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Strategies for differential sensory responses mediated through the same transmembrane receptor

Reza Yaghmai[1,3] and Gerald L.Hazelbauer[1,2,4]

Departments of [1]Genetics and Cell Biology and [2]Biochemistry/Biophysics, Washington State University, Pullman, WA 99164-4660, USA

[3]Present address: George Washington School of Medicine, George Washington University, 2300 Eye Street, Washington, DC 20037, USA

[4]Corresponding author

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Trg mediates chemotaxis of *Escherichia coli* to galactose and ribose by recognition of respective, sugar-occupied binding proteins. Although both attractants act through one transmembrane receptor, maximal response is ~50% greater to ribose. This phenomenon was investigated by mutational analysis of a 20-residue segment of Trg implicated in ligand interaction and signalling. Among 17 defective receptors, responses to the two chemoattractants were reduced equivalently for seven and differentially for 10, in some cases reversing the preference order. Mutational substitutions with equivalent effects occurred throughout the segment, but those with a greater effect on galactose or ribose response were segregated to the amino-terminal two-thirds or the carboxy-terminal one-third, respectively, a segregation corresponding in large part to a functional division based on signalling phenotypes. A model for binding protein-mediated recognition revealed two strategies for differential responses. The wild-type preference for ribose probably reflects a balance of receptor affinities and a limiting supply of binding proteins. Mutants with reversed preference probably have differentially reduced receptor affinities and those with an accentuated ribose preference probably have altered signalling abilities. Two-step recognition of ligand allows functional separation of detection and response.

Key words: bacterial chemotaxis/chemoreceptors/mutational analysis/periplasmic binding proteins/signal transduction

Introduction

Chemotaxis in *Escherichia coli* is mediated by a family of four transmembrane receptor proteins [see Bourret *et al.* (1991) and Hazelbauer *et al.* (1990) for detailed information and primary references]. Three of the four receptors recognize periplasmic, ligand-binding proteins in their ligand-occupied conformations and thus mediate taxis to the respective small molecule ligands. The receptor Tar binds ligand-occupied, maltose-binding protein (as well as the small molecule aspartate), while Tap recognizes ligand-occupied, dipeptide-binding proteins. The receptor Trg interacts with two different, ligand-occupied proteins, galactose-binding protein and ribose-binding protein. The fourth receptor, Tsr, does not appear to recognize any protein ligand, but only the small molecule serine. The receptors are homodimers of two 60 kDa subunits (Milligan and Koshland, 1988), organized into a periplasmic domain that binds ligand, a membrane domain containing two transmembrane segments from each subunit and a cytoplasmic domain that initiates intracellular signalling and mediates sensory adaptation by covalent modification at several, specific methyl-accepting glutamyl residues. Crystallographic analysis of the periplasmic domain of Tar_s, from *Salmonella typhimurium*, has revealed an elongated dimeric structure in which two subunits, each a four-helix bundle, interact along an interface between helices 1 and 1′ (Milburn *et al.*, 1991). Aspartate is bound in a site at the dimer interface near the membrane-distal end of the bundles, interacting with residues from both subunits. Structural (Milburn *et al.*, 1991) and mutational (Yaghmai and Hazelbauer, 1992) analyses implicate the membrane distal, 1–1′ interface as crucial in the conformational change that initiates transmembrane signalling. The four transmembrane segments are clustered, with helices 1 and 1′ in close proximity (Burrows, 1991; Lynch and Koshland, 1991; Pakula and Simon, 1992); interaction between subunits also occurs in the cytoplasmic domain (Mowbray *et al.*, 1985).

Mutational substitutions that affect specifically interaction of ligand with Tar, Tsr or Trg cluster in two limited segments of the aligned sequences of the periplasmic domain (Park and Hazelbauer, 1986; Kossman *et al.*, 1988; Lee *et al.*, 1988; Wolff and Parkinson, 1988; Lee and Imae, 1990; Gardina *et al.*, 1992), indicating that ligand interaction sites are similarly placed in all those receptors. For Tar and Tsr, the residues identified by mutational analysis as crucial for binding of aspartate or serine correspond to side chains that interact with aspartate in the crystal structure of Tar_s. Models for structures of the periplasmic domain of Tar_E and Trg based on sequence alignment with Tar_s reveal that residues identified as important for interactions with occupied binding proteins are at the very distal end of the four-helix bundle. The site for interaction of Tar_E with maltose-binding protein is adjacent to, but slightly membrane-distal from, the aspartate binding site. Computer-assisted docking of the binding protein and receptor suggests a plausible way in which this interaction might occur (Stoddard and Koshland, 1992). Occupancy at the two distinct binding sites for aspartate and for maltose-binding protein appears to generate signals of comparable nature, but different magnitude (Mowbray and Koshland, 1987). Trg also mediates differential tactic response to two distinct attractants, galactose and ribose (Hazelbauer and Harayama, 1979), but for both attractants recognition is by means of interaction of the receptor with two distinct sugar-binding proteins. These two large polypeptides might be expected to interact with the receptor in similar ways. However, the specific Trg residues that participate in ligand interaction are not identical for the two binding proteins, since a single amino acid substitution in Trg can eliminate response to galactose

without affecting response to ribose (Park and Hazelbauer, 1986). In this study we focus on the differential ability of the two sugar attractants to generate sensory response mediated by the receptor Trg and demonstrate that response can be altered to the same or different extents for the two ligands by single-residue substitutions in a ligand interaction region of the receptor, in some cases reversing the relative strengths of the two attractants.

Results

Differential responses mediated by the receptor Trg

Trg mediates response to two different attractants, galactose and ribose, by recognition of the respective, sugar-occupied binding proteins. Although these two attractants act through a common receptor, they are not of equal strength; that is to say that the magnitude of response is greater to one attractant (ribose) than to the other (galactose). We measured chemotactic response of cell populations in spatial gradients and of individual cells stimulated by temporal gradients, as well as assessing the magnitude of transmembrane signalling by the receptor *in vivo*, and found a consistent difference between the two attractants. Maximal response to ribose was ~50% greater than maximal response to galactose as documented by the data for the chemotactically wild-type strain shown in Figures 1–3. Figure 1 shows chemotactic response in the capillary assay (Adler and Dahl, 1973) of motile cells migrating in spatial gradients of the two attractant sugars. Half-maximal accumulation occurred at approximately the same concentration for the two sugars, but maximal accumulation was greater in response to ribose. This difference is also evident in previously published studies (Hazelbauer and Adler, 1971; Adler *et al.*, 1973; Hazelbauer and Harayama, 1979; Koman *et al.*, 1979). Response of individual wild-type cells to temporal step gradients of galactose or ribose from no sugar to each of a range of final concentrations was determined using tethered cells in a flow chamber (Figure 2A). Responses were quantified by measuring the time required for cellular adaptation to the step increase (Berg and Tedesco, 1975). Curves that fit the data resemble simple binding isotherms (note the logarithmic scale on the abscissa would create a sigmoidal shape for a simple hyperbolic function). The origin of the curves shown in Figure 2 and the significance of the pair of curves for the response to galactose will be considered in the following section. The features to be noted here are that the concentrations of the two sugars that elicited the half-maximal response were approximately equal, but the maximal response was substantially greater for stimulation by ribose than by galactose. The data in Figure 2A, as well as previous observations (Hazelbauer and Harayama, 1979; Koman *et al.*, 1979; Park and Hazelbauer, 1986), demonstrate that responses of chemotactically wild-type cells to temporal gradients of galactose or ribose are similar in concentration dependence but differ in magnitude.

We assessed the molecular response of Trg to stimulation by galactose and ribose by observing the increase in adaptational methylation induced by ligand occupancy (Figure 3). Attractant binding to the periplasmic domain of a chemoreceptor has two effects on the cytoplasmic domain; one is transient, the other is persistent. The transient effect influences activity of a soluble protein kinase and thus changes the cellular content of the phosphorylated form of

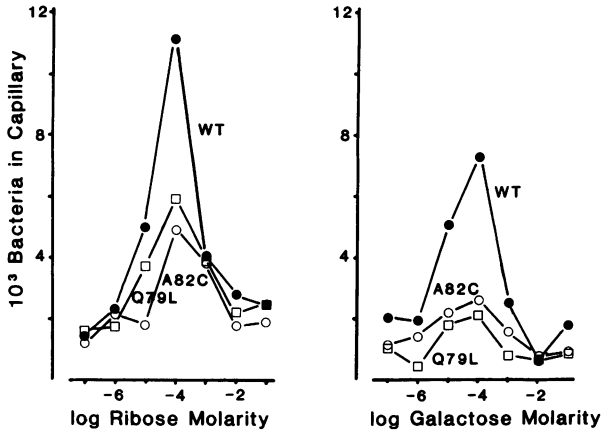

Fig. 1. Response of mutant and wild-type cells to spatial gradients in the capillary assay. Accumulation at 30°C of bacteria in 1 μl microcapillaries from a suspension in a buffer at 3×10^7 cells/ml is plotted versus the concentration of sugar initially in the capillary. Cultures were grown at 35°C to $\sim 2 \times 10^8$/ml in H1 minimal salts medium containing 0.4% of the sugar to which taxis was tested. Strains were CP593 with the following *trg* genes inserted in the *lac* region: wild-type *trg* (●, WT), *trg-A82C* (○, A82C) and *trg-Q79L* (□, Q79L).

a protein that controls flagellar function (Bourret *et al.*, 1991). The effect is transient because the receptor adapts to continued occupancy by increased methylation of several methyl-accepting sites in the cytoplasmic domain. The increase in methylation is the persistent effect of ligand occupancy. The magnitude of transmembrane signalling is reflected in the degree of receptor methylation. The level of methylation on a receptor population can be assessed by SDS–PAGE and immunoblotting with receptor-specific antiserum (Yaghmai and Hazelbauer, 1992), because the electrophoretic mobility of receptor polypeptides is increased slightly by each methyl group (Nowlin *et al.*, 1988), creating a pattern of electrophoretic bands in which the degree of methylation among a population of receptor proteins is reflected in the proportion of more rapidly migrating bands. Figure 3 shows immunoblot patterns of Trg from cells in the absence of stimulant and in the continued presence of saturating concentrations of galactose or ribose. The distribution of the several electrophoretic forms of unstimulated Trg corresponds to a low average number of methyl groups per polypeptide. Stimulation by galactose or ribose shifts the distribution to more rapidly migrating forms, corresponding to increased methylation, but the shift is more pronounced for ribose. In agreement with other assays, the magnitude of transmembrane signalling measured by the extent of adaptational methylation was greater for stimulation by ribose than by galactose.

Differential responses and two-step recognition

The most informative assay of chemotactic behavior is the measurement of response of individual cells to temporal gradients. A striking feature of the differential responses documented by this assay (Figure 2A) is that the principal difference between responses to ribose and to galactose, as well as between responses of wild-type and mutant cells (see following section), is in the magnitude of maximal responses not in the concentration dependence. For attractants recognized directly by a receptor, this would indicate differences primarily in the efficiency of signalling induced

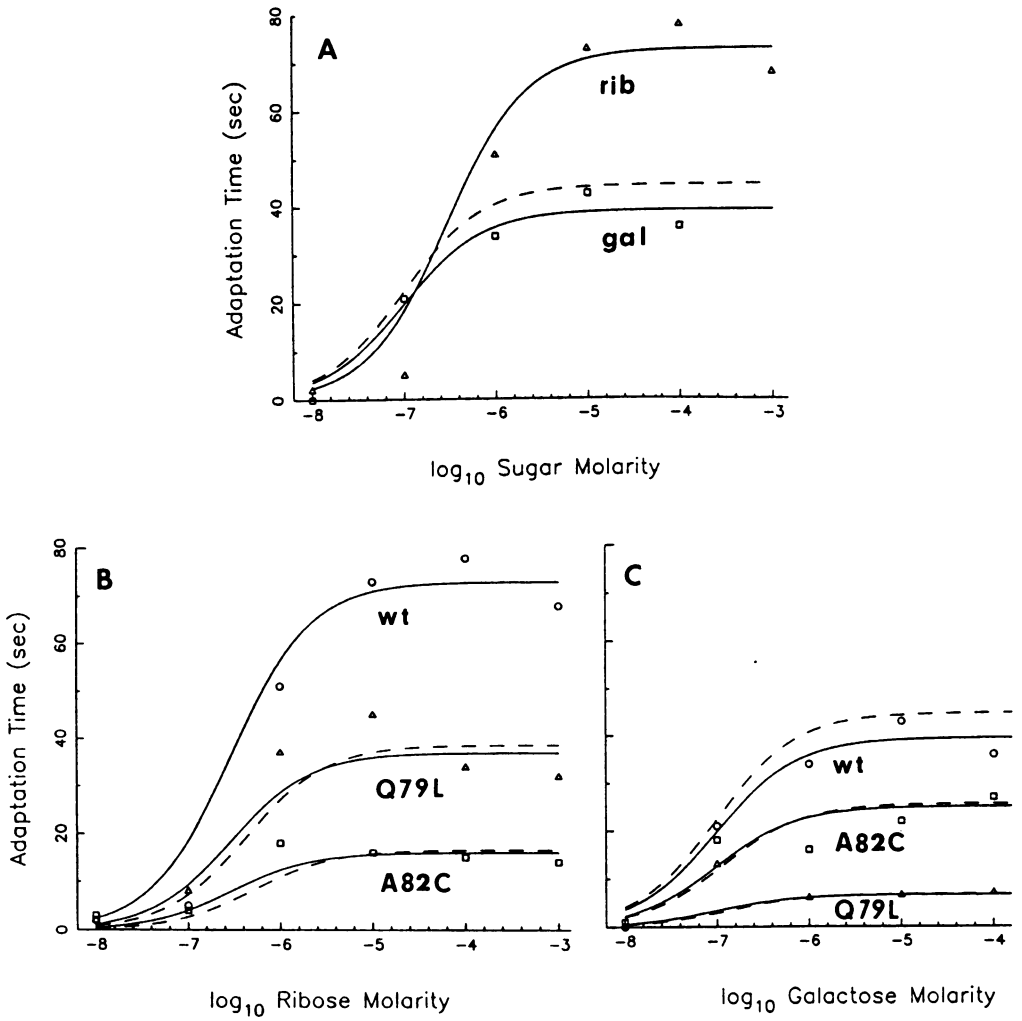

Fig. 2. Responses of mutant and wild-type cells to temporal gradients. Cells grown as described in Figure 1 were tethered and tested at room temperature for responses to temporal gradients from no sugar to the concentration indicated. Cells respond by inhibition of clockwise rotation of the flagellar motor to produce exclusively counterclockwise rotation and are adapted when the usual pattern of alternation between directions of rotation is reestablished. A minimum of 10 cells was observed and an average adaptation time (time at which the first complete clockwise rotation occurred) was determined. Standard deviations ranged from 5 to 12 s, depending on the magnitude of the response, except for wild-type response to ribose at $10^{-6}-10^{-4}$ M for which they were 15–20 s. Responses by a Δ(*trg-100*) strain, due primarily to temperature effects, averaged 10 s for ribose-grown cells stimulated by ribose and 14 s for galactose-grown cells stimulated by galactose and were subtracted from response times to yield the values shown. The curves were generated from a dose–response relationship and appropriate parameters (see text). **A**. The curves for galactose response were calculated using the same proportionality constant as for response to ribose (dashed line) or by adjusting the proportionality constant to provide a best fit to the data (solid line). **B** and **C**. The curves for Trg-A82C and Trg-Q79L were fitted to the data by adjusting K_2, the association constant of Trg and occupied binding protein (dotted line), or p, the proportionality constant (solid line). Strains and symbols as for Figure 1.

by ligand binding not in the affinity of receptor for ligand. For attractants recognized by receptors in two steps via binding proteins, reduced efficiency of signalling by the occupied receptor would also have its principal effect on the magnitude of the maximal response, but involvement of an intermediate component in recognition can, under specific conditions, introduce an alternative origin for the reduced responses shown in Figure 2. The crucial factor is the total amount of binding protein in the periplasm. This determines the maximal possible concentration of sugar-occupied binding protein and thus could limit the fraction of receptor sites occupied if the maximal concentration were lower than that necessary to saturate the receptor. This appears to be the case for Trg and the two sugar binding proteins it recognizes. As described in Materials and methods, estimates for periplasmic concentrations of galactose- and ribose-binding proteins in appropriately induced cells are 230 and 410 μM, respectively. We used a model for indirect recognition (see Materials and methods) to calculate apparent dissociation constants of 530 and 420 μM for the complex of Trg with the respective sugar-occupied binding proteins, values in the same range as an estimate for the dissociation constant of occupied maltose-binding protein and Tar (Manson *et al.*, 1985). Although these values are only rough estimates, it seems clear that binding protein-mediated responses would be limited by the amount of binding protein. Could these limitations explain the characteristic difference in maximal response to galactose and ribose? We addressed the possibility by using our model for two-step recognition and previously determined values for the sugar affinity and cellular content of the binding proteins to generate predicted dose–response curves. When we assumed the same efficiency of signalling for both sugar-occupied binding proteins, the predicted responses (Figure 2A, ribose curve

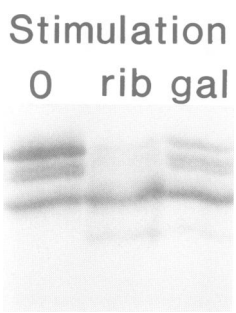

Fig. 3. Signalling by wild-type Trg in response to a saturating stimulus of ribose or galactose. Methylation state was used to assay signalling *in vivo*. The figure is an immunoblot using anti-Trg serum of a strain lacking active chromosomal transducer genes, but harboring a plasmid carrying a wild-type copy of *trg*. Only the region including Trg is shown; the lowest band in the left-hand lane has an apparent M_r of 60 000. Cells were removed from actively growing cultures in tryptone broth with no added sugar (0), with saturating amounts of ribose (rib) or of galactose (gal).

and dashed galactose curve) corresponded quite well with the experimental data. A relatively minor 12% reduction in signalling efficiency for the galactose protein (solid line galactose curve) made that correspondence precise. Thus the particular features of limited amounts of binding proteins can create differential magnitudes of responses that correspond to those we observed experimentally, implying no need to consider the possibility of significantly different signalling efficiencies. However, the quantitative correspondence may be misleading. If the value used for the dissociation constant of ribose from the occupied binding protein is reduced by 20%, a change within the error of the measurement, the difference between plateau values for the two dose–response curves is reduced to one-third of the difference shown in Figure 2A, and thus the theoretical curves would no longer correspond to the experimental difference. An alternative approach to investigating differential responses is mutational analysis.

Mutational analysis of differential responses

To what degree are components of binding and signalling in Trg shared by the two protein ligands and to what degree are they independent? We addressed this issue by mutational analysis of a segment of Trg implicated in ligand interaction and intramolecular signalling. Residues 69–88 of Trg span a segment of the periplasmic domain that includes a cluster of positions in the aligned sequences of Trg, Tar and Tsr, at which mutational substitutions affect ligand interaction specifically (Park and Hazelbauer, 1986; Kossmann *et al.*, 1988; Lee *et al.*, 1988; Wolff and Parkinson, 1988; Lee and Imae, 1990; Mowbray and Koshland, 1990; Gardina *et al.*, 1992). We used a mixture of mutagenic oligonucleotides corresponding to codons 69–88, containing on average one randomly placed nucleotide change per molecule to create a collection of altered Trg proteins. Analysis of the signalling properties of these substituted receptors (Yaghmai and Hazelbauer, 1992) revealed that the region spanning residues 71–79 had properties of a signalling segment, since specific single amino acid substitutions in that region caused a persistent transmembrane signal that mimicked the effect of ligand occupancy, presumably by inducing or favoring the conformational change usually generated by ligand binding; while the segment from residue 79–87 was found to be important for productive ligand interaction and linkage to the signalling segment. We investigated whether amino acid substitutions in residues 69–88 affected responses to galactose and ribose equivalently or differentially. Semi-solid agar swarm plates were used to assay the ability of the altered proteins to mediate taxis towards each of the sugars (Figure 4). Wild-type Trg mediates formation of a sharp ring that moves from the point of an inoculation with a characteristic rate. Defective Trg proteins result in rings that are less sharp and/or move more slowly. Experience with many *trg* mutations allows us to conclude that this assay is a sensitive and reliable way to characterize defects in Trg. The patterns shown in Figure 4 are representative examples of reproducible differences between wild-type and mutant strains. Tactic ability was assayed for strains in which a mutated *trg* had been introduced into a chromosome deleted of wild-type *trg*, but carrying normal copies of all other transducer and chemotaxis genes. Among substituted proteins that were not substantially altered in stability, 17 were less efficient than the wild-type protein in mediating the formation of chemotactic rings in response to gradients of galactose or ribose. For seven of the defective receptors, responses to the two sugars, as judged by the size and sharpness of the rings in comparison to those formed through the action of wild-type Trg, were affected to approximately the same extent and those responses ranged from weak to undetectable. Ten receptors were differentially defective in mediating taxis to the two sugars, four more defective in galactose taxis and six more defective in ribose taxis. For one (Trg-G81W) of the former class and three (Trg-A82C, Trg-R85L and Trg-E88K) of the latter class the differential nature of the defects was relatively modest, but introduction into three of those receptors (Trg-G81W, Trg-A82C and Trg-E88K) of a second amino acid substitution, which by itself had little effect on function (R.Yaghmai and G.L.Hazelbauer, in preparation), accentuated differences between galactose and ribose responses (Figure 4). There was a striking segregation between the two classes of mutations that caused differential defects. Those more defective in response to galactose were distributed from positions 74–81 and those more defective in response to ribose were distributed from residues 82–88 (Table I). The first grouping corresponds in large part to the signalling segment identified in the study discussed above (Yaghmai and Hazelbauer, 1992), while the latter set is located in a region important for ligand interaction and linkage to the signalling segment. The significance of this distribution will be considered in the Discussion.

We selected a representative from each of the differentially defective classes for a detailed analysis of dose–response characteristics. For the class in which galactose response was more strongly affected, Trg-Q79L was chosen as a stable protein exhibiting an intermediate level of defect; for the class in which ribose response was more defective, Trg-A82C was selected as a stable protein with a substitution at a different position than the previously characterized Trg-R85H (Park and Hazelbauer, 1986). Although the defect in Trg-A82C was modest, as assayed by formation of chemotactic rings by cells with a single, chromosomal copy of the gene, other data, including the phenotype of cells carrying the altered gene on a multicopy plasmid and the phenotype of cells with a single copy of *trg-A82C-S84I*, indicated that the altered protein had a significantly

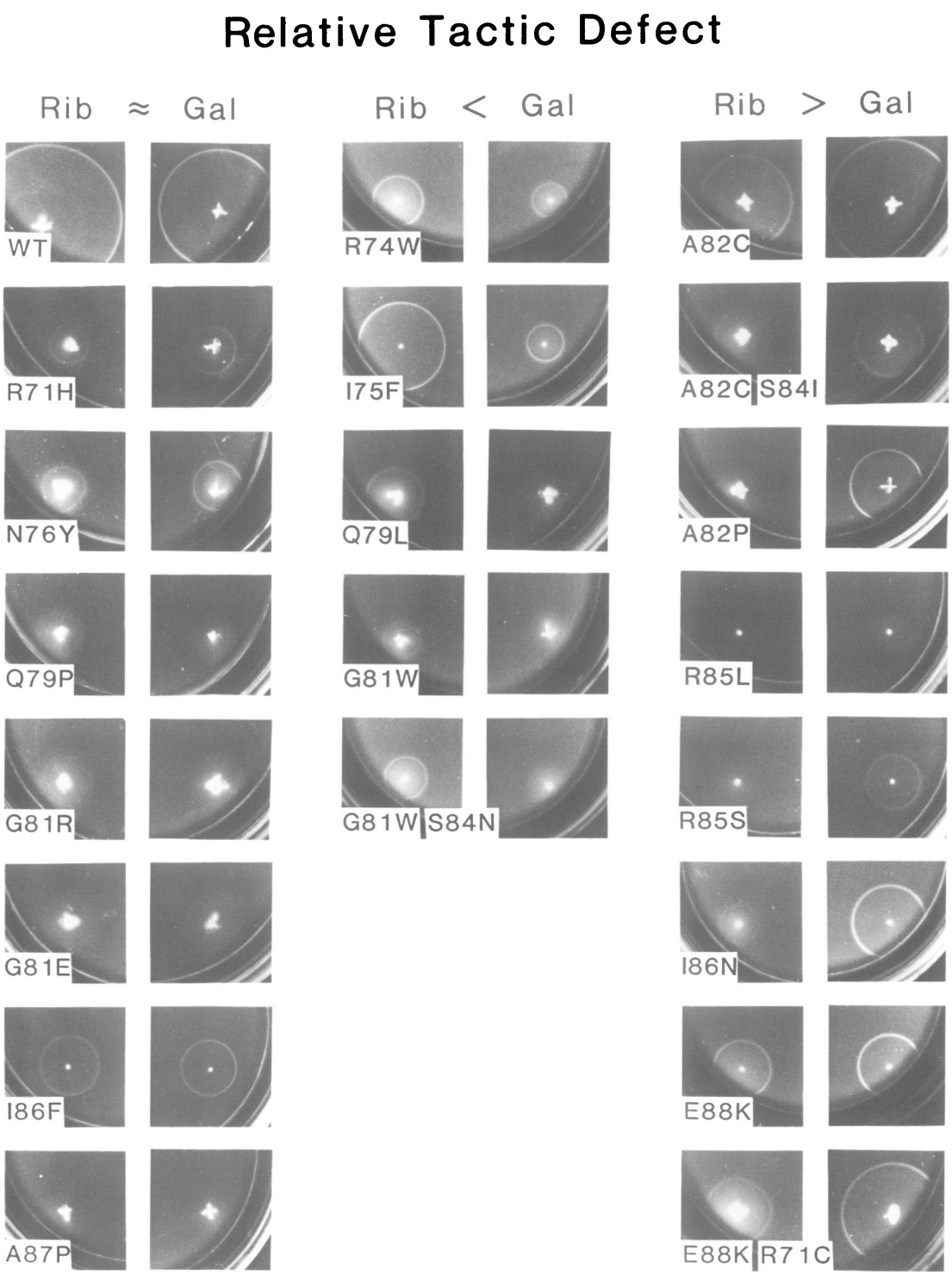

Fig. 4. Chemotaxis mediated by wild-type and altered Trg proteins. Motile, growing cells carrying a single chromosomal copy of *trg* in the *lac* region and normal chromosomal genes for all other chemotaxis components were inoculated into semisolid agar plates containing minimal salts, required amino acids and a limiting amount of ribose (0.1 mM) or galactose (0.05 mM) and incubated at 35°C for 17 h. The strains are designated by the Trg protein produced, wild-type (WT) or with an amino acid substitution(s), e.g. Trg-R74W (R74W) or Trg-G81W-S84N (G81W S84N), and grouped according to the relative degree of perturbation of tactic response to the two sugars: approximately equal effect on responses to galactose (rib ≅ gal), a greater effect on response to galactose (rib < gal) or vice versa (rib > gal). The differences in ring size and sharpness were reproducible in repeated analysis.

differential defect. Tactic function was assessed first by the semi-quantitative capillary assay using cells containing a chromosomal copy of the mutated *trg* gene and a normal complement of all other taxis-related genes. As shown in Figure 1, both altered receptors were less efficient than the normal protein at mediating taxis to either galactose or ribose. Although the two mutants exhibited only marginally different responses to the two sugars, the slightly greater

Table I.

Residue	% Solvent accessibility[a]	Secondary structure[a]	Substitution	Signalling phenotype[b]	Tactic defect[c]
R71	0	helix	H	occupancy mimic	r ≅ g
R74	2	helix	W	wild-type	r < g
I75	1	helix	F	wild-type	r < g
N76	0	helix	Y	occupancy mimic	r ≅ g
Q79	10	helix	L	occupancy mimic	r < g
			P	insensitive	r ≅ g
G81	0	helix	E	insensitive	r ≅ g
			R	insensitive	r ≅ g
			W	insensitive	(r < g)*
A82	0	helix	C	insensitive	(r > g)*
			P	insensitive	r > g
R85	20	helix	L	insensitive	(r > g)
			S	insensitive	r > g
I86	36	loop	F	insensitive	r ≅ g
			N	insensitive	r > g
A87	56	loop	P	insensitive	r ≅ g
E88	50	loop	K	wild-type	(r > g)*

[a]Information for the comparable position in Tar_s (Milburn *et al.*, 1991). Solvent accessibility data calculated by S.-H.Kim (personal communication) for the ligand-bound form of Tar_s using the method of Lee and Richards (1971).
[b]Data from Yaghmai and Hazelbauer (1992; in preparation).
[c]Data from Figure 4. Parentheses indicate a modest difference in the extent of defect; an asterisk indicates that introduction of a second, neutral change accentuates this difference. See Figure 4 and text.

response of Trg-Q79L to ribose and the slightly greater response of Trg-A82C to galactose were consistent with the pattern observed in formation of tactic rings. Assay of response to temporal stimulation by tethered cells (Figure 2B and C) provided clear documentation of the differential nature of the functional defects in Trg-Q79L and Trg-A82C. As observed in the capillary assay, both altered proteins exhibited a reduced effectiveness in relation to the wild-type receptor. In addition, the mutational substitutions had differential effects on sensory responses to gradients of the two sugars. Trg-Q79L exaggerated the wild-type differential between response to ribose and galactose. The single amino acid substitution in Trg-A82C reversed the rank order of the differential response. For this altered receptor, galactose elicited a stronger response than ribose. The principal change engendered by both alterations was in the magnitude of response; there appeared to be little effect on the concentration required for a half-maximal response. We used two approaches to fit our dose–response equation to data for the mutant strains. In the first approach, we assumed that the mutational substitution altered the affinity of the receptor for occupied binding protein and thus varied that parameter while holding all other constants. In the second approach, we assumed that the substitutions altered the efficiency of signalling and thus varied the proportionality constant. Curves generated by altered affinity (dashed lines in Figure 2B and C) or by altered signalling efficiency (solid lines in Figure 2B and C) provided equally good fits to the data.

Discussion

Strategies for differential response

The observations documented here show that cells can respond differentially to two stimulants even though response to both is mediated by the same transmembrane receptor. There is a stronger chemotactic response by *E.coli* to ribose than to galactose and this preference can be reversed or accentuated by single amino acid changes in the Trg receptor. A notable feature is that the preferences are expressed as differences in the magnitude of maximal responses. What is the origin of such differential responses? An important factor is that the two sugars are not recognized directly by the receptor, but instead indirectly as the result of interaction of the respective sugar-occupied binding proteins with the receptor. Yet differential response does not involve completely independent binding sites on Trg for the two polypeptide ligands, since previous work showed that those sites overlap functionally and this work showed that they overlapped structurally. A saturating level of one Trg-linked attractant reduces response to the other and vice versa (Adler *et al.*, 1973; Strange and Koshland, 1976; Park and Hazelbauer, 1986), in contrast to the minimal interference between the two Tar ligands, aspartate and maltose-binding protein (Mowbray and Koshland, 1987). Several single amino acid substitutions described in this study have drastic effects on chemotaxis to both galactose and ribose without generally disrupting receptor structure. The two sites appear to be in very similar positions on the surface of the receptor, but are distinguished by specific side chain interactions. Our analysis identified two ways in which a receptor with a single site for two protein ligands can mediate distinctly different magnitudes of maximal responses to two compounds bound by the proteins. One involves a balance between available binding protein and receptor affinity for the occupied binding protein, such that maximal response can be differentially limited. The other involves differential efficiencies of signalling generated by the interaction of two ligand-occupied proteins. As discussed in Results, the former provides the simplest explanation of differential responses by wild-type cells. Mutational substitutions that accentuate or reverse the differential response of wild-type cells could affect receptor affinity or signalling. Curve fitting cannot distinguish the two possibilities (Figure 2B and C), but interpretation in light of the recently determined structure of the periplasmic domain of the chemoreceptor Tar_s provides insight.

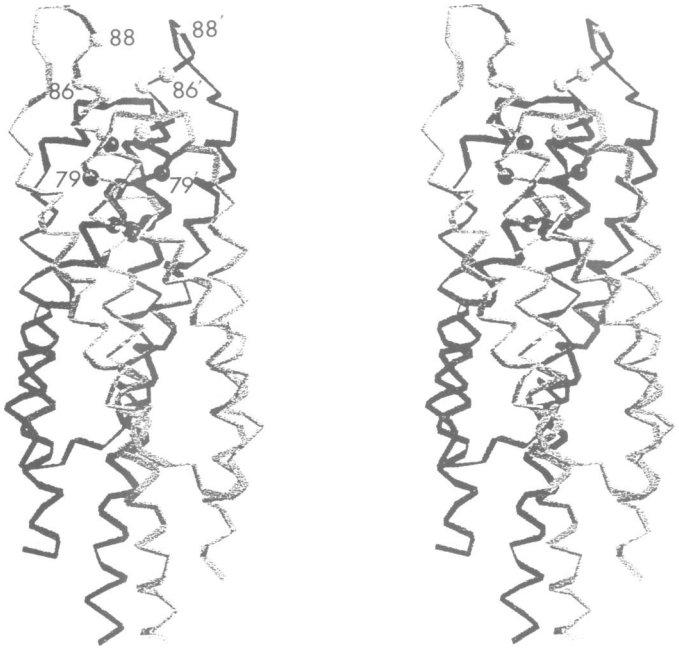

Fig. 5. Stereo view of the C_α structure of the ligand-binding domain of Tar_s. The dimer of two four-helix bundles described in Milburn *et al.* (1991) is shown with the membrane-distal end at the top. The two subunits are distinguished by different shading. The amino termini of the subunits, corresponding to the beginnings of helices 1 and 1′, are at the bottom center and the carboxyl termini, corresponding to the ends of helices 4 and 4′, are at the bottom on each side. For clarity the first seven residues of each subunit have been left out since their positions in the crystal are thought to be artifactual. C_α positions in helices 1 and 1′ and the adjoining loop that correspond to substitutions in Trg that affect galactose response more than ribose response (R74, I75, Q79 and G81) are marked with dark balls, those corresponding to substitutions that affect ribose response more than galactose response (A82, R85, I86 and E88) are marked with light balls and selected positions are labeled with residue number.

Differential mutational defects and the three-dimensional structure of the ligand-binding domain

The three-dimensional structure of the ligand-binding response of Trg can be modeled using the known structure of the corresponding domain of Tar_s (Milburn *et al.*, 1991) and alignment of chemoreceptor sequences (Hazelbauer, 1988). The striking correspondence of positions in Tar and Trg at which mutational substitutions disrupt interaction with binding proteins (Gardina *et al.*, 1982; Yaghmai and Hazelbauer, 1992) provides confidence in the validity of this modeling, particularly in the ligand interaction region relevant to the studies presented here. The alpha-carbon structure of the ligand binding domain of Tar_s (Milburn *et al.*, 1991) is shown in Figure 5 with indications of positions at which mutational substitutions in Trg have differential effects on responses to galactose and ribose. The secondary structure and percent solvent accessibility for Tar_s positions corresponding to Trg substitutions considered in this study are listed in Table I. The table also summarizes the relative effect of each substitution on response to galactose and ribose as documented in Figure 4 and the signalling phenotype as determined previously (Yaghmai and Hazelbauer, 1992). In that study we found that specific substitutions at residues 79, 81, 82 and 85−87 reduced the ability of Trg to signal upon stimulation by ribose (the attractant used in those studies), creating 'insensitive' receptors and that specific substitutions in Trg at residues 71, 72 and 75−79 induced transmembrane signalling in the absence of stimulation and thus mimicked ligand occupancy.

Substitutions in Trg that have a greater effect on response to galactose than to ribose are all at positions buried at the subunit interface created by packing of helices 1 and 1′ (Table I and Figure 5). This makes it unlikely that these side chains interact directly with polypeptide ligands and thus substitutions at those positions could reduce receptor affinity only by a general structural disruption. For two protein ligands binding to the same receptor surface it would be relatively difficult to create differential changes in affinity by such general disruption. Instead the alterations are more likely to reduce the efficiency with which ligand binding initiates intramolecular signalling, consistent with the suggested role for this region in signalling (Milburn *et al.*, 1991; Yaghmai and Hazelbauer, 1992). How could an alteration in a signalling region have a greater effect on signals generated by one of the two ligands? The signalling pathways for the two stimuli might be sufficiently distinct to be differentially affected by specific substitutions. Alternatively, the pathways might be essentially the same, but because maximal occupancy by galactose-binding protein is more limited than for ribose-binding protein, at any given proportion of the limited maximal occupancies galactose signals would be weaker. Substitutions that made signalling more difficult could have a proportionately greater effect on response to galactose than to ribose. Our mutational analysis indicates substantial, if not complete, overlap in the signalling pathways. Substitutions with greater effect on galactose than ribose taxis still reduce response to both attractants, albeit unequally. Other substitutions in the signalling region, at the same or nearby positions, have equal and often substantial effects on response to both attractants. Thus we favor the notion that occupancy by either ligand initiates essentially the same signal. Substitutions that increase the energy barrier for the signalling conformational change or reduce the extent of the change by altering the initial or final state could reduce the weaker galactose signal proportionately more than the stronger ribose signal.

Substitutions in Trg that have a greater effect on ribose than on galactose response occur in the final, membrane-distal turn of helix 1 and in the loop connecting helices 1 and 2. At three of the four comparable positions in Tar_s, the residue side chains are substantially exposed to solvent (Table I and Figure 5) and thus would be available for physical interaction with ligand. The position corresponding to A82, three residues from the carboxy-terminus of helix 1, is completely buried in Tar_s even though the following position (83) is substantially solvent-accessible (22%). Position 82 in Trg may be more exposed than the comparable residue in Tar_s, since in Trg-A82C the cysteine exhibited rapid and complete intersubunit, oxidative crosslinking upon addition of oxidant, but no crosslinking without addition (Burrows, 1991) and significant reactivity to sulfhydryl reagents *in vivo* (Yaghmai, 1991). Alternatively, the side chain at Trg position 82 may be buried and thus not involved directly in binding, but instead be particularly important in providing a structural coupling between the nearby binding residues and the subunit interface where the signalling conformational change is initiated. In any case, the four positions where substitutions reduce ribose taxis more than galactose taxis are probably involved directly or indirectly in physical interaction with ligand, contributing substantially to receptor affinity for ribose-binding protein and less significantly to affinity for galactose-binding protein. Thus interpretations based on the three-dimensional structure of the ligand-binding domain suggest that the mutational substitutions that reduce ribose response relative to galactose response have differential effects on affinities for the two ligands while substitutions that reduce galactose response relative to ribose response have effects on signalling.

Differential response based on features of two-step recognition

The receptor Trg mediates differential responses to the two chemoattractants galactose and ribose. Our data indicate that it does so without utilizing two independent binding sites for the corresponding sugar-occupied binding proteins. Instead it appears that response magnitude differs, even though concentration dependence is quite similar, as a result of characteristic features introduced by two-step recognition of the two sugars. The limiting effect on maximal response of having a binding protein concentration in the same range as the dissociation constant of the complex of receptor and occupied binding protein has been noted in studies of maltose-binding protein and Tar (Manson *et al.*, 1985; Mowbray and Koshland, 1987). Our study of differential responses mediated through a common receptor site introduces the notion that this limitation could have functionally useful consequences, specifically an uncoupling of dose dependency from receptor occupancy. In the situation studied here, the relatively high affinity of binding protein for sugar and low affinity of receptor for occupied binding protein means that an alteration of the affinity of receptor for occupied binding protein affects response only minimally in its concentration dependence, but substantially in its magnitude. Thus preferences of one attractant versus another could be changed without necessarily reducing the ability to detect the less preferred.

In *E.coli*, of the six recognition sites that bind chemoattractants, four occur on soluble binding proteins, rather than directly on the transmembrane receptors. It is not clear whether this high frequency of indirect recognition indicates a functional advantage for the arrangement. The possibility of altering response magnitude independent of sensitivity of detection could be such an advantage. It will be interesting to see whether two-step recognition is utilized similarly in other receptor systems.

Materials and methods

Strains and plasmids

CP362 and CP593 are both derivatives of *E.coli* K12 strain OW1 (Ordal and Adler, 1974). CP362 is Δ(*tsr-7028*), Δ(*tar-tap)5201*, Δ(*trg-100*) and thus has no active transducer genes (Park and Hazelbauer, 1986). CP593 is Δ(*trg-100*) Lac^+ and was derived from CP177 (Park and Hazelbauer, 1986) by P1 transduction from a Lac^+ strain. pMG2 is a pUC13 derivative containing *trg* under the control of its native promoter (Nowlin *et al.*, 1988). Generation and identification of mutations in *trg* codons 69–88 has been described in Yaghmai and Hazelbauer (1992). Strains with single copies of mutated *trg* genes integrated into the *lac* region were created by selecting for integration of pUC13-*trg* hybrids into *lac* in a temperature-sensitive *polA* strain, transducing the integrated plasmid to CP593 and screening for resolution and loss of the plasmid (Park and Hazelbauer, 1986).

Chemotaxis assays

Assays of strains with a single copy of *trg* integrated into the *lac* region were performed as described in the following references: semisolid agar swarm plates (Hazelbauer *et al.*, 1989); capillary assay and tethered cell assay (Park and Hazelbauer, 1976). The immunoblot assay was performed with CP362/pMG2 grown in tryptone broth at 35°C in the absence or presence of 0.2% sugar (13 mM ribose or 11 mM galactose). At a density of $\sim 2.5 \times 10^8$ cells/ml (in the midst of logarithmic growth), a sample of 2.5×10^7 cells was added to trichloroacetic acid (10% final concentration). Precipitated material was separated by polyacrylamide (11%, 0.073% *bis* acrylamide) gel electrophoresis (Hazelbauer and Harayama, 1979), electroblotted onto nitrocellulose, treated with anti-Trg serum and a peroxidase-coupled secondary antibody (Bio-Rad, Richmond, CA), and incubated with hydrogen peroxide and 4-chloro-1-naphthol.

A mathematical model of dose–response relationships for indirectly recognized chemoattractants

Response of individual cells to temporal step gradients of galactose or ribose involves a two-step recognition process: (i) binding of sugar to a binding protein and (ii) association of the sugar-occupied binding protein with Trg. We modeled the process assuming that the binding steps were independent and Trg did not bind unoccupied binding protein. Thus:

$$S + B + T \overset{K_1}{\rightleftharpoons} SB + T \overset{K_2}{\rightleftharpoons} SBT$$

where S = sugar, B = binding protein, T = transducer (receptor) and K_1 and K_2 are association constants. As shown for directly recognized chemoattractants (Berg and Tedesco, 1975), response (R) is considered to be proportional to the fraction of receptor sites occupied (F_0). The proportionality constant (p) relates the fraction occupied to response time (R) in seconds. Variation in this constant is equivalent to the variation in efficiency of signalling. Response is expressed as follows:

$$R = pF_0 = p\,\frac{K_1 K_2 [B_T] [S]}{1 + K_1[S] (1 + K_2[B_T])}$$

where $[B_T]$ = total concentration in the periplasm of binding protein and $[S]$ = concentration of sugar added to tethered cells. For response of wild-type cells to galactose, the values $K_1 = 7 \times 10^6\ M^{-1}$, $K_2 = 1.9 \times 10^3\ M^{-1}$ and $B_T = 2.3 \times 10^{-4}$ M were derived, respectively, from a published dissociation constant (Miller *et al.*, 1980), a calculation using the relationship $K_R = K_1 (1 + K_2 B_T)$ where K_R is the inverse of the concentration of stimulating sugar at which response is half-maximal (Figure 2) and values for periplasmic content of the binding protein (Koman *et al.*, 1979) and the volume (0.15 fl) of the periplasm (Hazelbauer, 1979). For response of wild-type cells to ribose, the values $K_1 = 1.7 \times 10^6\ M^{-1}$, $K_2 = 2.4 \times 10^3\ M^{-1}$ and $B_T = 4.1 \times 10^{-4}$ M were derived as for galactose except that K_1 was an average determined in the course of a previous study (Koman *et al.*, 1979). A value for the proportionality constant 'p' was calculated using the maximal value of R for ribose (73 s) and F_0 at saturating ribose.

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References

Adler,J. (1973) *J. Gen. Microbiol.*, **74**, 77–91.
Adler,J., Hazelbauer,G.L. and Dahl,M.M. (1973) *J. Bacteriol.*, **115**, 824–847.
Berg,H.C. and Tedesco,P.M. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 3235–3239.
Bourret,R.B., Borkovich,K.A. and Simon,M.I. (1991) *Annu. Rev. Biochem.*, **60**, 401–441.
Burrows,G.G. (1991) Ph.D. thesis, Washington State University, WA, USA.
Hazelbauer,G.L. (1979) In Inouye,M. (ed.), *Bacterial Outer Membranes: Biogenesis and Function*. John Wiley and Sons Inc., New York, pp. 449–473.
Hazelbauer,G.L. (1988) *Can. J. Microbiol.*, **34**, 466–474.
Hazelbauer,G.L. and Adler,J. (1971) *Nature*, **230**, 101–104.
Hazelbauer,G.L. and Harayama,S. (1979) *Cell*, **16**, 617–625.
Hazelbauer,G.L., Park,C. and Nowlin,D.M. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 1448–1452.
Hazelbauer,G.L., Yaghmai,Y., Burrows,G.G., Baumgartner,J.W., Dutton,D.P. and Morgan,D.G. (1990) In Armitage,J.P. and Lackie,J.M. (eds), *Biology of the Chemotactic Response*. Cambridge University Press, Cambridge, UK, pp. 107–134.
Koman,A., Harayama,S. and Hazelbauer,G.L. (1979) *J. Bacteriol.*, **138**, 824–847.
Kossmann,M., Wolff,C. and Manson,M.D. (1988) *J. Bacteriol.*, **170**, 4516–4521.
Lee,B.K. and Richards,F.M. (1971) *J. Mol. Biol.*, **55**, 379–400.
Lee,L. and Imae,Y. (1990) *J. Bacteriol.*, **172**, 377–382.
Lee,L., Mizuno,T. and Imae,Y. (1988) *J. Bacteriol.*, **170**, 4769–4774.
Lynch,B.A. and Koshland,D.E., Jr (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 10402–10406.
Manson,M.D., Boos,W., Bassford,P.J., Jr and Rasmussen,B.A. (1985) *J. Biol. Chem.*, **260**, 9727–9733.
Milburn,M.V., Privé,G.G., Milligan,D.L., Scott,W.G., Yeh,J., Jancarik,J., Koshland,D.E., Jr and Kim,S.-H. (1991) *Science*, **254**, 1342–1347.
Miller,D.M., III, Olson,J.S. and Quiocho,F.A. (1980) *J. Biol. Chem.*, **255**, 2415–2471.
Milligan,D.L. and Koshland,D.E., Jr (1988) *J. Biol. Chem.*, **263**, 6268–6275.
Mowbray,S.L. and Koshland,D.E., Jr (1987) *Cell*, **50**, 171–180.
Mowbray,S.L. and Koshland,D.E., Jr (1990) *J. Biol. Chem.*, **265**, 15638–15643.
Mowbray,S.L., Foster,D.L. and Koshland,D.E., Jr (1985) *J. Biol. Chem.*, **260**, 11711–11718.
Nowlin,D.M., Bollinger,J. and Hazelbauer,G.L. (1988) *Proteins*, **3**, 102–112.
Ordal,G.W. and Adler,J. (1974) *J. Bacteriol.*, **117**, 509–516.
Pakula,A.A. and Simon,M.I. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 4144–4148.
Park,C. and Hazelbauer,G.L. (1986) *J. Bacteriol.*, **167**, 101–109.
Strange,P.G. and Koshland,D.E., Jr (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 762–766.
Stoddard,B.L. and Koshland,D.E., Jr (1992) *Nature*, **258**, 774–776.
Wolff,C. and Parkinson,J.S. (1988) *J. Bacteriol.*, **170**, 4509–4515.
Yaghmai,R. (1991) Ph.D. thesis, Washington State University, WA, USA.
Yaghmai,R. and Hazelbauer,G.L. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 7890–7894.

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