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Transmitter and receiver modules in bacterial signaling proteins

(signal transduction/regulation/protein structure/evolution/chemotaxis)

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ABSTRACT **Prokaryotes are capable of sophisticated sensory behaviors. We have detected sequence motifs in bacterial signaling proteins that may act as transmitter or receiver modules in mediating protein–protein communication. These modules appear to retain their functional identities in many protein hosts, implying that they are structurally independent elements. We propose that the fundamental activity characterizing these domains is specific recognition and association of matched modules, accompanied by conformational changes in one or both of the interacting elements. Signal propagation is a natural consequence of this behavior. The versatility of this information-processing strategy is evident in the chemotaxis machinery of *Escherichia coli*, where proteins containing transmitters or receivers are linked in "dyadic relays" to form complex signaling networks.**

Cell sensory systems mediate a wide variety of adaptive behaviors involving changes in gene expression or cell movement. Their information-processing capabilities often rival those of neural circuits and include such tasks as signal amplification, integration of multiple inputs, sensory adaptation, and excitatory and inhibitory interactions with other signaling pathways. Yet the circuit elements of intracellular signaling systems are individual molecules, principally proteins. What molecular mechanisms enable these relatively simple components to form such sophisticated communication systems?

Comparisons of "two-component regulatory systems" in bacteria have revealed sequence motifs that might promote communication between prokaryotic signaling elements (1). These simple signaling systems are comprised of a "sensor" protein that detects environmental stimuli and a "regulator" protein that controls expression of particular genes (2). The carboxyl termini of sensors are similar in sequence over a length of about 200 amino acids, whereas the amino termini of regulators are similar over a length of about 100 amino acids. Since regulator function appears to be modulated by information transmitted from the sensor, these shared sequence motifs could represent discrete "transmitter" and "receiver" (T/R) modules that mediate protein–protein communication. Receiver sequences have also been found in the CheB and CheY components of the *Escherichia coli* chemotaxis machinery (2, 3), and a corresponding transmitter has been identified in the CheA protein (ref. 4, unpublished results). These findings suggest that transmitter and receiver modules might be a characteristic feature of other signaling systems as well.

If bacteria make extensive use of T/R modules, we would expect receivers to be specifically tuned to their cognate transmitters to avoid crosstalk between signaling pathways. Indeed, evidence for low-level interference of this type has been reported (2). Thus, some signaling systems might employ communication modules that differ from the canonical sequences but nevertheless retain structural features characteristic of transmitters and receivers. To test this idea we used T/R probes with relaxed match criteria to examine proteins for unorthodox transmitters or receivers.

We found sequences resembling T/R modules in many bacterial proteins with known or suspected signaling roles, and we conclude that transmitters and receivers, although variable in primary structure, play similar and important roles in many prokaryotic signaling pathways. These modules may also be employed in other situations, such as active transport, where transient protein–protein interactions and induced conformational changes are involved. We propose that transmitters and receivers are formally analogous to dyadic mathematical operators, which interact in pairwise fashion to transform information from one frame of reference to another (5). Accordingly, proteins containing these modules can be linked into complex "dyadic relays" with diverse signaling capabilities.

METHODS

Consensus Sequences for T/R Modules. We derived consensus sequences from the canonical T/R proteins (1),* using published alignments (2) and the following rules: If the same amino acid occurred in 90% or more of the sequences at a particular position, it was included as an absolute identity. Otherwise, if at least 50% of the amino acids at corresponding positions belonged to the same chemical family, a symbol for that family was used in the consensus. Family groups were as follows (in the standard one-letter symbols): acidic and amidic (D, E, N, Q), basic (H, K, R), polar (A, G, P, S, T), nonpolar (I, L, M, V), aromatic (F, W, Y), and cysteine (C). If more than half of the aligned sequences had a gap at a given position, nothing was added to the consensus. Otherwise, a wild-card symbol was used.

Alignment Scores for T/R Modules. Estimating the significance of sequence comparisons between proteins is difficult (18). We devised an alignment measure that relates an observed consensus match to that expected of an "average" *E. coli* protein 10% longer than the consensus. Unlike a true significance measure, larger scores will be more likely with longer proteins.

Alignments were evaluated with the BestFit program of the University of Wisconsin GCG package (19), which provides two scores: "% similarity" (S), the proportion of residue pairs that meet the match criteria, and "quality" (Q), an aggregate of individual match and gap scores. Q scores were computed at four different gap penalties (-3.5, -3.0, -2.5, -2.0), using the following alignment values: $+1.4$ (absolute/absolute); $+0.8$(absolute/functional); $+0.5$(functional/func-

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Abbreviations: T/R, transmitter and receiver; MCP, methyl-accepting chemotaxis protein.

*CheA (unpublished data), CpxA (6), DctB (1), EnvZ (7), NtrB (8), PhoR (2), and VirA (2) contain transmitters; CheB (9), CheY (10), DctD (1), NtrC (11), OmpR (12), PhoB (13), SfrA (14), Spo0A (15), Spo0F (16), and VirG (17) contain receivers.

tional); −0.2 (mismatch); 0.0 (wild-card matches); indicated gap penalty (gap); and −0.3 (per gap residue). Q and S scores for randomized sequences were nearly independent, so we defined an empirical metric, R, as

$$R = Max\left[\left(\frac{S}{S_{max}}\right) \times \left(\frac{Q}{Q_{max}}\right)^{E}\right]^{1/2},$$

where Max was the largest value as a function of gap penalty; E, which forces congruence of the two distributions, was about 0.42; and S_{max} and Q_{max} were the scores obtained when the consensus sequence was compared against itself.

A normally distributed population of R scores was generated from alignments with 100 random targets of length 10% greater than the probe and amino acid composition typical of *E. coli* (ref. 18; our own database). The observed mean, r, and standard deviation, ∂r, were used to calculate an alignment score as a normal deviate of R,

$$A = (R - r)/\partial r.$$

Computer Analyses. Database searches and initial sequence comparisons were done on a VAX 8600. Final statistics were generated with an Excel template on a Macintosh II microcomputer.

RESULTS AND DISCUSSION

We constructed T/R consensus sequences that symbolically marked sites of functional, as well as absolute, conservation (Fig. 1). Our receiver consensus had 118 residues, 39 of which were absolute, and four of which were wild cards. Our transmitter consensus was 216 residues in length but had 41 wild-card sites and only 39 absolute positions. Alignment scores were then determined for every prokaryote protein in the National Biomedical Research Foundation data bank and compared to a control set obtained from jumbled transmitter and receiver consensus sequences. The distributions of control scores closely fit those expected for random sequences. In contrast, the experimental distributions were detectably nonrandom (data not shown). Part of this difference was due to the canonical proteins used to construct the consensus probes, all of which had scores above 5. However, proteins with alignment scores in the range of 2–5 also occurred in excess of chance, suggesting that they might contain unorthodox T/R modules.

To determine whether there might be a functional basis for the skewed distribution of T/R scores, we examined bacterial proteins with high scores for possible roles in cellular signaling processes. We also translated genes from the European Molecular Biology Laboratory and GenBank DNA databases whose products were genetically or biochemically related to interesting proteins in the National Biomedical Research Foundation data bank. In all, 1139 prokaryote proteins were inspected, and about 10% of them had T/R alignment scores above 1.8, an arbitrarily chosen cutoff. However, one-third of those proteins exceeded the cutoff score for both receiver and transmitter alignments, and the regions of optimal alignment generally overlapped, implying structural similarity between transmitter and receiver modules. In such cases, the higher score was used to classify the module. Since the DNA libraries were not inspected in systematic fashion, and since many of the proteins with scores above the cutoff are undoubtedly spurious, we will not present a comprehensive list, but rather we will discuss selected examples of proteins with putative T/R modules (Fig. 2).

Unorthodox T/R Modules in Regulatory Proteins. Some of the proteins shown in Fig. 2 appear to be members of "two-component regulatory systems," as defined by Nixon *et al.* (2). In the canonical examples the "sensor" is typically a transmembrane protein with a transmitter in its cytoplasmic domain. The "regulator" is a cytoplasmic protein in which a receiver is joined to a DNA-binding domain targeted to specific promoters. Proteins with unorthodox T/R modules clearly fit this paradigm.

UhpA and UhpB regulate the expression of UhpT, a hexose-phosphate permease (32). UphA is a soluble factor required for UhpT transcription and contains a receiver at its

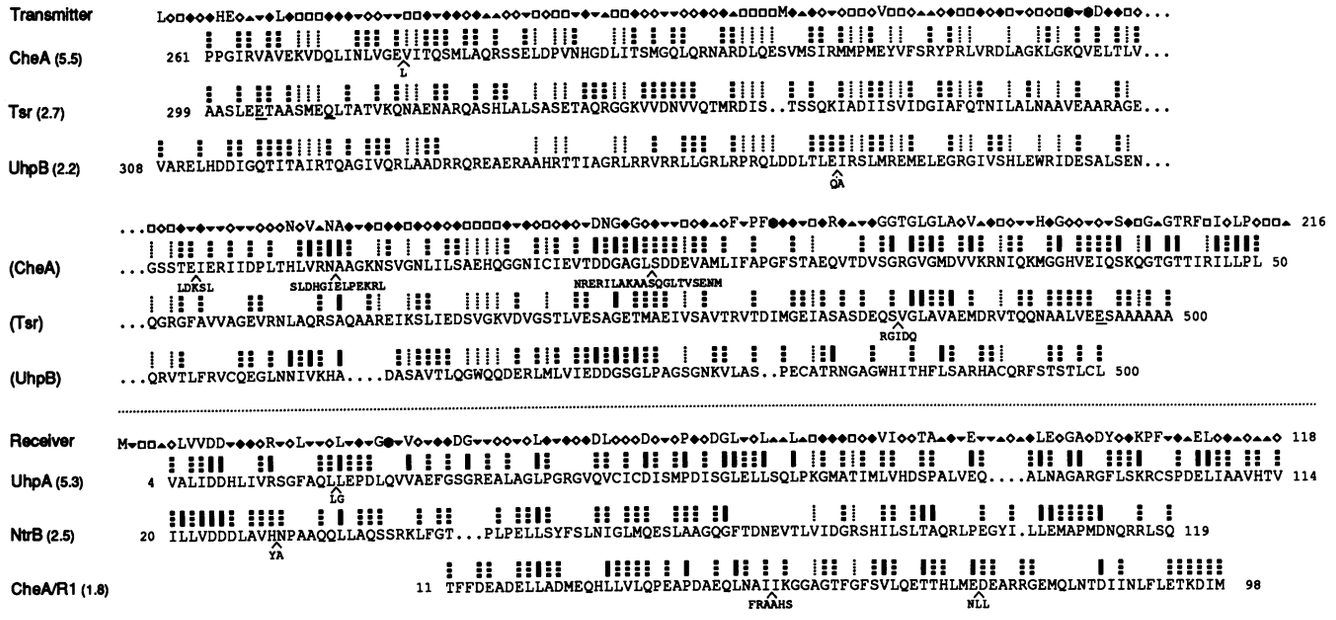

FIG. 1. Alignments of transmitters and receivers with the consensus sequences. Consensus residues are indicated by one-letter amino acid code or one of the following symbols: ▾, acidic or amidic (D, E, N, Q); ▴, basic (H, K, R); ◆, polar (A, G, P, S, T); ◇, nonpolar (I, L, M, V); ⬟, aromatic (F, W, Y); □, wild-card. Three representative transmitter and receiver modules are shown, with alignment scores indicated in parentheses after the protein names. Gaps in the alignments are indicated by dots; insertions are displayed in small type beneath carets. Three types of matches are indicated: absolute (▌); family (⋮); and wild-card (⋮). Underlined residues in Tsr indicate the methylation sites involved in sensory adaptation.

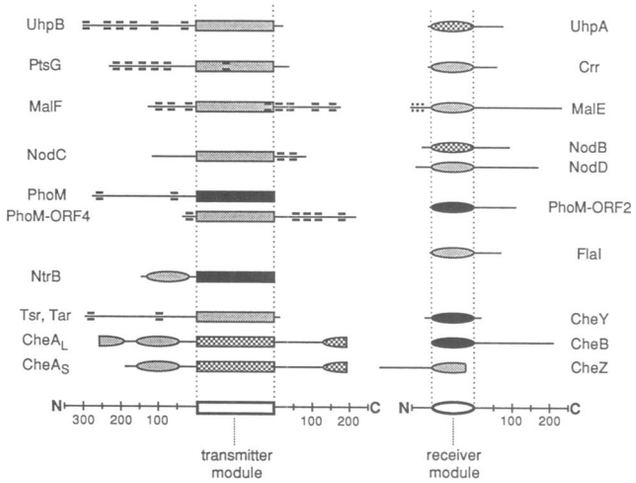

FIG. 2. Unorthodox T/R modules in bacterial proteins. Shown are examples of proteins with T/R scores above the 1.8 cutoff that might belong to matched T/R pairs or module families: UhpB and UhpA (20); PtsG (21) and Crr (22); MalF (23) and MalE (24); NodC (25) and NodB, NodD (26); PhoM, PhoM-ORF2 and PhoM-ORF4 (27); FlaI (28); NtrB (18); Tsr (29), Tar (30), CheA (unpublished data), CheB and CheZ (9) and CheY (10). The degree of shading indicates the approximate alignment score of the module: light = 1.8–3.0; medium = 3.0–6.0; dark = above 6.0. Exact scores for each protein are given in the text. Possible membrane-spanning segments, identified by the algorithm of Eisenberg *et al.* (31), are indicated by short horizontal bars. Pairs of small squares at the beginning of MalE denote a cleavable hydrophobic leader needed for transport to the periplasmic space. The receiver modules in NtrB and CheA were identified by dot matrix comparisons and subsequent selective alignment tests with the BestFit program. The scales at the bottom of the figure are calibrated in amino acid residues to show the lengths of each protein on either side of its modules. Transmitters are represented as 200 residues in length, receivers as 100 residues in length.

amino terminus (A = 5.3; Fig. 1). UhpB is an inner membrane component that activates UhpA in response to exogenous glucose 6-phosphate and contains a transmitter at its carboxyl terminus (A = 2.2; Fig. 1). This portion of the UhpB molecule appears to lack membrane-spanning segments and might be located in the cytoplasm, where it could communicate with UhpA. In the absence of UhpB function, high levels of UhpA lead to constitutive expression of UhpT (32), implying that UhpA may be activated by crosstalk from other transmitters, as has been suggested for NtrC (2). Since UhpA is ordinarily activated by UhpB, the UhpB transmitter presumably functions in the canonical fashion even though it is rather different from the consensus.

PtsG ("glucose-specific enzyme II") is a membrane-associated component of the phospho*enol*pyruvate-dependent glucose phosphotransferase uptake system (PTS) (33). Crr ("glucose-specific enzyme III") is a cytoplasmic protein that interacts with PtsG to catalyze glucose phosphorylation and uptake. Crr also plays important roles in global regulation. Its phosphorylated form activates Cya (adenylate cyclase), whereas its unphosphorylated form directly inhibits other transport systems (34, 35). The carboxyl-terminal half of PtsG contains a transmitter motif (A = 1.9); the amino-terminal half of Crr contains a receiver motif (A = 2.5). Although neither module greatly resembles the canonical ones, they may make a matched pair that communicates information on glucose availability to other components of the global regulatory machinery.

The FlaI protein is required for the expression of flagellar functions and mediates catabolite repression effects on motility and chemotaxis (28). It contains a receiver module at its amino terminus (A = 2.5) and may respond to regulatory signals from as-yet-unidentified transmitter proteins.

NodB, NodC, and NodD are *Rhizobium melliloti* proteins involved in the formation of nitrogen-fixing root nodules in legumes (36). NodD [actually several similar proteins (37)] is a transcriptional activator that responds to environmental phenolic compounds indicating the presence of a suitable plant host (38) and contains a receiver module at its amino terminus (A = 2.8). NodB and NodC, whose expression is regulated by NodD, might make up a T/R pair. NodC appears to be an integral membrane protein with a transmitter (A = 2.7) located in the middle of the molecule, just ahead of several potential membrane-spanning segments. NodB contains a receiver at its amino terminus (A = 3.2) and might respond to sensory signals from NodC.

The PhoM operon produces several proteins implicated in the control of alkaline phosphatase expression (39). PhoM appears to be an integral membrane protein, with a near-canonical transmitter (A = 9.4) located at its carboxyl terminus. PhoM-ORF4 also appears to be a membrane protein, and it contains an unorthodox transmitter near its amino terminus (A = 2.6). Either or both of these putative "sensors" might communicate with PhoM-ORF2, which contains a receiver at its amino terminus (A = 9.4) and presumably serves a regulatory function.

NtrB communicates information about nitrogen availability to NtrC, which in turn controls expression of glutamine synthetase (40). Unlike other canonical sensors, it is not membrane associated (1), and it receives its sensory input from other signaling proteins rather than directly from the environment. The amino terminus of NtrB contains a receiver module (A = 2.5) that may be responsible for regulating its transmitter activity, either in response to sensory signals or as part of a feedback circuit. Keener and Kustu (41) have recently demonstrated that NtrB is capable of autophosphorylation. It seems likely that the phosphate acceptor site is located within the NtrB receiver domain (see below). Proteins such as NtrB, which have coupled receivers and transmitters, should be versatile signaling elements and may be a characteristic feature of signaling systems with multiple inputs or feedback controls.

Dyadic Relays in Chemotaxis. The chemotaxis machinery of *E. coli* provides a striking example of how T/R modules can be linked in dyadic relays to form complex signaling networks (Fig. 3).

Most chemotactic responses are mediated by methyl-accepting inner membrane proteins (MCPs), which have a periplasmic receptor domain that monitors the chemical environment and a cytoplasmic signaling domain that controls the flagellar motors (30). The signaling regions of MCP molecules contain a transmitter module (see Tsr and Tar in Fig. 2; A = 2.7). Unlike canonical sensors, MCP molecules undergo adaptation, which enables cells to detect temporal changes in chemoeffector concentrations. Adaptation involves addition or removal of methyl groups at specific glutamic or glutamine residues in the cytoplasmic domain (42). These methylation sites, which are evidently capable of modulating MCP signaling properties, are located at each end of the transmitter module (see underlined residues in the Tsr alignment, Fig. 1). They appear to be an embellishment of the basic transmitter motif, conferring feedback control of module activity without an accompanying receiver.

MCP signals are transmitted to the flagella by a network of cytoplasmic proteins including CheA, CheW, CheY, and CheZ. CheY probably controls switching behavior by interacting directly with the flagellar motors, whereas CheZ may act to antagonize CheY function (43, 44). Both proteins contain receivers that might be involved in intercepting or interpreting transducer signals. CheA and CheW are thought to interact with the MCP transducers to relay sensory signals

to CheY and CheZ (45). In addition, signals generated through CheA and CheW appear to function in feedback control of CheB, the enzyme that removes MCP methyl groups during sensory adaptation (46). CheW has no obvious T/R modules. However, CheA has not only a canonical transmitter (Fig. 1) but three potential receivers (Fig. 2). The modular complexity of CheA is consistent with a central role in processing chemotactic signals, and it might serve to integrate sensory inputs, not only from MCP modules, as shown in Fig. 3, but from other sensory pathways as well. For example, chemotactic responses to glucose and other phosphotransferase system sugars might involve communication between the transmitters in sugar-specific enzymes II (see PtsG in Fig. 2) and one of the CheA receiver modules.

Recent biochemical studies indicate that sensory signaling in the chemotaxis system may involve protein phosphorylation cascades (47–49). We suggest that T/R modules play important roles in these reactions. For example, CheA is known to autophosphorylate, and the likely site of modification is within its amino-terminal receiver module (48, 50). The placement of this module is particularly intriguing, as it distinguishes the large and small forms of the CheA protein, which are produced from different in-frame translational start sites in the cheA structural gene and might have different functions (Fig. 2) (ref. 51, unpublished results). Phosphate groups on CheA are subsequently transferred to CheB and CheY and probably serve to regulate their functional activities (47). Phosphorylated CheY may produce clockwise rotation of the flagellar motors; phosphorylated CheB may have elevated methylesterase activity. The sites of phosphorylation are most likely within the CheB and CheY receiver modules. In contrast, the receiver in CheZ is probably not a phosphate acceptor, but rather serves to accelerate the loss of phosphates from CheY (47). Finally, it appears that the carboxyl-terminal CheA receiver may accept sensory input from the MCP transmitters (48). This might activate the CheA transmitter, thereby controlling its autophosphorylation rate, and in turn the flow of phosphate through CheB and CheY.

How Transmitters and Receivers Might Work. We view transmitters and receivers as discrete functional units capable of mediating protein–protein communication. Signaling transactions between proteins containing matched T/R modules would involve three steps (Fig. 4): (*i*) sensory activation of the transmitter; (*ii*) interaction of the activated transmitter with its cognate receiver in the target protein and concomitant structural modification of the receiver; and (*iii*) modulation of the functional activity of the target protein by the modified receiver.

Module Interactions. Simple lock-and-key interactions between modules could provide a unifying mechanism for these disparate activities. Transmitters and receivers may have

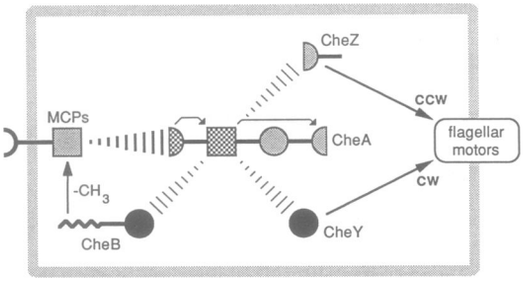

FIG. 3. Possible communication links between T/R modules in chemotaxis proteins. Transmitters are represented by boxes, receivers by circles or semicircles. The degree of shading roughly indicates the extent of similarity to the canonical sequences, as indicated in Fig. 2. Because of its modular complexity, CheA probably plays a central role in processing chemosensory information. CW and CCW, clockwise and counter-clockwise.

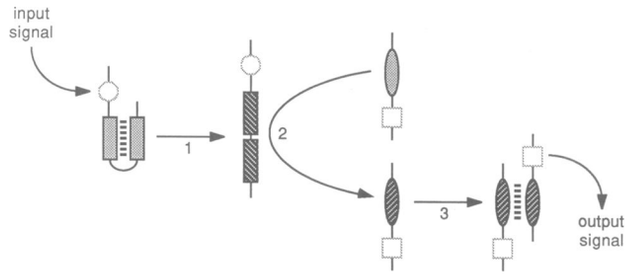

FIG. 4. Possible mechanisms of T/R transactions in dyadic relays. Transmitters are represented by rectangles, receivers, by ovals; other input or output domains in host proteins are represented by circles or squares, respectively. Dark shading indicates modules that have been activated by sensory input. Signaling proteins containing T/R modules could communicate through dyadic relays in which transmitters, activated by sensory input (step 1), modulate receivers (step 2), leading to an output signal (step 3). In complex networks, input and output operations may be mediated by transmitter and receiver modules coupled in the same signaling element.

acquired this property from a common ancestral sequence capable of self-recognition and aggregation. First, T/R modules bear resemblances in overall structural organization and amino acid composition that hint of a common ancestor. Second, transmitters are roughly twice the size of receivers, suggesting that they may be related by a duplication event. Thus, we predict specific interactions between matched transmitter and receiver modules and between related or identical receivers.

Transmitter-Mediated Receiver Modifications. Information transfer from transmitter to receiver module must involve a structural modification of the receiver. Phosphorylation events have been implicated in the NtrB–NtrC (42, 52) and chemotaxis (47, 50) systems, but other covalent or noncovalent modifications could serve the same purpose. Receiver modifications must be readily reversible; otherwise, activated receivers would continue to relay sensory signals in the absence of stimuli. Moreover, receiver deactivation should be relatively rapid to permit the system to deal effectively with transitory stimuli.

Receiver Control of Host Protein Activity. The functional activity of a protein containing a receiver module must be regulated by receiver modification state. Since receivers appear to be evolutionarily discrete elements that function in many different proteins, it appears these modules may be structurally independent entities, capable of controlling host protein activity without resorting to stereospecific interactions with host protein sequences. We propose that this control is effected through association or dissociation of the host protein subunits mediated by interaction of their receiver modules (Fig. 4). Thus, the primary effect of receiver modifications such as phosphorylation might be to influence the ability of the modules to aggregate.

Host Protein Control of Transmitter Activity. Transmitters in soluble proteins are probably controlled by a receiver in the same molecule, as appears to be the case for NtrB and CheA. Transmitters located in the cytoplasmic domains of transmembrane proteins are probably regulated by conformational changes propagated across the membrane from a periplasmic receptor domain. Transmembrane signaling is poorly understood, and there could conceivably be a variety of mechanisms based on this general theme. One simple possibility is shown in Fig. 4, in which the two receiver-like halves of a transmitter are paired in the inactive state. Transmembrane signals could activate the transmitter by disrupting this self-pairing.

Do T/R Modules Have Other Uses? We have suggested that T/R modules mediate specific protein–protein contacts that

result in conformational changes to at least one of the interacting partners. These properties could in principle be applied to many biological situations. For example, the MalF and MalE components of the ATP-driven maltose transport system comprise a functional T/R pair (Fig. 2). During uptake, maltose molecules are transferred from MalE, a periplasmic maltose-binding protein, to the membrane-associated MalF/MalG proteins, and subsequently to the cytoplasmic side of the membrane (53). The putative transmitter in MalF (A = 2.7) coincides with a probable periplasmic domain in the molecule (54) and could conceivably interact with the receiver in MalE (A = 1.8) to induce maltose-bound molecules to give up their ligand to the membrane-associated transport components.

Predicting Module Interactions. The mechanism modules employ for specific recognition may be a relatively simple one—for example, complementary interactions between α-helical segments. Unfortunately, three-dimensional structures have not been determined for any transmitter or receiver protein. Until such information is available, further study of T/R primary structures, particularly the unorthodox modules described in this report, may reveal sequence motifs associated with pairing specificity, and should prove generally useful in predicting the biochemical behavior of signaling proteins.

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1. Ronson, C. W., Nixon, B. T. & Ausubel, F. M. (1987) *Cell* **49,** 579–581.
2. Nixon, B. T., Ronson, C. W. & Ausubel, F. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7850–7854.
3. Stock, A., Koshland, D. E., Jr., & Stock, J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7989–7993.
4. Stock, A., Chen, T., Welsh, D. & Stock, J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1403–1407.
5. Gibbs, J. W. (1901) *Vector Analysis* (Yale Univ. Press, New Haven, CT).
6. Weber, R. F. & Silverman, P. M. (1988) *J. Mol. Biol.*, in press.
7. Mizuno, T., Wurtzel, E. T. & Inouye, M. (1982) *J. Biol. Chem.* **257,** 13692–13698.
8. MacFarlane, S. A. & Merrick, M. (1985) *Nucleic Acids Res.* **13,** 7591–7606.
9. Mutoh, N. & Simon, M. I. (1986) *J. Bacteriol.* **165,** 161–166.
10. Matsumura, P., Rydel, J. J., Linzmeier, R. & Vacante, D. (1984) *J. Bacteriol.* **160,** 36–41.
11. Buikema, W. J., Szeto, W. W., Lemley, P. V., Orme-Johnson, W. H. & Ausubel, F. M. (1985) *Nucleic Acids Res.* **13,** 4539–4555.
12. Nara, F., Matsuyama, S.-I., Mizuno, T. & Mizushima, S. (1986) *Mol. Gen. Genet.* **202,** 194–199.
13. Makino, K., Shinagawa, H., Amemura, M. & Nakata, A. (1986) *J. Mol. Biol.* **190,** 37–44.
14. Drury, L. S. & Buxton, R. S. (1985) *J. Biol. Chem.* **260,** 4236–4242.
15. Kudoh, J., Ikeuchi, T. & Kurahashi, K. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2665–2668.
16. Trach, K. A., Chapman, J. W., Piggot, P. J. & Hoch, J. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7260–7264.
17. Winans, S. C., Ebert, P. R., Stachel, S. E., Gordon, M. P. & Nester, E. W. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8278–8282.
18. Doolittle, R. F. (1981) *Science* **214,** 149–159.
19. Deveraux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395.
20. Friedrich, M. J. & Kadner, R. J. (1987) *J. Bacteriol.* **169,** 3556–3563.
21. Erni, B. & Zanolari, B. (1986) *J. Biol. Chem.* **261,** 16398–16403.
22. Nelson, S. O., Schuitema, A. R. J., Benne, R., van der Ploeg, L. H. T., Plijter, J. S., Aan, F. & Postma, P. W. (1984) *EMBO J.* **3,** 1587–1593.
23. Froshauer, S. & Beckwith, J. (1984) *J. Biol. Chem.* **259,** 10896–10903.
24. Duplay, P., Bedouelle, H., Fowler, A., Zabin, I., Saurin, W. & Hofnung, M. (1984) *J. Biol. Chem.* **259,** 10606–10613.
25. Jacobs, T. W., Egelhoff, T. T. & Long, S. R. (1985) *J. Bacteriol.* **162,** 469–476.
26. Egelhoff, T. T., Fisher, R. F., Jacobs, T. W., Mulligan, J. T. & Long, S. R. (1985) *DNA* **4,** 241–248.
27. Amemura, M., Makino, K., Shinagawa, H. & Nakata, A. (1986) *J. Bacteriol.* **168,** 294–302.
28. Bartlett, D., Frantz, B. & Matsumura, P. (1988) *J. Bacteriol.* **170,** 1575–1581.
29. Boyd, A., Kendall, K. & Simon, M. I. (1983) *Nature (London)* **301,** 623–626.
30. Krikos, A., Mutoh, N., Boyd, A. & Simon, M. I. (1983) *Cell* **33,** 615–622.
31. Eisenberg, D., Schwarz, E., Komaromy, M. & Wall, R. (1984) *J. Mol. Biol.* **179,** 125–142.
32. Weston, L. A. & Kadner, R. J. (1987) *J. Bacteriol.* **169,** 3546–3555.
33. Stock, J. B., Waygood, E. B., Meadow, N. D., Postma, P. W. & Roseman, S. (1982) *J. Biol. Chem.* **257,** 14543–14552.
34. Saier, M. H., Jr., & Roseman, S. (1976) *J. Biol. Chem.* **251,** 6598–6605.
35. Saier, M. H., Jr., & Roseman, S. (1976) *J. Biol. Chem.* **251,** 6606–6615.
36. Rossen, L., Shearman, C. A., Johnston, A. W. B. & Downie, J. A. (1985) *EMBO J.* **4,** 3369–3373.
37. Honma, M. A. & Ausubel, F. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8558–8562.
38. Djordjevic, M. A., Redmond, J. W., Batley, M. & Rolfe, B. G. (1987) *EMBO J.* **6,** 1173–1179.
39. Wanner, B. L., Wilmes, M. R. & Young, D. C. (1988) *J. Bacteriol.* **170,** 1092–1102.
40. Hirschman, J., Wong, P.-K., Sei, K., Keener, J. & Kustu, S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7525–7529.
41. Keener, J. & Kustu, S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4976–4980.
42. Khery, M. R. & Dahlquist, F. W. (1982) *J. Biol. Chem.* **257,** 10378–10386.
43. Parkinson, J. S., Parker, S. R., Talbert, P. B. & Houts, S. E. (1983) *J. Bacteriol.* **155,** 265–274.
44. Segall, J. E., Ishihara, A. & Berg, H. C. (1985) *J. Bacteriol.* **161,** 51–59.
45. Stewart, R. C. & Dahlquist, F. W. (1987) *Chem. Rev.* **87,** 997–1025.
46. Springer, M. S. & Zanolari, B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5061–5065.
47. Hess, J. F., Oosawa, K., Kaplan, N. & Simon, M. I. (1988) *Cell* **53,** 79–87.
48. Oosawa, K., Hess, J. F. & Simon, M. I. (1988) *Cell* **53,** 89–96.
49. Parkinson, J. S. (1988) *Cell* **53,** 1–2.
50. Hess, J. F., Oosawa, K., Matsumura, P. & Simon, M. I. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7609–7613.
51. Smith, R. A. & Parkinson, J. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5370–5374.
52. Ninfa, A. J. & Magasanik, B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5909–5913.
53. Shuman, H. A. & Treptow, N. A. (1985) in *The Enzymes of Biological Membranes*, ed. Martonosi, A. N. (Plenum, New York), 2nd Ed., Vol. 3, pp. 561–575.
54. Boyd, D., Manoil, C. & Beckwith, J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8525–8529.