JOURNAL OF BACTERIOLOGY, Nov. 1997, p. 6714–6720 Vol. 179, No. 21
0021-9193/97/$04.00+0

High- and Low-Abundance Chemoreceptors in *Escherichia coli*: Differential Activities Associated with Closely Related Cytoplasmic Domains

XIUHONG FENG, JAMES W. BAUMGARTNER,† AND GERALD L. HAZELBAUER*

Department of Biochemistry and Biophysics, Washington State University, Pullman, Washington 99164-4660

Received 2 June 1997/Accepted 22 August 1997

In *Escherichia coli*, two high-abundance chemoreceptors are present in cellular dosages approximately tenfold greater than two low-abundance receptors. In the absence of high-abundance receptors, cells exhibit an abnormally low tumble frequency and the ability of the remaining receptors to mediate directed migration in spatial gradients is substantially compromised. We found that increasing the cellular amount of the low-abundance receptor Trg over a range of dosages did not alleviate these defects and thus concluded that high- and low-abundance receptors are distinguished not simply by their different dosages in a wild-type cell but also by an inherent difference in activity. By creating hybrids of the low-abundance receptor Trg and the high-abundance receptor Tsr, we investigated the possibility that this inherent difference could be localized to a specific receptor domain and found that the cytoplasmic domain of the high-abundance receptor Tsr conferred the essential features of that receptor class on the low-abundance receptor Trg, even though it is in this domain that residue identity between the two receptors is substantially conserved.

Methyl-accepting chemoreceptor proteins mediate the chemotactic response of *Escherichia coli* to an array of attractants and repellents (5, 9, 10). Related proteins have been detected in a substantial number of bacterial species (20), and it appears that such proteins are generally components of the sensory systems that direct motile bacteria to favorable environments. The hallmarks of this chemoreceptor family are a highly conserved region (~40 residues) involved in intracellular signaling and adjoining conserved regions containing methyl-accepting glutamyl residues that are covalently modified in the process of sensory adaptation. Both of these regions are located in the carboxyl-terminal half of the chemoreceptor. Studies of chemoreceptors from *E. coli* and *Salmonella typhimurium* have implicated the involvement of the highly conserved region in the control of a noncovalently associated histidine kinase, CheA (5, 25), and have identified two distinct actions: (i) basal activation of the kinase and (ii) modulation of kinase activity in response to a change in receptor occupancy. Basal activation, effected by unoccupied or adapted receptors, is in essence a steady-state signaling that determines the cellular content of the phosphorylated response regulator CheY (phospho-CheY). Phospho-CheY interacts with the flagellar switch to induce clockwise (CW) rotation by the otherwise counterclockwise (CCW)-rotating motor. In a wild-type cell, basal activation creates a level of phospho-CheY that results in a balance between CCW and CW flagellar rotation that creates a corresponding alternation between smooth swimming and tumbling. This alternation causes the cell to trace a random walk. Modulation of kinase activity from its level of basal activation occurs through receptors that have undergone a change in receptor occupancy but have not yet adapted. It alters the content of phospho-CheY, and thus the CCW/CW balance, and hence the frequency of tumbles, biasing the random walk to direct the cells to a more favorable environment.

In *E. coli*, chemoreceptor Tsr mediates taxis toward serine, Tar mediates taxis toward aspartate and maltose, Trg mediates taxis toward ribose and galactose, and Tap mediates taxis toward dipeptides (see references 5, 9, and 10 for original references). All but Tap are known to mediate the response to one or more receptor-specific repellents. Very recently a fifth receptor, Aer, has been identified (4, 27). Since characterization of this probable mediator of oxygen taxis, apparently present in small cellular amounts, is still at an early stage, it will not be considered further. The four receptors share a common organization in which two transmembrane segments bracket a periplasmic domain of ~150 residues and connect it to a cytoplasmic domain of ~300 residues. Residue identity among aligned sequences of the four periplasmic domains is at the margin of statistical significance, but it is nearly 60% for the cytoplasmic domains (9).

In a wild-type cell, some chemoreceptors are present in significantly greater amounts than others. There are approximately 10-fold more copies of Tsr or Tar than there are of either of the low-abundance receptors, Trg and Tap (12, 34). Cells that contain the low-abundance receptor Trg but lack the two high-abundance receptors are unable to accumulate in capillaries containing chemoattractants recognized by Trg (29, 31). This inability to migrate effectively in chemical gradients of Trg-linked attractants was initially interpreted to mean that the pathway of signaling from Trg to the flagellar motor included passage of the signal through one or the other high-abundance receptor (29, 31). However, cells containing only Trg responded to temporal gradients of galactose and ribose, indicating that this low-abundance receptor represented a parallel and equivalent signaling component (11), albeit one that was ineffective in mediating chemotactic migration of cells lacking high-abundance receptors. In the present study, we have investigated why Trg-mediated taxis is inefficient in a cell lacking high-abundance receptors.

(Portions of this work were performed by J. W. Baumgartner

* Corresponding author. Phone: (509) 335-2174. Fax: (509) 335-9688.

† Present address: Dept. of Neurobiology, Amgen Corp., Thousand Oaks, CA 91320.

TABLE 1. Strains used in this study

Strain	Parent, genotype, and/or phenotype
OW1	*ara-14 his-4 lacY1 leuB6 rpsL136 thi-1 thr*(Am)*-1 tonA31 tsx-78 xyl-5*
CP177	OW1 Δ*trg-100 zdb*::Tn*5*
CP361	OW1 Δ*trg-100* Δ*tsr-7028 zdb*::Tn*5*
CP362	OW1 Δ*trg-100* Δ*tsr-7028* Δ(*tar-tap*)*5201 zdb*::Tn*5*
CP419	OW1 Δ(*tar-tap*)*5201*
CP425	OW1 Δ(*tar-tap*)*5201* Δ*tsr-7028*
CP594	CP362, Lac^+
CP607	CP362(pGB1)
CP618	CP177(pGB1)
HB1537	CP361(pGB1)
HB1337	CP594 *lacZ*::*tsr*
HB1338	CP594 *lacZ*::*trg*
HB1341	CP594 *lacZ*::*tsrg*
HB1342	CP594 *lacZ*::*trsr*

in partial fulfillment of the requirements for a Ph.D. from Washington State University, Pullman, 1992.)

MATERIALS AND METHODS

Strains and plasmids. Strains used were derivatives of *E. coli* K-12 and are listed in Table 1. The plasmid pGB1 is a derivative of pKK223-3 containing the *lacI*^{q} gene and the *trg* coding sequence fused to the *tac* promoter (7). We constructed *trsr* and *tsrg* by recombining *Eco*RI-*Nde*I fragments of a plasmid containing *tsr* and of a plasmid containing *trg* in which a single nucleotide change in codon 266 created an *Nde*I site. The alteration in *trg* caused an amino acid change from Gln to His but did not appear to perturb receptor function (3). The *trg*-containing plasmid was a derivative of pMG2 (22) in which the *Nde*I site of the pUC13 vector had been eliminated by digestion with that endonuclease, treatment with mung bean nuclease, and ligation of the blunt ends. In this construct, *trg*, under the control of its natural promoter, was bracketed by a unique *Hin*dIII site on its 5′ side and a unique *Eco*RI site on its 3′ side. The *tsr*-containing plasmid was a combination of the pUC19 vector lacking its *Nde*I site and a 2.7-kb fragment carrying *tsr* under the control of its native promoter. That fragment originated from pAB125, a derivative of pAB100 (6) provided by M. I. Simon (California Institute of Technology), that contained a Tn*5* insertion to the 5′ side of *tsr*. The *tsr*-containing *Hin*dIII fragment of pAB125 was introduced into pBR322, the resulting plasmid was cleaved with *Hpa*I, an *Eco*RI linker was introduced, the DNA was cleaved with *Bgl*II, and the resulting *tsr*-containing fragment was introduced between the *Eco*RI and *Bam*HI sites of pPLc2833 (Pharmacia). In this construct, pAI58, with *tsr* under the control of its native promoter, was bracketed by an *Eco*RI site on its 5′ side and a *Hin*dIII site on its 3′ side, the opposite of *trg* in pMG2. The placement of *Eco*RI and *Hin*dIII sites in relation to *tsr* was inverted by isolating the *tsr*-containing *Eco*RI-*Hin*dIII fragment from pAI58 that had been treated with those two enzymes plus *Pst*I (to cleave the similarly sized vector fragment), creating blunt ends with DNA polymerase, ligating the blunt-ended fragment into *Sma*I-cleaved M13mp19, screening for the desired orientation by determining patterns of cleavage with appropriate restriction enzymes, and transferring the 2.7-kb *tsr*-containing *Hin*dIII-*Eco*RI fragment to pUC19 lacking its natural *Nde*I site (see above) to create pJB9. Recombination of *Eco*RI-*Nde*I and *Nde*I-*Eco*RI fragments of pMG2 lacking the vector *Nde*I site and pJB9 yielded pJB13 (*tsrg*) and pJB14 (*trsr*). In all four plasmids the receptor gene was bracketed by segments of *lac* DNA, and thus it was possible to select for recombination of each plasmid into the *lac* region and subsequent resolution of the plasmid (24), resulting in placement of each respective hybrid and intact gene, under the control of its native promoter, in the *lac* region of a strain otherwise lacking the chemoreceptor genes.

Behavioral assays. Semisolid-agar swarm plates containing minimal salts, required amino acids at 0.5 mM, 50 μg of ampicillin per ml (if the strain harbored a plasmid conferring resistance to this antibiotic), and either 0.05 mM galactose, 0.1 mM ribose, or 0.1 mM serine plus 1 mM glycerol were inoculated with highly motile, mid-exponential-phase cultures in tryptone broth and placed in a humid incubator at 35°C (14). Rates of ring formation were determined by measuring ring diameters each hour for at least 6 h, beginning soon after a ring was first evident (3 to 4 h after inoculation). Rotational phenotypes and responses to temporal gradients were determined using cells grown at 35°C to mid-exponential phase in H1 minimal salts (13) containing required amino acids at 1.0 mM and 0.2% ribose. Cells were harvested, treated to shear their flagella, tethered to a glass surface by using antibodies to flagellin, washed extensively in 10 mM potassium phosphate (pH 7.0)–0.1 mM EDTA–1 mM sodium succinate–1 μM methionine, observed by videomicroscopy at 35°C, and recorded on videotape (24). Solutions containing a repellent or attractant were added to the tethered cells, and changes in rotational mode were noted.

Quantitative immunoblotting. Samples of cultures grown at 35°C in minimal salts-ribose medium (described above) containing 50 μg of ampicillin per ml and a specific concentration of isopropyl-β-D-thiogalactopyranoside (IPTG) were taken at mid-exponential phase, added to ice-cold trichloroacetic acid (TCA; final concentration, ~8%), incubated on ice for at least 30 min, pelleted by centrifugation, resuspended in electrophoresis sample buffer, boiled for 5 min, fractionated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (11% polyacrylamide, 0.074% *N*,*N*′-methylenebisacrylamide; pH 8.2) (22), electroblotted onto nitrocellulose membranes (Schleicher and Schuell, Inc., Keene, N.H.), treated with anti-Trg serum and a peroxidase-coupled secondary antibody (Bio-Rad, Richmond, Calif.), and incubated with hydrogen peroxide and 4-chloro-1-naphthol. The amount of Trg in each sample was determined by quantifying the intensity of staining with a densitometer (Molecular Dynamics, Inc.) and relating that intensity to a standard curve constructed from samples of pure Trg on the same immunoblot.

Labeling with [*methyl*-^{3}H]methionine and dual analysis by immunoblotting and fluorography. Labeling in vivo with ^{3}H-methyl groups was performed essentially as described previously (8). Cultures grown as described for determination of rotational phenotypes were harvested by centrifugation and submitted to at least five cycles of suspension and pelleting with a chemotactic wash solution (10 mM potassium phosphate buffer, pH 7.0; 0.1 mM EDTA). Cells were suspended to 9×10^7 per ml in the chemotactic wash solution containing 200 μg of chloramphenicol per ml and 25 mM sodium succinate and then placed in tubes in a 30°C water bath. After 10 min, 30 μCi of L-[*methyl*-^{3}H]methionine (Amersham Corp., Arlington Heights, Ill.) (27 Ci/mmol) was added to a final concentration of 2.9 μM. 30 minutes later, an attractant, repellent, or buffer control was added, and incubation was continued for 30 min before ice-cold TCA was added to ~8%. Samples representing ~4.5×10^7 cells were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting as described above. After being recorded by video photography, the stained blot was treated with En^{3}Hance Spray (New England Nuclear, Boston, Mass.) by three cycles of spraying and drying, placed on preflashed X-ray Hyperfilm (Amersham Corp.), and stored at −70°C until development of the film.

Protease accessibility. Spheroplasts were created by a modification of the method described by Randall and Hardy (26). Samples were removed from highly motile mid-exponential-phase cultures growing at 35°C in tryptone broth containing 100 μg of ampicillin per ml, and cells were pelleted at 14,000 rpm in an Eppendorf 5415 microcentrifuge and suspended in ice-cold 0.1 M Tris-acetate (pH 8.2)–0.5 M sucrose–5 mM EDTA at approximately 10^9 per ml. Twenty microliters of a 2-mg/ml solution of lysozyme was added to 250 μl of cell suspension, and this mixture was immediately added to 250 μl of ice-cold sterile H_2O. After incubation on ice for 5 min, 10 μl of 1 M $MgSO_4$ was added to stabilize the spheroplasts, which were pelleted as described above, suspended in an equal volume of 0.25 M Tris-acetate (pH 8.2)–0.25 M sucrose–10 mM $MgSO_4$, divided into two equal portions, and pelleted again. One sample, providing intact spheroplasts, was suspended in the previous solution containing 4 M urea, and the other sample, providing lysed spheroplasts, was suspended in 0.25 M Tris-acetate (pH 8.2)–10 mM EDTA–8 M urea and, after 2 min, diluted with an equal volume of 0.25 M Tris-acetate (pH 8.2)–20 mM $MgSO_4$–0.5 M sucrose-2% β-octyl glucoside. To portions of each sample containing material equivalent to 4.5×10^7 cells was added trypsin in 10 mM Tris-acetate (pH 7) to a final concentration of 0.25 mg per ml or an equivalent volume of buffer alone. After 15 min at room temperature, phenylmethylsulfonyl fluoride was added to a concentration of 1.5 mM, TCA was added to ~8%, and samples were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting.

RESULTS

Trg-mediated chemotaxis. Trg-mediated responses in cells lacking the high-abundance receptors are undetectable by the capillary assay (29, 31) but can be detected by assaying the formation of chemotactic rings on semisolid-agar plates containing galactose or ribose (14) (Fig. 1). Thus, in the absence of high-abundance receptors, Trg can mediate migration in a spatial gradient. As in the capillary assay, Trg-mediated taxis in the plate assay was substantially improved by the presence of one or both high-abundance receptors (Fig. 1).

Receptor dosage. A simple explanation for ineffective Trg-mediated taxis in cells lacking high-abundance receptors would be that in such cells there is an abnormally low ratio of total receptor proteins to the other components of the chemosensory system. We assessed this possibility by increasing Trg dosage over a range of values by using a plasmid carrying *trg* under the control of a modified *lac* promoter. Rotational phenotypes and rates of migration for chemotactic rings on plates

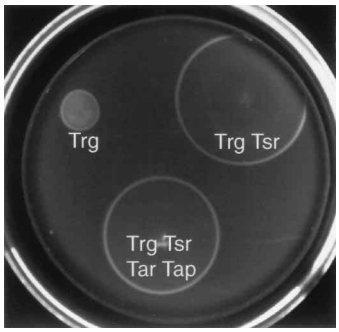

FIG. 1. Chemotactic response mediated by Trg in the absence or presence of other chemoreceptors. Strains containing only Trg (CP425), Trg and Tsr (CP419), or Tsr, Tar, Trg, and Tap (OW1) were inoculated into a semisolid-agar plate containing 100 μM ribose, and the plate was photographed 12 h later. The differences among ring sizes and sharpness were reproducible in repeated analyses. A very similar pattern of responses was observed when the attractant was galactose rather than ribose.

containing galactose or ribose were determined at various levels of Trg induction (Fig. 2), beginning at a dosage of Trg roughly equivalent to the total amount of all chemoreceptors in a wild-type cell (12). As shown in Fig. 2, increasing the amount of Trg did not improve the tactic response or correct the CCW bias characteristic of cells lacking high-abundance receptors. In cells containing high-abundance receptors, Trg-mediated responses were improved by increased amounts of Trg to a maximum at approximately 10^4 Trg monomers per cell and were reduced at higher dosages. In both cellular backgrounds, increased Trg resulted in increased CCW bias, an effect also observed for cells containing increased amounts of a high-abundance chemoreceptor (19). It is notable that no matter what the cellular dosage of Trg, cells containing high-abundance receptors exhibited more effective Trg-mediated taxis than cells lacking those receptors. Thus, it appears that high-abundance receptors have an influence on rotational bias, and correspondingly on tactic efficiency, that cannot be provided by titrating the amount of the low-abundance receptor Trg.

Hybrids of a low- and a high-abundance receptor. The chemoreceptors of *E. coli* have a common domain organization. Does the difference between a high-abundance receptor and a low-abundance receptor, documented in the previous section, reside in a specific domain? We addressed this by creating Tsr-Trg hybrid receptors at a fusion joint in the cytoplasmic domain 42 residues distal to the charged residue that marks the cytoplasmic boundary of the second transmembrane segment. The site corresponds to a natural *Nde*I site in *tsr* (and in *tar*) used previously to create functional Tar-Tsr (17), Tar-EnvZ (33), and Trg-EnvZ (3) fusions. The fusion site is close to the middle of the polypeptide chain, separating essentially all of the conserved region of the cytoplasmic domain from the part of the protein in which primary structure is minimally conserved. In an alignment of Tsr and Trg sequences (9), identity between aligned residues was 66% for the 272 residues on the carboxy-terminal side of the fusion joint but only 14% for the 257 residues on the amino-terminal side. Even though the actual numbers of residues on the two sides of the fusion joint are slightly different, we will refer to the amino-terminal and carboxy-terminal "halves" of the fusion proteins. Following the convention of Krikos et al. (17), we designated the protein created from the fusion of the amino-terminal half of Trg and the carboxy-terminal half of Tsr as Trsr, the opposite construction as Tsrg, and the respective genes as *trsr* and *tsrg*.

Cellular content of hybrid proteins. We used immunoblotting with anti-Trg serum to assess the content of receptor in cells containing *lac*-integrated copies of *tsr*, *trg*, *tsrg*, or *trsr* as the sole chemoreceptor gene (Fig. 3, upper row). Antibodies in the anti-Trg serum recognize Tsr, albeit at a 10- to 15-fold lower efficiency than they recognize Trg (21). Since production from the two respective chromosomal genes under the control of their natural promoters results in a cellular content of 10- to 15-fold more Tsr than Trg (12), the difference in reactivities balances the difference in cellular contents to yield approximately equivalent immunoblot staining for cells producing only Tsr or only Trg from the respective genes (Fig. 3, upper half; compare Tsr and Trg). Cells producing Tsrg exhibited similar levels of staining, consistent with the combination of greater production from the *tsr* promoter and a reduced number of Trg-specific epitopes. For Trsr, the combination of low-level production from the *trg* promoter and the lack of important epitopes in the carboxy-terminal domain of Trg resulted in a low level of immunoblot staining under our standard conditions. However, these cells contained sufficient receptor to exhibit clear immunoblot staining at higher ratios of antiserum to cellular material (data not shown), to mediate efficient Trsr-mediated taxis (see below), and to provide intensities of labeling with ^{3}H-methyl groups comparable to those of other receptors (Fig. 3, lower row). These observations indicate that the cellular content of Trsr is similar to that of Trg. The multiple bands observed in electrophoretic displays of chemoreceptors in Fig. 3 are not the result of proteolysis but instead

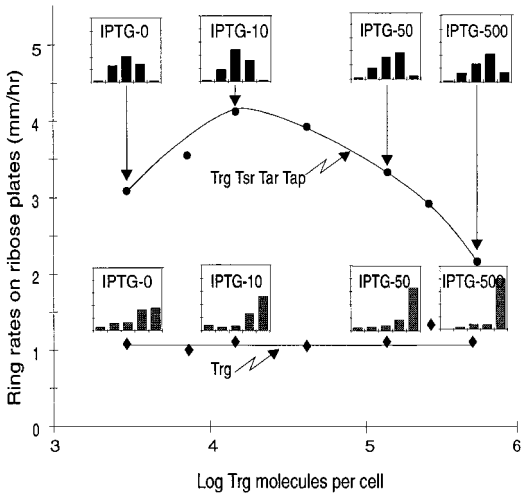

FIG. 2. Effect of cellular content of Trg on chemotactic ability and rotational bias. Strains CP607 (Trg) and CP618 (Trg Tsr Tar Tap) harboring plasmid pGB1 carrying *trg* under the control of the *tac* promoter were inoculated into semisolid-agar plates containing 100 μM ribose and 0, 5, 10, 20, 50, 100, or 500 μM IPTG. Rates of ring expansion (average of two measurements) are plotted versus content of Trg as determined by quantitative immunoblotting. At IPTG concentrations of 20, 50, 100, and 500 μM, the cellular content of Trg in CP618 (containing Tsr, Tar, and Tap as well as Trg) was sufficiently high that Trg could be detected by immunoblotting with anti-Trg without interference due to cross-reaction with the other receptors (18), and the cellular dosages determined were very similar for CP607, which contains only Trg. At lower IPTG concentrations, it was not possible to determine Trg content in CP618 because of cross-reaction with the other receptors, but since at higher levels of induction the amount of Trg in CP607 was essentially the same as in CP618, the Trg dosages determined for CP607 have been assigned to both strains. Patterns of flagellar rotation at selected IPTG levels were analyzed. The histograms show the relative proportions of rotating cells in five categories, displayed from left to right as follows: exclusively CW, predominantly CW with reversals, reversing frequently with no evident directional bias, predominantly CCW with reversals, and exclusively CCW.

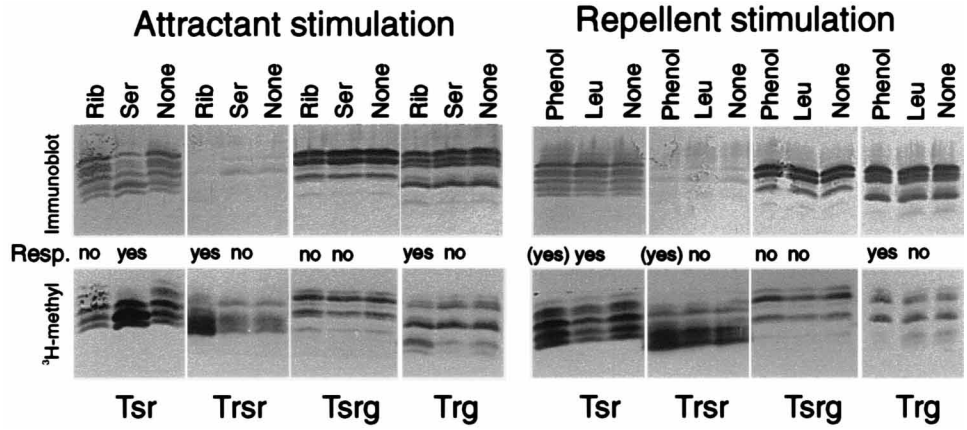

FIG. 3. Patterns of covalent modification and changes in response to temporal stimulation. Strains deleted for all four native chemoreceptor genes but containing a single receptor gene inserted in the *lac* operon that coded for Tsr (HB1337), Trsr (HB1342), Tsrg (HB1341), or Trg (HB1338) were labeled with 1-[*methyl*-^{3}H]methionine and stimulated with 10 mM ribose (Rib), 10 mM serine (Ser), 5 mM phenol, 10 mM leucine (Leu), or buffer (None). Samples were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting with anti-Trg. The two upper panels show the immunoblot pattern in the region around an apparent M_r of 60,000. The blots were treated with a fluorographic spray and placed on X-ray film. The bottom panels show fluorographs corresponding to the immunoblots in the upper panels. In the space between the upper and lower panels, we have indicated by "yes" or "no" whether addition of the attractant or repellent caused a response (Resp.). Exposure times were 5 h for the Tsr samples and 100 h for the others.

reflect the influence of functionally important covalent modifications on the electrophoretic mobilities of Tsr and Trg in an SDS-polyacrylamide gel (22, 28). Some mutationally altered chemoreceptors are prone to proteolytic degradation in vivo, but this was not the case for the two fusion proteins. Examination of immunoblots stained at higher levels than those shown in Fig. 3 revealed that the fusion proteins and the natural receptors were comparably resistant to such degradation.

Localization of hybrid proteins. We assessed membrane localization of hybrid receptors by determining patterns of accessibility to protease added externally to spheroplasts. Correct insertion of Tsr or Trg in the cytoplasmic membrane places ~150 residues of the amino-terminal domain in the periplasm and ~300 residues of the carboxy-terminal domain in the cytoplasm. Treatment of spheroplasts with trypsin in combination with 4 M urea to disrupt the otherwise protease-resistant periplasmic domain resulted in proteolytic digestion of the exposed part of a chemoreceptor, leaving a protected fragment corresponding to the second transmembrane segment plus the cytoplasmic domain. Destruction of the membrane barrier by using detergent before protease treatment resulted in digestion of this fragment. In such protease accessibility assays (Fig. 4 shows representative data), Tsrg and Trsr exhibited quantitative conversion to the expected protected fragments, indicating that the entire cellular population was appropriately inserted in the cytoplasmic membrane.

Modifications of hybrid proteins. Adaptational covalent modifications of the hybrid receptors were assessed by examining electrophoretic patterns of immunoblots and fluorographs of ^{3}H-methyl group-labeled protein (Fig. 3). Both hybrid proteins exhibited patterns consistent with essentially normal deamidation and methylation. As observed for Tar-Tsr fusions (17, 32), the characteristic electrophoretic pattern of a methylated form was a function of the cytoplasmic domain of the respective hybrid protein: the pattern exhibited by Tsrg resembled that of Trg, and the pattern exhibited by Trsr resembled that of Tsr. Chemoreceptors adapt to persistent attractant or repellent occupancy by compensatory increases or decreases in methylation, respectively. These are exhibited in electrophoretic patterns of ^{3}H-methyl group-labeled receptors as an increased intensity of labeling and as an increased proportion of more-rapidly migrating (more highly methylated) forms of the protein (adaptation to attractant) or as a decreased intensity and a decreased proportion of more-rapidly migrating forms (adaptation to repellent) and, in immunoblots, as changes in relative intensities among the more highly methylated and less highly methylated electrophoretic species. As documented in Fig. 3, such changes were observed for Tsr and Trg stimulated by the appropriate attractant (serine or ribose, respectively) or repellent (leucine or phenol, respectively), but no reproducible changes occurred upon addition of a compound not recognized by the particular receptor. Recognition of the attractant serine and of the attractant plus binding protein complex of ribose and ribose-binding protein is known to be mediated by the periplasmic domains of Tsr and Trg,

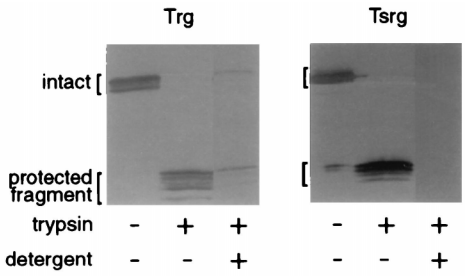

FIG. 4. Protease accessibility of a natural and a hybrid chemoreceptor. The figure shows an immunoblot of samples of spheroplasts formed from cells harboring plasmid-borne *trg* or *tsrg* and lacking chromosomal copies of chemoreceptor genes but otherwise wild type for chemotaxis. In each panel, the left lane contained a sample of intact, untreated spheroplasts, the middle lane contained a sample treated with trypsin in the presence of 4 M urea, and the right lane contained a sample of spheroplasts subjected to osmotic lysis and treatment with 1% β-octyl glucoside as well as trypsin and urea. Each lane represents material from an equivalent amount of spheroplasts. The upper brackets (intact) mark the position near an apparent M_r of 60,000 of the multiple electrophoretic forms of intact chemoreceptors, and the lower brackets (protected fragment) mark the position near an apparent M_r of 40,000 of the multiple electrophoretic forms of the segment of a chemoreceptor protected from externally added protease. The stained material in the lower region of the left lane of the Tsrg panel represents spillover from the middle lane, not degradation of intact receptor. Patterns similar to those shown here were observed for Tsr and Trsr.

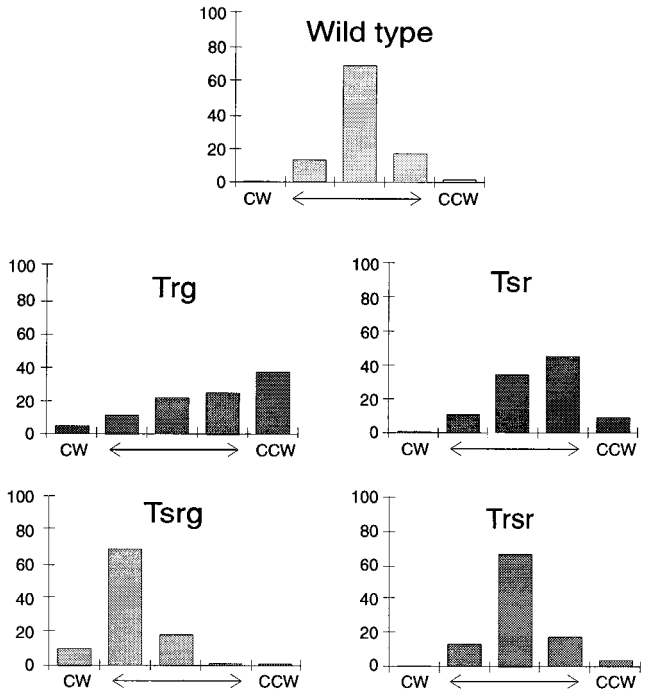

FIG. 5. Rotational phenotypes. The four strains used for the analysis in Fig. 3 plus the chemotactically wild-type strain OW1 were tethered and analyzed for rotational phenotypes by observing at least 100 rotating cells, for 10 to 15 s each, and classifying the behavior into one of five categories, which are displayed from left to right in each histogram as follows: exclusively CW, predominantly CW with occasional reversals, reversing frequently with no evident directional bias, predominantly CCW with occasional reversals, and exclusively CCW (30). The data presented are averages of two independent determinations that yielded very similar distributions.

respectively. Consistent with this domain function, Trsr exhibited increased methylation in response to stimulation by ribose but not serine. The magnitude of the increase upon ribose stimulation was much greater than that observed for intact Trg; it resembled the substantial change characteristic of Tsr stimulated by serine. In contrast, Tsrg exhibited no significant change in methylation upon stimulation with either attractant.

Steady-state signaling and tactic response. In comparison to wild-type cells, those containing only Tsr or only Trg exhibited a moderate or a substantial CCW bias, respectively. Cells containing Trsr exhibited a rotational bias essentially indistinguishable from that of wild-type cells, and cells containing Tsrg exhibited an abnormal bias skewed to CW (Fig. 5). The ability of hybrid receptors to mediate tactic responses in the absence of other chemoreceptors was examined by using temporal and spatial gradients. Tethered cells were submitted to a gradient consisting of from zero to a saturating concentration of an attractant or repellent. Glycerol is a repellent that acts through all four well-characterized chemoreceptors (23). We observed strong repellent (CW) responses to a gradient consisting of from 0 to 0.5 M glycerol in cells containing Trg, Tsr, or Trsr. Tsrg mediated a weaker but distinct response. Stimulation of Trg- or Trsr-containing cells by ribose or galactose resulted in an immediate CCW response of the entire population. Tsr-containing cells responded to stimulation by serine, but Tsrg-containing cells did not. Migration in spatial gradients was tested by using semisolid-agar plates (Fig. 6). Trsr mediated a response to galactose and ribose but not to serine, as expected for a hybrid protein with the ligand recognition domain of Trg. The responses mediated by this hybrid protein were significantly better than those mediated by Trg itself. In contrast, the complementary hybrid, Tsrg, mediated no detectable response to serine even though the serine recognition site in the hybrid receptor would be expected to be intact (16).

DISCUSSION

Low- and high-abundance receptors. Why do cells lacking the two high-abundance chemoreceptors exhibit low tumble frequencies and an inability to accumulate in capillary assays in response to spatial gradients of attractants recognized by a still-present low-abundance receptor (29, 31)? We addressed this question by first establishing that such cells were not actually incapable of migrating in spatial gradients of attractants recognized by the low-abundance receptors Trg but simply required an appropriately sensitive assay for detection of their reduced tactic abilities (Fig. 1). This assay and analysis of rotational phenotypes were used to investigate the effect of increased dosage of a low-abundance receptor in Trg-only cells (Fig. 2). It was striking that the amount of Trg in Trg-only cells had no effect on tactic efficiency over a range of Trg dosages greater than 100-fold and did not cause the substantial CCW bias to become more balanced. This observation led to the important conclusion that high- and low-abundance receptors are distinguished not simply by different dosages in a wild-type cell but also by an inherent difference in activity.

Hybrid receptors. We created hybrids of a low- and a high-abundance chemoreceptor to investigate whether the inherent difference between the two receptor classes could be localized to a specific receptor domain. We found that the cytoplasmic domain of the high-abundance receptor Tsr conferred the essential features of that receptor class on the low-abundance receptor Trg. Replacement of the carboxy-terminal half of Trg with the 66% identical carboxy-terminal half of Tsr created the hybrid Trsr. As the sole cellular receptor, Trsr established a wild-type tumble frequency and mediated migration in gradients of Trg-linked attractants comparable to that of cells containing high-abundance receptors. This hybrid protein, a low-abundance receptor in terms of copy number, exhibited the activities of a high-abundance receptor and thus appeared to be an "improved" form of Trg. Very similar observations have been made for the product of the homologous fusion of the low-abundance receptor Tap and the high-abundance receptor Tar (35). Thus, the determinants that confer the ability of a sole cellular chemoreceptor to set a physiologically useful tumble frequency and to mediate efficient taxis appear to reside in the cytoplasmic domain of the high-abundance receptor, even though it is in this domain that residue identity is substantially conserved between the two receptor classes. Among the nonconserved residues in the cytoplasmic domain, some must con-

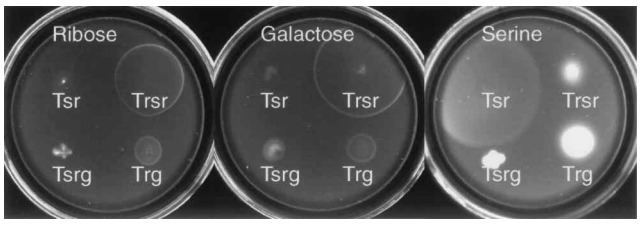

FIG. 6. Chemotactic responses mediated by wild-type and hybrid receptors. The strains used for the analysis shown in Fig. 3, each of which contains a single chemoreceptor gene inserted in the *lac* region, were inoculated into semisolid-agar plates containing 100 μM ribose, 50 μM galactose, or 100 μM serine plus 1 mM glycerol. The plates were placed in a humid incubator at 35°C and photographed 12 h later.

tribute to an activity or to an interaction that occurs in one receptor class but not in the other and allows (or blocks) appropriate basal activation of the kinase.

The high-abundance receptor character of Trsr suggested that the complementary fusion, Tsrg, should exhibit features of a low-abundance receptor and thus be unable to establish a wild-type tumble frequency or mediate effective chemotaxis when present as the sole cellular chemoreceptor. This was in fact the case, but the nature of Tsrg abnormalities implied that this hybrid receptor was not simply the low-abundance version of a serine receptor but also lacked functional linkages between its heterologous domains. The Tsr-Trg combination did not create a drastically unstable or nonnative protein, since it was present in the cell at the expected level (Fig. 3), was inserted normally into the membrane (Fig. 4), was sufficiently native to serve as a substrate for methylation and demethylation (Fig. 3), and mediated a detectable repellent response to a temporal gradient of glycerol. However, functional defects were substantial. Tsrg did not mediate a behavioral response to either spatial (Fig. 6) or temporal (see Results) gradients of serine, nor did it mediate changes in covalent modification in response to stimulation by temporal gradients of receptor-specific attractants or repellents (Fig. 3). In addition, Tsrg generated a substantial CW bias for cells in which it was the sole cellular receptor. A consistent explanation for these observations is that the combination of the amino half of Tsr and the carboxy half of Trg does not allow functional coupling of the receptor domains. If the two halves were correctly folded but not functionally coupled, then the hybrid could be proteolytically stable, correctly localized, and methylated, but ligand occupancy would not affect kinase activity or methylation level, and thus the receptor would not mediate a tactic response. This uncoupling might also explain the CW bias conferred by the hybrid, since studies of fragments of chemoreceptor Tsr in vivo have indicated that in the absence of the periplasmic domain, the cytoplasmic domain produces a CW signal (1). The detectable repellent response to glycerol mediated by Tsrg might occur in the absence of functional domain coupling if glycerol acted directly on the cytoplasmic domain, a notion consistent with the ability to stimulate all chemoreceptors (23). A spontaneous fusion of the highly conserved regions of two chromosomally adjacent receptor genes created a hybrid of the high-abundance receptor Tar and the low-abundance receptor Tap that emitted a dominant CCW signal, perhaps reflecting aberrant coupling between heterologous segments (30).

Recognition sites for repellents. Information about recognition site for chemorepellents is limited. Characterization of Tar-Tsr hybrids placed the leucine recognition site of Tsr on the amino-terminal side of the *Nde*I-created fusion joint (17). This led to the prediction that Trsr, lacking the amino-terminal half of Tsr, would be insensitive to leucine, which was what we observed (Fig. 3). The complementary hybrid, Tsrg, unable to respond to serine (perhaps because of the domain uncoupling described in the previous section), was correspondingly insensitive to leucine (Fig. 3).

Phenol stimulates several—or perhaps all—chemoreceptors but induces different responses, in terms of sign and magnitude, in each (15, 37). Yamamoto et al. (37) reported that Trg mediated a substantial repellent response to phenol, detected by both induced tumbling and demethylation, whereas Tsr mediated a possible repellent response, detected by some increase in tumbling but not as a change in methylation. We confirmed the Trg-mediated repellent response to phenol. The lack of a similar response mediated by the functional hybrid Trsr implies that the phenol recognition site is not contained in the amino-terminal half of Trg present in that protein. For the functionally defective hybrid Tsrg, stimulation by phenol did not cause a discernible change in distribution of methylated forms in cells containing chromosomally integrated *tsrg* (Fig. 3) but did cause a detectable shift to less-highly methylated forms in cells with a high dosage of Tsrg resulting from a plasmid-borne gene (2). Thus, it appears that phenol acts through the carboxy-terminal half of Trg. We detected a marginal Tsr-mediated response to phenol, but it appeared to be an attractant response (Fig. 3). The hybrid Trsr also exhibited a marginal attractant response to phenol. All of these observations are consistent with the notion that phenol acts by affecting the cytoplasmic domain of chemoreceptors.

Activity differences in closely related domains. What is the origin of the functional difference between the cytoplasmic domains of Tsr and Trg? The cytoplasmic domain participates in two functionally important sets of interactions, one with CheA and CheW (kinase control) and the other with CheR and CheB (adaptation). A difference in either or both could account for the difference between high- and low-abundance receptors. If the cytoplasmic domain of a low-abundance receptor were less efficient at basal activation of the kinase than the same domain of a high-abundance receptor, then in the absence of high-abundance receptors (or their relevant domains) the steady-state level of phospho-CheY would be low, resulting in a low tumble frequency. Alternatively, if adaptational methylation were less efficient for low-abundance receptors than for high-abundance receptors, adaptation might lag sufficiently such that the average level of phospho-CheY would be depressed, resulting in a low tumble frequency. There are several indications that low- and high-abundance receptors differ in properties related to adaptation. In the absence of high-abundance receptors, adaptation to Trg-mediated stimuli is slow (13), so slow that for large temporal changes in receptor occupancy, adaptation does not occur even over extended periods of observation (12, 23, 37). In vitro studies of Tsr and Tar have led to the conclusion that a conserved carboxy-terminal pentapeptide confers much of the ability of those receptors to interact with the methyltransferase and thus to be methylated (36). Since this pentapeptide is present in high-abundance receptors but absent in low-abundance receptors, Wu et al. (36) suggested that methylation of Trg or Tap occurs not by independent interaction with the methyltransferase but rather via the binding of this enzyme to a neighboring high-abundance receptor. Our observations show that this indirect process is not required for Trg to be methylated, since in cells devoid of high-abundance receptors, Trg is methylated and exhibits changes in extent of methylation upon stimulation by an attractant or a repellent (Fig. 3). However, methylation of low-abundance receptors by methyltransferase bound to neighboring high-abundance receptors might result in modification rates unattainable in the absence of high-abundance receptors. In fact, the apparent extent of methylation of the low-abundance receptor Tap was substantially increased by grafting onto that receptor the pentapeptide-containing, carboxy-terminal sequence of the high-abundance receptor Tar (35).

In any case, direct comparison of high- and low-abundance receptors by in vitro assays of methylation and kinase activation is the next logical step toward understanding the difference between these two classes. The difference could be informative in understanding receptor action, since the mechanism of signal amplification in bacterial chemotaxis is not yet understood and, even for large changes in occupancy, low-abundance receptors require significant amplification to influence cellular behavior in an environment with a substantial majority of other receptors.

ACKNOWLEDGMENTS

This work was supported by grant GM29963 from the National Institutes of Health to G.L.H.

We thank Akihiro Iida and Dawn Nowlin for performing some of the steps in the creation of pJB9.

REFERENCES

1. **Ames, P., and J. S. Parkinson.** 1994. Constitutively signaling fragments of Tsr, the *Escherichia coli* serine chemoreceptor. J. Bacteriol. **176:**6340–6348.
2. **Baumgartner, J. W.** 1992. The functional role of the transmembrane domains of sensory receptors from *Escherichia coli*. Ph.D. thesis. Washington State University, Pullman.
3. **Baumgartner, J. W., C. Kim, R. E. Brissette, M. Inouye, C. Park, and G. L. Hazelbauer.** 1994. 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. J. Bacteriol. **176:**1157–1163.
4. **Bibikov, S. I., R. Biran, K. E. Rudd, and J. S. Parkinson.** 1997. A signal transducer for aerotaxis in *Escherichia coli*. J. Bacteriol. **179:**4075–4079.
5. **Bourret, R. B., K. A. Borkovich, and M. I. Simon.** 1991. Signal transduction pathways involving protein phosphorylation in prokaryotes. Annu. Rev. Biochem. **60:**401–411.
6. **Boyd, A., K. Kendall, and M. I. Simon.** 1983. Structure of the serine chemoreceptor in *Escherichia coli*. Nature (London) **301:**623–626.
7. **Burrows, G. G., M. E. Newcomer, and G. L. Hazelbauer.** 1989. Purification of receptor protein Trg by exploiting a property common to chemotactic transducers of *Escherichia coli*. J. Biol. Chem. **264:**17309–17315.
8. **Engström, P., and G. L. Hazelbauer.** 1980. Multiple methylation of methyl-accepting chemotaxis proteins during adaptation of *E. coli* to chemical stimuli. Cell **20:**165–171.
9. **Hazelbauer, G. L.** 1988. The bacterial chemosensory system. Can. J. Microbiol. **34:**466–474.
10. **Hazelbauer, G. L.** 1991. Bacterial chemoreceptors. Curr. Opin. Struct. Biol. **2:**505–510.
11. **Hazelbauer, G. L., and P. Engström.** 1980. Parallel pathways for transduction of chemotactic signals in *Escherichia coli*. Nature (London) **283:**98–100.
12. **Hazelbauer, G. L., and P. Engström.** 1981. Multiple forms of methyl-accepting chemotaxis proteins distinguished by a factor in addition to multiple methylation. J. Bacteriol. **145:**35–42.
13. **Hazelbauer, G. L., and S. Harayama.** 1979. Mutants in transmission of chemotactic signals from two independent receptors of *E. coli*. Cell **16:**617–625.
14. **Hazelbauer, G. L., C. Park, and D. M. Nowlin.** 1989. Adaptational "cross-talk" and the crucial role of methylation in chemotactic migration by *Escherichia coli*. Proc. Natl. Acad. Sci. USA **86:**1448–1452.
15. **Imae, Y., K. Oosawa, T. Mizuno, M. Kihara, and R. M. Macnab.** 1987. Phenol: a complex chemoeffector in bacterial chemotaxis. J. Bacteriol. **169:**371–379.
16. **Jeffery, C. J., and D. E. Koshland, Jr.** 1993. Three-dimensional structural model of the serine receptor ligand-binding domain. Protein Sci. **2:**559–566.
17. **Krikos, A., M. P. Conley, A. Boyd, H. C. Berg, and M. I. Simon.** 1985. Chimeric chemosensory transducers of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82:**1326–1330.
18. **Lee, G. F., M. R. Lebert, A. A. Lilly, and G. L. Hazelbauer.** 1995. Transmembrane signaling characterized in bacterial chemoreceptors by using sulfhydryl cross-linking *in vivo*. Proc. Natl. Acad. Sci. USA **92:**3391–3395.
19. **Liu, J., and J. S. Parkinson.** 1989. Role of CheW protein in coupling membrane receptors to the intracellular signaling system of bacterial chemotaxis. Proc. Natl. Acad. Sci. USA **86:**8703–8707.
20. **Morgan, D. G., J. W. Baumgartner, and G. L. Hazelbauer.** 1993. Proteins antigenically related to methyl-accepting chemotaxis proteins of *Escherichia coli* detected in a wide range of bacterial species. J. Bacteriol. **175:**133–140.
21. **Nowlin, D. M., D. O. Nettleton, G. W. Ordal, and G. L. Hazelbauer.** 1985. Chemotactic transducer proteins of *Escherichia coli* exhibit homology with methyl-accepting proteins from distantly related bacteria. J. Bacteriol. **163:**262–266.
22. **Nowlin, D. M., J. Bollinger, and G. L. Hazelbauer.** 1987. Sites of covalent modification in Trg, a sensory transducer of *Escherichia coli*. J. Biol. Chem. **262:**6039–6045.
23. **Oosawa, K., and Y. Imae.** 1983. Glycerol and ethylene glycol: members of a new class of repellents of *Escherichia coli* chemotaxis. J. Bacteriol. **154:**104–112.
24. **Park, C., and G. L. Hazelbauer.** 1986. Mutations specifically affecting ligand interaction of the Trg chemosensory transducer. J. Bacteriol. **167:**101–109.
25. **Parkinson, J. S.** 1993. Signal transduction schemes in bacteria. Cell **72:**857–871.
26. **Randall, L. L., and S. J. S. Hardy.** 1986. Correlation of competence for export with lack of tertiary structure of the mature species: a study *in vivo* of maltose-binding protein in *E. coli*. Cell **46:**921–928.
27. **Rebbapragada, A., M. S. Johnson, G. P. Harding, A. J. Zuccarelli, H. M. Fletcher, I. B. Zhulin, and B. L. Taylor.** 1997. The Aer protein and serine chemoreceptor Tsr independently sense intracellular energy levels and transduce oxygen, redox, and energy signals for *Escherichia coli* behavior. Proc. Natl. Acad. Sci. USA **94:**10541–10546.
28. **Rice, M. S., and F. W. Dahlquist.** 1991. Sites of deamidation and methylation in Tsr, a bacterial chemotaxis sensory transducer. J. Biol. Chem. **266:**9746–9753.
29. **Silverman, M., and M. Simon.** 1977. Chemotaxis in *Escherichia coli*: methylation of *che* gene products. Proc. Natl. Acad. Sci. USA **74:**3317–3321.
30. **Slocum, M. K., N. F. Halden, and J. S. Parkinson.** 1987. Hybrid *Escherichia coli* sensory transducers with altered stimulus detection and signaling properties. J. Bacteriol. **169:**2938–2944.
31. **Springer, M. S., M. F. Goy, and J. Adler.** 1977. Sensory transduction in *Escherichia coli*: two complementary pathways of information processing that involve methylated proteins. Proc. Natl. Acad. Sci. USA **74:**3312–3316.
32. **Tatsuno, I., L. Lee, I. Kawagishi, M. Homma, and Y. Imae.** 1994. Transmembrane signalling by the chimeric chemosensory receptors of *Escherichia coli* Tsr and Tar with heterologous membrane-spanning regions. Mol. Microbiol. **14:**755–762.
33. **Utsumi, R., R. E. Brissette, A. Rampersaud, S. A. Forst, K. Oosawa, and M. Inouye.** 1989. Activation of bacterial porin gene expression by a chimeric signal transducer in response to aspartate. Science **245:**1246–1249.
34. **Wang, E. A., K. L. Mowry, D. O. Clegg, and D. E. Koshland, Jr.** 1982. Tandem duplication and multiple functions of a receptor gene in bacterial chemotaxis. J. Biol. Chem. **257:**4673–4676.
35. **Weerasuriya, S., B. Schneider, and M. D. Manson.** Chimeric chemoreceptors in *Escherichia coli*: signaling and methylation properties of Tar-Tap and Tap-Tar hybrids. Submitted for publication.
36. **Wu, J., J. Li, G. Li, D. G. Long, and R. M. Weis.** 1996. The receptor binding site for the methyltransferase of bacterial chemotaxis is distinct from the sites of methylation. Biochemistry **35:**4984–4993.
37. **Yamamoto, K., R. M. Macnab, and Y. Imae.** 1990. Repellent response functions of the Trg and Tap chemoreceptors of *Escherichia coli*. J. Bacteriol. **172:**383–388.