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Repellent Response Functions of the Trg and Tap Chemoreceptors of *Escherichia coli*

KIMIKO YAMAMOTO,[1] ROBERT M. MACNAB,[2] AND YASUO IMAE[1]*

Department of Molecular Biology, Faculty of Science, Nagoya University, Chikusa, Nagoya 464-01, Japan,[1] and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511[2]

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The chemoreceptors responsible for the repellent response of *Escherichia coli* to phenol were investigated. In the absence of all four known methyl-accepting chemoreceptors (Tar, Tsr, Trg, and Tap), cells showed no response to phenol. However, when Trg, which mediates the attractant response to ribose and galactose, was introduced via a plasmid, the cells acquired a repellent response to phenol. About 1 mM phenol induced a clear repellent response; this response was suppressed by 1 mM ribose. Thus, Trg mediates the repellent response to phenol. Mutant Trg proteins with altered sensing for ribose and galactose showed a normal response to phenol, indicating that the interaction site for phenol differs from that for the ribose- and galactose-binding proteins. Tap, which mediates the attractant response to dipeptides, mediated a weaker repellent response to phenol. Tsr, which mediates the attractant response to serine, mediated an even weaker response to phenol. Trg and Tap were also found to function as intracellular pH sensors. Upon a pH decrease, Trg mediated an attractant response, whereas Tap mediated a repellent response. These results indicate that all the receptors in *E. coli* have dual functions, mediating both attractant and repellent responses.

Bacteria show chemotaxis by modulating their swimming pattern; an increase in attractant concentration or a decrease in repellent concentration causes a transient increase in smooth swimming, whereas changes in the opposite direction cause a transient increase in tumbling. For the mediation of responses to a variety of chemical stimuli, *Escherichia coli* has four kinds of methyl-accepting receptors, called Tar, Tsr, Trg, and Tap (for reviews, see references 13 and 30). Tar mediates attractant responses to aspartate and maltose and a repellent response to Ni^{2+}, Tsr mediates an attractant response to serine and a repellent response to leucine, Trg mediates attractant responses to ribose and galactose, and Tap mediates an attractant response to dipeptides. These receptors interact directly with amino acid attractants but require specific binding proteins for the interaction with sugars and dipeptides. The closely related species *Salmonella typhimurium* has four related receptors, called Tar, Tsr, Trg, and Tip (13, 30), which differ in some cases from the corresponding receptors of *E. coli*. For example, in *S. typhimurium*, Tar is defective in mediating the maltose response (16), the function of Tip has not yet been identified (24), and Tap is believed to be absent (15).

The structural genes for most of these receptors have been cloned and sequenced. Amino acid sequences deduced from the DNA sequences indicate considerable similarities among these receptors (13, 30). They are transmembrane proteins with molecular masses around 60 kilodaltons and are organized in the membrane with a small N-terminal cytoplasmic region, a transmembrane segment, a periplasmic domain, a second transmembrane segment, and a cytoplasmic domain (10, 14, 23). The periplasmic domain is responsible for ligand binding, whereas the cytoplasmic domain is responsible for production of the signal by which the swimming pattern is modulated via protein phosphorylation reactions (9, 21). The cytoplasmic domain contains the methylesterification sites, which are responsible for behavioral adaptation (29).

Although most chemical stimuli are mediated by a single receptor, some are mediated by two or more receptors. One example is glycerol, which causes a repellent response in *E. coli* if any one of the receptors is present (18, 19). Another example is weak acids, such as acetate and benzoate, to which Tsr mediates a repellent response but Tar mediates an attractant response (6, 22). Krikos et al. (9) showed that the cytoplasmic domains are responsible for mediating these opposite responses, consistent with the finding that these weak acids act by decreasing the cytoplasmic pH (6, 22).

Phenol is also a complex chemoeffector; *E. coli* shows an attractant response to it, whereas *S. typhimurium* shows a repellent response (11, 32). We showed previously (4) that the attractant response in *E. coli* is mediated by Tar but that in *S. typhimurium* this Tar function is dominated by the function of an unidentified receptor that mediates a repellent response to phenol. Since Tar-deficient mutants of *E. coli* showed only a weak repellent response to phenol, we suggested that *E. coli* had no receptors for mediating a repellent response to phenol.

We have investigated whether any transducer in *E. coli* has the ability to mediate a repellent response to phenol. Such analyses have revealed several interesting properties of Trg and Tap.

MATERIALS AND METHODS

Bacterial strains and plasmids. All strains used are derivatives of *E. coli* K-12 and are listed in Table 1, together with the plasmids used.

Transformation to prepare plasmid-containing cells was carried out as described previously (12).

Chemicals. Phenol and D-ribose were obtained from Wako Pure Chemicals, Osaka, Japan; synthetic L-serine was obtained from ICN Pharmaceuticals, Inc., Plainview, N.Y.; glycyl-L-leucine was obtained from Peptides Institute, Inc., Osaka, Japan; isopropyl-β-D-thiogalactopyranoside was obtained from Sigma Chemical Co., St. Louis, Mo.; L-[*methyl*-^{3}H]methionine (12 Ci/mmol) was obtained from Dupont NEN Research Products, Boston, Mass.

Cell growth. Cells were grown at 30°C with shaking in

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype	Source (reference)
Strains		
RP487	*che*$^+$	J. S. Parkinson (26)
RP4324	Δ(*tar-tap*)*5201*	J. S. Parkinson (26)
AW660	*tsr-12 tar trg-1*	J. Adler (8)
AB1200	Δ(*tar-tap*)*5201* Δ*tsr7028*	M. I. Simon (9)
HCB339	Δ(*tar-tap*)*5201* Δ*tsr7028* *trg*::Tn*10*	H. C. Berg (35)
CP474	Δ*trg100* (pCP31)	G. L. Hazelbauer (20)
Plasmids		
pCP31	*trg*$^+$	G. L. Hazelbauer (20)
pCP32	*trg-19*	G. L. Hazelbauer (20)
pCP33	*trg-8*	G. L. Hazelbauer (20)
pAK101	*tar*$^+$	M. I. Simon (9)
pNM33[a]	*tsr*$^+$	N. Mutoh
pVB8[b]	*lacI*^{q} P_{tac} *tap*$^+$	V. Blank

[a] Constructed by exchanging the *Eco*RI-*Hin*dIII fragment of pAB100 (9) with the corresponding fragment of pBR322 (N. Mutoh and M. I. Simon, unpublished data). Provided by N. Mutoh of Aichi Prefectural Colony Research Institute, Kasugai, Japan.

[b] V. Blank, Ph.D. thesis, University of Konstanz, Konstanz, Federal Republic of Germany, 1987. Provided by M. D. Manson of Texas A&M University, College Station.

tryptone broth (1% tryptone, 0.5% NaCl) supplemented with 0.5% (vol/vol) glycerol. When necessary, 27 mM ribose was added to ensure full induction of the ribose-binding protein, which is necessary for ribose taxis (7). Ampicillin was added to a concentration of 50 μg/ml to cells containing plasmids.

In the case of HCB339 cells containing pVB8, the Tap content was increased by growing the cells for 5 h in the above medium supplemented with 1 mM isopropyl-β-D-thiogalactopyranoside.

Measurement of chemotactic response. Temporal stimulation was used for the measurement of chemotactic responses to various stimuli. Cells in motility medium (10 mM potassium phosphate buffer, pH 7.0, 0.1 mM EDTA, 10 mM sodium DL-lactate supplemented with 20 μM methionine) were used at 25°C, and changes in the smooth-swimming fraction were measured photographically as described previously (18). In some experiments, the pH of the motility medium was adjusted to 6.0 or 7.4.

Measurement of methylation levels of receptors. Methylation of receptors was analyzed as described previously (19). In brief, cells in motility medium supplemented with 200 μg of chloramphenicol per ml were incubated with 10 μM [*methyl*-^{3}H]methionine (0.5 Ci/mmol) at 30°C for more than 35 min. Various chemoeffectors were then added, and incubation was continued. Samples were withdrawn at intervals and mixed with ice-cold trichloroacetic acid (final concentration, 5%). Radioactivity incorporated into receptors as methylesters was measured by a vapor-phase equilibrium procedure (31). For this purpose, the cells were collected on a cellulose nitrate membrane filter (pore size, 0.45 μm) and washed with ice-cold 5% trichloroacetic acid. The filter was then put in a 1.5-ml Eppendorf microcentrifuge tube. After the addition of 0.2 ml of 1 M NaOH, the tube was immediately transferred to a scintillation vial containing 2.4 ml of ACSII (Amersham Corp., Arlington Heights, Ill.), and the vial was sealed tightly. After overnight incubation at 37°C, the tube was removed and the radioactivity in the vial was measured in a scintillation counter.

Analysis of methylation-banding patterns of receptors. Methylation of receptors was carried out as described above, except that the cells were incubated with 2 μM [*methyl*-^{3}H]methionine (12 Ci/mmol). For HCB339(pCP31), the concentration of [*methyl*-^{3}H]methionine was increased to 6 μM. After incubation at 30°C for 40 min, various effectors were added, and the incubation was continued for 10 or 15 min. Methylation-banding patterns were obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the method of Slonczewski et al. (27).

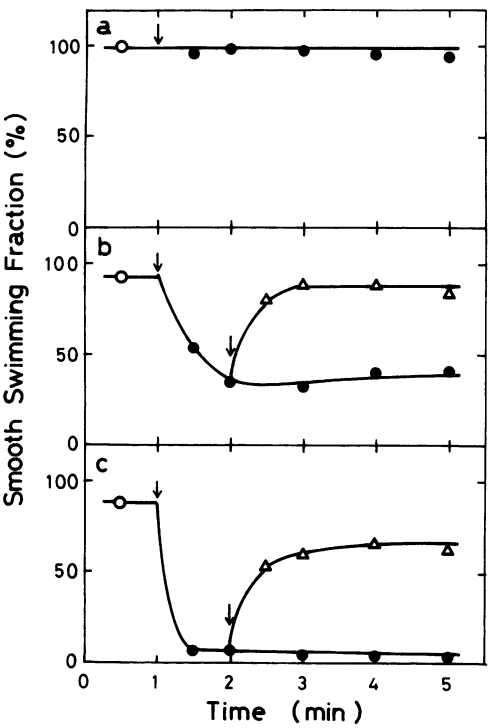

FIG. 1. Role of Trg in the repellent response to phenol. At the time indicated by the first arrow, phenol was added to a concentration of 5 mM (●), and at the second arrow, ribose was added to a concentration of 1 mM (△). Changes in the smooth-swimming fraction were monitored at 25°C. (a) HCB339 (*tar tap tsr trg*). (b) AB1200 (*tar tap tsr*). (c) HCB339(pCP31) (*trg*$^+$).

RESULTS

Phenol sensing by wild-type *E. coli* Trg protein. In a previous paper (4), we reported that the Tar protein of *E. coli* mediates an attractant (i.e., smooth-swimming) response to phenol and that cells lacking Tar show a weak repellent (i.e., tumbling) response to phenol. To investigate whether or not this repellent response is mediated by one of the known receptors, we have examined the swimming behavior of mutants with defects in these receptors. HCB339, which lacks all four receptors and swims smoothly, showed no response to 5 mM phenol (Fig. 1a). However, AB1200, which retains Trg but also swims smoothly, showed a moderate repellent response to 5 mM phenol, with little or no adaptation (Fig. 1b); this response was suppressed by 1 mM ribose, a Trg-mediated attractant (Fig. 1b). These results suggest that the presence of Trg is important for the repellent response to phenol.

The amount of Trg is normally 1/5 to 1/10 that of Tar or Tsr (2), which might explain why the response to phenol in AB1200 was not stronger. We therefore increased the amount of Trg by the use of a multicopy Trg-encoding

plasmid, pCP31. The transformant, HCB339(pCP31), showed a strong repellent response to 5 mM phenol, with no adaptation (Fig. 1c); the response was considerably suppressed by 1 mM ribose, and the suppression also continued without adaptation. Attractants for the other (absent) receptors, such as 1 mM aspartate (Tar), 1 mM serine (Tsr), and 1 mM glycylleucine (Tap), did not suppress the phenol response (data not shown). It is noteworthy that the attractant response to 1 mM ribose in the cells lasted for more than 10 min without adaptation (data not shown).

The response to phenol was further tested with tethered cells. Unstimulated HCB339(pCP31) showed mainly counterclockwise rotation; 5 mM phenol caused a clear increase in clockwise rotation, and subsequent addition of 1 mM ribose restored the counterclockwise rotation (data not shown). A control strain [HCB339(pAK101)] which has Tar and therefore shows an attractant response to phenol (4) showed transient counterclockwise rotation in response to phenol. Thus, the observed tumbling response in cells containing Trg is truly a chemotactic response of clockwise rotation and not just the impairment of motility that phenol can cause (4).

Phenol sensing by mutant Trg proteins. Park and Hazelbauer (20) have isolated two different *trg* mutants; one is *trg-19*, which causes a defect only in galactose sensing, and the other is *trg-8*, which causes defects in both ribose and galactose sensing. The phenol responses of HCB339 transformed with plasmids carrying either these mutant *trg* genes or the wild-type gene were indistinguishable; at phenol concentrations of 0.5 mM or higher, these cells showed a strong repellent response. Thus, the recognition site for phenol is not identical to that for ribose- and galactose-binding proteins.

Modulation by other receptors of phenol sensing by Trg. An unusual feature of the repellent response to phenol in cells with Trg as the sole transducer was the absence of adaptation (Fig. 1). However, when Tsr and Trg were both present at wild-type levels as in RP4324, the cells showed significant adaptation (Fig. 2a). This effect of Tsr was reduced when the amount of Trg was increased by introducing pCP31 (Fig. 2a). These results suggest that Trg itself has almost no adapting ability to phenol (or ribose, not shown) but that the presence of other receptors can result in adaptation to phenol.

Wild-type cells show a Tar-mediated attractant response to phenol (4). When the amount of Trg was increased by use of plasmid pCP31, however, the cells showed a repellent response with partial adaptation (Fig. 2b). Thus, the relative amounts of Tar and Trg in the cells determine whether they show an attractant or a repellent response to phenol. Even with elevated amounts of Trg, the presence of other receptors aids in the behavioral adaptation to phenol.

Changes in the methylation level of Trg by phenol. The carboxy methylation of specific glutamic acid residues located in the cytoplasmic domain of receptors is the basis of adaptation to many chemical stimuli (29). In the case of HCB339(pCP31), which has elevated Trg levels and no other receptors present, 5 mM phenol or 1 mM ribose did not cause any detectable changes in methylation level (not shown), consistent with the absence of adaptation to phenol and ribose in this strain. RP4324, which has the normal amounts of Tsr and Trg and shows some adaptation to phenol, also showed no detectable changes in the methylation level of receptors by phenol, whereas 10 mM serine caused a clear increase (data not shown). Since most of the receptors in these cells are presumed to be Tsr, this indicates that the methylation level of Tsr is not affected by phenol.

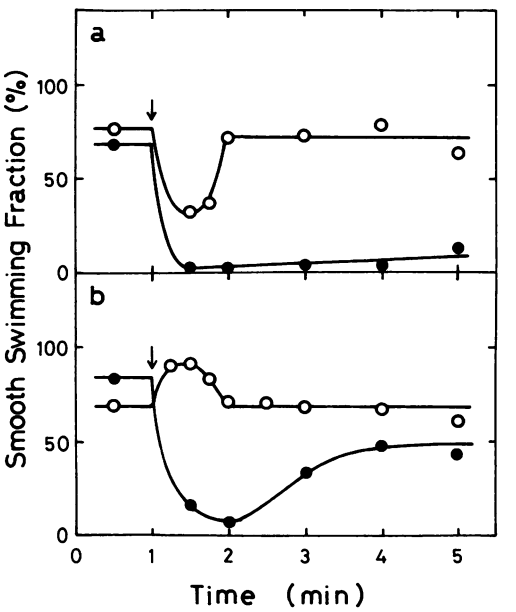

FIG. 2. Effect of Tsr and Tar on the phenol response of cells with low and high Trg levels. (a) RP4324 (*tar tap*) (○) and RP4324 (pCP31) (*trg*$^+$) (●). (b) RP487 (wild type) (○) and CP474 (Δ*trg*) (pCP31) (*trg*$^+$) (●). At the time indicated by the arrow, phenol was added to a concentration of 5 mM, and changes in the smooth-swimming fraction were monitored at 25°C.

Interestingly, when the amount of Trg was increased in this strain by the use of plasmid pCP31, detectable changes in methylation level occurred, with phenol causing a decrease and ribose causing an increase (Fig. 3).

On sodium dodecyl sulfate-polyacrylamide gels, Trg migrates as a set of bands that correspond to different numbers

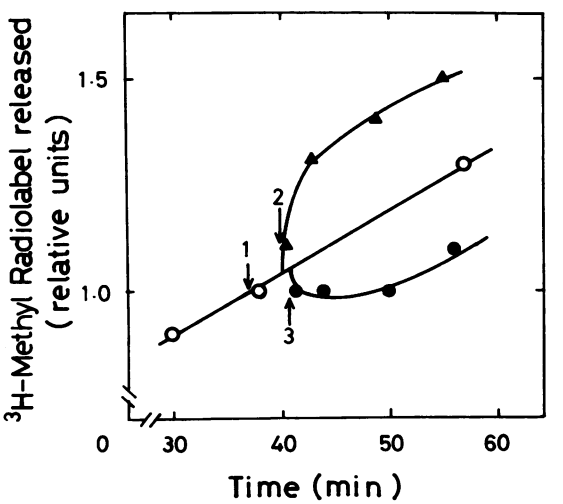

FIG. 3. Effect of phenol or ribose on the methylation levels of receptors in the absence of Tar in strain RP4324(pCP31). Cells were incubated with [*methyl*-^{3}H]methionine in the presence of chloramphenicol and separated into three samples. At the times indicated by arrows 1, 2, and 3, deionized water (○), 10 mM ribose (▲), and 10 mM phenol (●), respectively, were added. Radiolabeled amounts incorporated into receptors as methylesters (measured as methanol released by alkaline hydrolysis) were normalized to the amount at the time deionized water was added.

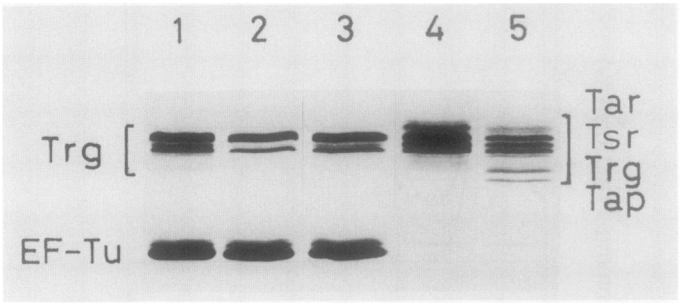

FIG. 4. Changes in the methylation-banding pattern of Trg caused by the addition of phenol or ribose. HCB339(pCP31) (*trg*$^+$) cells were incubated with 6 μM [*methyl*-^{3}H]methionine at 30°C for 40 min and then for another 10 min after the addition of deionized water (lane 1) or 5 mM phenol (lane 2). Ribose was added to a concentration of 10 mM to the cells treated with 5 mM phenol for 5 min, and the cells were incubated for an additional 10 min (lane 3). The samples were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. The methylation banding patterns of other receptors in RP487 (wild type, lane 4) and CP474 (overproduced Trg, lane 5) under unstimulated conditions are shown for comparison. In these experiments, the concentration of methionine was 2 μM, and the exposure time was reduced by one-half to avoid overlapping of bands. The bands at the lower molecular weight (ca. 43,000) are from elongation factor Tu (EF-Tu).

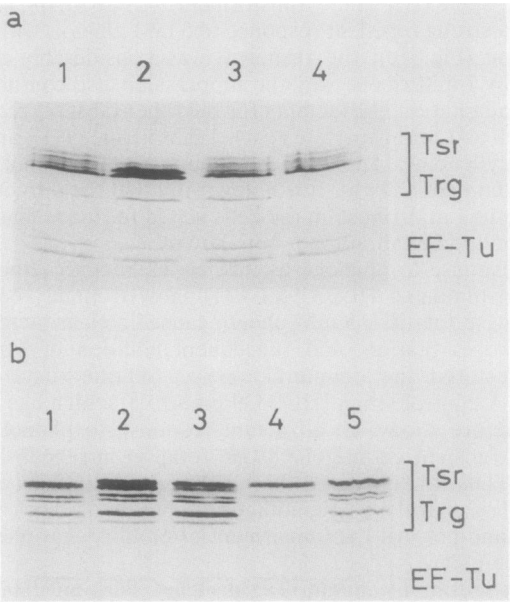

FIG. 5. Methylation banding patterns of receptors in cells with both Tsr and Trg. Experimental conditions were similar to those for Fig. 4. (a) RP4324 (*tar tap*). (b) RP4324(pCP31) (*trg*$^+$). Lane 1, Deionized water; lane 2, 10 mM serine; lane 3, 10 mM ribose; lane 4, 5 mM phenol; lane 5, 5 mM phenol plus 10 mM ribose.

of methylated glutamic acid residues (5, 17). Trg in HCB339(pCP31) was clearly methylated in the absence of any effectors and migrated as a set of bands (Fig. 4, lane 1) which covered a much narrower mobility range than the bands of other receptors (Fig. 4, lanes 4 and 5).

Phenol (5 mM) caused the faster-migrating bands of Trg in HCB339(pCP31) to disappear (Fig. 4, lane 2), while the subsequent addition of 1 mM ribose caused a significant reappearance (Fig. 4, lane 3). Thus, although the overall changes in the methylation level of Trg in this strain were small, phenol did cause some demethylation, and ribose did cause some methylation.

In the case of RP4324, which has the normal amount of Tsr and Trg, neither phenol (Fig. 5a, lane 4) nor ribose (lane 3) caused much change in the methylation pattern, but serine caused a strong increase in the faster-migrating bands in the upper group (lane 2). When the amount of Trg was increased by introducing pCP31, the addition of phenol (Fig. 5b, lane 4) caused a decrease not only in the faster-migrating bands in the upper group but also in the other bands; the subsequent addition of ribose caused a reappearance of these bands (lane 5). Thus, the presence of other receptors resulted in a significant demethylation in Trg by phenol even with elevated Trg levels. It is noteworthy that the addition of ribose or serine to RP4324(pCP31) caused very similar changes in the banding pattern of the methylated receptors (Fig. 5b, lanes 2 and 3), although serine caused more increase in the upper group.

Phenol sensing by Tap and Tsr. We reported previously (4) that AW660, which has defects in Tar, Tsr, and Trg, showed a weak tumbling response to phenol. This response was suppressed by the addition of 1 mM glycylleucine, a Tap-mediated attractant (15) (data not shown), suggesting that Tap might be involved in mediating this repellent response.

To test this, we used HCB339(pVB8), with the Tap content maximized by growing the cells in the presence of isopropyl-β-D-thiogalactopyranoside. Phenol caused a significant tumbling response, although this required a concentration of 3 mM or more (Fig. 6a), significantly higher than that (ca. 0.5 mM) at which cells with Trg showed a full response. Subsequent addition of 1 mM glycylleucine suppressed the tumbling response (data not shown). Thus, Tap is effective for mediating a repellent response to phenol, although less so than Trg.

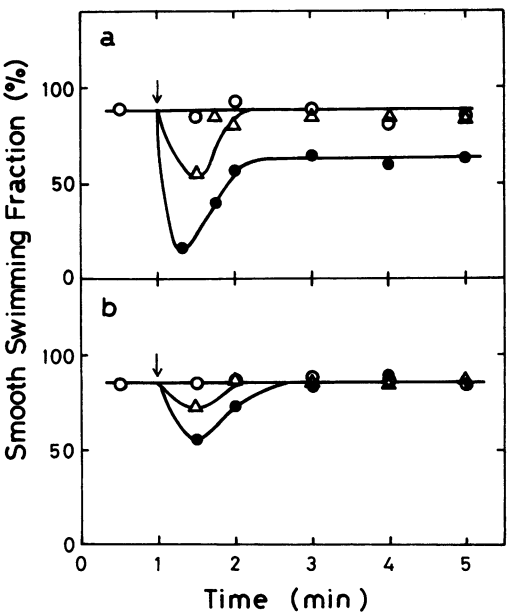

FIG. 6. Repellent response to phenol at pH 7.4 mediated by Tap or Tsr. At the time indicated by the arrow, phenol was added to a concentration of 1 (○), 3 (△), or 5 mM (●), and the changes in the smooth-swimming fraction were monitored at 25°C. (a) HCB339(pVB8) (*tap*$^+$). (b) HCB339(pNM33) (*tsr*$^+$).

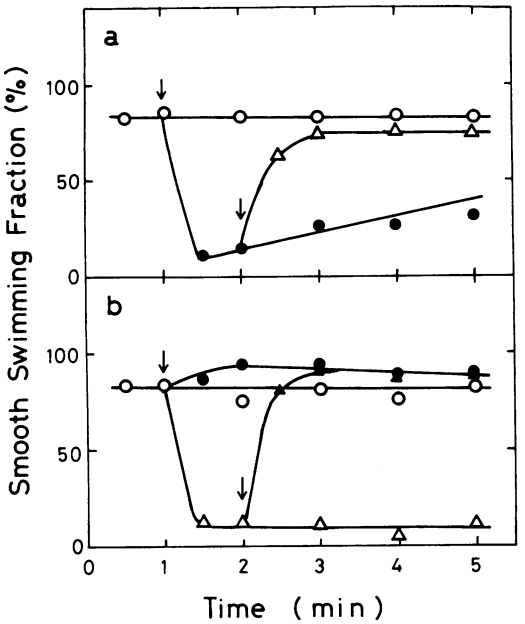

FIG. 7. Intracellular pH sensor activities of Tap and Trg. Responses were measured at pH 6.0. (a) HCB339(pVB8) (*tap*$^+$). Symbols: ○, no addition; ●, 10 mM benzoate at the first arrow; △, 1 mM glycylleucine at the second arrow. (b) HCB339(pCP31) (*trg*$^+$). Symbols: ○, no addition; ●, 10 mM benzoate at the first arrow; △, 1 mM phenol at the first arrow; ▲, 10 mM benzoate at the second arrow.

Although the methylation level of Tsr in RP4324 was not affected by phenol (Fig. 5), cells of HCB339(pNM33), with elevated Tsr levels, showed a weak tumbling response to 5 mM phenol at pH 7.4 (Fig. 6b). Thus, Tsr is a very weak repellent receptor for phenol. No response was seen at a lower pH (6.0), and so the Tsr-mediated response is pH dependent, unlike those mediated by Trg or Tap (not shown).

Internal pH sensor activity of Tap and Trg. Tar and Tsr both function as intracellular pH sensors, although they produce opposite behavioral responses (9). We found that Tap and Trg are also intracellular pH sensors. HCB339 (pVB8) showed incessant tumbling in response to 10 mM benzoate at pH 6.0 (Fig. 7a); this response was completely suppressed by 1 mM glycylleucine. The same treatment in HCB339(pCP31) induced a smooth-swimming response (Fig. 7b) which could be made more pronounced by the prior addition of phenol. Addition of benzoate at pH 7.4 did not induce any response in either Tap- or Trg-containing cells (data not shown). Thus, Tap appears to be a Tsr-like pH sensor, and Trg appears to be a Tar-like pH sensor. Similar results were obtained with 30 mM acetate, although in this case, even HCB339 cells showed significant tumbling after incubation for several minutes at pH 6.0, as has been reported by Wolfe et al. (34).

DISCUSSION

Wild-type *E. coli* cells show an attractant response to phenol which is mediated by the Tar receptor, whereas in the absence of Tar they show a repellent response (4). We have now shown that all of the remaining receptors (Trg, Tap, and Tsr) contribute to this repellent response to some degree. Quantitative analysis of the response of cells with elevated levels of only one of each of these receptors revealed that Trg is most active, Tap is less active, and Tsr is least active in this regard. In wild-type cells, the amounts of Trg and Tap are 1/5 to 1/10 those of Tar and Tsr (13, 30), and so the abundance of Tar as an attractant receptor could account for the net attractant response to phenol in wild-type cells. Consistent with this idea is the observation that increasing the amount of Trg in wild-type cells causes them to show a repellent response to phenol. Thus, we conclude that the amount of Tar relative to Trg (and, to a lesser degree, Tap and Tsr) determines whether *E. coli* cells respond to phenol as an attractant or a repellent.

The activity of Trg for phenol sensing is not necessarily affected by the loss of its activity for ribose and galactose sensing, suggesting that different sites are utilized for mediating each of these stimuli. The phenol-sensing abilities of Trg and Tap are not affected by the pH of the medium in the range from 6.0 to 7.4. Since the pK_a of phenol is 10.0, it is almost entirely in the neutral form in the pH range studied. Therefore, unlike the repellent response to weak acids such as benzoate, the repellent response to phenol is not caused by alteration of the intracellular pH, which is closely regulated by the cell (28). In contrast, the Tsr-mediated response to phenol shows a pH dependence, increasing with increasing pH of the medium. This may indicate that the phenol-sensing site in Tsr is located in its periplasmic domain.

A characteristic property of Trg is its failure to effect adaptation to any type of stimulus when it is the only receptor present (Fig. 1). Consistent with this, neither ribose nor phenol induce significant changes in the methylation level of Trg (Fig. 4). This is not a consequence of the abnormally high amount of Trg in these cells, since cells with the normal amount of Trg also show no adaptation to ribose (1). However, when other receptors are present together with Trg (even at elevated levels of the latter), cells show significant adaptation to both ribose and phenol (Fig. 2), accompanied with changes in the methylation levels of receptors (Fig. 3 and 5). These results may be interpreted by the idea of crosstalk between different kinds of receptors, as has been discussed previously (3, 25). In this connection, it is interesting that ribose and serine caused quite similar changes in the methylation-banding patterns of cells with Tsr and an elevated amount of Trg (Fig. 5b).

The cytoplasmic domains of Tar and Tsr in *E. coli* function as intracellular pH sensors, although their properties are opposite; Tar mediates an attractant response to a pH decrease, whereas Tsr mediates a repellent response (9). Trg and Tap were also found to be active as intracellular pH sensors with opposite properties, Trg being a Tar-like pH sensor and Tap being a Tsr-like pH sensor. This suggests that the cytoplasmic domain of Tap may resemble that of Tsr rather than Tar, even though the *tap* gene is immediately adjacent to the *tar* gene on the chromosome and has been postulated to result from a gene duplication event (33).

Although we have shown that the (normally masked) repellent response of *E. coli* to phenol is primarily mediated by Trg, it remains to be seen whether, in *S. typhimurium*, the dominant repellent response to phenol is mediated by the homologous receptor.

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