Transmembrane Signalling by a Hybrid Protein: Communication from the Domain of Chemoreceptor Trg That Recognizes Sugar-Binding Proteins to the Kinase/Phosphatase Domain of Osmosensor EnvZ

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Received 16 July 1993/Accepted 13 December 1993

Chemoreceptor Trg and osmosensor EnvZ of *Escherichia coli* share a common transmembrane organization but have essentially unrelated primary structures. We created a hybrid gene coding for a protein in which Trg contributed its periplasmic and transmembrane domains as well as a short cytoplasmic segment and EnvZ contributed its cytoplasmic kinase/phosphatase domain. Trz1 transduced recognition of sugar-occupied, ribose-binding protein by its periplasmic domain into activation of its cytoplasmic kinase/phosphatase domain as assessed in vivo by using an *ompC-lacZ* fusion gene. Functional coupling of sugar-binding protein recognition to kinase/phosphatase activity indicates shared features of intramolecular signalling in the two parent proteins. In combination with previous documentation of transduction of aspartate recognition by an analogous fusion protein created from chemoreceptor Tar and EnvZ, the data indicate a common mechanism of transmembrane signal transduction by chemoreceptors and EnvZ. Signalling through the fusion proteins implies functional interaction between heterologous domains, but the minimal sequence identity among relevant segments of EnvZ, Tar, and Trg indicates that the link does not require extensive, specific interactions among side chains. The few positions of identity in those three sequences cluster in transmembrane segment 1 and the short chemoreceptor sequence in the cytoplasmic part of the hybrid proteins. These regions may be particularly important in physical and functional coupling. The specific cellular conditions necessary to observe ligand-dependent activation of Trz1 can be understood in the context of the importance of phosphatase control in EnvZ signalling and limitations on maximal receptor occupancy in binding protein-mediated recognition.

Bacterial cells recognize features of their environment and transduce that recognition into internal signals that evoke appropriate responses. Intracellular signalling in bacteria occurs in many cases by a common biochemistry (for recent reviews, see references 3, 29, and 31). In these systems, often called two-component regulatory systems, a protein kinase places a phosphate from ATP at one of its histidyl residues and that phosphate is subsequently transferred to an aspartyl residue of a response regulator, causing activation of the regulator. For many signalling systems, the kinase resides in the cytoplasmic domain of a transmembrane sensor protein and the regulator protein interacts with promoter DNA to control expression of specific genes. A well-characterized example of such a system is the EnvZ-OmpR pair of *Escherichia coli* that regulates, in response to osmotic pressure, the expression of two genes, *ompC* and *ompF*, coding for outer membrane porins (3, 11, 29). EnvZ is the transmembrane sensor protein, and OmpR is the phosphorylation-activated DNA-binding protein (1, 9, 15). EnvZ has a transmembrane

domain made up of two segments, one near the amino terminus and the other near the middle of the polypeptide chain, a periplasmic domain thought to be involved in sensing osmotic pressure (32), and a cytoplasmic domain that has both kinase and phosphatase activity specific for OmpR (10, 18). Mediation by EnvZ of dephosphorylation of OmpR-phosphate may involve action of EnvZ as an enzyme or as an activator of an autophosphatase activity of OmpR. In this article, we refer to the phosphatase activity of EnvZ without implying one or the other of these possibilities. Several lines of evidence indicate that EnvZ acts as a dimer (37, 38).

The sensory system that mediates chemotaxis in *E. coli* is more complex. Specific references to the information that follows can be found in recent reviews (3, 14). There are four transmembrane chemoreceptors, but these proteins do not contain a kinase in their cytoplasmic domains. Instead, receptor cytoplasmic domains interact with a common, soluble kinase protein. Those receptor domains are also involved in sensory adaptation mediated by changes in the extent of methylation at several specific glutamyl residues. Two target proteins are phosphorylated and hence regulated by the kinase: one interacts with flagellar motors to influence the pattern of swimming, and the other is a methyl esterase that acts, as part of the adaptation mechanism, on glutamyl methyl esters of the receptor. Each of the four chemoreceptors recognizes different ligands. Three interact with ligand-occu-

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pied periplasmic binding proteins. The receptor Trg binds galactose- and ribose-binding proteins. Tap recognizes dipeptide-binding protein, and Tar interacts with maltose-binding protein. In addition, Tar binds aspartate directly, and the fourth receptor, Tsr, binds serine. Chemoreceptors are homodimers (25) in which each subunit has a transmembrane domain of two transmembrane segments, a periplasmic, ligand-recognition domain and a cytoplasmic, signalling domain. There is substantial identity among the amino acid sequences of the four receptors in their cytoplasmic domains but little in their periplasmic or transmembrane domains (13), yet mutational analyses of ligand binding imply that there is a common three-dimensional organization even in those domains (12, 19, 21, 22, 28, 34–36).

A shared *NdeI* restriction site in *tar* and *tsr* allowed creation of a hybrid gene that produced a chimeric chemoreceptor in which all but the first 43 residues of the 35-kDa cytoplasmic domain of Tar were replaced by the corresponding segment of Tsr (20). This chimeric receptor functioned in a manner indistinguishable from that of the natural Tar protein. Thus, transmission of sensory signals from the periplasmic and transmembrane domain derived from one chemoreceptor to the cytoplasmic domain derived from another occurred successfully. A more radical replacement was performed by using a natural, conveniently placed *NdeI* site in *envZ* to create a fusion of *tar* and *envZ* analogous to the *tar*-*tsr* fusion (33). EnvZ and the chemoreceptors share a common transmembrane disposition and both are dimers, but there is little in common in their primary structure. The resulting Tar-EnvZ product, called Taz1, linked the periplasmic and transmembrane domains of Tar plus 43 residues of the cytoplasmic domain of that receptor to the cytoplasmic kinase/phosphatase domain of EnvZ. The chimeric protein transduced recognition of aspartate by the periplasmic domain of Tar into activation of the *ompC* promoter in a process that required OmpR and presumably involved increased phosphorylation of that regulatory protein (33).

However, one aspect of Taz1 function was inexplicably different from that of Tar. The intact chemoreceptor responds to stimulation not only by aspartate but also by maltose as the result of recognition by the receptor of sugar-occupied maltose-binding protein. Yet, although stimulation of Taz1 by aspartate produced significant activation of the *ompC* promoter, stimulation by maltose did not (33). Was some unusual feature of binding protein-mediated recognition responsible for this difference? We have investigated this issue by characterizing the functional activity of a hybrid receptor analogous to Taz1 in which the chemoreceptor segment is derived from Trg, a receptor that recognizes only sugar-binding protein ligands. We show that under the proper conditions the hybrid Trz1 protein can be observed to transduce recognition of occupied sugar-binding protein into activation of the *ompC* promoter.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *rbsB102::Tn10* insertion was introduced into RU1012 (33) by P1 transduction, inactivating *rbsB*, the gene for ribose-binding protein, by insertion of the transposon and inactivating *rbsK*, the gene for ribokinase (16), by a polar effect, creating strain CP919. This strain contains $\Phi(\text{ompC-lacZ})_{10-25}$ and $\Delta\text{envZ::Km}^r$ (33). In addition, as a derivative of MC4100, which is *flhD5301* (30), the strain is nonmotile and should not express the genes related to motility and chemotaxis, including the chemoreceptor genes. Transformation with pRB020 (see below) or pAI12, a

FIG. 1. Diagrams of Trz1 with relevant features indicated. The amino-terminal 265 residues of Trg (Trg') and the carboxy-terminal 230 residues of EnvZ ('EnvZ) are represented by the open and striped ribbons, respectively. Positions are indicated for the transmembrane segments of Trg' (TM-1 and TM-2; solid ribbons) and the phospho-accepting histidyl residue (H) of 'EnvZ. (A) Primary structure of Trz1 with a scale of residue numbers; (B) disposition of Trz1 across the cytoplasmic membrane (dotted lines).

pACYC184 derivative (16) carrying intact *rbsB* and *rbsK* expressed constitutively from a plasmid promoter, created CP920 and CP924, respectively. Transformation of CP920 with pAI12 created CP921. Plasmid pRB020 codes for Trz1. It is essentially plasmid pGB1 (4), a derivative of pKK223-2 that carries *trg* under the control of the *tac* promoter and also carries *lacI^q*, in which the segment *trg* that codes for the cytoplasmic domain of Trg is replaced by the comparable segment of *envZ*, creating a gene that codes for the protein diagrammed in Fig. 1. The construction of pRB020 required that pGB1 be treated to remove its sole *NdeI* site. This was accomplished by digestion with that endonuclease and then treatment with mung bean nuclease and ligation. Elimination of the *NdeI* site, which is located outside of the genes carried on the plasmid, did not have a discernible effect on the LacI-mediated expression of *trg*. The pGB1 derivative lacking the *NdeI* site was cleaved with *AflIII* and *StuI*, and the resulting 0.7-kb fragment of *trg* was replaced by one in which an *NdeI* site had been previously introduced at codons 266 and 267 by oligonucleotide-directed mutagenesis, to create pJB34. Plasmids pDR200 (8) and pJB34 were digested with *HindIII* and *NdeI*, and the 1-kb fragment of the former was joined to the 7-kb fragment of the latter to yield pRB020.

Methods. The immunoblot procedures used were described by Morgan et al. (26) and used antiserum raised to purified Trg or purified EnvZ, anti-rabbit immunoglobulin G raised in goats and coupled to horseradish peroxidase, and 1-chloro-4-naphthol as a chromogenic substrate for the peroxidase. The assay of β -galactosidase and the units of activity used were described by Miller (24).

RESULTS

Construction of a *trg-envZ* hybrid. We aimed to create a hybrid gene between *trg* and *envZ* that coded for a chimeric protein analogous to the Taz1 protein produced by the *tar-envZ* hybrid (33). The hybrid gene and fusion protein are diagrammed in Fig. 1. We introduced an *NdeI* site into *trg* at the position corresponding to the naturally occurring *NdeI* site in *tar* and *tsr*. Codon 266 of *trg* was changed from CAA to CAT, creating the sequence recognized by *NdeI*, CATATG, at codons 266 and 267. The single base substitution changed Gln-266 to His-266, a substitution that did not discernibly

FIG. 2. Immunoblots using anti-EnvZ and anti-Trg. Strains harboring, as indicated, plasmid pRB020 (pTrz1), pAI12 (pRBP), or pGB1 (pTrg) were grown in Luria broth plus 100 μ g of ampicillin per ml and 25 μ g of chloramphenicol per ml as appropriate, with or without IPTG (1 mM) as indicated. In the mid-logarithmic phase, samples containing 1.25×10^7 cells were harvested into trichloroacetic acid (final concentration, approximately 10%) and subjected to sodium dodecyl sulfate (SDS)-12% polyacrylamide gel electrophoresis, and duplicate samples were analyzed by immunoblotting using anti-EnvZ (A) or anti-Trg (B). The positions of Trg (60 kDa) and Trz1 (54 kDa) are indicated, and only the relevant portions of the immunoblots are shown. The bands above the position of Trz1 in panel A are not related to EnvZ since they occurred in a strain lacking that protein (lanes 1 and 2) as did a band below the region shown (33). The bands shown in panel B were the only ones visible on that immunoblot. Strains tested were CP919 (pTrz⁺ pRBP⁻ pTrg⁻), CP920 (pTrz⁺ pRBP⁻ pTrg⁻), CP921 (pTrz⁺ pRBP⁺ pTrg⁻), CP924 (pTrz⁻ pRBP⁺ pTrg⁻), and HB1032 (pTrz⁻ pRBP⁻ pTrg⁺).

affect the ability of Trg to mediate the chemotactic response to ribose or galactose as assayed on semisolid agar plates. Position 266 is the 44th in the sequence of the cytoplasmic domain that begins with the arginine that marks the cytoplasmic boundary of transmembrane segment 2. In *envZ*, the *NdeI* site comprises the codons for His-222 and Met-223, and His-222 is the 43rd residue of the EnvZ cytoplasmic domain (7). Thus, joining a 5' segment of *trg* with a 3' segment of *envZ* at the *NdeI* site created an in-phase coding sequence for a Trg-EnvZ hybrid protein of 495 residues. By using the convention of Utsumi et al. (33), we termed this protein Trz1. The amino-terminal 265 residues of Trz1 are derived from Trg and include the entire periplasmic domain, both transmembrane segments, and 43 residues of the cytoplasmic domain. The remaining 230 residues constitute the carboxy-terminal kinase/phosphatase domain of EnvZ.

The Trz1 protein. The *trz1* gene was constructed in a plasmid derived from pGB1 (4) in which the gene was under the control of *tac*, a modified *lac* promoter-operator, and the plasmid contained a copy of the *lacI^q* gene. In the absence of induction of the *tac* promoter, cells harboring pGB1 contain approximately fivefold more Trg than a cell with a single chromosomal copy of the gene, and full induction with isopropyl- β -D-thiogalactopyranoside (IPTG) increases that amount more than 100-fold (27). The *trz1*-containing plasmid, pRB020, was introduced into a host lacking a chromosomal copy of *envZ* and lacking detectable amounts of Trg or other chemoreceptors (Fig. 2B) as the result of a *flhD* mutation. Production of Trz was assessed by immunoblot analysis using anti-Trg and anti-EnvZ (Fig. 2). In IPTG-induced cells, a band of approximately 54 kDa was recognized by both antisera. We concluded that this band represented Trz1 because its size corresponded to the predicted value of 54.5 kDa, it was recognized by antisera to Trg and to EnvZ, and induction of the promoter controlling *trz1* expression substantially increased its cellular content.

Ligand-induced signalling by Trz1. We assessed the signaling properties of Trz1 by monitoring expression of an *ompC-lacZ* fusion gene in a strain in which production of β -galactosidase activity from that gene was known to be dependent on EnvZ and OmpR function (33). The chromosome of this strain contained the fusion gene, a deletion in *envZ*, and a transposon insertion in *rbsB*, the gene for ribose-binding protein, and the cells produced no native chemoreceptors (Fig. 2B). The strain was transformed with a plasmid carrying *rbsB*, one carrying *trz1*, or both plasmids. The various strains were assayed for Trz-dependent expression from the *ompC* promoter by measuring β -galactosidase activity after growth in minimal medium in the absence or presence of sufficient ribose to saturate the ribose-binding protein and thus to provide maximal occupancy of Trz1 by ligand-occupied sugar-binding protein. Expression from the *ompC* promoter as measured by β -galactosidase activity was very low for the host strain and for its derivatives harboring plasmid-borne *rbsB* or *trz1*, whether or not ribose was present (Fig. 3A). Cells containing both *rbsB* and *trz1* on compatible plasmids exhibited the same low level of β -galactosidase activity in the absence of ribose, but the presence of ribose resulted in a level of activity more than 20-fold higher (Fig. 3A). The stimulation of expression from the *ompC* promoter required a sugar recognized by ribose-binding protein; neither galactose nor lactose resulted in any change in activity (data not shown). This requirement for ligand-occupied, ribose-binding protein indicated that the stimulation was not due simply to the presence of the EnvZ-derived kinase/phosphatase domain of Trz1 but required its activation by ligand-induced transmembrane signalling from the polypeptide recognition site of Trg in the periplasmic domain.

The extent of stimulation was a function of the concentration of ribose (Fig. 4). Response was half maximal at approximately 15 μ M ribose and essentially saturated at concentrations exceeding 1 mM. In comparison to the chemotactic responses to ribose mediated by intact Trg (36), the dose-response relationship for Trz1 was shifted to higher sugar concentrations by approximately a factor of 100. This might reflect less efficient signalling by the hybrid receptor but also likely reflects the particular nature of the assay used to measure stimulation mediated by Trz1. Unlike the assay of chemotactic behavior using tethered cells, in which Trg-mediated response to added ribose can be quantified at the time of addition, quantification of Trz1-mediated activation of gene expression requires substantial periods of time after the addition of ribose for sufficient β -galactosidase to be produced. It seems likely that ribose added at low concentrations to cell cultures would be depleted in the course of 2 h of active growth, i.e., the conditions used for the studies summarized in Fig. 4, and thus the level of β -galactosidase activity in those cells would reflect exposure to ribose for only some fraction of the incubation time, resulting in an apparent shift in the dose-response curve to higher concentrations. We tested this idea directly by determining β -galactosidase activity at various times after the addition of ribose. For concentrations of 100 μ M or higher, activity increased linearly with time, while at 10 μ M ribose, activity increased only at the first time point at 30 min and then decreased gradually. We interpret this to mean that at the higher concentrations of ribose, cellular transport and metabolism had no significant effect on the active concentration of the sugar, while at lower concentrations those processes reduced the initial concentration rapidly enough to truncate or make undetectable the period over which the added ribose was present to act as a stimulus.

Requirements for effective activation of the *ompC* promoter by Trz1. Ligand-induced stimulation of β -galactosidase pro-

FIG. 3. Signalling through Trz assessed by measuring expression of an *ompC-lacZ* fusion gene. Strains described in the legend to Fig. 2, all of which carry a chromosomal *ompC-lacZ* fusion gene, were grown to the mid-logarithmic phase in minimal salts plus 0.4% glycerol and 25 μ g of ampicillin per ml and 7.5 μ g of chloramphenicol per ml as appropriate without or with 0.2% (13 mM) ribose and tested for β -galactosidase activity. The values plotted on the bar graphs either represent averages of two to four separate experiments each done with triplicate measurements (A) or are representative of several similar experiments (B). Error bars indicate standard deviations. For tests shown in panel A, no IPTG was added to the cultures, and thus the cellular content of Trz in cells harboring pTrz was low. For tests shown in panel B, 1 mM IPTG was added to the cultures, and thus the cellular content of Trz was approximately 100-fold higher.

duction from the *ompC-lacZ* fusion gene was strongly influenced by the amount of Trz1 present in the cells. The data shown in Fig. 3A were obtained by using cells in which the modified *lac* promoter that controls expression of plasmid-borne *trz1* was not induced; no IPTG was added to these cultures. Since the plasmid also carries the *lacI^a* gene, expression from the multiple copies of *trz1* in the absence of inducer resulted in a low cellular content of Trz (Fig. 2). Production of intact Trg without induction of the same promoter in the same plasmid results in approximately 750 molecules per cell, and induction with IPTG increases cellular content more than 100-fold (4). The relative intensities in the immunoblot shown in Fig. 2 indicate that induction by IPTG produces a similarly substantial increase in the cellular content of Trz1. However,

FIG. 4. Concentration dependence of ribose-induced signalling through Trz. Cells were inoculated into minimal salts plus 0.2% glucose, ampicillin, and chloramphenicol and allowed to grow for approximately one generation, at which time ribose was added to the indicated concentration. After 2 h of additional growth, a sample of the culture was tested for β -galactosidase activity. The activity present in the absence of added ribose was subtracted from the measured activity to produce the value plotted. All values except those between 133 μ M and 6.7 mM are averages of values from two independent experiments. The curve shown was generated from a model of dose-response relationships for indirect recognition of ligand (36) by using values of K_1 of 1.7×10^6 , K_2 of 2.4×10^3 , and B_7 of 8×10^{-4} and a transformation factor that expressed the effective ribose concentration as 1.18% of the concentration initially added.

this increased level of Trz1 did not result in a higher level of ribose-induced activation of the *ompC* promoter (Fig. 3B). In fact, cells containing ribose-binding protein, induced for a high content of Trz1 and stimulated with ribose, exhibited only half of the β -galactosidase activity of the same cells containing low levels of Trz (compare the rightmost bars in Fig. 3A and B). Strikingly, this moderate level of activity was not much different from the activity in the absence of ribose or from the activity of cells with a high content of Trz1 but lacking ribose-binding protein entirely (Fig. 3B, third paired set of bar graphs). It appears that, in cells with a high content of Trz1, the *ompC* promoter is activated to a moderate extent but that activation does not require ligand occupancy of the periplasmic domain and is little increased by such occupancy. Possible explanations for these observations will be considered in Discussion.

DISCUSSION

The results described here demonstrate that the hybrid protein Trz1, containing the periplasmic and transmembrane domains of the chemoreceptor Trg and the cytoplasmic domain of the osmosensor EnvZ, is functional for intramolecular transmembrane signalling. Recognition of occupied sugar-binding protein by the chemoreceptor-derived periplasmic domain resulted in altered activity of the kinase/phosphatase-containing cytoplasmic domain, as assayed by expression from the *ompC* promoter. Transmembrane signalling by a chemoreceptor-osmosensor hybrid was documented previously by Utsumi et al. (33) for Taz1, a fusion of the chemoreceptor Tar and EnvZ on which the design of Trz1 was based. Signalling by Trz1 reinforces the conclusion of the earlier study that chemoreceptors and the osmosensor EnvZ share a common mechanism of intramolecular transmembrane signal transduction. Control of the enzymatic activity of the EnvZ fragment by two

FIG. 5. Alignment of primary structure in selected regions of Tar, Trg, and EnvZ. The two regions containing residue identities (boxed) among the three sequences are shown. TM-1 of EnvZ was put in register with the previously aligned chemoreceptor sequences (13) by aligning the arginines (not shown) that mark the amino-terminal (cytoplasmic) border of the extended run of hydrophobic and neutral residues. The segment of EnvZ beginning just after TM-2 was put in register with the short cytoplasmic fragments of the chemoreceptors that are present in Taz1 and Trz1 (cytoplasmic fragment) by aligning the arginines that mark the carboxy (cytoplasmic)-terminal border of TM-2. For each segment, a single gap in the EnvZ sequence created the best correspondence with the chemoreceptor sequences.

different chemoreceptor segments, only marginally related in primary structure, raises the issue of the structural bases for this transduction.

Signalling through hybrid receptors. It is thought that binding of ligand to the periplasmic domain of a chemoreceptor results in a conformational change in the transmembrane domain that in turn causes an alteration in the cytoplasmic domain. Such alterations might involve simple movements of the two cytoplasmic subunits relative to each other or to the membrane, more complex conformational rearrangements within or between the subunits, or some combination of the two. Whatever the details of the process, the functional activity of Taz1 and Trz1 indicates that a functionally similar intraprotein signalling occurs in the hybrid receptors. Thus, the structure of those proteins must involve sufficiently firm physical coupling between the chemoreceptor and kinase domains such that a conformational change in the transmembrane domain would induce a change in the kinase domain. The very different primary structures of chemoreceptors and EnvZ argue against a physical coupling involving substantial packing of side chains at the interface of the two heterologous segments. Instead, coupling might involve fused units of extended secondary structure (for example, α -helices) that required little packing of side chains between heterologous segments. However, even this latter organization could involve some packing that thus might be reflected in conserved residues at critical positions. With this in mind, we examined the aligned sequences (13) of the segments of Tar and Trg present in Taz1 and Trz1. There were only a few scattered positions with identical amino acids in the periplasmic domain, in transmembrane segment 2 (TM-2), or in the short amino-terminal piece on the cytoplasmic side of transmembrane segment 1 (TM-1; see Fig. 1 for the positions of these segments). In contrast, the 27-residue TM-1 and the 45-residue cytoplasmic section from TM-2 through the fusion joint, the part of the segment of chemoreceptors called the linker (2), contained identical residues at, respectively, 30 and 33% of the positions (Fig. 5). Could these short segments be involved in the physical coupling of the two parts of the hybrid receptors? Examination of the EnvZ sequence provided a tantalizing correlation. An alignment of the sequences of EnvZ and the two chemoreceptors revealed few positions at which the EnvZ residue was the same as that in one or both chemoreceptors. However, over half of those matches clustered in TM-1 and the 45-residue segment of the linker. In

these two segments, matches between EnvZ and a particular chemoreceptor occurred at 22 to 26% of the positions (Fig. 5). In the alignment of the sequences, 15% of the positions in TM-1 and 18% of the positions in the linker fragment contained identical amino acids in all three proteins. Except for two positions at which arginines mark the boundary of a transmembrane segment, these are the only positions in which all three sequences are matched. The importance of the linker region in signalling between the heterologous segments of the hybrid receptors is also indicated by mutational analysis of Taz1 (17). Coupling between the two segments was disrupted by single-residue substitutions in Taz1 corresponding to locked signal mutations in *tsr* (2) or by substitution of the nine Tar residues at the Tar-EnvZ junction with the corresponding residues of EnvZ.

Collins et al. (6) noted a similarity between the linker regions of chemoreceptors, particularly Tsr, and the transmembrane sensor kinase NarX, a putative nitrate sensor, in terms of both modest but significant residue identities and occurrence of mutational substitutions that disrupted signalling. These observations prompted the authors to suggest that NarX and the chemoreceptors might share certain features of signal transduction. Alignment of the NarX linker sequence to the sequence shown in Fig. 5 revealed that the four sensor proteins had identical residues at five of the eight positions at which Tar, Trg, and EnvZ exhibit matches.

Factors influencing sugar-binding protein-mediated stimulation of hybrid receptors. Tar recognizes not only aspartate but also sugar-occupied, maltose-binding protein. However, the Taz1 fusion protein, which contained the ligand-recognition domain of Tar, mediated increased expression of the *ompC* promoter upon stimulation by aspartate but not upon stimulation by maltose (33). The demonstration in this work that Trz1 can transduce sugar-binding protein-mediated sensing of ribose into activation of *ompC* expression indicates that there is no fundamental feature of indirect recognition of attractant through receptor interaction with occupied binding protein that prohibits activation of a chemoreceptor-osmosensor hybrid. Instead, we believe that the particular characteristics of indirect recognition and of the kinase/phosphatase signalling domain in the hybrid receptor create specific requirements for effective transduction of ligand recognition via a binding protein into activation of the regulatory protein OmpR. An important observation is that ligand binding to the

Taz1 hybrid receptor has been shown to cause increased expression from the *ompC* promoter by inhibiting phosphatase activity, not by stimulating kinase activity (37). In some *in vivo* conditions, this would mean that much of the EnvZ phosphatase of Taz1 would have to be inhibited by ligand occupancy before cellular levels of OmpR-phosphate, and thus expression from the *ompC* promoter, would rise discernibly. Such a requirement could contribute to a significant degree to the high concentrations of aspartate, approximately 1,000-fold above the $5 \mu\text{M}$ K_d of the Tar-aspartate complex (5), necessary to detect ligand-dependent activation of *ompC* expression by Taz1 (33), although the reduction of low concentrations of aspartate by transport and metabolism during the time course of the assay, a phenomenon discussed above, probably also contributes. A requirement for substantial occupancy is particularly crucial for binding protein-mediated recognition. The content of binding protein in the periplasm defines the upper limit for concentration of the ligand (occupied binding protein) available to occupy receptor. In cells with chromosomal copies of the relevant genes, this means that the maximum possible proportion of occupied receptor is substantially less than 100% since the concentration of sugar-binding protein in the periplasm is near the value for the K_d for the complex of receptor and occupied sugar-binding protein (23, 36). For cells with increased amounts of receptor as the result of high-level expression from plasmid-borne genes, an excess of receptor over sugar-binding protein would also limit maximal occupancy. These considerations provide a way to interpret some experimental observations. The lack of Taz1-mediated increase in *ompC* expression upon stimulation by maltose (33) was likely the result of insufficient maltose-binding protein, produced from a single chromosomal copy of the gene, in relation to the dissociation constant of the receptor-binding protein complex and to the high cellular content of plasmid-encoded Taz1. The substantial ligand-dependent expression of *ompC* mediated by Trz1 (Fig. 3A) was observed in cells containing a low amount of the hybrid receptor and a high amount of ribose-binding protein, produced from multiple copies of a plasmid-borne gene. High-level production of Trz1 resulted in increased expression of *ompC* that was essentially independent of ligand occupancy (Fig. 3B). This could reflect an increased and highly buffered level of OmpR-phosphate that occurred because the vast excess of plasmid-produced enzyme over chromosomally produced substrate (OmpR) resulted in a shift in the normal balance between the reactions of phosphorylation and dephosphorylation.

Implications. The chemoreceptors and the osmosensor EnvZ of *E. coli* are among the best characterized of a large family of bacterial transmembrane sensor proteins that act by coupling recognition of the environment to the control of protein phosphorylation. These receptors share important features of intramolecular signal transduction that are independent of the stimulant, whether small molecule, protein, or osmotic pressure, and that allow creation of functional proteins by mixing modules with little relationship at the level of primary structure. It seems likely that these shared features extend to other members of the sensor family.

ACKNOWLEDGMENTS

We thank John David Lawson for obtaining some of the data included in Fig. 3 and Reza Yaghmai and Kwanghwan Jung for involvement in the early stages of investigating Trz1 activity.

This work was supported by grants from the National Institutes of Health (GM29963 to G.L.H. and GM19043 to M.I.), by a United States-Korea Cooperative Grant (INT-8807392 from the National Science Foundation) to G.L.H. and by a grant from the Ministry of

Science and Technology of Korea and the Korea Telecommunication Co. to C.P.

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