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COMMUNICATION MODULES IN BACTERIAL SIGNALING PROTEINS

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CONTENTS

ENVIRONMENTAL AWARENESS IN BACTERIA

Bacteria live in precarious environments. Nutrient and toxin levels, acidity, temperature, osmolarity, humidity, and many other conditions can change rapidly and unexpectedly. To survive, the cells must constantly monitor external conditions and adjust their structure, physiology, and behavior

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accordingly. An elaborate network of environmental sensors and response regulators underlies these adaptive behaviors. These signaling proteins typically communicate by means of two distinctive domains, termed transmitters (T) and receivers (R) (87). These "communication modules" handle a wide variety of signaling tasks, including host detection and invasion leading to symbiosis (58) or pathogenesis (116, 218); metabolic adaptation to changes in carbon (72), nitrogen (86), electron acceptor (97), and phosphate (207) sources; physiological responses to changes in medium osmolarity (123); chemotaxis (185); and stress-induced differentiation, such as sporulation (25) and fruiting-body formation (232). A detailed list of references is given in Table 1. In this article we discuss the structural and functional properties that enable transmitters and receivers to propagate sensory signals between proteins and describe ways of combining these versatile devices to assemble complex signaling circuits.

COMMUNICATION MODULES

The "Two-Component" Paradigm

The simplest bacterial signaling systems have two protein components: a "sensor," often located in the cytoplasmic membrane, that monitors some environmental parameter; and a cytoplasmic "response regulator" that mediates changes in gene expression or locomotion in response to sensor signals (Figure 1) (138, 157). Sensors contain a carboxy-terminal transmitter motif of roughly 240 amino acids. Response regulators share an amino-terminal receiver motif of about 120 residues (87). These modules are associated with a variety of unrelated domains that function as "input" and "output" elements. Input domains of sensor proteins modulate transmitter activity. Receivers

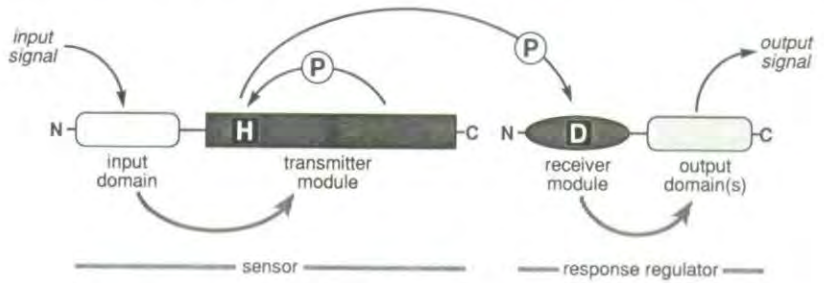

Figure 1 The "two-component" paradigm for sensory signaling via communication modules. Information flows through noncovalent controls exerted by one domain upon another (crosshatched arrows) and by phosphorylation reactions (arrows labeled P) involving histidine (H) and aspartate (D) residues. The convention of representing transmitters by rectangles, receivers by ovals, and input or output domains by rounded rectangles is also used in all subsequent figures.

activate or inhibit their associated output domains on receiving transmitter signals. The only demonstrated mechanism of communication between transmitters and receivers involves phosphorylation and dephosphorylation reactions (see references in Table 1). Transmitters have an autokinase activity that attaches phosphoryl groups from ATP to a histidine residue, from which they are subsequently transferred to an aspartate residue in the target receiver. Receiver phosphorylation modulates the activity of its adjoining output domain. Receiver dephosphorylation occurs by several routes and interrupts these regulatory responses. These transactions are mediated by specific recognition mechanisms that limit crosstalk from other signaling pathways.

It is increasingly evident that prokaryotic signaling systems are rarely as simple as this two-component paradigm. Most have multiple components, interconnections with other regulatory circuits, and feedback loops or other complex signaling properties. Nevertheless, elaborate communication networks also contain proteins with transmitter and receiver modules, which we regard as circuit elements for building more sophisticated signaling devices.

Module Hunts

We derived consensus sequences from best-fit alignments of the canonical transmitter and receiver sets (87) and used them to scan the GenBank and EMBL DNA data bases, translated in all six reading frames, for other prokaryotic module-containing proteins. We used a previously described scoring system based on relaxed sequence-matching criteria (87) to estimate the resemblance of polypeptide stretches to the canonical probe sequences. Proteins with scores above an arbitrarily high cutoff are listed in Table 1. The modules were loosely categorized as orthodox (closely resembling the canonical set, including absolute identities at key residue positions [described below]) or unorthodox (lacking one or more of these canonical features). Except for the CheA family, the signaling activities of proteins with unorthodox modules are poorly understood. Orthodox modules will command most of our attention in this review.

Communication modules have been found in a variety of gram-positive and gram-negative bacteria, and the list continues to grow. Extrapolating from the available sequence record, *Escherichia coli* alone may contain 40–50 transmitter–receiver pairs (187; our calculation). Although we found no examples of archaebacterial modules, there is much less sequence information on these organisms. Several groups have reported initial success in using consensus oligonucleotide probes to search directly for module-encoding genes by DNA hybridization (132, 142, 147). This should be a useful approach in archaebacteria as well. The widespread occurrence of communication modules in prokaryotes implies that they are evolutionarily ancient devices that have proven useful in diverse signaling applications.

Table 1 Bacterial signaling proteins containing communication modules

Adaptive system	Communication module types[a]			Organisms and references[b]
	T	T + R	R	
Cell cycle	—	—	FlbD[c]	Ccr (152)
Chemotaxis	**CheA**[d,e]	—	**CheY**[e], **CheB**	Bsu (15, 52), Ccr[e,f], Eae[g] (34), Eco (88, 110, 131), Rme[h], Pae (175), Sty (169, 171, 182, 183)
Host interactions				
Symbiosis	NodV	—	NodW	Bja (58)
Virulence	PhoQ	—	PhoP	Eco (85), Sty (117)
	Xcc2	—	Xcc1	Xca (142)
	—	BvgS[i]	BvgA	Bbr (124), Bpe (10, 181), Bpar (11)
	—	LemA	—	Psy (14, 66)
	—	RpfC	—	Xca (195)
	—	**VirA**	**VirG**	Arh (46), Atu (94, 219)
	—	—	LcrB	Rsp[j]
	—	—	MrkE	Kpn (3)
Metabolite utilization				
Carbon	DctB	—	DctD	Rle (156), Rme (75)
	PgtB	—	PgtA	Sty (229, 231)
	UhpB	—	UhpA	Eco (51), Sty (72)
	—	—	GlpR[k]	Pae (164)
	—	—	**FPr**[c]	Sty (53)
	—	—	TctD	Sty (217)
Nitrogen	NifR2	—	NifR1	Rca (79)
	NtrB	—	**NtrC**	Atu (209), Bpa (138), Eco (121), Kpn (103), Rme (24, 191), Val (105)
	—	—	AdgA[c]	Rca[l]
Phosphate	**CreC**[m,n]	—	**CreB**[m,n]	Eco (4)
	PhoR	—	**PhoB**[o]	Bsu (166, 167), Eco (108, 109), Kpn (92), Pae (8), Sdy (92)
Nitrogen fixation	**FixL**	—	**FixJ**, ORF138[n]	Aca (82), Bja (9), Rme (37)
	NifL	—	—	Avi[p]
	NtrY	—	NtrX	Aca (144)
Photosynthesis	—	—	RegA	Rca (168)
Redox changes	*HydH*	—	HydG	Eco (188), Sty (29)
	NarQ, NarX	—	NarL	Eco (150, 180)
	—	**ArcB**	**ArcA**[q]	Eco (43, 74)
	—	—	HoxA	Aeu (44)
	—	—	HupR1	Rca (153)
Stress from				
Antibiotics	—	RteA	RteB	Bth (176)
	—	—	YecB[r]	Eco (126), Pfl (91)
Heavy metals	*CutS*	—	CutR	Sli (201)
	—	—	PcoR	Eco (23)

Table 1 (*Continued*)

Adaptive system	Communication module types[a] T	T + R	R	Organisms and references[b]
Osmolarity	**EnvZ**	—	**OmpR**	Eco (32, 223), Sty (96)
	—	**BarA**	—	Eco (132)
Starvation	*AgrORF2*[x]	—	AgrA	Sau (146)
	DegS	—	**DegU**	Bsu (62, 90)
	—	AsgA	—	Mxa[t]
Turgor pressure	KdpD	—	KdpE	Eco(206)
Stress leading to				
Antibiotic synthesis	VanS	—	VanR	Efa (12, 61)
Capsule synthesis	**AlgR2**[d]	—	**AlgR1,** AlgB	Pae (40, 57, 159, 222)
	—	RcsC	RcsB	Eco (189)
Fruiting-body formation	—	**FrzE**[d]	FrzZ[n,u]	Mxa (111)
Gene transfer	ComP	—	ComA	Bsu (212)
	CpxA[v]	—	**ArcA**	Eco (43, 210)
Heterocyst formation	—	—	PatA[c,w]	Asp (95)
Sporulation	**KinA**[x], **KinB**[y]	—	**Spo0A, Spo0F**	Bsu (87, 147, 200)
Unknown	GrsBORF[z]	—	—	Bbre (203)
	MtuORF[aa]	—	—	Mtu (230)
	PleC	—	—	Ccr[bb]
	UspT[cc]	—	UrpT[cc]	Eco (65, 154)
	—	—	BmeORF[dd]	Bme (122)
	—	—	CacORF[ee]	Cac (60)
	—	—	CtrORF[ee,ff]	Ctr (174)
	—	—	LinORF	Lin[gg]
	—	—	PetHORF	Ssp7002 (162)
	—	—	RcaORF[hh]	Rca (38)
	—	—	SspORF[ii]	Ssp6803 (28)
	—	—	TrpX[jj]	Pae (59)
	—	—	YbcA[kk]	Eco (130), Sty[ll]

[a] Italicized protein names indicate tentative relationships often inferred from incomplete sequence data. Boldface print indicates proteins that are known to phosphorylate: AlgR1 (159); AlgR2 (159); ArcA, ArcB (E. Lin, personal communication); BarA (132); CheA, CheB, CheY (64); CpxA (P. Silverman, personal communication); CreB, CreC (5); DegS, DegU (129); EnvZ (71); FixJ, FixL (56); FPr (53); FrzE (112); KinA (147); KinB (J. Hoch, personal communication); NtrB (86); NtrC (135); OmpR (70); PhoB, PhoR (106); Spo0A, Spo0F (147); VirA (69, 76); VirG (77).

[b] Organism abbreviations: Aca, *Azorhizobium caulinodans;* Aeu, *Alcaligenes eutrophus;* Arh, *Agrobacterium rhizogenes;* Asp, *Anabaena* sp.; Atu, *Agrobacterium tumefaciens;* Avi, *Azotobacter vinelandii;* Bbr, *Bordetella bronchiseptica;* Bbre, *Bacillus brevis;* Bja, *Bradyrhizobium japonicum;* Bme, *Bacillus megaterium* IAM1030; Bpa, *Bradyrhizobium parasponiae;* Bpar, *Bordetella parapertussis;* Bpe, *Bordetella pertussis;* Bth, *Bacteroides thetaiotaomicron;* Cac, *Clostridium acetobutylicum;* Ccr, *Caulobacter crescentus;* Ctr, *Chlamydia trachomatis;* Eae, *Enterobacter aerogenes;* Eco, *Escherichia coli;* Efa, *Enterococcus faecium;* Kpn, *Klebsiella pneumoniae;* Lin, *Leptospira interrogans;* Mtu, *Mycobacterium tuberculosis;* Mxa, *Myxococcus xanthus;* Pae, *Pseudomonas aeruginosa;* Pfl, *Pseudomonas fluorescens;* Psy, *Pseudomonas syringae* pv. *syringae;* Rca, *Rhodopseudomonas capsulata;* Rle, *Rhizobium leguminosarum;* Rme, *Rhizobium meliloti;* Rsp, *Rhizobium* sp.; Sau, *Staphylococcus aureus;* Ssp7002, *Synechococcus* sp. PCC 7002; Ssp6803, *Synechocystis* sp. PCC 6803; Sdy, *Shigella dysenteriae;* Sli, *Streptomyces lividans;* Sty, *Salmonella typhimurium;* Val, *Vibrio alginolyticus;* Xca, *Xanthomonas campestris* pv. *campestris*.

[c] Unorthodox receiver: AdgA, CtrORF (no acid pocket); FlbD (no acid pocket or conserved lysine); FPr (several gaps to align); PatA. (No D equivalent to CheY D57. Our algorithm aligns PatA N311 with the 'D57' residue in the orthodox receiver consensus. However, the published alignment has PatA D313 in this position [95].)

[d] Unorthodox transmitter: AlgR2 (resembles partial transmitter; includes conserved H and block N); CheA, FrzE (sequence surrounding autophosphorylated histidine does not resemble block H and is distantly spaced from block N).

[e] Former names in *B. subtilis:* CheN (for CheA), CheB (for CheY).
[f] L. Shapiro, personal communication; incomplete sequence.
[g] Incomplete sequence.
[h] R. Schmitt, personal communication.
[i] Other names: BvgC, Vir.
[j] Upadhyaya, N. M., Scott, K. F., Dart, P. J. 1991. Rhizobium genes *lcrB* and *lcrD* involved in pigeonpea (*Cajanus cajan* Mill sp.) leaf curl induction show homology with *E. coli* regulatory genes (*ompR* and *fnr*) responsive to environmental stimuli. Unpublished GenBank entry M38698.
[k] GlpR is probably allelic to AgmR (164).
[l] Willison, J. C. 1991. Essential genes of *Rhodobacter capsulatus* and *Escherichia coli* RT possibly involved in nitrogen metabolism. Unpublished GenBank entry X59399.
[m] Former names in *E. coli:* PhoM (for CreC), PhoM ORF2 (for CreB).
[n] Phenotype uncertain.
[o] Current *B. subtilis* name: PhoR.
[p] Blanco, G., Bali, A., Kennedy, C., Woodley, P. 1992. Molecular analysis of the *nifL* gene of *Azobacter vinelandii*. Unpublished GenBank entry X64832.
[q] Former names: Dye, SfrA, FexA, Msp, Seg.
[r] Current *Pseudomonas fluorescens* name: GacA; former *E. coli* name: UvrC ORF2.
[s] Deduced from incomplete sequence reported in Stock et al (186).
[t] L. Plamann, personal communication.
[u] D. Zusman, personal communication.
[v] Former names: EcfB, Ssd, Eup.
[w] The only known example of a C-terminal receiver that is not part of a TR protein.
[x] Former name: SpoIIJ.
[y] J. Hoch, personal communication.
[z] ORF near *Bacillus brevis grsB*.
[aa] ORF near *Mycobacterium tuberculosis* gene for 19-kD antigen.
[bb] Sharma, P., Wang, S. P., Schoenlein, P. V., Ely, B. 1992. Genes affecting polar organelle development in *Caulobacter crescentus*. Unpublished GenBank entry M91449.
[cc] Partial sequence data.
[dd] ORF near *Bacillus megaterium* gene for glucose dehydrogenase.
[ee] ORF near *Clostridium acetobutylicum cba*.
[ff] ORF in *Chlamydia trachomatis* plasmid.
[gg] Ding, M., Yelton, D. B. 1990. ORF near *Leptospira interrogans leuB*. Unpublished GenBank entry M59431.
[hh] ORF near *Rhodopseudomonas capsulata petG;* incomplete sequence.
[ii] ORF near *Synechococcus* sp. PCC 7002 *psaE;* possibly incomplete sequence.
[jj] Our nomenclature; ORF near *Salmonella typhimurium trpAB* operon.
[kk] Former name in *E. coli:* YdnY. Name in *Salmonella typhimurium:* FimZ.
[ll] Swenson, D. L., Clegg, S. 1992. Analysis of a *fimA-lacZ* fusion in *Salmonella typhimurium*. Unpublished GenBank entry M90677.

Despite their prevalence in bacteria, transmitters and receivers are scarce in eukaryotes. One intriguing candidate is phytochrome, which serves an important signaling function in plants. It contains a transmitterlike domain and possibly autophosphorylates (163). A protein kinase from rat mitochondria was recently found to contain an orthodox transmitter, although it is not yet clear whether it autophosphorylates at a histidine residue (147a). Interestingly, its protein target, branched-chain α-ketoacid dehydrogenase, is phosphorylated at a serine rather than an aspartate residue (147a). A reverse open reading frame adjacent to the UBI1 gene of *Saccharomyces cererisiae* encodes an orthodox receiver motif (R. Baum, personal communication). Its function, if any, is unknown, although its presence seems unlikely to be a mere coincidence. There are also tantalizing parallels, both structural and functional, between bacterial receivers, as exemplified by the CheY protein of *E. coli*, and the Ras family of signal-transducing proteins in eukaryotes (13,

27). Receiver modules, like their G-protein counterparts, can be regarded as molecular switches whose state is regulated by reversible conformational changes and an intrinsic timing mechanism (185).

Modular Designs and Evolution

Transmitter- and receiver-containing proteins exhibit a variety of module arrangements (Figure 2). Receivers can occur alone (R), in tandem (RR), or in combination with different output domains (RO), most of which have regulatory functions and can themselves be grouped into subfamilies on the basis of sequence similarity. Proteins in the RO_I group have unrelated output domains with disparate functions. For example, Spo0A regulates gene expression leading to sporulation (147), whereas CheB is a methylesterase that covalently modifies chemoreceptors during sensory adaptation to chemotactic stimuli (179). Output domains of the RO_{II} subfamily appear to have DNA-binding roles. Some of these response regulators (OmpR, PhoB, and VirG) bind to specific target sequences upstream from the promoters they regulate (78, 104, 107, 145, 149, 202). The RO_{III} group is still poorly understood but may involve a DNA-binding function as well, since the output domain of FixJ resembles the promoter recognition region of sigma factors (80). The RO_{IV} subfamily contains two linked output domains. The C-terminal domain binds at enhancer sequences in the vicinity of the regulated target genes (190, 211), whereas the central domain interacts with the σ^{54} form of RNA polymerase holoenzyme to promote formation of open transcription complexes (213).

Most sensor proteins are located in the cytoplasmic membrane with their transmitters projecting into the cell. Nearly all of them have two membrane-spanning segments (186; our computational analyses), but at least one, UhpB, has more (72). Their periplasmic domains are structurally unrelated and have diverse receptor functions. For example, EnvZ senses osmolarity changes (198), KdpD is thought to respond to turgor pressure (47), and UhpB detects periplasmic hexose phosphates (72, 216). The periplasmic region need not be a receptor, however. ArcB, a redox sensor, may detect stimuli through interaction with an element of the respiratory chain within the cytoplasmic membrane (73). Its small periplasmic domain may serve strictly as an anchor. Similarly, FixL, a heme-binding protein that monitors oxygen, is anchored to the membrane, but its receptor domain is on the cytoplasmic side (125). A few transmitter-containing proteins, such as NtrB and DegS, are soluble and contain N-terminal domains that presumably have input roles, although this has not yet been established (62, 103). Finally, some signaling proteins contain both kinds of communication modules (Table 1).

Communication modules are often joined to adjacent input or output domains by apparently flexible connectors such as Q-linkers (221), AP-rich

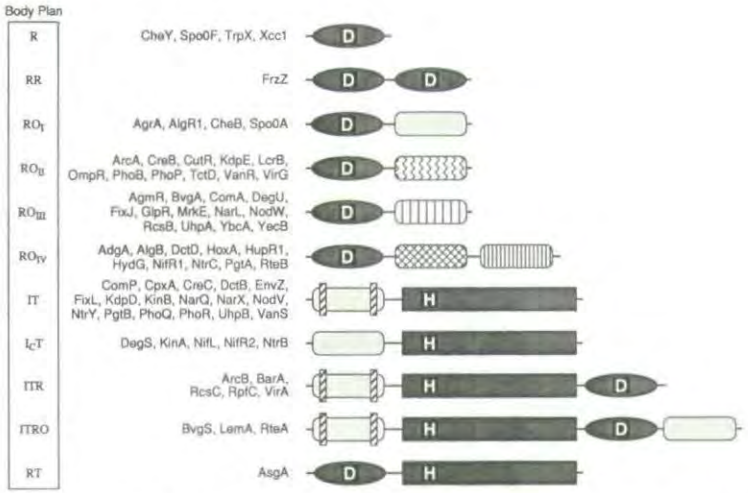

Figure 2 Arrangements of orthodox communication modules in signaling proteins. The shorthand body plan notation lists domains or modules in order from the N terminus, using the symbols I (input domain), T (transmitter), R (receiver), and O (output domain). Most input domains contain membrane-spanning segments (indicated by shaded bars); those that have none are denoted Ic (cytoplasmic). The output domains of RO$_I$ proteins are dissimilar, whereas those within each of the other RO groups are related in sequence but different between groups.

linkers (151), or charge-rich linkers (55). For example, the receiver and output domains in many response regulators are joined by Q-linkers, as are the input domain and transmitter of NtrB (221). AP-rich linkers can be found between the histidine phosphorylation site and transmitter of FrzE (111) and between the transmitter, receiver, and C-terminal domains of BvgS (11). Charge-rich linkers join the P_1, P_2, and transmitter domains of CheA (our observations; see below). We suggest that pliable connections are a common structural feature of transmitter and receiver proteins and are important to their signaling activities. We will refer to such regions as "flexible linkers" in subsequent sections.

To explore possible evolutionary relationships among communication modules, we carried out a dendrographic analysis of 91 receivers and 53 transmitters by using the PileUp program of the University of Wisconsin Genetics Computer Group Sequence Analysis Package, version 7.1 (41). Abridged versions of the trees are shown in Figure 3 and illustrate several important features. First, some clusters can be rationalized in terms of function, such as the modules associated with nitrogen utilization. Transmitters of NtrB, NifR2, and NtrY are similar in sequence, as are their cognate receivers in NtrC, NifR1, and NtrX. Second, some clusters are correlated with protein body plan. For instance, modules of the ITR and ITRO proteins BvgS, BarA, LemA, RpfC, RcsC, and ArcB all fall into the same transmitter and receiver

groups. Third, some transmitter clusters have matching receiver clusters, even though there is no obvious functional or design basis for the clustering. NarX, DegS, and UhpB have similar transmitters, and the receivers in their partner response regulators, NarL, DegU, UhpA, are also clustered. Similarly, the CpxA, CreC, EnvZ, KdpD, and PhoR transmitters are clustered, as are the receivers in ArcA, CreB, OmpR, KdpE, and PhoB, their response regulator partners. Finally, there are several exceptions to these three generalizations. The FixJ receiver, for example, clusters with its nitrogen-related counterparts, whereas the FixL transmitter does not.

We draw two general conclusions from these observations. First, communication modules may have been disseminated by relatively recent lateral transmission. Proteins in *E. coli* and *Bacillus subtilis*, organisms that probably diverged more than a billion years ago (39), often contain very similar modules (e.g., the transmitters in NarQ, NarX, UhpB, ComP, and DegS; the receivers in NarL, UhpB, and DegU). Given the extensive variability among modules

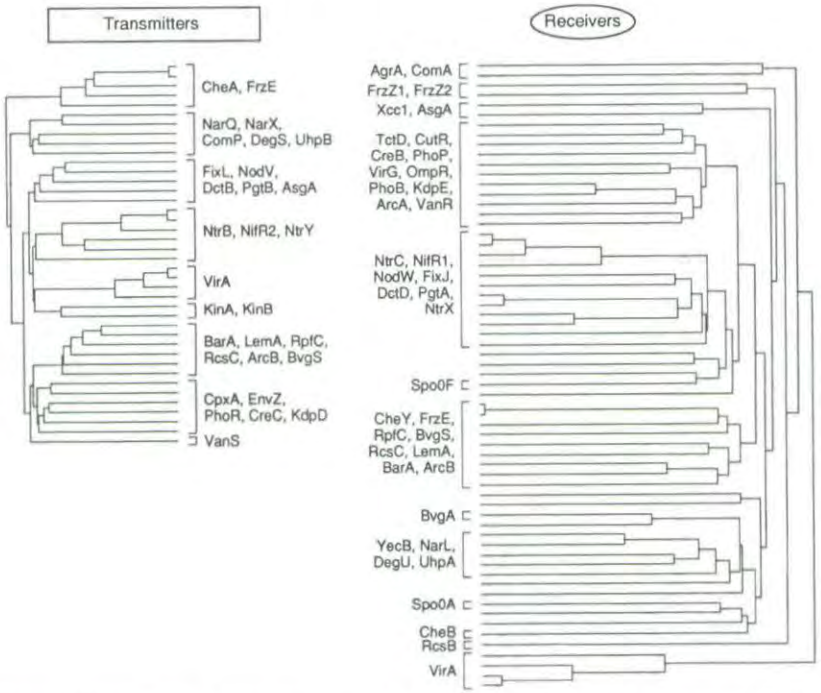

Figure 3 Dendograms showing sequence relatedness of selected transmitters and receivers. The total length of the horizontal lines connecting two proteins is inversely proportional to the extent of similarity between their modules. Proteins in each similarity cluster are listed in order from top to bottom. When a group contains examples of the same protein from different species, the name is listed only once.

within a species, it seems unlikely that sequence similarity between species could result from either divergent or convergent evolution. We note that some module-containing proteins are encoded on naturally occurring plasmids, which could serve as vehicles for horizontal transfer to disparate taxa (12, 46, 94, 176). Second, sequence relatedness is not highly correlated with communication specificity. For example, the ComP and DegS transmitters are similar in sequence but communicate with very dissimilar receivers (ComA, DegU). Thus, it may be difficult, on the basis of sequence comparisons alone, to discern the determinants responsible for communication specificity. We return to this issue later.

RECEIVERS

Receiver modules recognize and interact with their cognate transmitters, accept signals from them, and then modulate output activity. We assume that these transactions involve changes in receiver phosphorylation state, even though this has not been explicitly demonstrated for many of the proteins listed in Table 1.

Phosphorylation and Dephosphorylation Reactions

Once a transmitter has autophosphorylated, the transfer of phosphate from histidine to receiver is most probably catalyzed by the receiver itself rather than by the transmitter. Two key findings support this view. First, a proteolytic fragment of CheA that contains phosphohistidine but lacks other parts of the transmitter is sufficient for transphosphorylation of CheB and CheY (63). Second, CheB, CheY, PhoR, NtrC, and presumably other response regulators can phosphorylate themselves by using phosphoramidate and other small molecule phosphate donors (48, 100). Thus, receivers are enzymes in their own right, capable of using various phosphate sources, including appropriately presented phosphohistidines, as substrates for in vivo autophosphorylation.

The in vitro half-lives of phosphorylated receivers vary greatly, from a few seconds for CheB and CheY (64, 224) to several hours for OmpR (70) and VirG (77). Receiver dephosphorylation also appears autocatalytic, because it can take place in the absence of other proteins. Like the autophosphorylation reaction, it is dependent on Mg^{2+} or other divalent cations and is effectively halted on denaturation (161, 215). This autophosphatase activity confers a characteristic half-life on the activated receiver. As discussed below, transmitters may modulate this activity to control receiver phosphorylation levels.

Structure–Function Relationships: the CheY Model

The crystal structures of the *Salmonella typhimurium* (184) and *E. coli* (205) CheY proteins, which correspond to individual receiver modules, have been determined at high resolution. All orthodox receivers contain highly conserved

aspects of primary structure and may be built along similar lines. A recently obtained low-resolution X-ray structure for Spo0F, a single-domain receiver protein from *B. subtilis*, resembles that of CheY in all major respects (J. Hoch, personal communication). Four highly invariant residues, which correspond to Asp-12, Asp-13, Asp-57, and Lys-109 in CheY, play central roles in the phosphorylation and signaling activities of receiver modules. We adopt the convention of using the position of a CheY residue in single quotes (e.g., 'Asp-57') when referring to the corresponding residue in other receivers, which differ slightly in overall length and register.

CheY is an α/β barrel containing five sets of alternating β strands and α helices connected by short loops or turns (Figure 4*a*) (184, 205). The β strands (1–5) align in parallel to form a hydrophobic inner core with the α-helical segments (A–E) wrapped around the outside of the molecule. The N and C termini are both at one end of this roughly cylindrical structure, and the functionally critical residues are at the opposite end. Aspartates 12, 13, and 57 lie close together to form an acid pocket into which the side chain of Lys-109 protrudes.

'Asp-57' has been shown by direct chemical analyses to be the phosphorylation site in CheY (161) and in VirG (77). Mutants with amino acid replacements at this position in various receiver proteins, including CheB (178), CheY (19), DegU (35), NtrC (S. Kustu, personal communication), OmpR (21), and VirG (77), are incapable of being phosphorylated and have no signaling activity. Thus, 'Asp-57' seems to be the main phosphorylation site in receiver modules. Whether some receivers contain different or

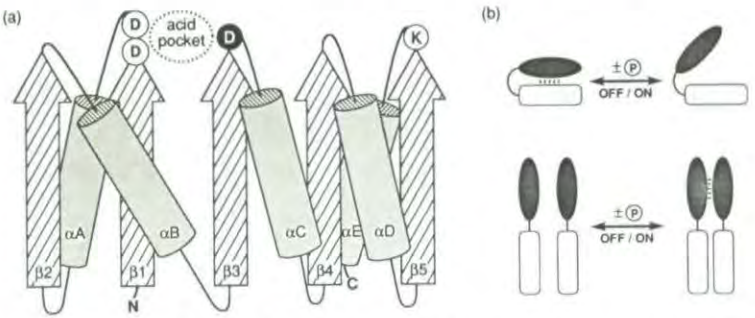

Figure 4 (a) Structural features of orthodox receivers based on the X-ray structure of CheY. Arrows represent strands, and cylinders represent α helices. In the native structure, an α-βbarrel, α helices αA and αE are close together and arrayed along with the other helices on the outer face of the barrel. The black circle indicates the aspartate that serves as the phosphorylation site; other important residues are indicated by white circles. (b) Two general mechanisms for controling output domain activity (OFF or ON) through changes in receiver phosphorylation state ±p. Receivers could modulate activity by direct interaction with the output domain (top) or by mediating subunit association (bottom).

alternative phosphorylation sites remains an open issue (19). A possible example is PatA, which contains a highly orthodox receiver except for an asparagine at 'Asp-57' (see Table 1, footnote c).

The acid pocket is a characteristic feature of orthodox receivers. 'Asp-13' is almost universally present, but about half of the receivers have a glutamate instead of an aspartate at position '12'. Many receivers, unlike CheY, also have an acidic residue at position '14'. Mutations affecting the acid pocket residues generally reduce phosphorylation ability. Depending on the location and nature of the mutant amino acid, some alterations reduce function and others lead to constitutive activity. Examples include CheB (178), CheY (19), DegU (35), NarL (45), OmpR (21), PhoB (225), Spo0A (173), and VirG (155). In CheY, Asp-13 and Asp-57 contribute to the binding of divalent cations, particularly Mg^{2+}, which plays a crucial role in CheY phosphorylation activities (101). The acid pocket residues may serve a similar function in other receivers.

'Lys-109' is found in all orthodox receivers and probably plays a fundamental role in their behavior. In unphosphorylated CheY, the ϵ-amino group of Lys-109 lies close to the carboxyl group of Asp-57 (205). An arginine replacement at this position results in a complete loss of function, even though the mutant protein can still be phosphorylated (99). Moreover, fluorine nuclear magnetic resonance (NMR) studies suggest that Lys-109 is repositioned in the known constitutively active CheY mutants (J. Falke, personal communication). These findings suggest that phosphorylation might displace the side chain of Lys-109 from the acid pocket, thereby triggering conformational changes in other parts of the molecule which regulate its activity. An Asp-to-Lys replacement at position '110' in DegU leads to hyperproduction of degradative enzymes (62) that could be caused by a similar mechanism of conformational change. Replacement of 'Lys-109' with arginine reduces both the phosphorylation and dephosphorylation ability of CheY (99) and CheB (177). These defects could stem directly from alterations in the chemistry of the acid pocket or indirectly from the effects of conformational changes.

Control over Output Activity in Response Regulators

Phosphorylation has been implicated, at least circumstantially, in controlling output activity for five of the six receiver subfamilies shown in Figure 2. Usually phosphorylation leads to an activation of output function, but in some response regulators the unphosphorylated form may also have a functional role. The two forms of DegU, for example, have different regulatory activities (36). Phosphorylation of CheY augments clockwise (CW) rotation of the flagellar motors, perhaps by enhancing its affinity for the switching machinery (19). Phosphorylation of CheB enhances its methylesterase activity (102).

Phosphorylation of PhoB (106), OmpR (50), and presumably VirG (155) enhances their ability to bind to specific regulatory sequences involved in transcriptional activation. Phosphorylation of NtrC enhances its DNA-binding ability (48, 214) and enables it to promote open-complex formation between target promoters and the σ^{54} form of RNA polymerase (213).

Receiver mutations that reduce phosphorylation ability usually block stimulus-induced changes in output activity. These changes often occur in acid pocket residues. Such mutations have been characterized in CheB (178), CheY (19), OmpR (21, 83), PhoB (225), and VirG (155). However, phosphorylation per se is probably not required for output activity. There are several examples of constitutively active receiver proteins that are phosphorylated poorly or not at all, at least in vitro. The Asp-13–to–Lys mutant of CheY is a poor substrate for phosphorylation but causes CW-biased flagellar rotation (19). A mutant OmpR with a glutamine replacement at 'Asp-57' is unphosphorylated in vitro and inactive in vivo, but second-site mutations at several positions within its receiver domain can restore transcriptional activity (20). In both of these cases, the constitutive activities of the mutant proteins are not dependent on their cognate transmitter proteins (CheA and EnvZ), although in vivo activation through crosstalk phosphorylation remains a remote possibility. Other examples of constitutively active and transmitter-independent receiver mutants have been described for CheB (177), DegU (35, 62), NarL (45), and Spo0A (139, 173).

Since the activating effects of response regulator phosphorylation can be mimicked by mutational changes, phosphorylation may be simply a device for inducing conformational changes. One such mechanism, involving displacement of 'Lys-109' from the acid pocket, has already been discussed, but in principle any sort of phosphorylation-induced deformation could be harnessed to regulate adjoining output domains. Although we know very little about how this control is accomplished, it does appear that receivers have developed both positive and negative strategies for controlling associated output domains.

Receiver modules in some response regulators seem to control their output domains through inhibition. The enzymatic activity of CheB is stimulated on proteolytic or genetic removal of its receiver module (170). Similarly, the isolated output domain of FixJ is transcriptionally competent, suggesting that the receiver negatively controls its activity (80). Phosphorylation presumably activates these proteins by releasing receiver-imposed output inhibition. The central and C-terminal domains of the RO$_{IV}$ response regulator subfamily (NtrC, DctD, etc.) closely resemble other regulatory proteins (NifA, GerE) that function without an attached receiver module, suggesting that negative controls might operate here as well (33, 42, 67). However, there is experimental evidence on both sides of the issue. The isolated central domain

of DctD is transcriptionally competent (68), whereas removal of the receiver module from NtrC does not stimulate its transcriptional activity (42; S. Kustu, personal communication).

The output domains in most response regulators may be under positive control, but the evidence so far is mainly circumstantial. For example, removal of the receiver module in OmpR (202) does not enhance the DNA-binding ability of its output domain, implying that the receiver is needed to activate output function. Consistent with this conclusion, only a few missense changes in the OmpR receiver lead to constitutive transcriptional activity (20). Unlike CheB, the OmpR control mechanism evidently cannot be circumvented by lesions that simply disrupt the receiver. Similar arguments can be made for constitutively active mutants of CheY (19), DegU (35, 62, 128), and Spo0A (139, 173).

Two general classes of mechanisms would enable receivers to exert positive or negative control over output function (Figure 4*b*). Control could involve direct interaction of the receiver with the output domain, promoted through specific contacts modulated by the phosphorylation state (Figure 4*b* top). Although conceptually simple, this requires that receivers coevolve with their output partners, constraining the generation of new communication specificities. Alternatively, output control might involve no direct contact between the receiver and the rest of the protein, in which case any particular receiver could in principle be functionally coupled to a variety of output domains. A simple mechanism for indirect control could be through phosphorylation-induced changes in the receiver aggregation state (87). For example, phosphorylation might cause receiver subunits to dimerize, thereby altering the function of their output domains by bringing them into closer proximity (Figure 4*b* bottom). The presence of flexible linkers joining receiver and output domains in many response regulators implies that some relative movement of the two domains may be necessary for proper control, but neither direct nor indirect models make explicit predictions about the importance of linker length. In fact, lengthening the linker of NtrC by four or eight residues had no effect on its function (221).

These control models make several different genetic predictions, but as yet little work has been done to test them. One approach would be to examine the effects of "module liberation" by severing the connection between receiver and output domain and expressing one or both of them as separate proteins. Indirect control via changes in subunit association requires that the receiver and output domain be physically linked, whereas direct control via specific contacts might not. A second approach would involve "module swaps" in which receivers and output domains are paired with new partners. If control were indirect, it should be possible to regulate an output domain with a heterologous receiver (and its cognate transmitter), whereas direct control

mechanisms should be abrogated in chimeric proteins. Two attempts at receiver swaps, CheY for CheB (R. Stewart, personal communication) and OmpR for NtrC (42), have yielded negative results. However, in both cases the transplanted receivers probably use a positive control strategy, whereas the displaced receivers may use a negative strategy, so the failure of the hybrid proteins to function properly may not provide a critical test of these models.

Several recent lines of evidence support the idea of control by receiver dimerization. VirG shows concentration-dependent DNA binding, suggesting that it acts as a multimer (148). NtrC binds to DNA as a dimer but tetramerizes on phosphorylation, leading to cooperative binding at target sites (48, 213, 214). It may be this enhanced binding, rather than phosphorylation per se, that activates NtrC for transcription (214). Phosphorylated OmpR can be trapped in higher-order aggregates by chemical crosslinking (133). Conceivably, phosphorylation enhances the tendency of OmpR to form multimers, which in turn enhances its binding affinity for regulatory sequences, leading to induction or repression of target promoters. Nakashima et al (133) have described two dominant receiver mutants of OmpR that are phosphorylated normally (which probably accounts for their dominance) but are defective in both phosphorylation-induced DNA binding and aggregation. Brissette et al (22) isolated two other OmpR mutants with similar properties. These four mutants have single missense changes in residues at the C-terminal end of α-helix D or at the N-terminal end of α-helix E. These might be regions involved in receiver dimerization contacts.

TRANSMITTERS

Transmitter modules recognize and interact with cognate receivers, emitting signals to them under input control. We assume that these transactions involve changes in transmitter phosphorylation activities, even though this has not been explicitly demonstrated for many of the proteins listed in Table 1.

Phosphorylation and Dephosphorylation Reactions

The only proven catalytic activity of transmitters is the intramolecular formation of a high-energy phosphohistidine (5, 50, 63, 77, 106, 134). As we discussed in the previous section, receivers probably promote their own phosphorylation, using phosphohistidine in cognate transmitters as a preferred substrate. Thus, the transmitter autophosphorylation reaction represents a major control point for regulating the flow of phosphate into receivers.

Several transmitter proteins also exhibit an apparent "phosphatase" activity that provides a second method for regulating the phosphorylation states of cognate receiver proteins. Examples are EnvZ/OmpR (1, 70) and the related Ta21/OmpR (228), PhoR/PhoB (106), and DegS/DegU (36, 194). In the

first three systems, the "phosphatase" reaction requires divalent metal ions and ATP, ADP, or nonhydrolyzable analogs of ATP. Similar requirements apply for NtrB and the nitrogen regulator protein, PII, which together accelerate the hydrolysis of phospho-NtrC (86, 135). Transmitter-dependent "phosphatase" activities have also been postulated for other systems whose chemistries are not yet well characterized, such as NarX/NarL (31), PhoP/PhoQ (118), and CreC/CreB (5).

The ability of the CheB (102), CheY (161), NtrC (86), PhoB (106), and presumably other receivers to catalyze their own dephosphorylation raises questions about the nature of the "phosphatase" activity ascribed to sensor proteins. Conceivably, transmitters may not catalyze removal of phosphoryl groups from receivers but, rather, may augment the receiver's intrinsic dephosphorylation activity, perhaps by manipulating the local conformation of the receiver phosphorylation site. CheZ, a component of the *E. coli* chemotaxis system, bears no resemblance to transmitters and provides precedent for this notion. It specifically accelerates the dephosphorylation of CheY but could be an allosteric regulator rather than a true phosphatase (64, 99).

Receiver proteins with no detectable autophosphatase ability, such as OmpR (70), may nevertheless possess a latent activity that is turned on through transmitter interactions. All orthodox receivers contain the acid pocket residues known to be vital for both autophosphorylation and autophosphatase activities in CheY (101). Relatively minor structural changes within this active site could dramatically alter the overall behavior of the module (99), so that a receiver with no basal phosphatase activity might be inducible under proper conditions.

Sequence and Structural Features

Orthodox transmitters contain short blocks of common sequence, similarly arranged but variably spaced (Figure 5). We refer to these motifs by their characteristic residues (blocks 'H', 'N', 'G1', 'G2', and 'F'). Stock et al (186) defined the first four; the fifth became apparent after alignment of many new transmitters available since their review. Block H, the most variable of the five regions, is located in the N-terminal half of the transmitter and includes the histidine residue that serves as the site of autophosphorylation. The other segments are located in the C-terminal half of the transmitter. Blocks G1 and G2 resemble glycine-rich portions of nucleotide-binding domains (158). They are separated by a spacer of somewhat variable length and composition, with block F roughly in its middle.

It is not uncommon for transmitters to lack one of these similarity regions. Important examples are the transmitters of the CheA family, which lack block H but contain a histidine near the N terminus that serves as the site of

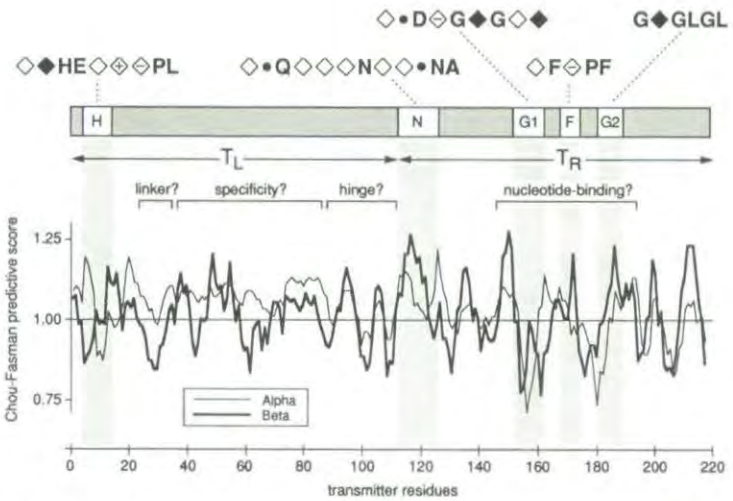

Figure 5 Structural features of orthodox transmitters. Five sequence motifs characteristic of transmitters are shown at the top. Letters indicate amino acids present in at least 70% of aligned transmitter sequences at that position. Diamond symbols indicate positions at which at least 50% of the amino acids belong to the same chemical family: white, nonpolar (I, L, M, V); black, polar (A, G, P, S, T); plus sign, basic (H, K, R); minus sign, acidic or amidic (D, E, N, Q). Black dots indicate wild card positions with less than 50% family conservation. An aggregate secondary-structure prediction and possible functions of key regions are also shown.

autophosphorylation (63). The region surrounding this residue bears little resemblance to the block H consensus. Other examples of missing motifs are block N in FrzE, block G1 in AgrORF2, and block G2 in NarQ and NarX (186; our own alignments). Clearly, no single subsequence is necessary or sufficient to define a transmitter.

Mutational studies of EnvZ and Taz1, a hybrid protein with the EnvZ transmitter coupled to the aspartate-binding domain of the Tar chemosensor (204), have provided information about the functional roles of these transmitter blocks. Replacements at the block H histidine eliminate both autophosphorylation and "phosphatase" activity (83, 227). However, mutational changes at other block H residues can affect either activity alone (2, 160), suggesting that local structure around the critical histidine influences the ratio of the two activities. Mutations in blocks N, G1, or G2 eliminate autokinase activity (84, 228). Some of the N and G1 lesions retain "phosphatase" activity (228), implying that blocks N and G1 may not be directly involved in that function. This finding and the fact that some transmitters lack one of the G blocks indicate that G1 and G2 may each comprise a nucleotide-binding site. Mutational changes in block F have not yet been described.

To gain further insight into the structural organization of transmitters, we aligned the sequences of 35 orthodox transmitters and averaged their Chou-Fasman (30) prediction scores for α-helix and β-sheet secondary structures (Figure 5). Assuming that transmitters with different primary structures have similar tertiary structures, the chance of correctly predicting secondary-structure features should increase with sample size. In fact, when applied to a set of 68 receivers, this approach unambiguously identified all five β segments and four of the five α segments in CheY, so it may be generally useful as a predictive tool. In any event, it makes some interesting suggestions about the functional architecture of transmitters.

First, transmitters may consist of two approximately equal subdomains, which we call T_L and T_R. Most consensus tracts (N, G1, F, G2) lie in T_R, whereas T_L is quite variable, except for block H. It seems likely that T_R contains catalytic determinants common to all transmitters. Perhaps T_L contains determinants that impart recognition specificity for target receivers.

Second, structure averaging implies that these two regions have distinctly different folding configurations (Figure 5). A prominent α-β-α motif surrounds block H, followed by a polar region with no apparent structure, which resembles a flexible linker (about residue 30 in the consensus). Between residues 40 and 90 it is difficult to discern features owing to the extensive sequence variability in this portion of T_L, but there are hints of α-β repeats similar to those in receivers. T_R, in contrast, seems to be rich in β strands punctuated by large tracts without obvious secondary structure. Blocks N and F are β structures, whereas blocks G1 and G2 are associated with unstructured segments that resemble the glycine loops characteristic of many ATP-binding sites (158).

Third, T_L and T_R may be connected by a hingelike linker. The T_L-T_R junction (residues 90–110) contains three unstructured regions separated by β strands. In individual transmitter sequences these unstructured tracts are typically proline-rich β turns βt. The prolines do not appear in the consensus because the region in which they are embedded is highly variable. Hinges in type I and type II cyclic AMP (cAMP)-dependent protein kinases and in cGMP-dependent protein kinases contain the related motif, βt-β-βt (192, 193, 197).

The parallels between transmitters and regulatable eukaryote kinases are striking (172, 196). Both kinds of enzymes have autophosphorylated intermediates, conserved and variable subdomains, multiple nucleotide-binding sites, and extremely low basal activities. One major difference is that the phosphate in the autophosphorylated eukaryote enzyme does not participate in subsequent transphosphorylations but, rather, serves a strictly regulatory role in preserving the active state of the enzyme (172). The eukaryote kinases all contain autoinhibitory domains connected to the active-site domain by a

flexible linker. Prior to autophosphorylation, the two domains are tightly apposed and the enzyme is quiescent. Autophosphorylation releases inhibition and activates the enzyme (172). By analogy, we propose that the putative hinge connecting T_L and T_R permits contacts between them that regulate the enzymatic activities of transmitters in much the same way. Further, block G2, which is involved in both autokinase and phosphatase activities, could be a functional analog of the cGMP-binding sites of cGMP-dependent protein kinases in eukaryotes (193), which play an important role in modulating enzyme conformation. ATP (or other nucleotide) bound to the G2 site might destabilize the association between T_L and T_R, thereby exposing both subdomains and leading to changes in autokinase or "phosphatase" activity. Since bound ATP serves as an allosteric effector and not as a substrate, this would explain why "phosphatase" stimulation occurs with nonhydrolyzable ATP analogs.

Input Control of Transmitter Activity in Sensors

Input domains in sensor proteins appear to modulate the autokinase and "phosphatase" activities of their adjoining transmitters to control the phosphorylation state of response regulators. Although there is scant evidence directly linking sensory input to changes in transmitter activity, the circumstantial case is compelling. For example, ArcB (73), EnvZ (2, 50, 70, 198), PhoR (106, 226), and VirA (26) sensor mutations that cause constitutive target gene expression in vivo cause elevated autokinase activities in vitro. With the wild-type sensors, environmental stimuli elicit similar changes in gene expression, presumably by stimulating transmitter autokinase activity (or inhibiting "phosphatase" activity). There are technical obstacles to measuring these transmitter activities in vivo, but two sensors, albeit altered ones, have been successfully coupled to stimuli in vitro. In the chimeric sensor Taz1, aspartate stimulates autokinase activity in a detergent-solubilized system (227). Anaerobiosis enhances autophosphorylation of a soluble fragment of FixL (125).

We postulate that transmitters could have two autokinase states resulting from "OFF" and "ON" conformations. The overall level of autokinase activity in a sensor population would reflect the proportion of transmitters in each signaling state. Stimuli would modulate autokinase activity by shifting the OFF/ON equilibrium. There is precedent for this mechanism in the methyl-accepting chemotaxis proteins (MCPs) that serve as chemoreceptors in *E. coli* and other bacterial species. Although MCPs do not contain orthodox transmitters, they exhibit intriguing parallels to sensor proteins. Like many sensors, MCPs are inner membrane proteins with distinct input and output domains. Moreover, they have two signaling states, one causing CW rotation and the other causing counterclockwise (CCW) rotation of the flagellar motors

(7). Thus, MCPs may provide useful insight into how a two-state signaling mechanism might operate in orthodox transmitter proteins.

Many sensor proteins probably have the same membrane topology and overall domain organization as MCP molecules do (Figure 6*a*). This has been demonstrated directly for CpxA (210), EnvZ (49), and VirA (113). Nearly all of the others also exhibit two potential membrane-spanning segments that should arrange the molecule into an N-terminal periplasmic receptor domain and a C-terminal cytoplasmic signaling domain. These input and output domains are joined by a nondescript "linker" segment emanating from the second transmembrane segment. This linker may play an important role in propagating conformational changes between the two domains during transmembrane signaling. A model, based on a suggestion by Stock et al (185), is diagrammed in Figure 6*b*. It suggests that the input and output domains of sensors may individually undergo transitions between relaxed and tense conformations. When the domains are rigidly coupled, a conformational

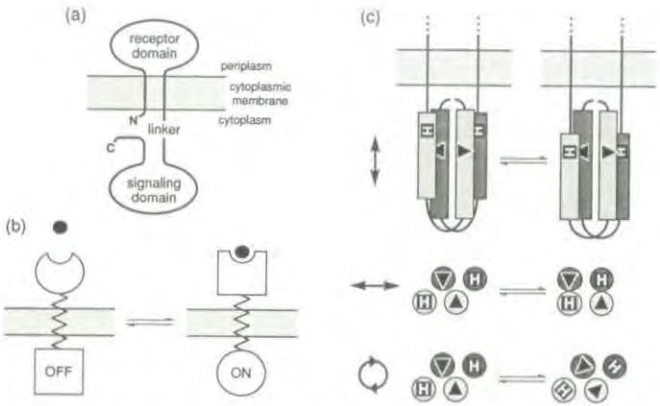

Figure 6 Models of membrane topology and transmembrane signaling in sensor proteins. (a) Membrane organization of MCP chemoreceptors. Many sensors have this topology, in which case receptor domain is synonymous with input domain and signaling domain is synonymous with transmitter module. (b) Possible mechanical coupling between periplasmic and cytoplasmic domains during transmembrane signaling. Each domain has relaxed (rounded) and tensed (squared) conformations. When one relaxes, the other is constrained to tense. The black circle represents a generic stimulus (chemoeffector, osmolarity, etc.). Stimulation shifts the receptor domain into the tensed state, leading to relaxation and activation of the signaling domain. In this particular model, OFF and ON specifically refer to the autokinase activities of transmitters. (c) Mechanisms for inducing conformational changes in transmitters through the linker and membrane-spanning segments of sensors. The transmitter is pictured as a dimer, with triangles to indicate the catalytic sites; the input domain is not shown. Linear or rotational movements of the linkers relative to each other could shift transmitters between the OFF and ON conformations. Only three of many possible movements are diagrammed.

change in one domain is necessarily accompanied by an opposing change in the other domain. As one domain relaxes, the other tenses.

The model proposes that the ON form of the transmitter corresponds to a conformationally relaxed state, whereas the stimulated input domain assumes a conformationally tensed state. It predicts that the unstimulated input domain inhibits autokinase activity. Thus, severing the connection to the input domain should activate the transmitter. This experiment has been performed by deleting part or all of the input domain in many different sensors, including ArcB (E. Lin, personal communication), CreC (5), EnvZ (1, 50, 71, 198), FixL (56), PhoR (106, 226), and VirA (26, 69, 77, 127). In every case the liberated transmitter exhibits substantial autokinase activity in vitro, corresponding to partly or fully induced signaling behavior in vivo.

Linker mutations could affect output activity in two different ways. Some might effectively uncouple the two domains, leading to constitutive signaling activity. Others might lock the transmitter in the inactive state by mimicking the conformational effects of input stimuli. Both types of linker defects have been found in MCP mutants (6). Linker mutations in transmitter-containing proteins also bias signal output in two directions. A linker mutant of EnvZ behaves as though locked in the OFF state (199), whereas four BvgS linker mutants seem to be in the ON state (115). Several linker mutations have been obtained in NarX by selecting for inducer-independent expression of target genes (31, 81). Collins et al (31) noted sequence similarities between the NarX and MCP linker regions and found that two of the NarX mutations had exact MCP counterparts, suggesting that these two classes of membrane receptors might very well use the same transmembrane signaling mechanism.

MCPs function as homodimers (119), but little is known about the quaternary organization of transmitter-containing proteins. EnvZ at least may also function as a dimer since EnvZ (or Taz1) mutants with different autophosphorylation defects exhibit intragenic complementation (227). Diploids in which one of the mutant proteins has a replacement at the conserved histidine and the other has a deletion of blocks N, G1, and G2 have substantial autokinase activity, indicating that an intact phosphorylation site in one subunit can interact productively with an intact catalytic site in another subunit. Since the cytoplasmic CheA protein, which contains an unorthodox transmitter, is also a dimer by several physical criteria (54), it is not unreasonable to suppose that a dimeric organization is the general rule for transmitter-containing proteins. Those attached to membrane-spanning segments might have the two subunits oriented in parallel (Figure 6*c*). The hingelike region in the middle of the transmitter could enable the subunits to fold in a way that aligns the catalytic region of one with the phosphorylation site in the other. This general arrangement might enable relatively minor conformational changes, propagated, for example, through the linker segment

during transmembrane signaling, to modulate transmitter activity by shifting the orientation of the interacting segments.

Transmembrane signaling is still poorly understood, even in MCP molecules. One recent study suggested that relative motion of the membrane-spanning segments in a single MCP subunit could trigger conformational changes in the cytoplasmic signaling domain (120). However, the X-ray structures of the unbound and aspartate-bound forms of the Tar receptor domain indicate that relative motion of the two subunits may be responsible for propagating conformational changes to the cytoplasmic domain (114). In either case, the membrane-spanning segments play obviously important roles because mutational changes in these segments often shift MCP molecules into CW- or CCW-biased signaling states (6, 141, 143). Mutations in the transmembrane portions of EnvZ produce analogous signaling defects, with some mutants locked in the autokinase OFF state and others in the ON state (160, 199). We conclude that the membrane-spanning segments in sensors with MCP-like structures probably move during transmembrane signaling. It is easy to imagine ways in which movements of the segment adjoining the linker could modulate the autokinase activity of transmitters, particularly if activity requires interaction between sensor subunits. A few specific examples are shown in Figure 6*c*

Is a two-state signaling model too simplistic for transmitters that also have "phosphatase" activity? Mutational changes in the EnvZ and Taz1 sensors often affect autokinase and "phosphatase" activities in reciprocal fashion (1, 2, 160, 198, 199, 228): when one is elevated, the other is diminished. Stimuli also modulate the ratio of the two activities. These findings are at least consistent with the possibility that the autokinase OFF conformation is the one with "phosphatase" activity. Until there is evidence to the contrary, we see no need to postulate the existence of more than two conformational states for transmitters.

TRANSMITTER–RECEIVER TRANSACTIONS

The signaling characteristics of communication module circuits should reflect the interplay of several parameters that govern-transmitter–receiver transactions. The transaction cycle of a simple circuit in which output signal is related directly to receiver phosphorylation state is diagrammed in Figure 7. Changes in receiver phosphorylation begin with activation and autophosphorylation of the transmitter in response to sensory input. Next, unphosphorylated receivers associate reversibly with the activated transmitter through specific recognition interactions. After engaging the transmitter, the receiver catalyzes transfer of the phosphate from the histidine residue to its own aspartate acceptor site. Phosphotransfer between phosphoramidate and

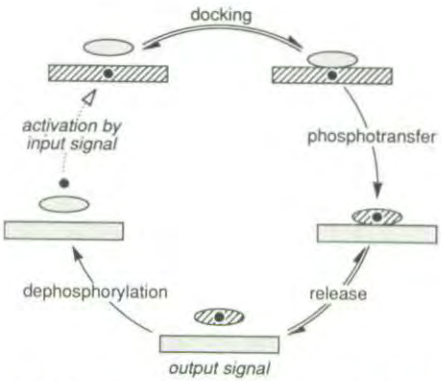

Figure 7 Critical events in the transmitter–receiver transaction cycle. Docking and release are controlled by reversible equilibria, whereas phosphotransfer from transmitter to receiver and subsequent dephosphorylation of the receiver may be effectively unidirectional. Hatched shading denotes modules in an activated conformation; black circles represent phosphoryl groups.

acyl phosphate should involve only a small enthalpy change, but phosphorylation-induced conformation changes could influence the reversibility of the reaction. Thus, receiver phosphorylation could have two consequences. First, it might reduce binding affinity for the dephosphorylated transmitter, facilitating its release and reentry into the signaling cycle. Second, it will lead to production of an output signal by one of the mechanisms discussed above (see Figure 4*b*). The change in response regulator activity should persist until the receiver loses its phosphate, either spontaneously, through its own autophosphatase activity, or through transmitter-stimulated dephosphorylation.

Communication Specificity

The docking interaction between transmitter and receiver and the subsequent phosphotransfer step will determine the overall precision of signal transmission. In most cases, docking affinity is probably substantially greater for cognate than for noncognate interactions, but the structural determinants responsible have not yet been identified. Since receivers are able to discriminate between small-molecule phosphodonors (48, 100), the local structure of the transmitter phosphohistidine probably contains all the information needed for substrate recognition. However, it seems likely that cognate modules also make specific contacts outside their substrate and catalytic sites. To look for structural determinants that might contribute to docking specificity, we examined sequence alignments within well-defined clusters of transmitters and receivers (see Figure 3). In related transmitters, residue identities that define the cluster generally occur near the center of the variable region in T_L, around block H, and preceding block N (data not shown). These regions

might participate in receiver recognition. A similar analysis of receiver families revealed no characteristic pattern of residue identities. If a distinct docking mechanism exists, it presumably would involve stereospecific contacts between exposed surface features of transmitters and receivers. The best candidates, in receivers at least, are the external α helices. Phosphorylation-induced conformational changes might shift the orientations of the contact sites to facilitate the docking and release steps. Phosphorylated transmitters should have high affinity for unphosphorylated receivers, whereas phosphorylated receivers should have low affinity for unphosphorylated transmitters. Unfortunately, these ideas cannot be tested until transmitter–receiver complexes have been explicitly demonstrated and characterized.

Crosstalk Happens

Receivers acquire phosphoryl groups not only from their cognate transmitters but also from heterologous transmitters and, in some cases, directly from small molecules such as phosphoramidate or acetyl phosphate. Most in vitro (5, 48, 70, 100, 136) and in vivo (5, 93, 132, 136) observations of such "crosstalk" have been made with skewed reactant stoichiometries. Under physiological conditions, crosstalk between the same signaling components may be negligible. However, in some systems crosstalk phosphorylation represents a normal and substantial part of the overall signal flow to a particular response regulator. PhoB, for example, can be phosphorylated by CreC (formerly PhoM), a "crosstalking" sensor, well enough to carry out its normal in vivo transcriptional activities (5). Similarly, KinA and KinB both activate Spo0A (147, 173) and NarQ and NarX both activate NarL (31, 150). Moreover, fluctuations in the intracellular levels of acetyl phosphate or acetyladenylate substantially influence the activity of CheY (220), PhoB (93, 208), and NtrC (48). These examples of in vivo crosstalk are undoubtedly physiologically meaningful, although deciphering the logic behind such "crossregulation" may prove difficult (207).

INTEGRATED CIRCUITS

Proteins that contain both a transmitter and a receiver (see Figure 2) offer the simplest examples of "integrated circuits" built from communication modules: ArcB *(E. coli)* controls gene expression under low-oxygen conditions (74); RcsC *(E. coli)* is involved in regulating capsular polysaccharide synthesis (189); BvgS *(Bordetella pertussis)* initiates a signal cascade leading to virulent infections in humans (181); RteA *(Bacteroides thetaiotaomicron)* regulates expression of tetracyline resistance genes (176); and VirA *(Agrobacterium tumefaciens)* senses phenolic compounds exuded from plant wounds and triggers an invasive response (219). These proteins all work in conjunction with conventional response regulators (ArcA, RcsB, BvgA, RteB, and

VirG, respectively). Four others have no known partners to date: LemA *(Pseudomonas syringae)* and RpfC *(Xanthomonas campestris)* are involved in virulence responses (66, 195); AsgA *(Myxococcus xanthus)* plays a part in starvation-induced differentiation (L. Plamann, personal communication); and BarA *(E. coli)* has no known physiological role but can activate OmpR via crosstalk (132).

Except for AsgA, all of these proteins contain a membrane-associated input domain connected to an orthodox transmitter followed by an orthodox receiver. (BvgS, LemA, and RteA also have C-terminal output domains, whose roles are not well understood). Two circumstantial observations indicate that the transmitters in these proteins may be targeted to the attached receivers as well as to the receivers in cognate response regulators. First, amino acid replacements at the 'Asp-57' position in the ArcB (73) and VirA (26) receivers cause loss of inducibility, implying that induction requires phosphorylation of this site. ArcB and VirA transmitters have demonstrated autokinase ability and are the most likely phosphodonors. Second, a flexible linker joins the transmitter and receiver in BvgS (11), consistent with the notion that the two modules make reversible contacts with one another, perhaps during association and phototransfer.

Autoinhibition and Switching

Interaction of transmitters and receivers in the same protein could lead to novel signaling properties. Most importantly, the receiver could function as a switchable autoinhibitory domain. In its unphosphorylated state it might bind to the transmitter, preventing autophosphorylation or interaction with response regulators. Phosphorylation of the receiver, either by the adjoining transmitter or by one in a different protein, might reduce its interaction affinity, thereby freeing the transmitter to participate in other signaling transactions. In addition, the phosphorylated receiver itself might transmit output signals, either directly or through a response regulator as in the phosphorelay circuitry of *B. subtilis* sporulation (25). In the absence of activating input signals, autoinhibition should lead to low basal output. An inducing signal of sufficient strength, routed through either the input domain or the receiver, would activate the transmitter, relieving inhibition. The decay rate of the phosphorylated receiver and its interplay with the input domain in regulating transmitter activity will determine the overall signaling effect. If the receiver is dephosphorylated slowly, the protein could operate as a high-gain switch. Once fully induced, its output would no longer depend on the continued presence of the stimulus. Different parameter combinations could engender other responses.

The ArcB and VirA proteins may exemplify such switching devices. When their C-terminal receivers carry amino acid replacements at the 'Asp-57' position, the mutant proteins are essentially noninducible (26, 73). However,

if the receivers are deleted, the proteins remain inducible but have reduced sensitivity (26, 73, 113). This suggests that the unphosphorylated receivers inhibit transmitter activity and that phosphorylation of the receiver (or its removal by deletion) releases inhibition. The normal role of these receivers is probably to augment stimulus sensitivity, perhaps by acting as low-threshold switches or signal amplifiers.

CheA and Chemotaxis

Communication modules are used in novel ways by *E. coli* and *S. typhimurium* for chemotactic signaling [see (18) and (185) for reviews]. These organisms swim by rotating their flagellar filaments: CCW rotation propels the cells forward, whereas CW rotation causes them to turn or tumble. MCPs and other chemoreceptors elicit chemotactic movements by altering the pattern of flagellar rotation in response to changes in attractant or repellent concentration. Motor responses occur within 200 ms of stimulus detection (165), a much shorter time scale than for the regulatory responses mediated by other transmitter and receiver proteins.

MCPs modulate the autokinase activity of CheA, containing an unorthodox transmitter, to control the phosphorylation states of two response regulators, CheB and CheY (16, 17, 137). Phospho-CheY interacts with the switching machinery to augment CW rotation of the flagellar motors (19). Phospho-CheB demethylates MCP molecules to bring about sensory adaptation (102). The phosphorylated forms of both effectors are very short lived, but there is no evidence for a "phosphatase" activity associated with CheA (64). Another protein, CheZ, specifically stimulates the loss of phosphate from CheY (64, 99). It is not known whether CheA plays any role in regulating that effect. Except for low-level crosstalk, CheB and CheY acquire their phosphate from CheA, whose autophosphorylation activity largely dictates the flux of phosphate through the signaling system. CheA has a low basal autophosphorylation rate that is stimulated several hundred-fold by MCP molecules in the CW signaling mode (16, 17, 137). MCPs in the CCW state inhibit CheA activity below its uncoupled rate. Another signaling component, CheW, plays an essential but still poorly understood role in coupling CheA to chemoreceptor control (16, 17, 98, 137).

CheA acts as the "central processing unit" in this signaling scheme. At a minimum it must sample and integrate MCP signaling activities over the entire population of receptor molecules. Moreover, by virtue of the large amplification factor in receptor coupling, CheA may be mainly responsible for the exquisite sensitivity of chemotactic responses. It must also control the relative flow of phosphate to CheB and CheY. Whether those proportions are "hardwired" into CheA or whether they can be modulated is an open question. Similarly, it is not known if CheA plays any role in regulating the CheY

dephosphorylation activity of CheZ or whether it is sensitive to physiological conditions leading to crosstalk. The basic design of CheA is so radically different from that of other transmitter-containing proteins that it could very well be capable of all these signaling functions.

CheA functions as a dimer and seems to be elongated (54). Sequence analyses, genetic studies, and protease cleavage patterns all indicate that the molecule is segmentally organized into domains with different signaling roles (Figure 8). The CheA transmitter is centrally located and flanked on each side by two additional regions, P_1 and P_2 at the N terminus and M and C at the C terminus. The transmitter itself has an unusual structure. T_R contains conventional N, G1, and G2 blocks, which presumably serve the same functions, namely ATP binding and catalysis, as in orthodox transmitters. The F block, however, is shifted toward G2 and differs somewhat from the consensus. Most importantly, the site of phosphorylation is not in T_L but, rather, at histidine 48 (63), within a sequence that bears virtually no resemblance to the consensus H block. Another unusual feature is segment C, which resembles the first half of a receiver module (87). It ends just short of the 'Asp-57' phosphorylation site in the consensus motif.

The CheA proteins of *E. coli* and *S. typhimurium* (respectively 654 and 671 residues) have nearly identical primary structures and are functionally interchangeable. Differences between them are confined to two regions, evidently not under functional selection, that resemble charge-rich linkers. One separates the P_1 and P_2 segments, and the other separates P_2 and T_L. In native CheA molecules, both of these putative linkers are readily cut by a variety of proteases, as is the boundary between T_R and M (T. Morrison & J.

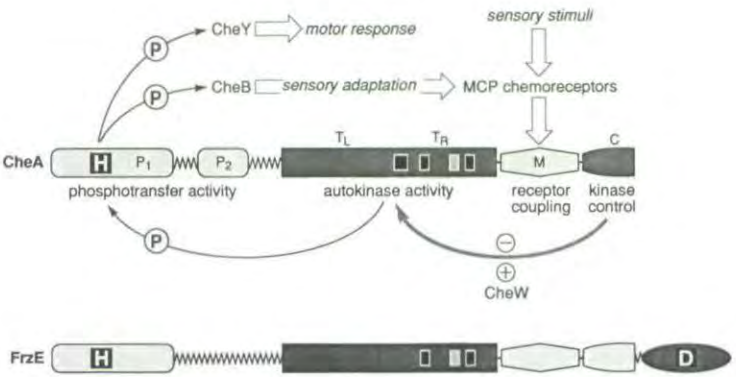

Figure 8 (Top) Domain organization and signal processing in CheA. Zigzag lines between domains represent flexible linkers. (Bottom) Domain organization of FrzE, a CheA homolog in *Myxococcus xanthus*.

Parkinson, in preparation). T_L also contains cleavage sites at positions corresponding to the linker and hinge features predicted in orthodox transmitters (see Figure 5).

The P_1 segment of CheA can be released by trypsin treatment and, if phosphorylated, serves as a phosphoryl donor for CheB and CheY (63). Thus, the CheA transmitter does not participate directly in this phosphotransfer reaction. Conversely, all of the structural information needed for recognition and interaction with CheB and CheY must be contained in the P_1 segment. Amino acid replacements at Ser-49 (63) or Arg-45 or Gly-53 (140; R. Bourret, personal communication) perturb both autophosphorylation and phosphotransfer ability, suggesting that residues in the immediate vicinity of the phosphorylation site contribute to its substrate recognition and specificity properties. The role of P_2 is still unclear because no P_2 mutations other than null mutations have been picked up in undirected mutant hunts. However, polypeptides containing the P_2 region are potent inhibitors of chemotaxis, implying that P_2 is capable of blocking an important signaling activity (T. Morrison & J. Parkinson, in preparation). CheY (and possibly CheB) may be the target of this inhibition effect, suggesting that the normal role of P_2 might be to assist the interaction between response regulators and the phospho-donor site in P_1.

Missense mutations that impair the uncoupled autophosphorylation rate of CheA have been isolated by random screening of nonchemotactic mutants (140) and by selecting phenotypic suppressors of *cheZ* deletion mutants (J. Liu & J. Parkinson, in preparation). A few of these mutants have changes in block N or near block G2, confirming that these segments are important for autokinase activity. In addition, two mutations that augment CheA activity lie just at the C-terminal end of G2 (J. Liu & J. Parkinson, in preparation), consistent with the idea that nucleotide binding at this site may stabilize an active transmitter conformation. However, most CheA mutants with reduced specific activity had mutations in the putative linker and hinge regions of T_L, implying that T_L plays some role in autophosphorylation. In orthodox transmitters, T_L must interact with T_R since it contains the histidine residue that is phosphorylated. Conceivably, the T_L segment of CheA retains this ability, perhaps as a means of modulating the autophosphorylation rate. Alternatively, T_L may interact with sequences around the autophosphorylation site in P_1, thereby bringing it to the catalytic center. The flexible linker between P_1-P_2 and the transmitter might be needed to facilitate such interactions.

Two complementary findings indicate that the M and C segments are involved in coupling CheA to chemoreceptor control. First, nonsense fragments lacking either the C segment or the M and C segments have normal specific activity for autophosphorylation but cannot be stimulated or inhibited by chemoreceptors (R. Bourret, personal communication). Second, a CheA

fragment consisting of the T_L-T_R-M-C segments can compete with intact CheA molecules for interaction with receptors (T. Morrison & J. Parkinson, in preparation).

Missense mutants have provided substantial insight into the functional roles of these segments in receptor coupling. Mutations throughout the M segment, obtained as allele-specific suppressors of CW-biased MCP mutants, cause moderate reductions in specific activity and more severe defects in receptor stimulation (J. Liu & J. Parkinson, in preparation). The allele-specific suppression patterns suggest that receptor molecules make direct contact with this region. Since this part of CheA is not needed for enzymatic activity, these mutations may somehow shift the molecule into an inactive conformation. Second-site mutations in the C segment can restore normal activity to these mutants, indicating that it could have been responsible for inhibiting their autokinase activity. Other mutations in the C segment, obtained as phenotypic (but not allele-specific) suppressors of CW- or CCW-biased MCP mutants, specifically alter the ability of CheA to respond to receptor-mediated stimulation or inhibition (J. Liu & J. Parkinson, in preparation). Most of these mutations affect only one of these processes, implying that two different control mechanisms are involved. Moreover, the lack of allele specificity in the suppression patterns of these mutants suggests that the C segment does not operate through direct contacts with the receptors. However, it might bind CheW, the accessory protein needed for receptor-coupled control of CheA. This possibility has not yet been tested.

Our working model of CheA-receptor coupling proposes that segment M interacts with the signaling region of MCP molecules, whereas segment C controls CheA autokinase activity. M might function as a conformational hinge that is manipulated by receptors to regulate transitions between the stimulated and inhibited conformations. The sequence resemblance of C to a receiver module suggests that it might function as a "pseudosubstrate" domain. Inhibition could involve direct contact between C and either the catalytic or phosphorylation site to block their interaction. The mechanism of receptor-mediated stimulation is not as simple. We imagine that the stimulated conformation of CheA binds CheW, using contact sites in segment C and possibly elsewhere in the molecule. It is conceivable that the role of CheW is merely to stabilize a highly active conformation, but so far it has not been possible to find CheA mutations that mimic this condition. More probably, CheW is accommodated in a stable complex near the active site and contributes to the catalytic mechanism, for example by enhancing the turnover rate or the affinity for ATP.

CheA, particularly its segmental organization and use of flexible linkers to join regions whose interactions must be regulated, represents a clever solution to a difficult signaling task. Homologs with essentially the same design have

been found in *B. subtilis* (52), *Caulobacter crescentus* (L. Shapiro, personal communication), *Enterobacter aerogenes* (34), and *Rhizobium meliloti* (R. Schmitt, personal communication), all of which resemble *E. coli* in size and mode of locomotion. *M. xanthus*, a longer bacterium that moves by gliding slowly on solid surfaces, has CheA homolog (FrzE) involved in cell aggregation and fruiting-body formation (111) (Figure 8, bottom). FrzE probably processes sensory information about nutrient conditions and cell density to regulate cell motility or the expression of other genes in the fruiting-body developmental pathway. It has no P_2 segment but, instead, contains a very long proline- and alanine-rich linker that connects the autophosphorylated histidine to the transmitter. FrzE also has at its C terminus an orthodox receiver module whose aspartate residue is the ultimate site of autophosphorylation in the protein (112). This receiver is connected by a short linker to the C segment, which bears little resemblance to its counterpart in CheA and may no longer serve as a kinase control device. Could this elaborate construct enable the cell to overcome the special problems inherent in diffusion and interaction of signaling components over long distances?

SUMMARY AND PROSPECTS

Bacterial signaling systems are often built with communication modules, i.e. domains that promote information transfer within or between proteins. Transmitters and receivers are probably ancient devices. However, owing to their utility in diverse signaling situations, they may have spread throughout the prokaryotic world, and possibly into eukaryotes, by horizontal transmission. Both kinds of modules are capable of autophosphorylation: transmitters form phosphohistidine, and receivers form phosphoaspartate, using transmitter phosphoryl groups as substrates. Adjoining input domains control transmitter phosphorylation. Receiver phosphorylation controls the activity of attached output domains. We are just beginning to understand the molecular mechanisms behind these signaling abilities. Some important areas for future study include the following.

1. Transmitter structure and function—the number of conformational states, their associated signaling activities, and how they are coupled to input control; the possible role of bound nucleotides in stabilizing different conformers; the contributions of conserved amino acid residues to various transmitter functions; the crystal structure of a typical transmitter.
2. Transmitter-receiver interactions—the structural basis for recognition specificity; how phosphorylation influences module affinities; the determinants controlling phosphotransfer rate; the extent of physiological crosstalk and its significance in different signaling systems.
3. Receiver control strategies—the nature of phosphorylation-induced con-

formational changes; the kinds of functional alterations leading to constitutively active receivers; the possible role of response regulator dimerization in output control; whether some receivers make stereospecific contacts with their output domains.

4. Variations on module themes—signaling properties of integrated circuit proteins; information flow in complex circuits with feedback loops, multiple inputs and outputs, etc.; modules in novel contexts such as the transmitter of CheA.

Molecular insights into these issues will provide better understanding of the adaptive signaling strategies available to prokaryotes and of the information-processing capabilities of proteins in general.

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