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 I. Tatsuno, M. Homma, K. Oosawa, I. Kawagishi, unpublished results.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.

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

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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 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-

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erodimer. Thr^{154} contributes aspartate contacts on the fourth periplasmic helix of Tar, which connects directly to TM2 and has been implicated in conformational changes associated with transmembrane signaling (*15*). The half-site containing Thr^{154} may be critical for signal propagation as well as ligand binding. A plasmid, pMK113-T154I$_{oc}$, expressing Tar containing both the T154I and 258$_{oc}$ substitutions (*16*) also complemented pRBB16-R73K (Fig. 2), indicating that mutations altering the fourth helix impair signaling only when they affect the full-length transducer.

Plasmid pMK113-229$_{oc}$ did not complement pRBB16-R73K or pRBB16-T154I, although the 229- and 258-residue fragments were present in similar amounts. This suggests that the segment of Tar between residues 229 and 258 is necessary to form functional heterodimers. The segment may participate in dimerization, membrane localization, or signal propagation.

One interpretation of these results is that a Tar heterodimer with only one cytoplasmic domain can maintain CheA activity (*17*) and that this activity is inhibited when the heterodimer binds aspartate (cis-inhibition). Because oligomers of the cytoplasmic domain are required for maximal stimulation of CheA activity in vitro (*8*), the cytoplasmic domains of two heterodimers might coordinately stimulate CheA. This stimulation would then be reversed by aspartate.

Alternatively, aspartate may inhibit the activity of CheA dimers not directly associated with the ligand-bound receptor molecule. According to this scenario (trans-inhibition), a signal generated at an aspartate-bound heterodimer could counter CheA activity maintained by full-length homodimers that cannot bind aspartate. Such a process could also account for amplification of attractant signals in a wild-type cell. Possible mechanisms for trans-inhibition, all of which would be facilitated by the clustering of chemoreceptors observed in *E. coli* (*18*), include direct communication among signaling domains in large receptor aggregates, propagated inactivation of multiple CheA dimers, or activation of the CheZ phosphatase (*4*) that degrades phosphorylated CheY protein.

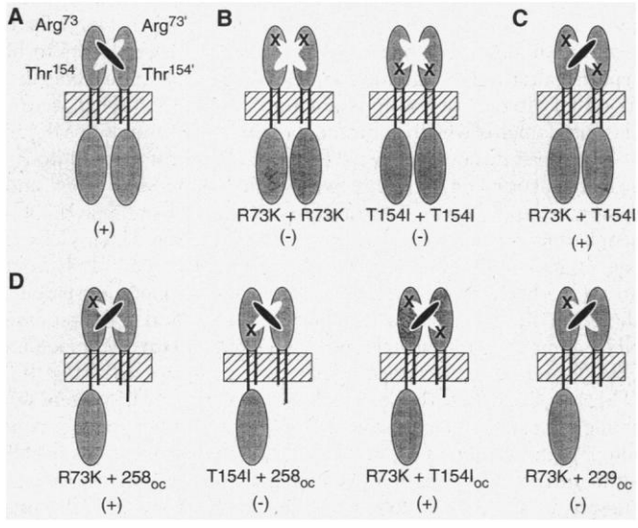

Fig. 1. Rationale for subunit complementation and summary of results. The aspartate response for each combination of proteins is shown as (+) or (−). (**A**) The wild-type dimer has two symmetrical aspartate-binding sites at the subunit interface. Because of negative cooperativity (*19*), aspartate (black oval) binds in only one or the other of the two possible orientations. (**B**) Expression of the same allele from both plasmids produces homodimers defective in equivalent half-sites in each subunit. Thus, neither homodimer has a functional aspartate-binding site. (**C**) In a cell expressing two different mutant Tar proteins, both homodimers (B) and heterodimers can form. A heterodimer defective in opposite halves of the binding site retains one complete binding site and mediates aspartate taxis. (**D**) The complementation pattern between full-length Tar proteins with binding defects and truncated Tar proteins is shown. In each case, homodimers (not shown) should form but will not respond to aspartate.

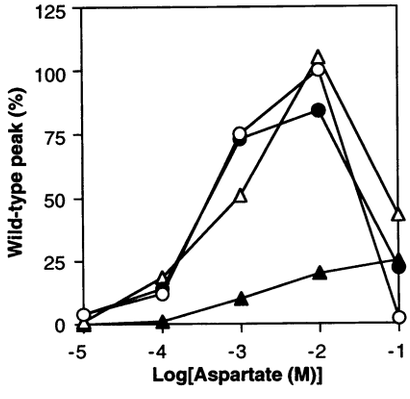

Fig. 2. Aspartate capillary assays with cells containing a truncated Tar subunit. Strain MM509 [*tsr*$^+$ Δ*tar-tap* (*9*)] doubly transformed with wild-type or mutant plasmids was tested for responses to different concentrations of aspartate (*9*). Plasmid combinations are: pRBB16 (*tar*$^+$) + pMK113-Δ*tar*, empty circles; pRBB16-R73K + pMK113-258$_{oc}$, filled circles; pRBB16-R73K + pMK113-T154I$_{oc}$, empty triangles; pRBB16-R73K + pMK113-Δ*tar*, filled triangles. The pMK113-Δ*tar* plasmid carries a deletion removing the promoter and 80% of the *tar* gene. All responses were normalized to the response to 1 mM serine, which is mediated by the chromosomally encoded Tsr receptor.

REFERENCES AND NOTES

1. Well-characterized receptors of this type include those for epidermal [Y. Yarden and J. Schlessinger, *Biochemistry* **26**, 1443 (1987)] and human [B. C. Cunningham *et al.*, *Science* **254**, 821 (1991)] growth factors and insulin [S. R. Hubbard, L. Wei, L. Ellis, W. A. Hendrickson, *Nature* **372**, 746 (1994)]. For a general review, see C.-H. Heldin, *Cell* **80**, 213 (1995).
2. M. V. Milburn *et al.*, *Science* **254**, 1342 (1991).
3. A. Krikos, N. Mutoh, A. Boyd, M. I. Simon, *Cell* **33**, 615 (1983).
4. J. B. Stock and M. G. Surette, in Escherichia coli *and* Salmonella: *Cellular and Molecular Biology*, F. C. Neidhardt *et al.*, Eds. (American Society for Microbiology Press, Washington, DC, ed. 2, 1996), pp. 1103–1129.
5. D. L. Milligan and D. E. Koshland Jr., *J. Biol. Chem.* **263**, 6268 (1988).
6. J. J. Falke and D. E. Koshland Jr., *Science* **237**, 1596 (1987).
7. D. L. Milligan and D. E. Koshland Jr., *ibid.* **254**, 1651 (1991).
8. A. G. Cochran and P. S. Kim, *ibid.* **271**, 1113 (1996).
9. P. Gardina, C. Conway, M. Kossman, M. Manson, *J. Bacteriol.* **174**, 528 (1992).
10. Mutations are denoted by a suffix indicating the substitution found in the altered protein For example, pMK113-R73K expresses Tar with arginine substituted by lysine at residue 73. The Tar-T154I substitution ($Thr^{154} \rightarrow Ile^{154}$) was as described [L. Lee and Y. Imae, *ibid.* **172**, 377 (1990)].
11. Plasmid pMK113 is a *tar*$^+$ Amp^r derivative of pBR322 (*9*). Plasmid pRBB16 is a *tar*$^+$ Tet^r derivative of pACYC184 [A. C. Y. Chang and S. N. Cohen, *ibid.* **134**, 1141 (1978)] constructed by R. Bourret (unpublished data). Approximately equal amounts of Tar are produced from each construct, as judged from immunoblots (*14*).
12. Strain VB12 is a derivative of strain RP437 [J. S. Parkinson, *ibid.* **135**, 45 (1978)] containing chromosomal transducer deletions (Δ*tsr-7021* Δ*tar-tap5201*). Strains lacking both Tar and Tsr (the serine receptor) are generally nonchemotactic. The experiments were repeated with the *recA56* strain, KO607 [K. Oosawa, N. Mutoh, M. I. Simon, *ibid.* **170**, 1326 (1988)], which produced the same complementation patterns.
13. Colonies that respond to aspartate form characteristic expanding rings in soft (0.325%) agar containing 0.1 mM aspartate (*9*). Chemotactic competence is judged by expansion rate and ring sharpness.
14. P. Gardina and M. Manson, unpublished results.
15. M. A. Danielson, H. P. Biemann, D. E. Koshland Jr., J. J. Falke, *Biochemistry* **33**, 6100 (1994); G. F. Lee, M. R. Lebert, A. A. Lilly, G. L. Hazelbauer, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3391 (1994). Signaling asymmetry with respect to the binding site was reported for a Tar-EnvZ fusion protein [Y. Yang, H. Park, M. Inouye, *J. Mol. Biol.* **232**, 493 (1993)].
16. A deletion derivative of this plasmid, lacking the sequence encoding the COOH-terminal 60% of the cytoplasmic domain, was also constructed. Both plasmids mediated identical responses, indicating that read-through of the UAA codon was insignificant.
17. I. Tatsuno, M. Homma, K. Oosawa, I. Kawagishi, *Science* **274**, 423 (1996); A. J. Wolfe, B. P. McNamara, R. C. Stewart, *J. Bacteriol.* **176**, 483 (1994).
18. J. R. Maddock and L. Shapiro, *Science* **259**, 1717 (1993).
19. H. P. Biemann and D. E. Koshland Jr., *Biochemistry* **33**, 629 (1994).

20. We thank R. Bourret for plasmid pRBB16 and the antibody to Tar. I. Kawagishi, K. Oosawa, and D. E. Koshland Jr. provided helpful suggestions. Supported by NIH grant GM39736.

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