different V_γ segments ($V_\gamma 2$ and $V_\gamma 4$) and two different J_δ segments ($J_\delta 1$ and $J_\delta 2$) are represented in the series. Deviations from the reported V_γ (*5*), J_δ, and C_δ (*6, 7*) sequences were minimal, with most clones exactly matching sequences described (Table 3). Analogously, the clones resulting from V_δ,C_γ PCR on thymus RNA showed the general structure V_δ-(D_δ)-J_γ-C_γ, again with proper splice junctions (Table 4). In this series of clones there were no deviations from the previously described sequences of $V_\delta 1$ (*6, 7*), J_γ (*5*), or C_γ (*11*).

In both groups of cDNA clones, single nonexhaustive screenings of M13 libraries derived from 10 μg of total thymus RNA yielded up to seven different clones in each case. Since duplicate clones were not observed, the total number of different γ-δ trans-rearrangement transcripts contained in this amount of RNA, which corresponds to approximately 10^6 thymocytes, must be considerably higher. Therefore, it is possible that a significant portion of all trans-rearrangements are actually transcribed.

A striking feature of both groups of cDNA clones is the overwhelming preponderance of clones showing open translational reading frames. In 10 of the 11 V_γ-(D_δ)-J_δ-C_δ clones and in all 9 V_δ-(D_δ)-J_γ-C_γ clones, an open reading frame is maintained across the junctional regions. These results imply that γ-δ trans-rearrangements which preserve the correct open translational reading frame are preferentially expressed over the more numerous trans-rearrangements that lack continuous open reading frames.

Two issues raised by our findings concern the molecular mechanism of trans-rearrangement and the possible functional role of the chimeric rearrangement products. By itself, the PCR procedure used to detect these rearrangements does not reveal the mechanism by which trans-rearrangements are created. However, for several reasons, it seems most likely that TCR trans-rearrangements are the result of chromosomal translocations. For example, balanced t(7;14)(p13;q11) chromosomal translocations have been observed at frequencies of 10^{-3} to 10^{-4} in phytohemagglutinin-stimulated lymphocytes from normal human peripheral blood (*3*). The breakpoints at 7p13 and 14q11 correspond to general chromosomal locations of the γ- and δ-TCR genes, respectively, suggesting that such translocations might represent the physical correlates of human γ-δ (and possibly γ-α) trans-rearrangements. Furthermore, at the molecular level, lymphocyte recombinase has been circumstantially implicated in the generation of chromosomal translocations in lymphoid malignancies (*2*). In fact, recombination in one example of another type of chromosomal aberration, an inv(14)(q11;q32) found in the SUP-T1 human T cell leukemia line, has been shown to involve two different antigen receptor genes, joining an immunoglobulin heavy chain V-region to a J-region of the α-TCR locus (*12*).

The presence of correct, uninterrupted translational reading frames in transcripts of chimeric gene rearrangements suggests that these rearrangements may direct the synthesis of functional TCR proteins. This interpretation is strengthened by the strong bias detected toward open rather than nontranslatable reading frames among the transcripts analyzed—a particularly significant finding in view of the general principle of allelic exclusion and preferential expression of the productive rearrangement observed in standard antigen receptor gene rearrangements (*1, 13*). If γ-δ trans-rearrangements are actually functional, chimeric V_γ-C_δ and V_δ-C_γ TCR polypeptide chains produced by them would be predicted to be qualitatively distinct, especially with respect to the local protein structure surrounding the hybrid junctional regions. In this way, γ-δ trans-rearrangements might contribute an additional component to the T cell receptor repertoire.

REFERENCES AND NOTES

1. G. D. Yancopoulos and F. W. Alt, *Annu. Rev. Immunol.* **4**, 339 (1986); F. W. Alt, K. Blackwell, G. D. Yancopoulos, *Science* **238**, 1079 (1987).
2. L. C. Showe and C. M. Croce, *Annu. Rev. Immunol.* **5**, 253 (1987); B. Tycko, T. C. Reynolds, S. D. Smith, J. Sklar, *J. Exp. Med.* **169**, 369 (1989).
3. J. P. Welch and C. L. Y. Lee, *Nature* **255**, 241 (1975); J. W. Beatty-DeSana, M. J. Hoggard, J. W. Cooledge, *ibid.*, p. 242; F. Hecht, B. K. McCaw, D. Peakman, A. Robinson, *ibid.*, p. 243; G. W. Dewald, K. J. Noonan, J. L. Spurbeck, D. D. Johnson, *Am. J. Hum. Genet.* **38**, 520 (1986).
4. R. Haars *et al.*, *J. Exp. Med.* **164**, 1 (1986); D. M. Pardoll *et al.*, *Nature* **326**, 79 (1987).
5. M.-P. LeFranc, A. Forster, R. Baer, M. A. Stinson, T. H. Rabbitts, *Cell* **45**, 237 (1986).
6. S. Hata, M. B. Brenner, M. S. Krangel, *Science* **238**, 678 (1987); E. Y. Loh, S. Cwirla, A. T. Serafini, J. H. Phillips, L. L. Lanier, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9714 (1988).
7. S. Hata *et al.*, *Science* **240**, 1541 (1988); K. Satyanarayana *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8166 (1988).
8. D. R. Littman *et al.*, *Nature* **326**, 85 (1987).
9. E. Loh, personal communication.
10. A. Forster, S. Huck, N. Ghanem, M.-P. LeFranc, T. H. Rabbitts, *EMBO J.* **6**, 1945 (1987); B. Tycko, J. D. Palmer, J. Sklar, unpublished observations for $V_\gamma 4$.
11. Y. Chien *et al.*, *Nature* **330**, 722 (1987).
12. C. T. Denny *et al.*, *ibid.* **320**, 549 (1986); C. T. Denny *et al.*, *Science* **234**, 197 (1986).
13. Y. Takihara *et al.*, *J. Exp. Med.* **169**, 393 (1989); M. J. Lacy, L. K. McNeil, M. E. Roth, D. M. Kranz, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1023 (1989).
14. R. K. Saiki *et al.*, *Science* **230**, 1350 (1985).
15. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
16. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 194–195.
17. M.-P. LeFranc and T. H. Rabbitts, *Nature* **316**, 464 (1985).
18. S. D. Smith *et al.*, *Cancer Res.* **44**, 5657 (1984).
19. H. Ohno *et al.*, *Jpn. J. Cancer Res.* **76**, 563 (1985).

20. We thank J. Rowley, E. Loh, and C. Morton for helpful discussion. Supported by a contract from the National Foundation for Cancer Research and NIH grant CA38621 (J.S.); and NIH National Research Service Award (B.T.); and an NIH Research Career Development Award (J.S.).

30 March 1989; accepted 27 June 1989

Activation of Bacterial Porin Gene Expression by a Chimeric Signal Transducer in Response to Aspartate

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The Tar chemoreceptor of *Escherichia coli* is a membrane-bound sensory protein that facilitates bacterial chemotaxis in response to aspartate. The EnvZ molecule has a membrane topology similar to Tar and is a putative osmosensor that is required for osmoregulation of the genes for the major outer membrane porin proteins, OmpF and OmpC. The cytoplasmic signaling domain of Tar was replaced with the carboxyl portion of EnvZ, and the resulting chimeric receptor activated transcription of the *ompC* gene in response to aspartate. The activation of *ompC* by the chimeric receptor was absolutely dependent on OmpR, a transcriptional activator for *ompF* and *ompC*.

ADAPTATION TO ENVIRONMENTAL changes is essential for the survival of free-living bacteria. *Escherichia coli* contains receptors in the cytoplasmic membrane for the detection of a variety of environmental signals (*1*). Methyl-accepting chemotaxis proteins (MCPs) are sensory transducers that monitor temporal changes of various chemoeffectors and respond to these changes by altering the swimming

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behavior of bacteria (*1, 2*). These chemoreceptors, such as Tar and Tsr (for aspartate and serine signaling, respectively), are transmembrane proteins that consist of three domains: the periplasmic, transmembrane, and cytoplasmic signaling domains (*3*). Chemoeffector binding at the periplasmic domain is thought to produce a signal that is propagated through the membrane to the cytoplasmic signaling domain and that is further processed by cytoplasmic components, eventually resulting in regulation of the rotation of the flagella. The function of both the Tar and Tsr chemoreceptors is modulated by methylation at specific sites within the signaling domain (*2, 3*).

Escherichia coli also adapts to changes in the osmolarity of the surrounding environment (*4*), and the reciprocal regulation of two genes for outer membrane porin proteins, *ompF* and *ompC*, is part of this adaptive process (*5*). Osmoregulation of *ompF* and *ompC* is under the control of the *ompR* and *envZ* genes (*5*). The OmpR protein is the positive transcriptional activator of *ompF* and *ompC* expression, and EnvZ is thought to act as an osmosensor, which modulates the function of OmpR (*5*). EnvZ is related to a class of proposed "sensor" proteins (which includes VirA, CpxA, PhoR, NtrB, CheA, and DctB) that are believed to sense particular environmental changes and modulate the function of their respective cytoplasmic effectors (VirG, SfrA, PhoB, NtrC, CheB/Y, and DctD, respectively) (*6*). EnvZ is an inner membrane protein (*7*) and can undergo autophosphorylation with subsequent phosphate transfer to OmpR in vitro (*8, 9*). Although there is not extensive sequence homology between EnvZ and MCPs such as Tar, the topological orientation of both proteins is similar (*7*); therefore, we examined whether EnvZ and Tar share a common mechanism of signal transduction across the membrane.

Both *tar* and *envZ* contain an Nde I site (CATATG) on the 3′ side of the region that encodes the second transmembrane domain. In *tar* this site exists between the codons for His^{256} and Met^{257}, where His^{256} corresponds to the 42nd amino acid residue after the second transmembrane domain (*3, 10*). In *envZ* the Nde I site occurs between the codons for His^{222} and Met^{223} where the His^{222} is the 43rd residue after the second transmembrane domain (*11*). Both Nde I sites are in phase with respect to each other, and the resulting Tar-EnvZ hybrid protein (encoded by pRB003, Fig. 1; and hereafter termed Taz1) thus consists of 484 amino acid residues. The NH_2-terminal 255 residues are derived from Tar, and the COOH-terminal 229 residues from EnvZ. The Tar portion consists of the periplasmic receptor domain (residues 1 to 6 and residues 38 to 188), the two transmembrane domains (TM1 from residue 7 to 37 and TM2 from residue 189 to 214), and the first 41 residues of the region after TM2 (residues 215 to 255), to which the COOH-terminal portion of EnvZ (residues 222 to 450, which in Taz1 become residues 256 to 484) is fused. The reciprocal hybrid protein (EnvZ-Tar) was also constructed, in which the COOH-terminal portion of EnvZ was replaced with the signaling domain of Tar at the Nde I site (encoded by pRB002; Fig. 1).

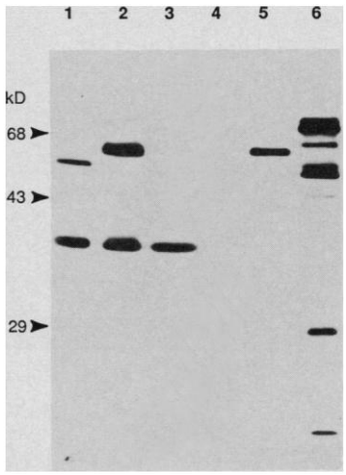

Fig. 2. Protein blot analysis of hybrid signal transducers. Total membrane proteins from RU1012 harboring the plasmids pAT428 (lanes 1 and 4), pRB003 (lanes 2 and 5), and pRB001 (lanes 3 and 6) were analyzed by SDS–polyacrylamide gel electrophoresis on a 12.5% polyacrylamide gel. Protein blot analysis with anti-EnvZ (1:500 dilution) (lanes 1, 2, and 3) and anti-Tar (1:250 dilution) (lanes 4, 5, and 6) was performed as described (*22*). The molecular mass standards are 68 kD, bovine serum albumin; 43 kD, lactoalbumin; and 29 kD, carbonic anhydrase. The band observed between the 43- and 29-kD markers in lanes 1, 2, and 3 is not related to EnvZ because this band can be observed even in strain RU1012.

The plasmid pRB003 was introduced into an *envZ* deletion strain, RU1012 (*12*). Membrane fractions were prepared from these transformants and submitted to protein blot analysis with antisera against EnvZ (anti-EnvZ) and Tar (anti-Tar). Taz1 was detected in relatively large amounts with both sera (Fig. 2) and had an apparent molecular mass of ~58 kD (the calculated molecular mass of Taz1 is 53 kD). In the membrane fraction of cells of strain RU1012 that had been transformed with *envZ* and *ompR* [plasmid pAT428 (7)], EnvZ was detected with anti-EnvZ at the position expected from its molecular mass [50 kD (*10*)] but was not detected with anti-Tar (Fig. 2). In the membrane fraction of strain RU1012 cells transformed with the *tar* plasmid pRB001, Tar was detected with anti-Tar but not with anti-EnvZ (Fig. 2); the appearance of multiple bands in this case with anti-Tar is probably due to different methylation states or proteolytic degradation of Tar, or both. Such multiple-banding patterns were not observed with Taz1, which suggests that the COOH-terminal signaling domain of Tar is probably responsible for this effect.

To investigate the signal transduction

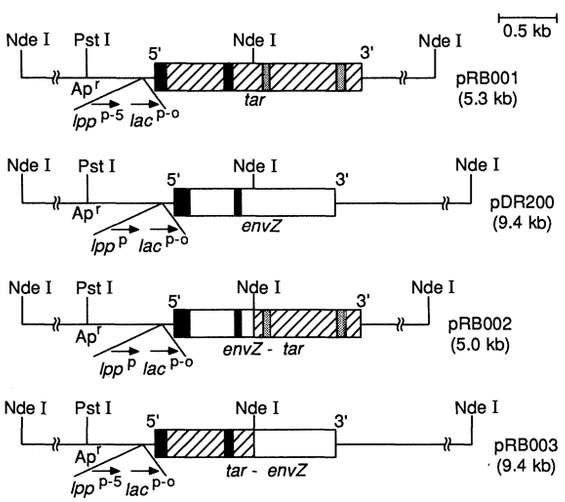

Fig. 1. Construction of plasmids pRB001, pRB002, and pRB003. The high expression vector pIN-III containing a mutated lipoprotein promoter (lpp^{p-5}) (*19*) was cut with Xba I and blunt-ended by Klenow treatment. The vector was then cut with Pst I and a 1-kb fragment containing the lpp^{p-5} promoter, and the *lac* promoter and operator (lac^{p-o}) region was isolated and cloned into the Pst I–Sma I sites of pNM13, which contains the entire coding sequence of *tar* including the Shine-Dalgarno (ribosome binding) sequence (*20*). The resulting plasmid is denoted as pRB001. Plasmid pRB002 was constructed by ligating a 2-kb Nde I fragment of pRB001 (containing the 3′-coding portion of *tar*) with a 3-kb Nde I fragment of pDR200 (containing the 5′-coding portion of *envZ*) (*7*). A 3-kb Nde I fragment of pRB001 (containing the 5′-coding portion of *tar*) was ligated with a 6.4-kb Nde I fragment of pDR200 (containing the 3′-coding portion of *envZ*) to form pRB003. The black boxes at the 5′ end and middle of the coding sequences represent putative signal sequences and sequences that encode the membrane-spanning region, respectively [in the case of *tar* this represents TM1 and TM2, respectively (*3*)]. The gray region indicates methylation sites of *tar*; striped region, DNA sequences from the *tar* gene; and open region, DNA sequences from the *envZ* gene. Abbreviations: lpp^{p-5}, modified consensus sequence at the −35 region of pIN-III (*19*); lpp^{p}, unmodified lipoprotein promoter (*17, 19*); lac^{p-o}, *lac* promoter-operator of pIN-III (*21*); and Ap^r, ampicillin resistance.

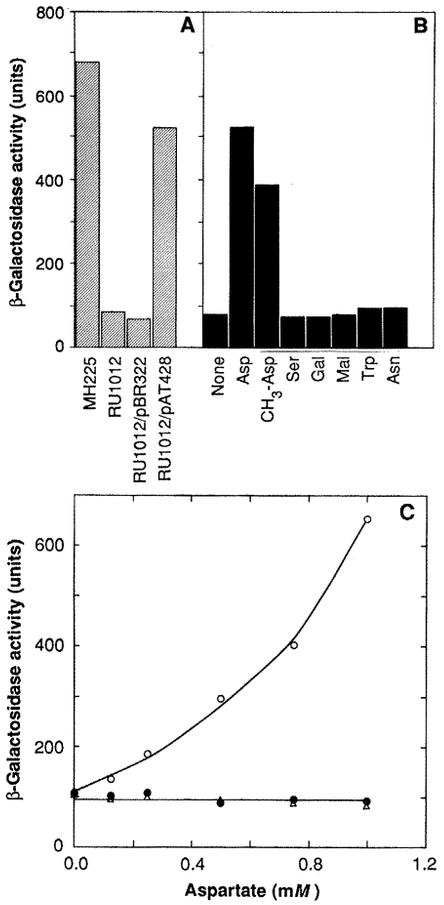

Fig. 3. EnvZ-dependent expression of *ompC-lacZ*. Cells were grown to mid-log phase in minimal medium A (*23*), and β-galactosidase activity of duplicate samples of each cell preparation was assayed (*23*). Duplicates agreed within ±10% of the plotted points, and similar results were obtained in six similar experiments. (**A**) Strains MH225 [Φ(*ompC-lacZ*) 10-25] (*24*), RU1012 (MH225, Δ*envZ*::Km^r), and RU1012 containing pBR322 or pAT428 (*envZ*, *ompR*) (7) were assayed. (**B**) RU1012 containing pRB003 was grown to mid-log phase in the absence (none) or the presence of aspartate (1 m*M*), CH_3-Asp (0.5 m*M*), serine (1 m*M*), D(+)-galactose (Gal) (20 m*M*), maltose (Mal) (6 m*M*), tryptophan (1 m*M*), or asparagine (1 m*M*) before β-galactosidase assay. (**C**) Strain RU1012 containing pRB001 (●), pRB002 (△), or pRB003 (○) was cultured in the presence of various concentrations of aspartate before β-galactosidase assay.

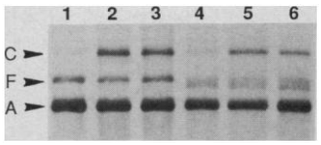

Fig. 4. Induction of OmpC protein by aspartate. Strain AT142 (Δ*envZ*::Km^r) (*12*) (lanes 1, 2, and 3) and TZ322 [Δ*envZ*::Km^r, Δ(*tar-tap* 5201)] (*12*) (lanes 4, 5, and 6), both containing pRB003, were cultured in minimal medium A (20 ml) containing methionine (20 μg/ml). At mid-log phase, cultures were filtered through a 0.45-μm Millipore membrane and washed with methionine-free minimal medium A. Cells were resuspended in minimal medium A containing either methionine (0.2 μg/ml) (lanes 1 and 4), methionine (0.2 μg/ml) and 1 m*M* aspartate (lanes 2 and 5), or methionine (0.2 μg/ml) and 1 m*M* CH_3-Asp (lanes 3 and 6). After a 30-min incubation at 37°C, 2-ml portions of the cultures were labeled with 20 μCi of [^{35}S]methionine (ICN-Radiochemicals; 1100 Ci/mmol) for 2 min. Outer membranes were prepared as described (*11*), and ^{35}S-labeled proteins were resolved on urea-SDS-polyacrylamide gels (*11*) and visualized by autoradiography. Approximately 10^5 cpm of ^{35}S was applied in each well. The positions of OmpC, OmpF, and OmpA are indicated by C, F, and A, respectively.

properties of Taz1, we monitored the expression of an *ompC-lacZ* fusion gene on the chromosome in strain RU1012 in response to the addition of aspartate in the medium. The β-galactosidase activity of strain RU1012 in minimal medium A (an intermediate osmolarity medium) was low in comparison to its parent strain MH225 (*envZ*$^+$) (Fig. 3A). However, when strain RU1012 was transformed with pAT428 (*ompR*, *envZ*), β-galactosidase activity increased approximately sixfold; β-galactosidase activity did not increase when RU1012 contained pBR322 (Fig. 3A). Thus, *ompC-lacZ* expression in strain RU1012 was dependent on EnvZ function. Strain RU1012 was then transformed with pRB001 (*tar*), pRB002 (*envZ-tar*), and pRB003 (*taz1*), and the β-galactosidase activity for each transformant was measured for cells grown in minimal medium A containing various concentrations of aspartate. β-Galactosidase activity of cells harboring pRB003 increased as the concentrations of aspartate increased (Fig. 3C). In contrast, in cells harboring either pRB001 or pRB002, β-galactosidase activity was not induced even in the presence of 1 m*M* aspartate (Fig. 3C). Thus Taz1 could complement EnvZ function in strain RU1012 in the presence of aspartate, whereas EnvZ-Tar could not complement EnvZ function, indicating that the COOH-terminal portion of EnvZ is required for *ompC* activation. Because the periplasmic domain of Tar is essential for aspartate binding (*13*), it appears that the signal received by the receptor domain of Tar is transmitted to the cytoplasmic signaling domain of EnvZ, which then modulates OmpR function to activate *ompC* transcription.

The effects of various other compounds on *ompC-lacZ* expression in RU1012 harboring pRB003 were investigated (Fig. 3B). Neither asparagine, serine, galactose, tryptophan, nor maltose increased β-galactosidase activity, whereas aspartate resulted in an approximately sevenfold increase in activity. The nonmetabolizable aspartate analog α-methyl-D,L-aspartate (CH_3-Asp) also increased β-galactosidase activity. These results indicate that Taz1 retains the ligand specificity of the Tar chemoreceptor. The reason for the inability of maltose, another chemoeffector for Tar (*14*), to increase β-galactosidase activity is not known. However, a motile derivative of strain RU1012 (*flaB*$^+$) showed a clear chemotactic response to maltose. This finding indicates that strain RU1012 probably contains a maltose binding protein and that the function of this protein is not affected by the removal of *envZ*.

To directly test the effects of Taz1 on the production of OmpC protein, we examined ^{35}S-labeled outer membrane proteins in strain AT142 (*ompC*$^+$, Δ*envZ*::Km^r) (*12*) carrying pRB003. Production of OmpC was induced by 1 m*M* aspartate or by 1 m*M* CH_3-Asp (Fig. 4). OmpC induction was also examined in a Tar-deletion strain, TZ322 [*ompC*$^+$, Δ*envZ*::Km^r, Δ(*tar-tap* 5201)] (*12*) carrying pRB003. The addition of 1 m*M* aspartate or 1 m*M* (CH_3-Asp) to this strain also increased OmpC production compared to the untreated control (Fig. 4), demonstrating that signal transduction by the Taz1 molecule was not mediated by heterodimers of Tar and Taz1.

OmpR is absolutely required for expression of *ompC* and *ompF* (*5*). To examine whether the induction of *ompC* expression by aspartate in the Taz sensory system was also dependent on OmpR function, we constructed an *ompR* mutant strain of RU1012 (K1108) (*12*), which was then transformed with pRB003. This strain showed low β-galactosidase activity in the absence (41 units) or in the presence (35 units) of 1 m*M* aspartate. In the same experiment, pRB003 in strain RU1012 (which contains a wild-type *ompR* gene) showed β-galactosidase activity of 80 and 525 units in the absence and presence of 1 m*M* aspartate, respectively. Thus, OmpR is required for *ompC* expression through Taz.

Although EnvZ has been proposed to act as an osmotic signal transducer (osmosensor) for the regulation of outer membrane porin expression (*5*), a signaling molecule that interacts with EnvZ has not yet been identified, and this has hampered direct studies of EnvZ function. Our results show that EnvZ and Tar share a common mechanism for signal transduction across the membrane, thus allowing the detailed molecular mechanism of signal transduction by EnvZ to be studied with the hybrid protein Taz1.

Recent studies on the Tar and Tsr proteins have indicated that the "linker" region distal to the second transmembrane domain is important for signal transmission (*15, 16*)

and that mutations in this region of Tsr result in so called "locked" or nonresponsive signaling output (*16*). An 80-residue sequence following the second transmembrane domain of EnvZ (residues 180 to 259) shows little sequence similarities to the linker regions of the Tar and Tsr chemoreceptors. Nevertheless, mutations in this region also appear to cause "locked" signal transduction: the *envZ*11 ($Thr^{247} \rightarrow Arg$) mutation results in a constitutive *ompC* phenotype (*17*). Furthermore, we have found that a mutation that changes His^{243} to Val resulted in unregulated *ompC* expression (*9*).

A switch region within the cytoplasmic signaling domain of Tsr has been proposed to modulate signaling conformations of this molecule (*16*). This region is between amino acids 371 and 420 of Tsr and is highly conserved between MCP molecules (*2, 3, 14*). A comparison between EnvZ and Tsr indicates a stretch of similar amino acids between residues 378 and 388 of Tsr (Leu-Ala-Leu-Asn-Ala-Ala-Val-Glu-Ala-Ala-Arg) and between residues 340 and 350 of EnvZ (Ala-Val-Ala-Asn-Met-Val-Val-Asn-Ala-Ala-Arg). This region in EnvZ is one of the conserved sequences shared by EnvZ-like molecules (*18*) and may serve a switching function.

REFERENCES AND NOTES

1. J. Adler, *Science* **166**, 1588 (1969); R. M. Mcnab, in Escherichia coli *and* Salmonella typhimurium, F. C. Neidhardt, J. L. Ingraham, B. Magasanik, K. B. Low, M. Schaechter, Eds. (American Society for Microbiology, Washington, DC, 1987), pp. 732–759.
2. R. C. Stewart and F. W. Dahlquist, *Chem. Rev.* **87**, 977 (1987).
3. A. Krikos, N. Mutoh, A. Boyd, M. Simon, *Cell* **33**, 615 (1983).
4. L. Csonka, *Microbiol. Rev.* **53**, 121 (1989).
5. S. Forst and M. Inouye, *Annu. Rev. Cell Biol.* **4**, 21 (1988).
6. C. W. Ronson, D. T. Nixon, F. M. Ausbel, *Cell* **49**, 579 (1987).
7. S. Forst, D. Comeau, S. Norioko, M. Inouye, *J. Biol. Chem.* **262**, 16433 (1987).
8. M. M. Igo and T. Silhavy, *J. Bacteriol.* **170**, 5971 (1988).
9. S. Forst, J. Delgado, M. Inouye, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
10. A. Krikos, M. P. Conley, A. Boyd, H. C. Berg, M. I. Simon, *ibid.* **82**, 1326 (1985).
11. D. E. Comeau, K. Ikenaka, K. Tsung, M. Inouye, *J. Bacteriol.* **164**, 578 (1985).
12. Strain RU1012 [Φ(*ompC-lacZ*) 10-25, Δ*envZ*::Km^r (kanamycin resistance)] was constructed by infection of strain MH225 [Φ(*ompC-lacZ*) 10-25] (*24*) with a P1 phage lysate of strain AT142 (Δ*envZ*::Km^r) [T. Mizuno and S. Mizushima, *J. Biochem. (Tokyo)* **101**, 387 (1987)] and selection of transductants that were white on MacConkey agar plates (Difco) containing kanamycin. The phenotype of strain AT142 is very low levels of OmpC and constitutive expression of OmpF. Strain K1108 was constructed from an *ompR* mutant strain W-2 [*ompR*, Φ(*ompC-lacZ*) 10-25] (R. Brissette and M. Inouye, unpublished data), which contained pAT2002 (*envZ*) (*11*; Mizuno and Mizushima above), by infecting with a P1 phage lysate of strain AT142, and the *ompR* and *envZ* double mutant was selected as white colonies on MacConkey plates containing kanamycin and ampicillin. Strain MH225 was transduced with P1 phage grown on one of these double mutants, and a white colony (K1108) was selected on MacConkey plates containing kanamycin. Strain K1108 was identified as the double mutant (*ompR*, Δ*envZ*::Km^r) because OmpC and OmpF were absent from outer membrane protein profiles, which is consistent with an $ompR^-$ and $envZ^-$ phenotype (*5*). In addition, K1108 containing plasmid pAT428 (*ompR*, *envZ*) was red on MacConkey plates but was white (that is, it could not be complemented) when the strain carried only the *envZ* gene (pAT2002). Strain TZ322 was constructed by infecting strain MM551 [strain RP437, Δ(*tar-tap* 5201)] [M. D. Manson, V. Blank, G. Brade, C. F. Higgins, *Nature* **321**, 253 (1986)] with a P1 lysate of strain AT142 and was selected for kanamycin resistance; transductants were then checked for their inability to swarm on low agar plates.
13. C. Wolff and J. Parkinson, *J. Bacteriol.* **170**, 4509 (1988); E. A. Wang and D. E. Koshland, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7157 (1980).
14. M. Manson and M. Kossman, *J. Bacteriol.* **165**, 34 (1986); M. Kossman, C. Wolff, M. Manson, *ibid.* **170**, 4516 (1988).
15. K. Oosawa and M. Simon, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6930 (1986).
16. P. Ames and J. Parkinson, *Cell* **55**, 817 (1988).
17. S.-I. Matsuyama, T. Mizuno, S. Mizushima, *J. Bacteriol.* **168**, 1309 (1986).
18. A. Stock, T. Chen, D. Welsh, J. Stock, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1403 (1988); C. W. Ronson *et al.*, *Nucleic Acids Res.* **15**, 7921 (1987).
19. S. Inouye and M. Inouye, *Nucleic Acids Res.* **13**, 3101 (1985).
20. K. Oosawa and M. I. Simon, unpublished data.
21. Y. Masui, J. Coleman, M. Inouye, in *Experimental Manipulation of Gene Expressions*, M. Inouye, Ed. (Academic Press, New York, 1983), pp. 15–32.
22. S. Forst, J. Delgado, G. Ramakrishnan, M. Inouye, *J. Bacteriol.* **170**, 5080 (1988).
23. J. H. Miller, in *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1972), pp. 352–355.
24. M. N. Hall and T. J. Silhavy, *J. Bacteriol.* **140**, 342 (1979).

25. We thank K. Tsung for discussions throughout this work, M. Simon for discussions at the early stages of this work and for anti-tar, M. Manson for strain MM551, and T. Mizuno for strain AT142. Supported by NIH grant GM19043-16 (to M.I.) and National Research Service Awards GM12350 (to A.R.) and GM1553 (to S.F.).

17 May 1989; accepted 17 July 1989

Acetylcholine and GABA Mediate Opposing Actions on Neuronal Chloride Channels in Crayfish

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A central principle of neural integration is that excitatory and inhibitory neurotransmitters effect the opening of distinct classes of membrane ionic channels and that integration consists of the summation of the opposing ionic currents on the postsynaptic membrane. In tangential cells of crayfish optic lobes, a hyperpolarizing, biphasic synaptic potential is produced by the concurrent action of acetylcholine and γ-aminobutyric acid (GABA). Acetylcholine hyperpolarizes the cell and increases chloride conductance. GABA depolarizes the cell by closing some of the same chloride channels. Therefore, in this case integration is achieved by the antagonistic actions of two transmitters on the same ionic channel.

TANGENTIAL (TAN1) CELLS ARE A ubiquitous class of centrifugal interneurons in the optic lobes of arthropods (*1*). TAN1 cells convey signals from dendrites in the second optic neuromere, the medulla externa, through a terminal arborization in the first optic neuromere, the lamina ganglionaris (Fig. 1A). The medulla is a rich source of acetylcholine (ACh) and GABA in several arthropods (*2*), including crayfish (*3, 4*). GABA-like immunoreactivity is observed in the axons of monopolar neurons (Fig. 1A) of the external chiasm, which project from the lamina to the medulla (Fig. 1B) and in columnar neurons within the medulla (Fig. 1C). The distribution of both transmitters is coextensive with the dendrites of TAN1 cells in the distal half of the medulla externa.

TAN1 cells respond to an increment of illumination with a graded, hyperpolarization [a postsynaptic potential (PSP)] of 10 to 20 mV, which decays to a smaller plateau (Fig. 2A) (*5*). The input conductance (G_n) of TAN1 cells change in parallel with the PSP; it increases at light onset (by 58 ± 18% SEM, $n = 10$) and then decreases toward a smaller value (17 ± 10% above resting G_n) (Fig. 2B).

Pharmacological analysis indicates that the light-elicited PSP and subsequent repolarization are mediated by ACh and GABA, respectively. Neuronal nicotinic antagonists such as pempidine and mecamylamine (*6, 7*) at micromolar doses reduce the PSP by 60 to 90%. Conversely, the GABA antagonist bicuculline (*8*) enhances the PSP and blocks the repolarization phase (Fig. 2C, lower trace). Bicuculline at 100 μ*M* increases the amplitude of the plateau phase by 245 ± 71% ($n = 6$) (±SEM).

ACh and GABA were pressure-injected into the medullary neuropile in the presence of 20 m*M* $CoCl_2$, which blocks synaptic

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