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Phenol Sensing by *Escherichia coli* Chemoreceptors: a Nonclassical Mechanism▽

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The four transmembrane chemoreceptors of *Escherichia coli* sense phenol as either an attractant (Tar) or a repellent (Tap, Trg, and Tsr). In this study, we investigated the Tar determinants that mediate its attractant response to phenol and the Tsr determinants that mediate its repellent response to phenol. Tar molecules with lesions in the aspartate-binding pocket of the periplasmic domain, with a foreign periplasmic domain (from Tsr or from several *Pseudomonas* chemoreceptors), or lacking nearly the entire periplasmic domain still mediated attractant responses to phenol. Similarly, Tar molecules with the cytoplasmic methylation and kinase control domains of Tsr still sensed phenol as an attractant. Additional hybrid receptors with signaling elements from both Tar and Tsr indicated that the transmembrane (TM) helices and HAMP domain determined the sign of the phenol-sensing response. Several amino acid replacements in the HAMP domain of Tsr, particularly attractant-mimic signaling lesions at residue E248, converted Tsr to an attractant sensor of phenol. These findings suggest that phenol may elicit chemotactic responses by diffusing into the cytoplasmic membrane and perturbing the structural stability or position of the TM bundle helices, in conjunction with structural input from the HAMP domain. We conclude that behavioral responses to phenol, and perhaps to temperature, cytoplasmic pH, and glycerol, as well, occur through a general sensing mechanism in chemoreceptors that detects changes in the structural stability or dynamic behavior of a receptor signaling element. The structurally sensitive target for phenol is probably the TM bundle, but other behaviors could target other receptor elements.

Chemotaxis, the movement of an organism toward or away from chemicals, is an important adaptive behavior of motile prokaryotes, such as *Escherichia coli*. Both bacteria and archaea employ chemoreceptors known as methyl-accepting chemotaxis proteins (MCPs) to monitor their chemical environments (3, 61). *E. coli* has four canonical MCPs: Tsr mediates attractant responses to serine and to the interspecies quorum signal, AI-2 (19, 47); Tar mediates attractant responses to aspartate and to maltose (17, 47); Tap mediates attractant responses to dipeptides and to pyrimidines (28, 31); and Trg mediates attractant responses to ribose and galactose (16). These transmembrane receptors function as homodimers of ~550-residue subunits, each comprising a periplasmic sensing domain, flanked by two membrane-spanning helices (TM1 and TM2), and a cytoplasmic kinase control domain (Fig. 1). The cytoplasmic region of MCPs consists of a signal-converting HAMP domain at the membrane interface and a long coiled-coil four-helix bundle (Fig. 1). The highly conserved membrane-distal tip of the cytoplasmic bundle contains residues that promote interactions with other MCP molecules, with the signaling kinase CheA, and with CheW, which couples CheA autophosphorylation activity to receptor control (4, 23, 50). CheA, in turn, regulates the phosphorylation state of CheY, which interacts with the switching machinery of the flagellar motors to control their direction of rotation. Low kinase activity allows counterclockwise (CCW) rotation, the default state, which drives forward swimming. High CheA activity leads to increased levels of phospho-CheY, which augments clockwise (CW) rotation of the motors, triggering tumbling motions that randomize the cell's heading.

As they swim about, *E. coli* cells track spatial gradients of attractant and repellent chemicals by modulating their tumble probability in response to temporal changes in chemoeffector levels (10, 29, 44). Ligand occupancy changes of the periplasmic domain promote conformational changes that modulate the structure or stability of the HAMP domain (42). HAMP, in turn, modulates the packing interactions between the helices of the kinase control bundle to regulate CheA activity (18). Temporal sensing depends on a sensory adaptation system that adjusts signal output through covalent changes to the receptor molecules. CheR, an MCP-specific methyltransferase, attaches methyl groups to several glutamic acid residues in the cytoplasmic domain (48); CheB, a methylesterase, opposes CheR action by hydrolyzing those glutamyl-methyl esters (49). The relative activities of these two enzymes regulate the steady-state level of receptor modifications. The conformational changes elicited by attractant stimuli make MCPs more favorable substrates for CheR; those elicited by repellent stimuli make MCPs better substrates for CheB. The ensuing methylation changes offset or cancel the stimulus signals, restoring receptor output to the prestimulus state. Thus, MCPs monitor temporal changes in chemoeffector levels by comparing current ligand occupancy with that in the recent past, recorded in the form of the receptor methylation state.

E. coli MCPs also mediate locomotor responses to changes

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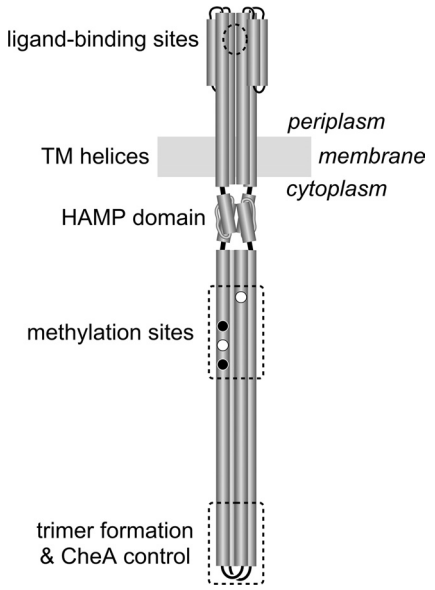

FIG. 1. Functional architecture of Tar and Tsr receptors. These molecules function as homodimers of ~550-residue subunits. The cylinders represent segments with α-helical secondary structure, drawn approximately to scale. The circles represent the four principal methylation sites in each subunit. The black circles denote glutamine residues that must first be deamidated by CheB before methylation; the white circles denote glutamic acid residues that undergo reversible CheR-mediated methylation. The methylation sites and kinase (CheA) control region operate as dynamic 4-helix bundles comprised of two interacting, antiparallel coiled coils.

in temperature (30), cytoplasmic pH (22, 43), and glycerol (39). The detection mechanisms for these stimuli are not well understood, but they probably target common elements of MCP molecules, because they are not specific to one class of receptor. Tar and Tsr, the most abundant MCP molecules in *E. coli*, both sense internal pH, glycerol, and temperature, although the signs of their individual signaling responses may be different. For example, Tar mediates an attractant response to a drop in internal pH, whereas Tsr mediates a repellent response to the same stimulus (25). The principal determinants for these opposing pH responses are several residues that differ in Tar and Tsr at the output end of the HAMP domain, adjoining the methylation helices (53).

All four of the orthodox MCPs in *E. coli* also sense the aromatic compound phenol, which is neither metabolized nor degraded by *E. coli*. Tar senses phenol as an attractant, whereas Tsr, Tap, and Trg sense phenol as a repellent (21, 56). The cell's overall behavioral response depends on the relative numbers of attractant-sensing Tar molecules and the other, repellent-sensing MCPs (21, 56). Despite different behavioral outputs, *E. coli* MCPs might have a common phenol detection mechanism. Those phenol-sensing determinants have not been identified.

Phenol and phenolic compounds are common industrial waste products and environmental pollutants, particularly in water sources (14, 33). Among methods for removing phenol from polluted sites, bioremediation approaches based on microbial metabolism are generally more efficient and more cost-effective than physicochemical methods (40). Moreover, chemotactic behavior could attract bacterial cells to pollutants, such as phenolic compounds, thereby enhancing bioremediation efficiency (40). For example, a chemotaxis-based approach that exploits ligand-induced changes in cell motility to create bacterial cells that seek and degrade pollutants has been proposed recently (46). Thus, understanding the mechanism of phenol sensing in chemotaxis could lead to useful new approaches for improving the biodegradation of this recalcitrant compound and its derivatives.

In this study, we investigated the mechanism of phenol sensing by the Tar and Tsr chemoreceptors of *E. coli*. We first devised a simple plate assay for phenol chemotaxis and then used it to assess phenol responses mediated by a variety of mutant Tar, Tsr, and hybrid receptors. Our findings suggest that MCPs detect phenol by a novel mechanism that does not involve a conventional ligand-binding site.

MATERIALS AND METHODS

Bacterial strains. All strains were derivatives of *E. coli* K-12 strain RP437 and contained the following markers relevant to this study: UU1250 [Δ*aer-1* Δ*tsr-7028* Δ(*tar-tap*)*5201* Δ*trg-100*] (5); UU1535 [Δ*aer-1* Δ(*tar-cheB*)*2234* Δ*tsr-7028* Δ*trg-100*] (7); UU1936 [*mutD5 zae*::Tn*10-13* Δ*aer-1* Δ*tsr-7028* Δ(*tar-tap*)*5201* Δ*trg-100*] (P. Ames and J. S. Parkinson, unpublished data).

Plasmids. The plasmids used in this work were pKG116, a derivative of pACYC184 (12) and pKMY297 (57) that confers chloramphenicol resistance and has a sodium salicylate-inducible expression/cloning site (11); pLC113, a relative of pKG116 that carries wild-type *tar* under salicylate control (5); pPA790, a derivative of pLC113 that encodes a Tar protein (Tar°) lacking residues 44 to 183 of the Tar periplasmic domain (15); pRR48, a derivative of pBR322 (9) that confers ampicillin resistance and has an expression/cloning site with a *tac* promoter and an ideal (perfectly palindromic) *lac* operator under the control of a plasmid-encoded *lacI* repressor, inducible by isopropyl-β-D-thiogalactopyranoside (IPTG) (51); and pRR53, a derivative of pRR48 that carries wild-type *tsr* under IPTG control (51).

The parental plasmids constructed for this work were pHP1, a derivative of pRR48 that carries a functional *tar* gene with a third-position change at codon 256 that removes an NdeI restriction site; pHP2, a derivative of pHP1 with a functional *tar* gene in which valine codons 200 to 202 were converted to alanine codons, creating a unique NotI restriction site; and pHP4, a derivative of pRR53 with a functional *tsr* gene in which codons 202 to 204 (corresponding to *tar* codons 200 to 202) were converted to alanine codons to create a unique NotI restriction site.

Plasmid mutagenesis. Site-directed plasmid mutations were generated by QuikChange PCR mutagenesis, as described previously (5). For all-codon mutagenesis, we used oligonucleotide primer pools that were fully degenerate, i.e., had equal frequencies of the four bases (A, C, G, and T) at all three base positions of the targeted codon. Candidate mutations were verified by sequencing the entire coding region of the targeted gene in the mutant plasmid. Random plasmid mutations were induced by propagating plasmids in the *mutD* strain UU1936, as described previously (27).

Construction of plasmids encoding hybrid receptors. To swap the periplasmic and transmembrane portions of chemoreceptors, we created a unique NotI restriction site in the TM2-encoding segment of receptor genes, either by site-directed mutagenesis (plasmids pHP2 and pHP4) or by primer-introduced sequences at the ends of PCR fragments (for *Pseudomonas* MCP genes). Introduction of the NotI site changed a VVV or VVL segment to AAA in Tar or Tsr, respectively, 6 residues before the TM2 aromatic anchor tryptophan. Both altered chemoreceptors exhibited wild-type function in tryptone and minimal phenol soft-agar assays. Hybrid plasmids were constructed by ligating plasmid fragments and PCR products that carried an NdeI site at the start of the coding sequence and a NotI site in the TM2 coding region at the same position in the coding region as the ones in plasmids pHP2 and pHP4. To swap the HAMP domains of chemoreceptors, we used the introduced NotI sites described above and a native or PCR primer-created NdeI site at the AS2 end of the HAMP-encoding segment of the *tar* and *tsr* genes. Receptor signaling domains were swapped in similar fashion, using the same NdeI site and a SalI site in the plasmid vector, just past the stop codon of the receptor gene.

The coding regions for three *Pseudomonas putida* MCPs (Pp0584, Pp2823, and

Pp5020) were PCR amplified from chromosomal DNA isolated from *P. putida* KT2440 cells provided by R. E. Parales, University of California—Davis.

Semisolid-agar assay for phenol chemotaxis. The phenol responses of plasmid-carrying strains were assessed on minimal soft-agar plates containing inducers (IPTG [50 to 100 μM] or sodium salicylate [0.4 to 0.6 μM]) and antibiotics (ampicillin [50 μg/ml] or chloramphenicol [12.5 μg/ml]) as appropriate. Minimal semisolid agar contained ~0.25% (wt/vol) agar and 0.5% (wt/vol) NaCl, plus the following final concentrations of components added sterilely after autoclaving the agar solution: 10 mM KPO_4, pH 7.0; 1 mM $(NH_4)_2SO_4$; 1 mM $MgCl_2$; 1 mg/liter thiamine HCl; 1 mM glycerol; and 0.1 mM threonine, methionine, leucine, and histidine. Note that the agar concentration is a critical factor for good resolution in these plate assays. We empirically determined the optimal agar concentration for each agar lot used in the study. Phenol-containing agar plugs were prepared by mixing molten 1% (wt/vol) agar with 0.1 M phenol and pouring the mixture into an empty petri dish to a depth of 5 mm. After allowing the phenol agar to harden, an 8-mm-diameter plug was excised with the large end of a plastic pipette tip and placed onto the surface of a minimal soft-agar plate that had been poured at least 12 h earlier. Cells were inoculated 2 centimeters from the center of the phenol plug by toothpick stab from fresh colonies. The assay plates were incubated at 30°C for 16 to 20 h.

Isolation of phenol-sensing Tar^o derivatives. Plasmid pPA790 was cycled through the *mutD* strain UU1936 to increase its frequency of spontaneous mutations. Independent plasmid pools were then transferred to strain UU1250, and for each pool, a line of about 10^5 transformant cells was placed on a soft-agar plate 2 cm from a phenol source. The selection plates were incubated at 30°C overnight, and cells near the phenol source were collected to initiate another selection cycle. Reconstruction experiments showed that each enrichment cycle produced a 100-fold increase in the frequency of phenol responders in the population. After three selection cycles, individual isolates were tested for their phenol responses, and their plasmids were sequenced.

Capillary assay for phenol chemotaxis. Capillary or Pfeffer assays were performed essentially as described previously (1). Briefly, cells were grown in tryptone broth (41) at 30 to 32.5°C to mid-exponential phase (optical density at 590 nm [OD_{590}], ~0.6), collected by centrifugation (4,000 to 5,000 × *g* for 5 min), washed twice in KEP motility buffer (41), resuspended in the same volume of KEP, and diluted with KEP to a final concentration of ~5×10^6 cells/ml. Drummond 1λ glass capillary tubes were flame sealed at one end and partly filled with phenol test solutions in KEP. Capillaries were placed in bacterial ponds at 30°C and removed after 30 min, and their bacterial contents were assayed by colony count.

Measurement of Tar and Tsr protein levels. Expression from plasmid derivatives was analyzed in strain UU1535, as described previously (5).

Protein modeling and structural display. Atomic coordinates for the Tsr-HAMP domain were generated from the Af1503-HAMP coordinates (Protein Data Bank [PDB] access number 2ASW) by the Swiss-Model server (36). Coordinates for the modeled transmembrane (TM) bundle of Tar were provided by G. L. Hazelbauer, University of Missouri. Structure images were prepared with MacPyMOL software (http://www.pymol.org).

RESULTS

Gradient plate assay for phenol chemotaxis. For routine assessments of phenol chemotactic responses, we used the colony morphologies of Tar or Tsr expression strains in minimal glycerol semisolid agar medium that contained a phenol source at the plate center (Fig. 2; see Materials and Methods for details). Strains were inoculated 2 cm from the center of the phenol source and allowed to grow for 16 to 20 h at 30°C. We then measured the distances from the site of inoculation to the colony edges closest to (D1) and furthest from (D2) the phenol source and computed a response index (RI) from those two values as follows: RI = D1/(D1 + D2). Colonies that were skewed toward the phenol source with RI values greater than 0.52 were deemed to show an attractant response (Fig. 2); colonies skewed away from the phenol with RI values less than 0.48 were considered to show a repellent response. Colonies with intermediate RI values (0.48 to 0.52) were considered to have neutral behavior with respect to the phenol gradient. The

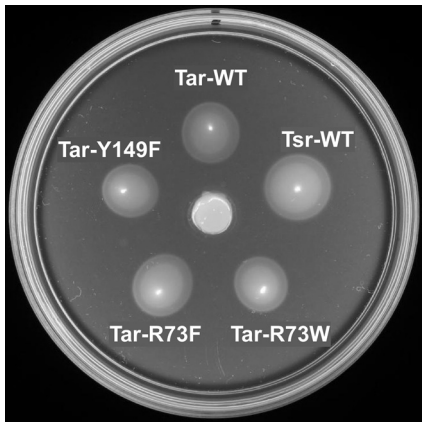

FIG. 2. Tar- and Tsr-mediated motility responses on soft-agar phenol gradient plates. UU1250 cells containing pHP1 (Tar-WT), pHP1 derivatives (Tar variants Y149F, R73F, and R73W), and pRR53 (Tsr-WT) were inoculated by toothpick stab from fresh transformants to a minimal soft agar plate containing a central agar plug of 100 mM phenol. The plate contained 100 μM IPTG to induce expression of the receptors and 50 μg/ml ampicillin to select for plasmid maintenance and was incubated for 20 h at 30°C. The response index values for these colonies are (reading clockwise from the top): 0.59, 0.47, 0.58, 0.62, and 0.63.

RI values given below are an average (± standard deviation) of at least three measurements. (Note that RI values measure the asymmetry of a colony, not its overall size, which under these assay conditions mainly reflects the cells' swimming behavior. Cells that swim with very low or very high tumbling rates spread more slowly in semisolid agar than do cells with balanced run-tumble behavior [6, 54, 58].) With these plate assays, wild-type Tar produced an attractant response to phenol (RI = 0.63 ± 0.05; n = 100), whereas wild-type Tsr produced a weak repellent response (RI = 0.45 ± 0.03; n = 10), behaviors consistent with previous reports of phenol responses mediated by these two receptors (21, 56).

For all behavioral studies, Tar or Tsr receptors were expressed from a regulatable plasmid in host strain UU1250, which carries deletions of all five MCP-related genes (*tar*, *tsr*, *tap*, *trg*, and *aer*). Thus, the experiments described below involved a single chemoreceptor species, expressed at normal physiological levels, in cells lacking all other receptor types.

Phenol sensing by Tar receptors with mutant ligand-binding sites. Residues R73 and Y149 are critical ligand-binding specificity determinants in the aspartate-binding pocket of Tar (8, 32, 55). To investigate whether the Tar aspartate-binding site might play a role in phenol sensing, we surveyed a collection of Tar mutants with random amino acid replacements at residue R73 or Y149 created by all-codon mutagenesis of codons 73 and 149 in the *tar* gene carried by plasmid pHP1. We created two plasmid libraries (denoted R73X and Y149X), transferred the mutant plasmids to the transducerless host strain UU1250, and screened on gradient plates for mutant plasmids that could not promote an attractant response to phenol.

Among more than 200 plasmids screened from each mutant library, 2% of the R73X and 3.5% of the Y149X plasmids could not promote a phenol response. These yields of nonfunctional *tar* genes are close to the expected frequency of nonsense codons from the all-codon mutagenesis. In addition to

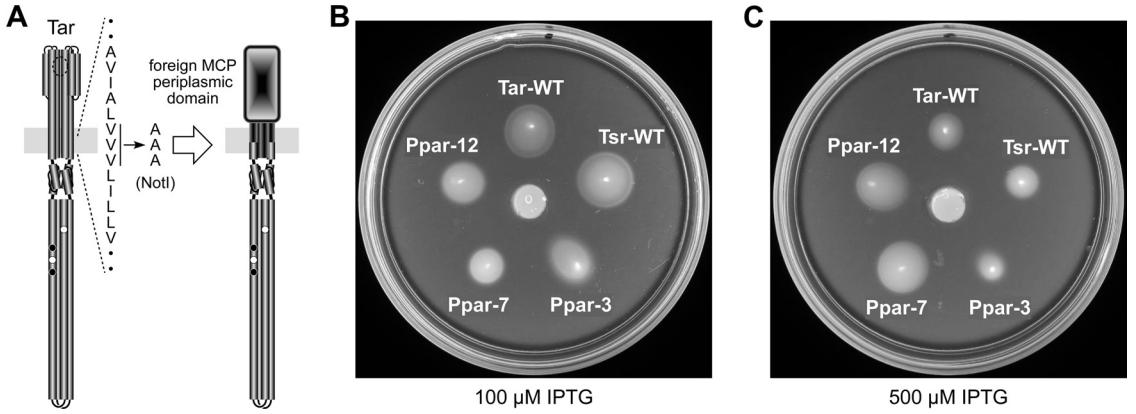

FIG. 3. Construction and properties of Tar hybrids with periplasmic domains from *P. putida* MCPs. (A) Hybrid construction scheme. The *tar* gene in plasmid pHP1 encodes a stretch of three valine residues near the cytoplasmic end of TM2. Those codons were converted to alanine codons to create a unique NotI restriction site. Coding regions for foreign periplasmic domains were spliced to *tar* at this new site. (B and C) Colony morphologies of UU1250 cells expressing different Tar hybrids on semisolid-agar phenol gradient plates containing 100 μM (B) and 500 μM (C) IPTG. The plates also contained 50 μg/ml ampicillin and were incubated for 16 h at 30°C.

nonsense mutants, some of the R73 mutants had a proline codon at the targeted site, whereas some of the Y149 mutants had a glutamine codon at the targeted site (data not shown). These results imply that only drastic structural changes of the aspartate-binding pocket were able to disrupt phenol sensing by Tar. More modest binding site lesions that completely destroy aspartate sensing (e.g., R73F and Y149F [data not shown]) had no effect on phenol sensing (Fig. 2). These results indicate that Tar does not sense phenol at the aspartate-binding site.

Phenol sensing by hybrid Tar receptors with a foreign periplasmic domain. To determine whether other determinants in the periplasmic domain of Tar might be responsible for phenol sensing, we replaced the Tar periplasmic domain, encoded by pHP1, with the corresponding portion of an MCP (Pp0584, Pp2823, or Pp5020) from *P. putida* KT2440. The splice junctions were near the cytoplasmic end of the membrane-spanning TM2 segment of Tar (Fig. 3A). In the transducerless host UU1250, two of the hybrid receptors (Ppar-3, containing the periplasmic domain of Pp0584, and Ppar-12, containing that of Pp5020) mediated attractant responses to phenol (Fig. 3B and C). Ppar-3 produced a strong response (RI = 0.74 ± 0.00) under moderate induction conditions (Fig. 3B); Ppar-12 produced a strong response (RI = 0.68 ± 0.00) at a higher inducer level (Fig. 3C). These findings suggest that the periplasmic domain of Tar contains no unique determinants necessary for sensing phenol as an attractant. Alternatively, phenol-sensing determinants may be common features of the periplasmic domains in other MCPs.

Phenol sensing by Tar receptors lacking a periplasmic domain. To determine whether the Tar periplasmic domain is essential for mediating an attractant response to phenol, we looked for phenol responses by a Tar derivative (Tar°) that lacks residues 44 to 183 of the periplasmic domain. Plasmid pPA790, which encodes Tar°, did not support an attractant response to phenol in strain UU1250 (Fig. 4). However, the Tar° receptor is known to be defective in activating the CheA kinase, which could account for the lack of a phenol response (15). Accordingly, we induced random mutations in pPA790 by growth in a *mutD* host, transformed UU1250 with a pool of mutant plasmids, and then enriched for phenol-responsive cells by three selection cycles on gradient plates (see Materials and Methods for details). Tar° derivatives obtained in this way

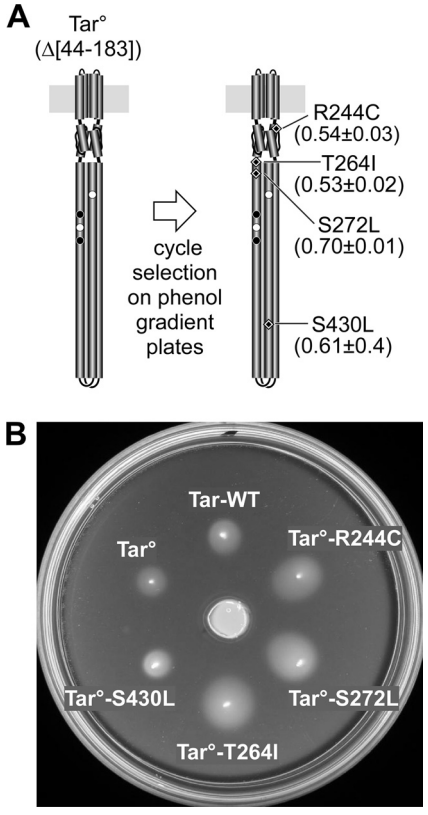

FIG. 4. Selection and properties of Tar° receptor mutants on soft-agar phenol gradient plates. (A) Summary of Tar° amino acid changes obtained by three cycles of selection for phenol attractant responses. The phenol response index values for each Tar° mutant derivative are shown in parentheses. (B) Colony morphologies of UU1250 cells expressing various Tar° derivatives. See the legend to Fig. 2 for additional details.

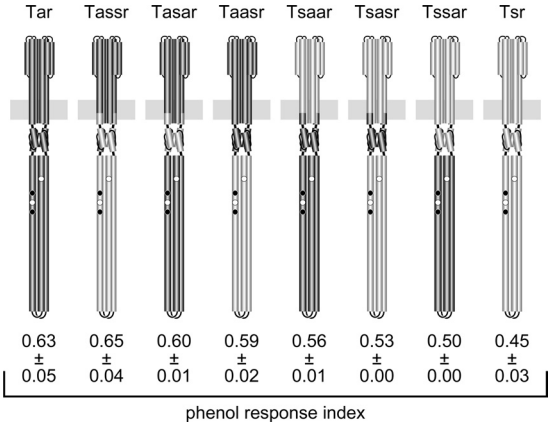

FIG. 5. Phenol responses mediated by Tar-Tsr hybrid receptors. See Materials and Methods for construction details. The three middle letters of the hybrid designation indicate the origin (T*a*r or Tsr) of the periplasmic domain and TM bundle, the HAMP domain, and the methylation bundle and kinase control tip. The RI values (bottom) represent the averages and standard deviations for three independent experiments.

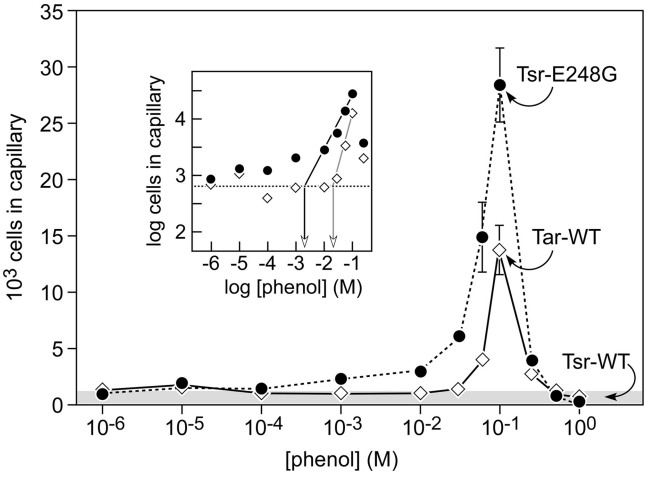

FIG. 6. Capillary assay of phenol attractant responses mediated by wild-type (WT) Tar and Tsr-E248G receptors. Plasmids expressing Tar-WT (pHP1), Tsr-WT (pRR53), and Tsr-E248G (pRR53 derivative) were transferred to strain UU1250 and tested for phenol chemotaxis as detailed in Materials and Methods. The data points represent averages of at least two independent experiments; the error bars indicate the standard deviations. Where error bars are not apparent, their size is less than that of the plot symbols. The shaded area represents the control accumulations due to background motility alone. The Tsr-WT response did not exceed this background range, and the individual data points are not shown. The inset contains a log-log plot for determining response threshold concentrations by back-extrapolating the rising portion of the curves to intercept the background motility level.

mediated attractant responses to phenol (Fig. 4). Each phenol-responsive derivative contained a single missense change that most likely enables Tar° to make CheA-active ternary signaling complexes (15). The activating mutations affect the cytoplasmic portion of the Tar° molecule (Fig. 4A) and demonstrate that the periplasmic portion of Tar is not required for phenol sensing (Fig. 4B).

Phenol sensing by Tar-Tsr hybrid receptors. To localize the Tar determinants responsible for sensing phenol as an attractant, we constructed a series of Tar hybrids with different segments swapped in from Tsr, which senses phenol as a repellent. Two junction points were used to make the hybrids, one in TM2 just before the HAMP domain (Tar 201/202) and one at the membrane-distal end of the HAMP domain that adjoins the methylation helices (Tar 256/257) (Fig. 5). We designate the hybrids by letters that indicate the origin (T*a*r or Tsr) of their three parts: periplasmic domain and TM bundle, HAMP domain, or methylation helices and kinase control domain). All three hybrids containing the Tar periplasmic domain (Tassr, Tasar, and Taasr) produced strong attractant responses to phenol (Fig. 5). However, hybrids containing the Tar HAMP domain (Tsaar and Tsasr) also produced attractant responses to phenol (Fig. 5). Only the Tssar chimera, which has neither the periplasmic nor the HAMP domain from Tar, failed to produce an attractant response to phenol. However, this hybrid receptor also failed to produce a repellent response to phenol, which suggests that the methylation/kinase control domains of Tar and Tsr may respond to input signals with slightly different sensitivities. We conclude that either the periplasmic domain and TM bundle of Tar or the HAMP domain of Tar is sufficient to mediate an attractant response to phenol in hybrid receptors, suggesting that both sides of the TM2-HAMP junction could contain phenol-sensing determinants.

Tsr receptor mutants that sense phenol as an attractant. Because the Tar HAMP domain can convert Tsr into an attractant sensor of phenol, we wondered whether a mutational alteration in the Tsr HAMP domain or in some other Tsr phenol-sensing determinant could reverse the sign of its phenol response. Accordingly, we subjected plasmid pRR53, which encodes wild-type Tsr, to *mutD* mutagenesis, transformed UU1250 with the plasmid pool, and selected for cells attracted to phenol on gradient plates. We characterized 11 independent isolates; all proved to have the same Tsr change: E248G. The Tsr-E248G receptor mediated a robust attractant response to phenol on gradient plates (RI = 0.64 ± 0.02) (not shown). However, because the plate assay involves multiple generations of cell growth, plate phenotypes could conceivably reflect phenol influences on colony growth. Thus, we compared the phenol responses of cells carrying wild-type Tar or Tsr-E248G in capillary assays, which measure the motility behaviors of nongrowing cells in diffusion-generated gradients. Both receptors promoted entry of cells into phenol-filled capillaries, with peak accumulations at 0.1 M phenol (Fig. 6). Tsr-E248G mediated a somewhat stronger response and a lower detection threshold than wild-type Tar (1 mM versus 10 mM) (Fig. 6, inset). These capillary responses demonstrate net up-gradient movements of cells in phenol gradients; the apparent attractant responses on gradient plates are not due to differential growth effects.

Tsr residue E248, a conserved feature of HAMP domains, resides at the start of the AS2 helix at the membrane-proximal end of the HAMP bundle. The signal output of Tsr-E248G is shifted toward the attractant-stimulated state, and it cannot mediate an attractant response to serine (59). The dynamic-bundle model of HAMP action (60) predicts that the HAMP bundle in Tsr-E248G has enhanced packing stability, resulting

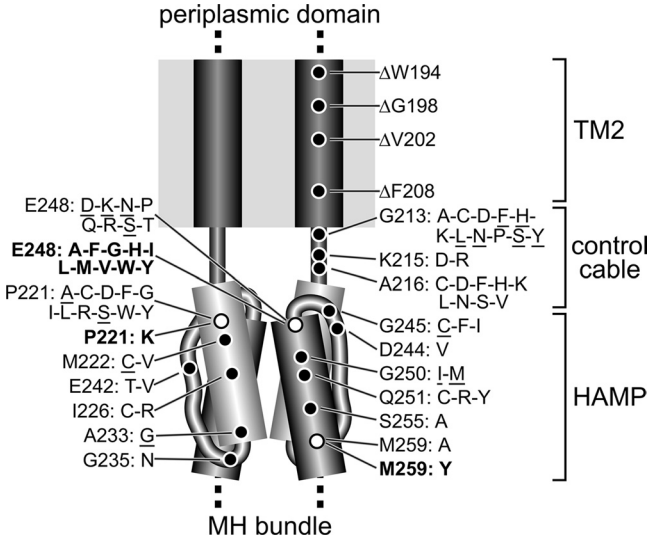

FIG. 7. Summary of mutant Tsr receptors screened for attractant responses to phenol in soft-agar gradient plates. The HAMP structural elements are as follows: AS1 helices (light-gray cylinders), AS2 helices (dark-gray cylinders), and connector segments (thin gray tubes). Amino acid replacements in the TM2-control cable-HAMP segments of Tsr, expressed from pRR53 or pPA114 derivatives, were tested in strain UU1250. The underlined replacements retain a repellent response to phenol; the replacements in boldface produced an attractant response to phenol; all other mutant receptors produced neutral responses to phenol. Replacements at three HAMP residues (white circles) gave rise to attractant responses. With the exception of some E248 lesions, all of these mutant receptors still mediate an attractant response to serine (24, 60).

in an elevated methylation response from the sensory adaptation system (59). Other amino acid changes at E248, particularly hydrophobic replacements, appear to cause similar signaling effects in Tsr (59). Accordingly, we screened other Tsr-E248 amino acid replacement mutants for phenol responses on gradient plates. Mutant receptors with hydrophobic (A, V, I, L, and M) or aromatic (F, Y, and W) residues mediated attractant responses to phenol (Fig. 7). With the exception of E248H, polar and charged amino acid replacements at E248 either preserved the Tsr wild-type repellent response to phenol or eliminated the response altogether (Fig. 7).

Lesions in the Tsr HAMP domain that mimic attractant signaling effects (ATT-mimic) elicit elevated methylation levels. Consequently, the mutant receptor's signal output can be restored to the wild-type set point position, but at the cost of reduced sensitivity to serine gradients (24, 59). Tsr residue P221, another conserved feature of HAMP domains, resides in the AS1 helix and interacts structurally with E248 (2, 13, 20). Amino acid replacements at P221 produce ATT-mimic signaling effects and impair, but do not abrogate, serine responses (59, 60). P221 replacements might augment HAMP packing by enhancing the stability of the AS1 helix. We screened Tsr-P221 mutants on phenol gradient plates but found only one more receptor that could mediate an attractant response: P221K. Interestingly, lysine is the only amino acid replacement at P221 that preserves the wild-type Tsr response to serine and causes no discernible change in Tsr output signals (60). We also screened a variety of other Tsr-HAMP mutants with ATT-mimic lesions in AS1, the connector, or AS2 but found only one (M259Y) that showed an attractant response to phenol (Fig. 7). Evidently, it takes a special change in the structure or stability of the Tsr-HAMP domain to reverse the sign of its phenol response.

The properties of Tar-Tsr hybrids indicated that the control cable segment joining TM2 to HAMP could be important for the sign of the phenol response. Accordingly, we examined, on phenol gradient plates, a number of Tsr ATT-mimic mutants that had lesions in TM2 or the control cable. None of the tested receptors mediated an attractant response to phenol (Fig. 7). All of these ATT-mimic receptors still mediate an attractant response to serine (24), indicating that their run-tumble swimming pattern can support gradient-tracking behavior. However, many of them no longer produced a discernible repellent response to phenol, suggesting attenuation of their ability to sense phenol as a repellent. Three mutant receptors that showed strong repellent responses to phenol (G213F [RI = 0.38], G213H [RI = 0.29], and G213L [RI = 0.32]) were converted to attractant sensors (RI = 0.61, 0.64, and 0.65, respectively) upon introduction of the E248G lesion, demonstrating that their control cable lesions had not irreversibly damaged any signaling determinants needed to sense phenol as an attractant.

DISCUSSION

Signaling elements in chemoreceptor molecules. MCP molecules comprise four modular signaling elements that communicate through bidirectional conformational interactions: (i) the ligand-binding domain and TM helices, (ii) the HAMP domain, (iii) the methylation helix bundle, and (iv) the protein interaction domain (Fig. 1) (18, 26, 52, 60). Adjacent modules are probably coupled in structural opposition, so that when the structural stability of an element changes during signal transmission, its immediate neighbors undergo a reciprocal change in stability (42, 52, 59, 60).

The ligand-binding domains of receptors represent stimulus-specific input points, but in principle, stimulus information could feed into receptor molecules at any later transmission step. The Tar and Tsr responses to cytoplasmic pH changes, for example, depend on several residues near the HAMP-MH1 junction (25, 53). Thermosensing, a property of all *E. coli* MCPs, could conceivably occur through temperature-dependent changes in the TM2-HAMP-methylation helix region (34–37). Repellent responses to glycerol and ethylene glycol, also mediated by multiple MCPs, could involve perturbations of membrane curvature and the relative positions of the TM helices (39). As discussed below, our studies of phenol responses mediated by Tar and Tsr suggest that MCP molecules may detect phenol stimuli by a mechanism similar to that of glycerol sensing.

Phenol-sensing determinants of Tar and Tsr. Several lines of evidence reported in this study demonstrate that the Tar ligand-binding domain, methylation bundle, and kinase control domain do not contain functionally important determinants for sensing phenol as an attractant. First, very few amino acid replacements at critical residues of the aspartate-binding pocket impaired the attractant response to phenol. Second, hybrid Tar molecules with periplasmic domains from *P. putida*

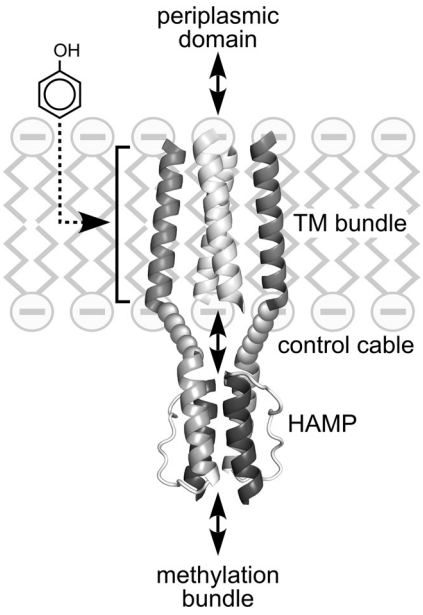

FIG. 8. Model for phenol sensing by Tar and Tsr. Bidirectional conformational interactions (double-headed arrows) between contiguous signaling elements transmit stimulus information to the kinase control tip of the receptor molecule. The shading convention for HAMP structural elements follows that of Fig. 7. Phenol might diffuse into the cytoplasmic membrane to influence the equilibrium position or structural stability of the transmembrane helices, thereby modulating the packing stability of the HAMP bundle.

MCPs or from Tsr, which senses phenol as a repellent, mediated attractant responses to phenol. Third, Tar° molecules, lacking all but a few residues of the periplasmic sensing domain, also mediated attractant responses to phenol when carrying an amino acid replacement in the cytoplasmic domain that enhanced CheA activation ability. Finally, the source of the methylation bundle and kinase control domain did not determine the sign of the phenol response in Tar-Tsr hybrids.

The Tar periplasmic domain plus transmembrane helices (TM bundle) sufficed to convert Tsr to an attractant sensor for phenol. In addition, the Tar HAMP domain produced an attractant response to phenol in Tar-Tsr hybrids. Moreover, some amino acid replacements in the Tsr HAMP domain, notably at residue E248, converted Tsr to a potent attractant sensor of phenol. Given that the Tar periplasmic domain itself is not required for phenol sensing, it follows that the TM bundle contains phenol-sensing determinants or imparts phenol-sensing ability to the adjacent HAMP domain. Conversely, if the HAMP domain does not detect phenol stimuli directly, its structure and dynamic behavior must potentiate phenol detection by the TM bundle (Fig. 8).

Possible mechanisms of phenol detection by Tar and Tsr. Phenol and other aromatic hydrocarbons interact with lipid membranes to affect their stability, fluidity, permeability, and other properties (38, 45). Thus, phenol might elicit signaling responses by diffusing into the cytoplasmic membrane and perturbing the membrane-spanning helices of MCP molecules. Phenol might reduce the stability of the TM helices or influence their preferred positions with respect to the hydrophobic core and head groups of the membrane lipids. Such structural changes could cause displacements of the TM2 helices that mimic those produced by more conventional attractant or repellent stimuli, which in turn modulate receptor output through changes in HAMP domain structure or stability.

The membrane model of phenol sensing requires that the TM helices of Tar and Tsr have different phenol-induced positions or structural sensitivities, because phenol causes output responses of opposite sign in these two receptors. In this scenario, amino acid replacements in the Tsr HAMP domain that reverse the sign of the phenol response must influence the prestimulus structure or position of the TM bundle. Alternatively, a dual-target model of phenol sensing offers a simpler mechanism for inverting the phenol responses. Phenol might, for example, perturb both the TM and HAMP bundles, but with different threshold sensitivities in different receptors. In Tar, the more sensitive element could be the TM bundle, whereas in Tsr, the more phenol-sensitive target might be the HAMP domain. The hydrophobic replacements at residue E248 that invert the Tsr phenol response have attractant-mimic signaling effects that are believed to stabilize the HAMP bundle (59, 60). Enhanced stability of the Tsr-HAMP bundle might render the Tsr TM bundle the more sensitive phenol target, thereby reversing the sign of the behavioral response. The results described in this report are consistent with both the single- and dual-target models of phenol sensing.

Behavioral responses to stimuli that reduce receptor stability. Recent studies suggest that bacterial chemoreceptors may convey stimulus information through changes in dynamic behavior rather than specific conformational differences (42, 59, 60). A dynamics-based view of signal transmission implies that receptors could be sensitive detectors of a variety of chemicals and physical conditions that perturb the structural stability of a target signaling element in the receptor molecule. The *E. coli* responses to temperature, cytoplasmic pH, glycerol, and phenol may be examples of stability-sensing behaviors. Notably, all transmembrane MCPs of *E. coli* mediate attractant or repellent responses to these stimuli, implying a common detection mechanism for each. Phenol-sensing behaviors should provide a good experimental model for elucidating these general stimulus detection mechanisms of chemoreceptors.

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