as the Tar-R73K/Tar-258_{oc} het-

Department of Biology, Texas A&M University, College Station, TX 77840, USA.

*To whom correspondence should be addressed.