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Mutation Plus Amplification of a Transducer Gene Disrupts General Chemotactic Behavior in *Escherichia coli*

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Transducers are transmembrane receptor proteins that generate intracellular signals on stimulation and participate in adaptation by appropriate changes in the level of methylation. The transducer mutation *trg-21* conferred a Trg⁻ phenotype and defective taxis to galactose and ribose but a normal response to other attractants when present in a single chromosomal copy. Amplification of *trg-21* by a multicopy plasmid made host cells generally nonchemotactic. The dominant phenotype resulted from a strong counterclockwise rotational bias of flagellar motors in Che⁻ cells. Apparently, the Trg21 transducer sends a continuous counterclockwise signal to flagella independent of tactic stimulation. It appears that the cell has a homeostatic capacity that is sufficient to compensate for the effect of mutant transducers produced from a single chromosomal copy of *trg-21*, but the capacity is exceeded in cells that have multiple copies of the gene. The Trg21 protein did not have a significant effect on methylesterase activity, indicating that the two global effects of a stimulated transducer, that is, on flagellar rotation and on modification enzymes, can occur independently. The mutant protein exhibited essentially normal turnover of methyl groups but had a drastic defect in deamidation which thus reduced the number of methyl-accepting sites. The *trg-21* mutation substitutes a threonine for Ala-419. This alanine is a conserved residue in all sequenced transducers and is in a region of the carboxy-terminal domain in which homology among the transducers is very high. The Trg21 phenotype implicates this conserved region in the generation of the excitatory signal which is directed at the flagella.

Chemosensory receptors mediate the influence of extracellular chemical signals on cellular behavior. The extent of cellular response is generally proportional to the magnitude of the stimulus. In many instances, proportionality is achieved by grading the duration of the response. This requires adaptation and restoration of the sensory system to a null state, even though the stimulating compound is still present. Mutational alterations that disrupt or uncouple adaptation mechanisms result in cells that are insensitive to normal controls and thus exhibit extreme behavior. For instance, in eucaryotic cells transformed by oncogenes from the usual controls over cell growth and division are uncoupled. An important group of oncogenes codes for altered forms of proteins that normally function in recognition and transduction of extracellular signals (13). In this report we describe a mutated gene of *Escherichia coli* that codes for a chemoreceptor uncoupled from normal adaptation mechanisms and that, like some oncogenes, perturbs general cellular behavior only on amplification of its cellular content.

There is a substantial body of information about sensory behavior and adaptation in the chemotactic system of *E. coli* (11, 29). An unstimulated, swimming cell traces a random walk made up of runs and tumbles which correspond, in wild-type cells, to counterclockwise and clockwise rotation, respectively, of the bacterial flagella. On stimulation the sensory system functions to bias the random walk. Increased occupancy of a ligand-binding site on the extracytoplasmic domain of a membrane-spanning receptor, which is called a transducer protein, induces the cytoplasmic domain of that protein to generate an excitatory signal. The signal shifts the rotational bias of the flagellar motors to a counterclockwise direction. The system is brought back to a null behavioral state, which is indistinguishable from unstimulated behavior, by an increase in the level of methylation of the excited transducer. Specific glutamates in the cytoplasmic domain are modified to form glutamyl methyl esters. An increase in occupancy of a ligand-binding site on a transducer activates glutamyl residues for a net increase in methylation and also affects modification enzymes globally, inhibiting the methylesterase (16, 19, 33) and possibly activating the methyltransferase. It appears that ligand binding and covalent modification send opposing inputs to the excitatory domain of the transducer. When the two inputs are balanced, adaptation is achieved. In an unstimulated or adapted state, flagellar motors switch directions frequently, methyltransferase and methylesterase are moderately active, and a constant but dynamic level of transducer methylation is maintained. Transducers have four to six methyl-accepting sites (15, 18, 32). Two of these glutamates become available as a result of deamidation of glutamines in a reaction catalyzed by the same enzyme that catalyzes demethylation (14). Rates of deamidation are affected by sensory stimuli in the same manner as rates of demethylation (28). It appears that adaptive balance is a function of charged versus neutral residues at the sites of covalent modification. Amides and methyl esters appear to be roughly equivalent as neutral residues, and thus the presence of two amides among the modification sites on a transducer may ensure that newly synthesized molecules are poised in an adaptation state between the two extremes (2).

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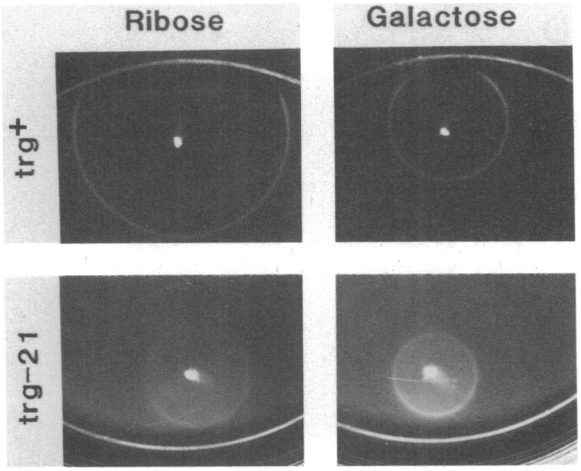

FIG. 1. Tactic responses of wild-type and *trg-21* mutant *E. coli* cells on semisolid agar swarm plates. Approximately 10^8 freshly grown, motile cells of *E. coli* OW1 (*trg*$^+$) or CP351 (*trg-21*) were used to inoculate plates containing 0.25% agar, minimal salts, the required amino acids, and 0.1 mM ribose or 0.05 mM galactose. Plates were photographed after incubation for 20 h at 35°C.

Four different transducers have been identified in *E. coli*. The Tsr protein binds serine (4, 12); Tar interacts with aspartate (4) and ligand-occupied maltose-binding protein (20); Trg recognizes ligand-occupied galactose- and ribose-binding proteins (10); and Tap is linked to a putative dipeptide-binding protein (22). Tsr and Tar are present at approximately 1,600 and 900 copies per cell, respectively, while the cellular content of Trg and Tap is substantially less (approximately 150 copies per cell [11]). We characterized a mutation, *trg-21*, that creates a Trg protein which is usually altered in transducer functions. When present in a single chromosomal copy, *trg-21* confers a partially defective Trg phenotype. However, when present on a multicopy plasmid, the mutated gene causes the host cell to become generally nonchemotactic.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strain AW721 is a derivative of AW562 identified as a *trg* mutant after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (C. B. Ball, Ph.D. thesis, University of Wisconsin, Madison, 1979). The mutant strain was a gift from J. Adler. The *trg-21* mutation contained in AW721 was introduced into a variety of strains by cotransduction with *zdb*::Tn*5* (7). The pBR322 hybrid plasmids carrying wild-type *trg* (pCP31) and *trg-21* (pCP34) have been described previously (24). Strains CP177 and CP362 are derivatives of OW1 containing, respectively, Δ*trg-100*, a deletion including the entire *trg* gene, or Δ*trg-100* plus Δ*tsr-7028* and Δ(*tar-tap*)*5201* (25).

Chemicals and enzymes. L-[*methyl*-^{3}H]methionine (10 to 15 Ci/mmol), L-[^{35}S]methionine (1,000 Ci/mmol), and [α-^{32}P]dATP (800 Ci/mmol) were obtained from New England Nuclear Corp., Boston, Mass. Enzymes and other supplies for recombinant DNA techniques were obtained from New England BioLabs, Inc., Beverly, Mass.

Assays and procedures. Procedures for growth and preparation of cells (10), semisolid agar swarm plates (25), tethering (10), in vivo methylation (6), preparation and radiolabeling of minicells (6), determination of nucleotide sequences (25), and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and fluorography (6) were as described previously. Steady-state evolution of [^{3}H]methanol was determined (16, 17) for a suspension of 4×10^8 cells per ml at 30°C in the presence of chloramphenicol (200 μg/ml) and 40 μCi of [*methyl*-^{3}H]methionine per ml at approximately 3 μM. Samples of 100 μl were removed at appropriate times, treated with 5% trichloroacetic acid, centrifuged, and analyzed for ^{3}H-labeled volatile compounds (16).

RESULTS

Behavior of *trg-21* mutants. When the *trg-21* mutation is present in the chromosomal copy of *trg*, the ability of the cell to respond to gradients of galactose or ribose is substantially reduced but not eliminated. The chromosomal mutation has no detectable effect on response to attractants recognized by transducers other than Trg. In capillary assays, the mutant cells do not accumulate in response to either galactose or ribose, even at concentrations 1,000-fold above the saturating values observed for wild-type cells (Ball, Ph.D., thesis). However, *trg-21* mutants form distinct, albeit abnormal, chemotactic rings on semisolid agar swarm plates containing galactose or ribose (Fig. 1). As noted previously (25), the swarm plate procedure is far more sensitive to marginal tactic abilities than is the capillary assay. The phenotype conferred by *trg-21* was changed dramatically by amplification of the cellular content of the mutated gene. Responses to galactose and ribose were no longer detectable; and responses to serine, aspartate, tryptone, and maltose were all drastically reduced in a chemotactically wild-type host harboring a multicopy pBR322–*trg-21* hybrid plasmid (Fig. 2). In contrast, a related pBR322-*trg*$^+$ plasmid did not perturb general tactic behavior. The effect of amplified *trg-21* was even more pronounced for a hybrid derivative of pUC13 (Fig. 2) which is known to exhibit a higher copy number per cell (34). The origin of the generally nonchemotactic phenotype was revealed by examination of motile behavior. Free-swimming Che$^-$ cells exhibited almost no tumbles, and tethered cells rotated almost exclusively in the counterclockwise, smooth-swimming direction. Specifically, introduction of pCP31, a pBR322-*trg*$^+$ hybrid plasmid, into the Che$^+$ strain CP177 created a strain that was unaltered in its Che$^+$ phenotype in which >90% of tethered cells exhibited normal, reversing behavior. The presence of pCP34, which carries *trg-21*, created a Che$^-$ strain with flagella locked in the counterclockwise mode; 100 of 103 tethered cells examined spun only counterclockwise. These observations indicate that the dominant Che$^-$ phenotype induced by amplification of the *trg-21* gene is the result of an altered rotational bias of the flagellar motors, which eliminates the possibility of attractant-mediated modulation of the balance between runs and tumbles. It appears that an amplified number of Trg21 proteins in a cell creates a strong counterclockwise signal, locking flagellar motors in one mode independent of either the presence of attractant stimuli or the adaptation state of the sensory system.

However, expression of dominance by *trg-21* does not require an increased cellular dosage of the mutated gene. In appropriate host cells dominance is exerted by a single chromosomal copy of *trg-21*. The crucial factor appears to be the extent to which cellular, homeostatic mechanisms can counterbalance the continuous counterclockwise signal generated by the Trg21 protein. The situation is illustrated in complementation tests with wild-type *trg* and *trg-21*. Introduction of a single copy of wild-type *trg* into the *lac* region of a strain with *trg-21* in the usual locus at 31 min created a

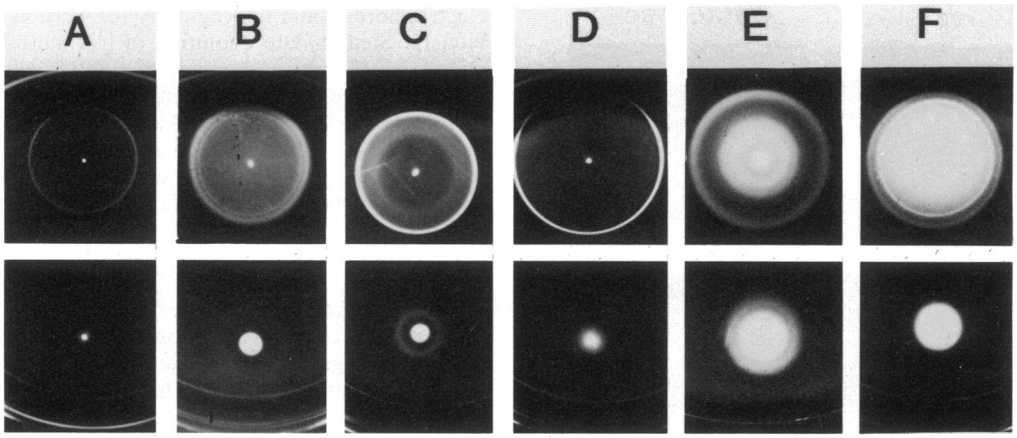

FIG. 2. Tactic responses of cells harboring hybrid plasmids carrying wild-type *trg* or *trg-21* on semisolid agar swarm plates. Conditions were as described in the legend to Fig. 1. *E. coli* CP177 cells harboring the pBR322-*trg*$^+$ hybrid plasmid pCP31 (upper row, panels A to E) or the pBR322–*trg-21* plasmid pCP34 (lower row, panels A to E) were used to inoculate plates containing minimal salts plus ribose (A), 0.1 mM serine and 1 mM glycerol as a source of energy (B), 0.1 mM aspartate and 1 mM glycerol as a source of energy (C), 0.1 mM maltose (D), or 1% tryptone (E). Panels F show responses of CP177 cells harboring a pUC13-*trg*$^+$ plasmid (upper row) or a pUC13-*trg-21* plasmid (lower row) on tryptone. Plates were photographed after incubation for 20 h (A to D) or after incubation for 8 h (E and F).

Trg$^+$ phenotype (construction of such strains has been described previously [21]). Thus, in otherwise chemotactically wild-type cells, *trg-21* was recessive to and complemented by the wild-type allele. However, in a strain with inactivated *tsr*, *tar*, and *tap* genes, *trg-21* was dominant over, and thus not complemented by, wild-type *trg*. The origin of this difference in complementation patterns can be understood in light of the substantial defect in behavioral homeostasis of these triple transducer mutants (8). When adapted and unperturbed, the triple mutants exhibit a pattern of rotational reversals that is relatively normal, but the mutants are greatly retarded in establishing a normal rotational balance after stimulation by attractants (8). Extended adaptation times are observed with stimuli for which adaptation is dependent on methylation (29), as well as with stimuli for which adaptation is independent of methylation (31), implying that adaptation is affected at the level of a common signal generated by all receptors rather than at the level of receptor modification. Thus, in cells defective in the normal control of excitatory signals, the Trg21 protein exerts a dominant effect, even at a low, normal dosage. This indicates that amplification per se does not induce the Trg21 protein to generate a counterclockwise signal, but rather, amplification increases the magnitude of the signal to a value that exceeds the homeostatic capacity of the cell.

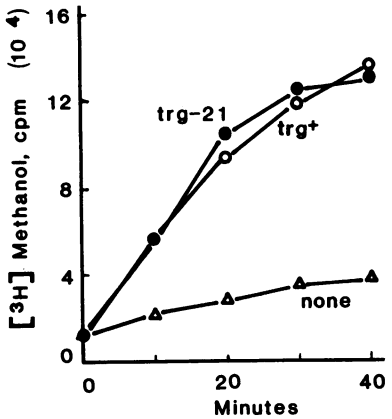

FIG. 3. Production of methanol by cells harboring plasmids carrying wild-type *trg* or *trg-21* (see the text for details). Cells tested were *E. coli* CP362, the strain missing all transducers (none); CP362 harboring pCP31 (*trg*$^+$); and CP362 harboring pCP34 (*trg-21*).

Activity of modification enzymes. Stimulation of a transducer by an increase in attractant occupancy has two documented, global effects on the tactic system: all flagellar motors receive counterclockwise signals and the methylesterase is inhibited (16, 19, 33). The Trg21 protein appears to send a continuous signal to the flagella but does not affect the modification enzyme. Methylesterase activity can be determined easily by monitoring the release of methanol, a product of demethylation (16). Determination of methanol production by cells made Che$^-$ by amplification of *trg-21* indicated that the mutant protein has little effect on methylesterase activity (Fig. 3). Methanol production by cells harboring a *trg*$^+$ or *trg-21* plasmid was indistinguishable for either an otherwise transducer-positive (data not shown) or transducer-negative (Fig. 3) host cell. Consistent with unperturbed methylesterase activity in cells made Che$^-$ by amplified *trg-21*, *methyl*-^{3}H-labeled forms of Tsr and Tar, displayed on SDS-polyacrylamide gel, did not exhibit the distinct enrichment for heavily methylated forms (3, 9, 17) that is characteristic for cells with reduced esterase activity (data not shown).

Covalent modification of the Trg21 protein. The *trg-21* mutation does not have a global effect on transducer modification, but it does have a specific and drastic effect on modification of the mutant gene product. The Trg21 protein is essentially not deamidated. In minicells containing an active methylesterase, but no methyltransferase, there was only a single electrophoretic form of [^{35}S]methionine-labeled Trg21 (Fig. 4B), corresponding to an unmodified polypeptide observed in cells without esterase or transferase (Fig. 4A). In contrast, the wild-type Trg protein in cells with esterase was distributed among one unmodified and three slower-migrating, deamidated forms (18) (Fig. 4B). Insensitivity to deamidation was characteristic of the entire population of Trg21 proteins, not just newly synthesized polypeptides detected by labeling with [^{35}S]methionine. In minicells harboring plasmids carrying *trg*, sufficient Trg protein is pro-

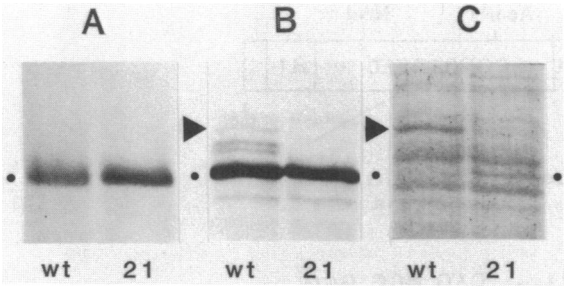

FIG. 4. Electrophoretic forms of wild-type and mutant Trg proteins. (A and B) Autoradiographs; (C) Coomassie brilliant blue staining of SDS-polyacrylamide gels. Only the region surrounding the position with an apparent molecular weight of 60,000 is shown. (A and B) [^{35}S]methionine-labeled polypeptides synthesized in minicells harboring pCP31 (labeled wt for *trg*$^+$) or pCP34 (labeled 21 for *trg-21*) and derived from *cheR cheB* (A) or *cheR cheB*$^+$ (B) cells. (C) Stained gel from which the autoradiograph in panel B was made. The position of the unmodified Trg protein is indicated by dots, and the position of the protein that was deamidated twice is indicated by arrowheads.

duced to make Trg polypeptides visible as stained bands on SDS-polyacrylamide gels (5). Such stained patterns revealed that in minicells with active esterase, the predominant form of normal Trg is deamidated twice, while only the unmodified form of Trg21 is visible (Fig. 4C). The Trg21 protein is not completely refractory to deamidation. Multiple cycles of stimulation of minicells by the general repellent glycerol (23) resulted in formation of detectable but limited quantities of deamidated forms of [^{35}S]methionine-labeled Trg21 (data not shown).

The Trg21 protein functions as a methyl-accepting substrate. In otherwise wild-type cells or in cells lacking the other transducer genes, Trg21 acquires *methyl*-^{3}H-labeled groups, under standard labeling conditions, to an extent not substantially different from that of normal Trg. However, the pattern of electrophoretic forms of *methyl*-^{3}H-labeled Trg21 is quite unlike the normal pattern (Fig. 5). Only two labeled bands appeared, one at the position of unmodified protein and the other at a position of lower apparent molecular weight. Results of an analysis to be described in detail elsewhere (D. M. Nowlin and G. L. Hazelbauer, manuscript in preparation) demonstrate that the two forms are the result of methylation of the Trg21 protein primarily at two sites, one at which methylation does not affect electrophoretic mobility of the polypeptide and the other at which methylation leads to increased mobility. Three methyl-accepting sites that are active in a wild-type protein are blocked in Trg21 because deamidation does not occur. There is no indication that the Trg21 protein induces altered modification of other transducer proteins, not even normal Trg produced in the same cell. *methyl*-^{3}H-labeling of the *trg*$^+$ *trg-21* partial diploid described above revealed a set of labeled bands corresponding to superposition of wild-type and *trg-21* mutant patterns (data not shown).

Identity of the mutational substitution. In vitro recombination experiments with *trg*-containing plasmids located the *trg-21* mutational change between an *Asu*I site and an *Nru*I site (Fig. 6) and demonstrated that this segment of the gene conferred both the dominant Che$^-$ feature and the defect in deamidation of the Trg21 protein. Determination of the nucleotide sequence of the entire restriction fragment revealed a single difference from the normal gene, a cytosine to thymine transition at position 1257, changing an alanine to threonine at position 419 of Trg (Fig. 6). This position is within a segment of extensive homlogy among all transducers. Over 90% of the amino acid sequence corresponding to Trg residues 370 to 424 is identical among the four transducers (2). Ala-419 is one of the conserved amino acids.

DISCUSSION

Dose-dependent dominance and cellular homeostasis. The *trg-21* mutation resembles a group of dominant Che$^-$ mutations that have been described previously (26). Those mutations, termed *cheD*s, all map within the *tsr* gene (3). Results of recent studies (3, 17) provide strong support for the notion that *cheD* mutations lock the Tsr transducer in an excited (counterclockwise-signaling) state that is insensitive to the adaptive action of methylation. The model suggests that the mutant protein sends a sustained counterclockwise signal to the flagella, eliminating tumbles and thus the possibility of tactic migration in response to any compound. However, nothing in this explanation is specific for the Tsr transducer. In a normal cell, an increase in ligand occupancy of any transducer can shift the rotational bias of the flagellar motors to an exclusively counterclockwise direction. Thus, it seems that any transducer could be altered to create a permanently signaling form. Yet, among hundreds of independent Che$^-$ and transducer mutants (26, 28), dominant Che$^-$ mutations have been identified previously in only one of the four transducer genes. The dose-dependent dominance of *trg-21* suggests an explanation. It appears that a normal cell has a distinct but limited capacity to compensate for the counterclockwise signal that is continuously generated by the Trg21 protein. The capacity is sufficient to compensate for the number of mutant proteins produced from a single chromosomal copy of *trg-21* but is exceeded by the quantity synthesized from multicopy plasmids carrying *trg-21*. We suggest that the same pattern would apply to analogous mutations in *tar* or *tap*. For *tsr* the number of *cheD* proteins produced from a single chromosomal gene is sufficient to perturb rotational balance. This difference is consistent with estimates of a relative cellular content of 11 Tsr:6 Tar:1 Trg:1 Tap (11). Our suggestion predicts that mutations conferring dominant Che$^-$ phenotypes would be found in *tar* and *tap*, as well as *trg*, if mutated genes were carried on multicopy plasmids. A survey of mutagenized hybrid plasmids has identified candidates for such mutation in *tar* (C. Wolff and J. S. Parkinson, personal communication).

What is the basis for the cellular capacity to compensate for a continuous excitatory signal from a mutant transducer?

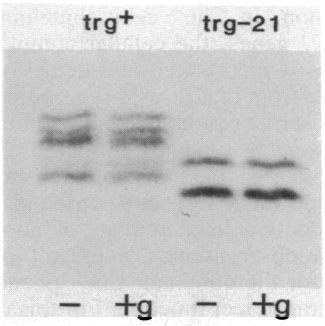

FIG. 5. *methyl*-^{3}H-labeled forms of wild-type and mutant Trg proteins. The figure is a fluorograph of a SDS-polyacrylamide gel of whole-cell extracts from the transducer deletion strain CP362 harboring pCP31 (*trg*$^+$) or pCP34 (*trg-21*). Cells were either unstimulated (–) or stimulated with 1 mM galactose (+g).

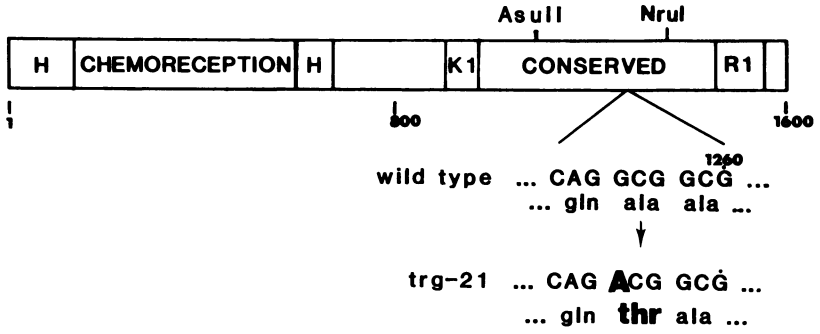

FIG. 6. The *trg-21* mutational change. The box diagram shows important features of the deduced amino acid sequence of the Trg protein. Abbreviations and explanations: H, hydrophobic, putative membrane-spanning regions; chemoreception, the ligand-binding, extracytoplasmic domain; K1 and R1, regions containing methyl-accepting and deamidation sites; conserved, the region of substantial homology among the four sequenced transducers. The scale is numbered in bases beginning with the first A of the coding sequence of *trg*. The positions of relevant restriction sites are indicated. The mutational change is indicated in boldface type.

It seems likely to be related to the normal mechanisms of signal destruction. Adaptation requires that signals be short-lived so that excitation does not persist after transducers cease the production of signals. In addition to any specific controls by the sensory system, the rate of decay is influenced by the concentration of the signal and of metabolically related species. Continued production of the signal over periods substantially longer than normal adaptation times could bring into play mass action and homeostatic relationships that would reduce the signal concentration. Cells unable to adapt normally because of defective modification enzymes exhibit distinct but limited capacity to shift the behavioral balance back toward the null state (27, 30). This capacity may reflect the same homeostatic influences on signals that could account for tolerance to low cellular doses of *trg-21*.

We have suggested that the Trg21 protein is locked in a signaling mode. An alternative explanation for the dominant Che^- phenotype conferred by amplified *trg-21* is that the mutant protein inactivates normal transducers by direct physical interaction in heterologous tetramers. A mutant that is missing all transducers exhibits exclusively smooth swimming behavior (8), and thus, a cell with all of its transducer proteins inactivated because of interaction with mutant polypeptides should exhibit a similar abnormal behavior. Although it is difficult to eliminate this possibility, several features of the Trg21 phenotype argue against it. The mutant protein itself is not inactive. It mediates a reduced but distinct tactic response (Fig. 1). In addition, the protein functions as an essentially normal substrate for methylation and demethylation. In Che^- cells containing an amplified number of *trg-21* genes, the cellular complement of transducer molecules is methylated and demethylated at a rate indistinguishable from that of wild type. Stimulation of such Che^- cells with serine results in changes in rates of demethylation that resemble changes observed in normal cells (Graham and Dahlquist, personal communication). Thus, the Trg21 protein and other transducer proteins present in the Che^- cells are active in a number of normal transducer functions. These observations are difficult to incorporate in an explanation for the dominant Che^- phenotype based on inactivation of transducer function. For this reason we favor the active signaling model to explain the Trg21 phenotype.

A role for the highly conserved region in signaling. Within the substantial region of sequence homology among the carboxy-terminal domains of the transducers, there is a segment of over 50 residues that is almost entirely conserved (2, 21). This conservation suggests that these residues could participate in functions common to all transducers: signal generation and interaction with modification enzymes. The data presented in this study establish that at least one conserved residue is directly linked to signal generation and imply that the highly conserved region includes the signaling domain.

Asymmetry of global effects induced by mutant transducers. A stimulated transducer has a global effect on two different targets. Stimulation shifts the rotational bias of all flagellar motors and alters the activity of the cellular complement of demethylase. The nature of the linkage of transducers to either target is not understood. The *trg-21* mutant provides an example in which only one of the two targets is affected. A threonine in place of Ala419 causes continual signaling to the flagella but has little effect on the methylesterase. Either the mutational substitution activates the linkage to only one target, implying that in the normal transducer different amino acids are involved in the generation of the two global effects, or the substitution generates a continuous signal that is strong enough to affect the flagella but not the enzymes, implying a difference in the sensitivity of the two targets or in the amplification within the two linkages. Some *cheD* mutants also exhibit an asymmetry in their chronic effects on the two global targets. For instance, *cheD192* has a drastic effect on rotational bias (3) but a minimal effect on esterase activity (17). The *trg-21* mutant seems to represent one extreme of a spectrum of dominant Che^- phenotypes, ranging from drastic effects on both global targets to a drastic effect on only the flagella.

The mutational substitution at position 419 creates a Trg protein with some, but not all, of the features of a transducer that is stimulated and not yet adapted. In the Trg21 protein, excitatory signaling and activation of the adaptation system are uncoupled. Flagellar motors receive a continuous counterclockwise signal, but this global signal is not accompanied by either a global effect on modification enzymes or, apparently, a specific activation of methyl-accepting groups for net methylation. Positive stimulation of a normal transducer reduces the rate of deamidation (28), and thus, the lack of deamidation of Trg21 may reflect an aspect of transducer structure that is locked in a stimulated state. It is interesting that Trg21 is blocked only for deamidation and not for demethylation, even though the same enzyme catalyzes both reactions at closely linked, chemically related sites. The features of Trg21 are consistent with a model for transducer stimulation in which ligand occupancy induces distinct

changes at more than one site on the intracellular domain of a transducer. Thus, a mutational substitution that mimics the excitatory change at one site would not necessarily affect other sites. This appears to be the case for the Trg21 protein.

Characterization of the *trg-21* mutation has provided insights into the bacterial sensory system. The data demonstrate that a transducer can generate the global excitatory change without generating adaptational changes and suggest that the cell has a homeostatic capacity that buffers the sensory system under certain abnormal conditions. The ability of an uncoupled chemoreceptor to disrupt general cellular behavior is a function of cellular homeostatic capacity. In *E. coli*, as well as in transformed eucaryotic cells (1), a crucial factor can be contributed by amplification of a mutated gene.

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