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Limited Homology Between Trg and the Other Transducer Proteins of *Escherichia coli*

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Transducers are transmembrane proteins that are central to the chemotactic system of *Escherichia coli*. The proteins transduce ligand recognition into an excitatory signal and function in adaptation as methyl-accepting proteins. The transducer genes *tsr*, *tar*, and *tap* have extensive homology with each other. However, previous studies revealed little indication of homology between those three transducer genes and a fourth gene, *trg*. We investigated the relationship between *trg* and the other genes by blot-hybridization experiments and the relationship between Trg and the other transducer proteins by immune precipitation and experiments with an antiserum raised to purified Trg protein. In experiments in which 35% mismatch would be tolerated, weak hybridization of *trg* was detected to a DNA fragment containing *tar* and *tap* but not to a fragment containing *tsr*. In experiments in which only 30% mismatch would be tolerated, no *trg* hybridization was apparent either to total chromosomal DNA or to DNA from hybrid plasmids carrying the other transducer genes. An anti-Trg serum formed immune precipitates with the Tsr and Tar proteins as well as with the Trg protein to which it was raised. We conclude that there is homology between Trg and the other transducers, but the homology is more limited than that shared among the other transducers. Furthermore, we found no indication of additional transducer genes closely related to *trg*. Thus, the *trg* gene is a somewhat distant cousin within a single transducer gene family of *E. coli*.

Motile bacteria make net progress along chemical gradients by altering their turning frequency in response to changes in concentration. In *Escherichia coli*, chemotactic behavior is the result of the functioning of a multicomponent sensory system that links receptors on the cell surface to the rotary motor of bacterial flagella (9). Tactic behavior can be divided into two parts, excitation and adaptation. In excitation, a change in occupancy of receptor sites results in a rapid shift in the normal balance between the two directions of flagellar rotation to rotation primarily or exclusively in one direction. Adaptation, the reestablishment of the normal balance, occurs seconds to minutes after excitation, depending on the nature and magnitude of the stimulus. Transmembrane proteins, called transducers, are central to both excitation and adaptation. Transducers interact directly with attractant molecules or with attractant-receptor protein complexes and generate the excitatory signals that affect flagellar rotation. Adaptation is correlated with covalent modification of the transducer molecules through which excitation has occurred. The modification is carboxyl methylation or demethylation of specific glutamyl residues in the transducer proteins.

Three different genes, *tsr*, *tar*, and *trg*, have been identified as coding for functional transducers in *E. coli*. The Tsr protein contains a receptor site for serine, the Tar protein binds aspartate as well as ligand-occupied maltose-binding protein, and the Trg protein mediates response to compounds recognized by the galactose- and ribose-binding proteins. Proteolytic maps of *methyl*-^{3}H-labeled peptides derived from transducer proteins reveal similarities between the Tsr and Tar proteins (4) but little similarity between the Trg protein and the other two transducers (6). The *tsr* and *tar* genes hybridize strongly to each other as well as to the *tap* gene, which codes for a transducer of unknown function (3). In contrast, no hybridization between *tar* and *trg* was detected in conditions allowing 25% mismatch (3). The amino acid sequences of Tsr, Tar, and Tap are ca. 80% homologous in their C-terminal halves (11). Thus, it seems likely that all three genes were

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derived from a common ancestral gene by gene duplication or fusion.

In this study, we have investigated the degree to which the *trg* gene and its product are related to the other transducer genes and their respective products. We found a distinct homology between the Trg protein and other transducers. However, the homology is much more limited than that shared among the other transducers.

MATERIALS AND METHODS

Bacterial strains and plasmids. HB233 is an *E. coli* K-12 strain that is wild type for chemotaxis. HB235, HB237, and HB238 are derivatives of HB233, carrying *trg-2*::Tn*10*, *tsr-49*, and *tar-52*Δ*1*, respectively. Each mutation results in the total lack of production of the respective transducer (7). The plasmids used are described in Fig. 1.

Chemicals. Restriction endonucleases were purchased from New England Biolabs, Inc., Beverly, Mass. and Bethesda Research Laboratories, Inc., Gaithersburg, Md. L-[*methyl*-^{3}H]methionine (12 to 15 Ci/mmol) and nick translation systems containing [α-^{32}P]dCTP (>600 Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass. Chloramphenicol, phenylmethylsulfonyl fluoride (PMSF), amino acids, and D-allose were from Sigma Chemical Co., St. Louis, Mo. All other chemicals were reagent grade.

Media. The growth media used were Luria broth, tryptone broth, and a minimal salts medium (7).

Preparation of DNA. Total chromosomal DNA from strain C600 was prepared from 300 ml of stationary culture in Luria broth. Cells were harvested by centrifugation and suspended in 65 ml of 0.1 M NaCl–50 mM Tris-hydrochloride (pH 8.1)–50 mM EDTA (STE). Sodium dodecyl sulfate (SDS) and protease K were added to final concentrations of 1% and 50 μg/ml, respectively. After agitation for 60 min at 35°C, the suspension of lysed cells was extracted three times with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1). Nucleic acids were precipitated from the phenol-extracted lysate with ethanol at 0°C and resuspended in 0.1× STE. This solution was made one time in STE and agitated for 60 min at 35°C in the presence of 50 μg of RNase A per ml. After extraction with phenol, chloroform, and isoamyl alcohol, the DNA was precipitated with ethanol and resuspended in 0.1× STE, the suspension was extracted with ether, and the DNA was once again precipitated with ethanol. Finally, the DNA was dried and suspended in 10 mM Tris–1 mM EDTA (pH 8.0). Plasmid DNA was prepared as described previously (6).

Analysis by blot hybridization. DNA was digested with restriction endonucleases under the conditions recommended by the manufacturers. A digestion mixture contained 0.5 μg of plasmid DNA plus 5 U of enzyme or 3.5 μg of chromosomal DNA and 20 U of enzyme. The fragments produced by digestion were separated by electrophoresis on agarose gels (6), denatured by alkaline treatment, and then electroblotted onto nitrocellulose filters. The filters were dried for 2 h at 80°C and then treated overnight at 49 or 55°C in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 10× Denhardt solution (Denhardt solution is 0.02% Ficoll, 0.02% bovine serum albumin, and 0.02% polyvinylpyrrolidone). Plasmid DNA used as a probe was labeled with [^{32}P]dCTP by nick translation as described by Rigby et al. (16) with a nick translation kit (New England Nuclear). The probe was heat

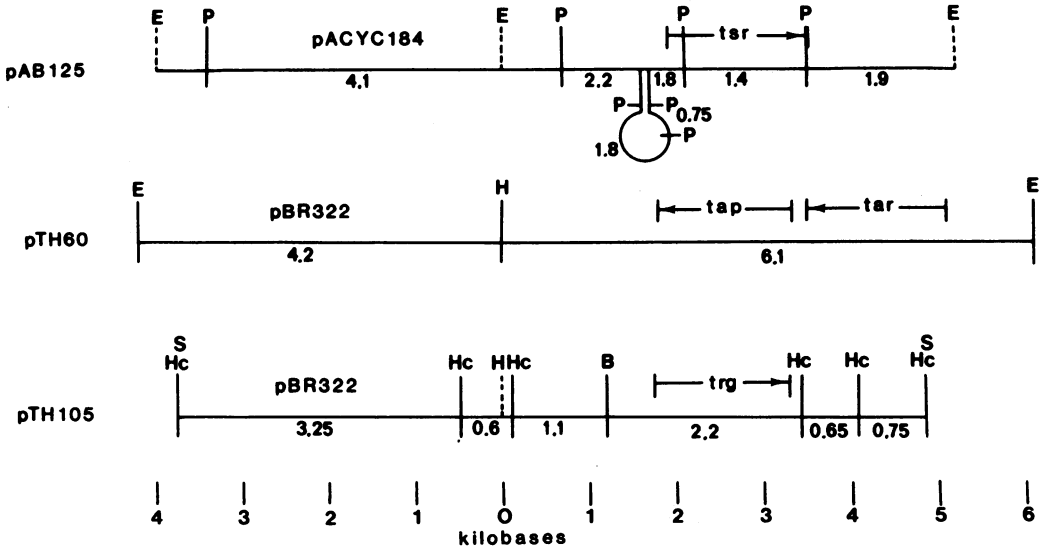

FIG. 1. Restriction maps of hybrid plasmid carrying transducer genes. The linear maps of these circular plasmids are positioned with the junction between vector and cloned insert at 0 kilobases. Restriction sites that were cleaved in the experiments in Fig. 2 are drawn as solid lines. Other sites are indicated with dashed lines. The size of fragments generated is indicated below each map. The stem-loop structure in the map of pAB125 represents Tn*5* inserted at the position indicated. The position and direction of transcription of the transducer genes are indicated above each map. Restriction endonuclease sites are symbolized as follows: E, *Eco*RI; P, *Pvu*II; H, *Hin*dIII; S, *Sal*I; Hc, *Hin*cII; B, *Bgl*II.

denatured just before use. Hybridization was carried out in 5× SSC–5× Denhardt solution–20 μg of sonicated and heat-denatured salmon sperm DNA per ml–20 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA–0.1% SDS for 48 h at 49 or 55°C. The filters were then washed at the hybridization temperature with 3.4× (49°C) or 3× (55°C) SSC–5 mM EDTA–0.2% SDS. Stringency conditions were determined by using the relationship of melting temperature to salt concentration and the percentage of G&C given by Schildkraut and Lifson (17) and the relationship of mismatch to melting temperature (−1.4°C in melting temperature for each 1% of mismatch) given by Hyman et al. (10).

Preparation of Trg for production of antisera. Minicells were prepared (5, 6) from 8 liters of stationary phase culture of TH1301, a minicell-producing strain carrying pTH57, a pBR322-*trg* hybrid plasmid (6). Each liter of culture yielded an amount of minicells equivalent to 60 to 80 optical density units per ml (560 nm) of a whole-cell suspension. Minicells were subjected to SDS-polyacrylamide gel electrophoresis in a discontinuous gel containing 11% acrylamide in the separation gel. The gel system was based on that of Laemmli (12), with the following modifications: the stacking gel contained 0.062 M Tris-phosphate, 0.1% SDS, and 5% acrylamide at pH 7.8. The separation gel had 25% of the concentration of bis-acrylamide used by Laemmli (12) and a pH of 8.2. The Tris-glycine running buffer was at pH 7.8. In this gel system, proteins with apparent molecular weights below 50,000 run with the ion front, but separation of the various forms of Trg is optimal. In fact, in this system, the slowest migrating form of Trg (band 1), representing polypeptide chains which have been modified twice in the CheB-dependent reaction and carry one or no methyl groups (6), was separated from all other proteins contained in the minicells. Band 1 was excised from 30 slab gels, 15 cm wide and 1 mm thick, which had been stained with Coomassie brilliant blue and dried onto filter paper. The excised strips were rehydrated in 20 mM Tris-hydrochloride (pH 7.8)–0.1% SDS–1 mM EDTA and placed at one end of a glass tube which was plugged in the middle with polyacrylamide of the same composition as the stacking gel described above. The tube was sealed at both ends with dialysis tubing and placed in an electrophoresis chamber. Upon electrophoresis, the protein moved through the plug into a small volume of 1 mM Tris-hydrochloride (pH 6.8)–0.1% SDS at the other end of the tube. The yield in this electrophoresis step, determined in separate experiments by using radioactive protein, was 80%. Approximately 100 μg of Trg protein was obtained and used for production of antisera in rabbits.

Antisera production. An initial injection of 10 μg of Trg protein in 0.5 ml of Hanks balanced salt solution, mixed thoroughly with 0.5 ml of complete Freund's adjuvant, was divided equally among the footpads of a rabbit. At 2-week intervals, injections of the same amount of antigen mixed with 0.5 ml of incomplete Freund adjuvant were given at two intramuscular sites. At 8 days after the fourth injection, the rabbit was bled, and the serum was collected and stored at −20°C.

Labeling with *methyl*-^{3}H. Cells were radioactively labeled with L-[*methyl*-^{3}H]methionine as described by Hazelbauer and Engström (7). At 30 min after the addition of isotope, the attractants serine, aspartate, and allose were added to a final concentration of 10 mM each. After 30 min, the suspensions were cooled in an ice bath and harvested by centrifugation.

Immune precipitation. *methyl*-^{3}H-labeled samples of 1.25×10^8 cells were solubilized by incubation for 10 min in 100 μl of 0.5% SDS–10 mM Tris-hydrochloride (pH 6.8) at 55 or 100°C. A 1-ml amount of 1% Triton X-100–10 mM Tris-hydrochloride (pH 7.4)–5 mM magnesium acetate–60 mM NH_4Cl–0.1 mM PMSF was added to the sample and centrifuged at 8,000 × *g* in an Eppendorf model 5412 centrifuge. A 50-μl amount of rabbit anti-Trg serum was added to the supernatant, and the mixture was incubated on ice for 15 to 30 min, frozen solid, and thawed at room temperature. The thawed solution was centrifuged as above, and the pellet was washed with the following series of solutions: (i) 0.15 M NaCl–0.5% Triton X-100–10 mM Tris-hydrochloride (pH 6.8)–5 mM EDTA–0.1 mM PMSF; (ii) the same as (i) but with 0.5 M NaCl; (iii) solution (i); (iv) 10 mM Tris-hydrochloride (pH 6.8); and (v) solution (iv). The final pellet is the immune precipitate. These pellets were suspended in 15 μl of electrophoresis sample buffer and boiled for 2 min. SDS-polyacrylamide gel electrophoresis was done essentially by the method of Laemmli (12) with the modifications described by Randall and Hardy (15) or those noted in an earlier section. Gels were treated for fluorography with En^{3}Hance (New England Nuclear), dried, and exposed to X-ray film at −70°C.

RESULTS

Hybridization experiments. We used pTH105, a pBR322 hybrid plasmid carrying *trg* (Fig. 1), as a radioactive probe in hybridization experiments. Under conditions similar to those used to reveal homology among the *tsr*, *tar*, and *tap* genes (3) in which 30% mismatch would be tolerated (55°C; 3.4× SSC), the probe hybridized with only one fragment generated by digestion of chromosomal DNA with *Eco*RI, *Hin*dIII, or *Pst*I, respectively (data not shown). In each case, the hybridized fragment had the length predicted for the restriction fragment that includes *trg* (1). Thus, we found no evidence for a gene in *E. coli* that possessed extensive homology with *trg*. Boyd et al. (3) reported that hybridization at 60°C of a *tar*-containing probe to restriction fragments of total chromosomal DNA did not reveal any candidates for *tar-trg* hybrids. However, the *trg* gene is located adjacent to the locus of termination of chromosomal replication, *terC*, and DNA from this region is under represented in samples of total chromosomal DNA. In fact, hybridization of the radioactively labeled *trg* probe to fragments of chromosomal DNA resulted in only modest labeling of the fragments containing *trg*. Thus, potential homologies between *trg* and the transducer genes *tsr*, *tar*, and *tap* were investigated directly by using hybrid plasmids carrying those genes. Appropriate restriction endonucleases were used to generate DNA fragments in which cloned genes were separated from vector DNA (Fig. 1), and

hybridization of a radioactive probe to the fragments was examined (Fig. 2). In a control experiment, hybridization was detected between radioactive probe and every fragment of the pBR322-*trg* plasmid (Fig. 2C). The pBR322 hybrid plasmid pTH60 (6) carries a 6.1-kilobase-pair (kbp) insert that includes both *tar* and *tap* (Fig. 1). At 55°C, the only hybridization observed was to the 4.2-kbp band of pBR322 DNA (data not shown). However, at the less stringent temperature of 49°C, the 6.1-kbp fragment was labeled by the *trg*-containing probe (Fig. 2B, the indicated band) but not by the vector alone (data not shown). We conclude that *trg* has a weak homology to either *tar* or *tap* or to both genes.

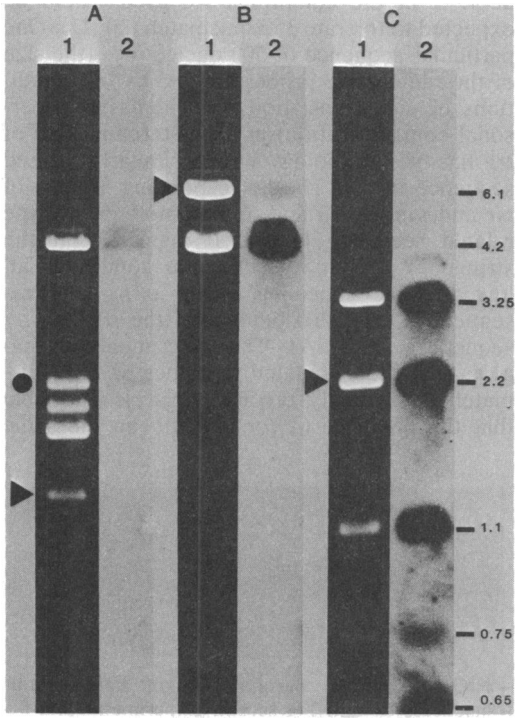

FIG. 2. Hyridization of a pBR322-*trg* probe to plasmids carrying transducer genes. Plasmid pTH105 (Fig. 1) was labeled by nick translation and was tested for hybridization to fragments of pAB125, which includes *tsr* (A); pTH60, which includes *tar* and *tap* (B); and pTH105, which includes *trg* (C). In each case, column 1 shows the separation on an agarose gel of fragments generated by the restriction endonuclease indicated in Fig. 1, and column 2 shows the autoradiographic pattern created by hybridization of the probe to the DNA fragments blotted onto nitrocellulose. The size in kilobases of some of the fragments is indicated on the righthand margin. In each pattern, the fragment containing the respective transducer gene is indicated by an arrow. In column A, lane 1, the dot indicates the fragment thought to contain ColE1 sequences homologous to pBR322.

The hybrid plasmid pAB100, in which a chromosomal fragment containing *tsr* is attached to pACYC184, carries a region on the cloned piece that hybridizes with pBR322 DNA (3). This region, probably derived from the ColE1 plasmid, is on the same *Pvu*II fragment as part of *tsr* and thus interferes with hybridization analysis. We avoided this difficulty by using pAB125 (3), a derivative of pAB100 with a Tn*5* insertion between the interfering region and the beginning of *tsr*. A 1.4-kbp fragment generated by *Pvu*II digestion of pAB125 contains almost the entire *tsr* sequence (Fig. 1). The first 167 nucleotides of the coding sequence are on a 1.8-kbp *Pvu*II fragment, whereas the final eight nucleotides are contained in a 1.4-kbp fragment. The ColE1-related sequences are on a separate 2.2-kbp fragment (Fig. 1). Even at the low stringency of 49°C hybridization, no labeling was detected of the 1.4-kbp *tsr* fragment, although the limited homology between pBR322 and the pACYC184 vector or the ColE1 region on the insert was reflected by labeling of the 4.2- and 2.2-kbp *Pvu*II fragments, respectively (Fig. 2A). Thus, there is not sufficient homology between *trg* and *tsr* to be detected by the procedures utilized.

Antiserum to the Trg protein. The hybridization experiments demonstrated that the *trg* gene does not have extensive homology to the other transducer genes. Antiserum raised to purified Trg protein provided a means of testing for limited homologies between that protein and the other transducers.

Trg protein was purified from minicells produced by a strain containing a pBR322-*trg* hybrid plasmid (6) and carrying null mutations in *tsr*, *tar*, and *tap* (7). By excising Trg bands from slab gels, we obtained sufficient Trg protein to raise antisera in rabbits. In this report, we describe the properties of one particular anti-Trg serum.

The serum precipitated Trg protein from the proteins of a *tsr tar tap* mutant strain carrying a pBR322-*trg* hybrid plasmid and thus containing about 10 times the amount of Trg protein found in a wild-type cell, whereas no protein was precipitated by serum taken from the same rabbit before immunization (Fig. 3). The anti-Trg serum was raised to protein purified from a strain carrying mutations that eliminate the production of the Tsr and Tar proteins (7), as well as Tap (3). Yet the serum precipitated *methyl*-^{3}H-labeled bands corresponding to the Tsr and Tar transducers from wild-type cells, as well as from strains carrying a null mutation (8) in *trg* (Fig. 4). Precipitation of *methyl*-^{3}H-labeled bands of transducer proteins from three null mutants, each lacking one of the functionally characterized transducers (Fig. 5), demonstrates that the antiserum recognizes both the Tsr and Tar pro-

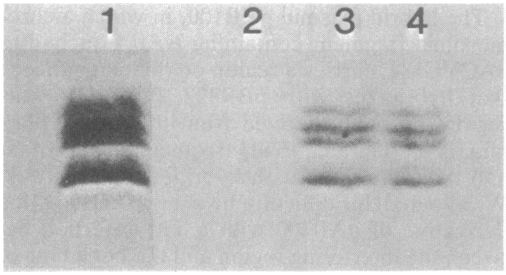

FIG. 3. Immune precipitation of Trg protein with anti-Trg serum. The figure shows the 60,000 M_r region of a fluorogram of an SDS-polyacrylamide gel prepared for maximum resolution of the bands of Trg protein. Trg protein labeled with [*methyl*-^{3}H]methionine was produced in TH1301, a *tsr tar tap* mutant containing pTH57, a pBR322-*trg* hybrid plasmid (6). Lane 1, a sample of whole, *methyl*-^{3}H-labeled cells. The four distinct bands represent band 1 to 4 (in descending order) as described in reference 6. That is half the amount used in the precipitation experiments of lanes 2 to 4. Lane 2, material precipitated by serum taken from the rabbit before immunization; lanes 3 and 4, material precipitated by anti-Trg serum. For the precipitations, the labeled cells were solubilized by 0.5% SDS at 55°C (lanes 2 and 3) or at 100°C (lane 4) and then treated with an excess of Triton X-100 before the antiserum was added. See the text for details.

teins. These two transducers were also precipitated from strains lacking Trg (Fig. 4), as well as from double mutants lacking Trg and either Tsr or Tar (data not shown). The immune precipitation of heterologous protein was observed whether membrane proteins were solublized by Triton X-100 at room temperature (data not shown) or by SDS at 55°C (Fig. 3, lane 3; Fig. 4, lanes 3 and 7; Fig. 5, lanes 2, 4, and 6) or at 100°C (Fig. 3, lane 4; Fig. 4, lanes 4 and 8). We conclude that there are antigenic determinants on the Trg protein that are also present on the Tsr and Tar proteins.

DISCUSSION

The data presented here indicate that there is homology among all of the known transducer proteins of *E. coli*. However, the homology between the *trg* gene and any of the three transducer genes previously shown to constitute a gene family (3, 11) is clearly less extensive than the homology those three genes exhibit to each other. This relationship is consistent with previous observations (3, 6) and identifies *trg* as a somewhat distant cousin within the transducer gene family.

The nucleotide sequence of *trg* is presently being determined in this laboratory. In accord with the observations reported here, we found sequence homology between some regions of the amino acid sequence deduced for the Trg protein and corresponding regions of the other transducers. However, this homology, as well as the homology between the *trg* nucleotide sequence and the sequences of the transducer genes, is significantly less than the homology among those other transducers or their corresponding genes.

The precise degree of homology between *trg* and the other transducer genes can be determined directly by comparison of the nucleotide sequences (2, 11). The patterns of homology are quite consistent with the results of hybridization experiments reported here and by Boyd et al. (3). The radioactive polynucleotides synthesized from probe template in the conditions we used are ca. 500 nucleotides in length (L. Thomashaw, personal communication). Hybridization at 49°C in the conditions we used would be expected to tolerate 35% mismatch (10, 17). One particular sequence of 500 nucleotides (the size of the radioactive probes created by our conditions of nick translation; L. Thomashaw, personal communication) in the C-terminal half of *trg* has 66% homology with a similarly located sequence in *tar*. The corresponding regions of *tsr* and *tap* are 63 and 61% homologous to the related sequence in *trg*. It appears that the stringency of our hybridization conditions allowed stable annealing of those *trg* and *tar* sequences (Fig. 2B) but not of the *trg* and *tsr* sequences (Fig. 2A). This discrimination suggests that the calculated tolerance of 35% mismatch is strikingly precise. It is also possible that the presence of *tar* and *tap* on the same

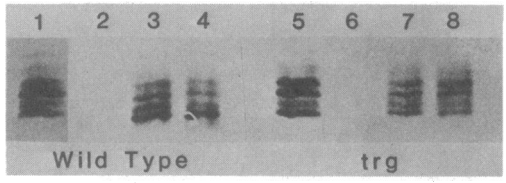

FIG. 4. Immune precipitation of heterologous transducers by anti-Trg serum. Experiments and figures are as described for Fig. 3. Lanes 1 to 4, material tested was *methyl*-^{3}H-labeled HB233, a wild-type strain for chemotaxis known to contain Tsr, Tar, and Trg proteins; lanes 5 to 8, material was labeled HB235, a derivative of HB233 lacking Trg protein as a result of a Tn*10* insertion in *trg*. A wild-type cell contains only 10% as much Trg protein as Tsr or Tar proteins, thus at this exposure the labeled Trg protein, located below the Tsr and Tar bands, is not visible on the fluorogram. Lanes 1 and 5, samples of the whole cells equivalent to half the amount of material used in the precipitation experiments; lanes 2 and 6, material precipitated with preimmune serum; lanes 3, 4, 7, and 8, material precipitated with anti-Trg serum. As described for Fig. 3, the material shown in lanes 3 and 7 was solubilized at 55°C, and the material shown in lanes 4 and 8 was solubilized at 100°C.

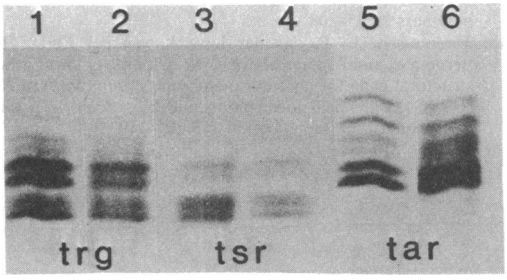

FIG. 5. Both Tsr and Tar proteins are precipitated by the anti-Trg serum. Experiments and figures are as described for Fig. 3. Lanes 1 and 2, material from the *trg* null mutant HB235; lanes 3 and 4, material from the *tsr* null mutant HB237; and lanes 5 and 6, material from the *tar tap* null mutant HB238. Odd-numbered lanes contain initial samples as in Fig. 3, and even-numbered lanes contain material precipitated after solubilization at 55°C. Similar precipitations of Tsr and Tar proteins were observed from strains that contained null mutations in *tar* and *trg* or *tsr* and *trg*, respectively.

fragment somehow enhanced the stability of hybrids with *trg* probes, and thus hybridization was detected to that fragment but not to the fragment containing the single *tsr* gene.

Homology between Trg protein and the other transducers was readily demonstrated by recognition of Tsr and Tar by anti-Trg serum. The strong cross-reactivity of Tsr and Tar with the serum implies that some strongly antigenic sites are shared by the transducer proteins. A few segments of the C-terminal region of Trg are 75% homologous to comparable regions of the Tsr or Tar proteins. Thus, these are likely to be the regions recognized by the cross-reacting serum.

A family of genes in which the members exhibit extensive homology in nucleotide sequence is a common observation in the study of eucaryotic cell biology. In contrast, few examples of gene families are known in procaryotes. Besides the family of genes for chemotactic transducers, the only other documented case in *E. coli* is a family of three homologous genes that each codes for a porin protein of the outer membrane (13, 14, 18). Frequently, eucaryotic genes within a family code for proteins that perform a common function but recognize different ligands. Often the common function of these proteins is related to the interaction of the eucaryotic cell with its environment. The transducer proteins of *E. coli* function as part of a procaryotic chemosensory system. Each protein binds a particular set of ligands and then functions in an identical way in the sensory system. The infrequent occurrence of gene families in procaryotes in comparison to eucaryotes may reflect the relatively modest requirements for single cell procaryotes to interact with chemicals or cells in their environment.

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