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Chemotaxis in *Escherichia coli*: Construction and Properties of λtsr Transducing Phage

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The *tsr* gene of *Escherichia coli*, located at approximately 99 min on the chromosomal map, encodes a methyl-accepting protein that serves as the chemoreceptor and signal transducer for chemotactic responses to serine and several repellents. To determine whether any other chemotaxis or motility genes were located in the *tsr* region, we constructed and characterized two λtsr transducing phages that each contain about 12 kilobases of chromosomal material adjacent to *tsr*. λtsr70 carries sequences from the promoter-proximal side of *tsr*; λtsr72 carries sequences from the promoter-distal side of *tsr*. Restriction maps of the bacterial inserts in these phages and Southern hybridization analyses of the bacterial chromosome indicated that the *tsr* gene is transcribed in the counterclockwise direction on the genetic map. Insert deletions were isolated in λtsr70 and transferred into the host chromosome to examine the null phenotype of *tsr*. All such strains exhibited wild-type swimming patterns and chemotactic responses to a variety of stimuli, but were specifically defective in serine taxis and otehr Tsr-mediated responses. In addition, UV programming experiments demonstrated that Tsr and several of its presumptive degradation products were the only bacterial proteins encoded by λtsr70 and λtsr72 that required host FlbB/FlaI function for expression. These findings indicate that there are probably no other chemotaxis-related genes in the *tsr* region. A series of *tsr* point mutations were isolated by propagating λtsr70 on a *mutD* host and used to construct a fine-structure map of the *tsr* locus. These mutations should prove valuable in exploring structure-function relationships in the Tsr transducer.

The *tsr* locus of *Escherichia coli* specifies a cytoplasmic membrane protein that serves as the sensory transducer for chemotactic responses to serine and several other compounds (24, 25, 29). The Tsr molecule is organized into a periplasmic and a cytoplasmic domain, connected by a single membrane-spanning segment (2, 14). The periplasmic domain contains chemoreceptor sites that monitor serine concentrations in the environment (2, 7). The cytoplasmic domain appears to generate signals of unknown nature that ultimately control the flagellar rotation pattern of the cell (5) and also contains several sites that can be reversibly methylated by the CheR and CheB enzymes (12). Changes in chemoreceptor occupancy trigger chemotactic responses by modulating the signaling activity of the cytoplasmic domain. Subsequent changes in transducer methylation state alter chemoreceptor affinity (31) and bring about sensory adaptation (11). Three other methyl-accepting chemotaxis proteins with similar properties are known in *E. coli*. Each mediates responses to a specific set of stimuli.

Mutational analyses of the Tsr protein have provided details about structure-function relationships in bacterial transducers (5, 19, 20, 23). Most *tsr* mutants are defective in responding to serine, temperature, and various repellents but have normal unstimulated flagellar rotation patterns and still respond to stimuli that are processed by other transducers (15, 19, 24). These mutants define the canonical Tsr$^-$ phenotype, which is assumed to represent the null condition. Other *tsr* mutants exhibit a general loss of chemotactic ability (Che$^-$) due to a pronounced counterclockwise (CCW) or clockwise flagellar rotational bias and may have lesions that lock the Tsr transducer in an active signaling mode (5, 19).

The *tsr* gene is located at 99 min on the *E. coli* genetic map, but the genetic context of this locus is still poorly understood. Since nearly all of the known chemotaxis and motility genes in *E. coli* are organized into cotranscribed groups, we examined the possibility that *tsr* is part of an operon or gene cluster containing previously undiscovered chemotaxis functions. In this report we describe the use of specialized λ transducing phages to clone and examine segments of the *tsr* region. Physical maps of the phage inserts demonstrated that the *tsr* gene is transcribed in the CCW direction on the genetic map. Deletion and point mutant derivatives of the transducing phages were used to construct a map of the *tsr* locus, and selected deletions were transferred into the bacterial chromosome to confirm the null phenotype of *tsr*. In all, approximately 12 kilobase pairs of chromosomal material on either side of the *tsr* locus were studied. Except for Tsr itself, the proteins encoded in this region did not appear to have chemotaxis-related functions.

MATERIALS AND METHODS

Strains. The bacterial strains used in this work were derivatives of *E. coli* K-12; most of them are close derivatives of RP437 (21). Primary strains are listed in Table 1. Other strains were constructed by transduction of desired mutations into one of the primary strains by using linked auxotrophic or drug resistance markers. Plasmids pLC8-9 (6), pAB100Δ131, pAB100Δ133, and pAB100Δ139 (3) were obtained from A. Boyd. λfla91 (25) was obtained from M. Silverman and carries the *c*I857 repressor mutation and a *tsr* insert derived from pLC8-9.

Medium. Cells were grown on tryptone (T) broth, plates,

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TABLE 1. Bacterial strains

Strain	Relevant markers	Reference or source
RP437	Wild type for chemotaxis	21
RP442	P2	21
RP449	*pel*	9
RP498	λfla91 *recA*	This work
RP526	*mutD5*	10
RP1507	*uvrA6* (λ *ind*) Δ(*flbB-flaH*)*4*	This work
RP1508	*uvrA6* (λ *ind*)	This work
RP3098	Δ(*flbB-flaH*)*4*	28
RP4532	Δ(*tar-tap*)*5201*	5
RP5501	λfla91	This work
RP5527	λfla91 *tsr-14*	This work
RP5543	Δ*tsr-9101*	This work
RP5546	Δ*tsr-9101 recA*	This work
RP5724	*tsr-501*::Tn*5*	This work
RP5838	Δ(*tar-tap*)*5201* Δ*tsr-7021*	This work

and swarm agar (18). Unless noted otherwise, all incubations were done at 35 to 37°C.

Isolation of λtsr strains. Specialized *tsr* transducing phage were obtained in four ways. Method I: RP498 was grown in T broth at 30°C to mid-logarithmic phase, transferred to 43°C for 30 min to induce the prophage, and incubated at 37°C until lysis was complete. Particles with large chromosomes were selected by plating on RP449 and kept for further study. Method II: RP5527 was induced as in method I, and the lysate was plated on RP5543. Plaque centers were picked to T swarm plates, and tsr^+ isolates were kept for further study. Method III: RP5501 was induced as in method I, and the lysate was fractionated by equilibrium centrifugation in a CsCl density gradient. Phage particles with high DNA contents were kept for further study. Method IV: RP437 was lysogenized with λtsr70Δ12 (see Fig. 4) and induced as in method I. The lysate was plated on RP442 to select Spi^- phages, which were kept for further study.

Candidate transducing phages produced by each of these methods were then tested for ability to complement the *tsr* defect of strain RP5546 (5). The DNA content of tsr^+ phage isolates was estimated from their relative plating efficiency on RP449 and RP437 and their rate of inactivation in 1.0 mM EDTA, pH 8.0, at 45°C (22). Chromosome length was subsequently determined more directly by summing the sizes of DNA fragments produced in restriction mapping experiments. The stability of the inserts in EDTA-sensitive isolates was assessed by plating individual plaques on T plates containing 0.8 mM EDTA (EDTA plates). Under these conditions, phage with a wild-type DNA content form plaques with an efficiency of approximately 10^{-4}. Isolates that formed plaques at higher efficiency were assumed to carry inserts with duplicated sequences and were discarded.

Deletion analysis of the *tsr* region. Spontaneous deletion derivatives of λtsr phage were selected on EDTA plates and analyzed for *tsr* function in complementation tests with RP5546. Selected deletions were transferred into RP437 by an integration-excision cycle (21). To isolate new *tsr* point mutations, λtsr70 was mutagenized by plaquing on RP526 and then replated on RP5838. Plaque centers containing infected cells were picked to T swarm plates to screen for Tsr^- or Che^- mutants as detailed previously (5, 26). Approximately 1% of the tested plaques carried *tsr* mutations. Phage-borne mutations were then transferred into RP4532 for mapping against λtsr deletion strains by previous methods (5, 26, 28).

Restriction analysis of phage DNA. Phage stocks were grown in RP5543 or RP5838 by standard methods (21), concentrated by polyethylene glycol precipitation, and banded in CsCl step gradients. Phage DNA was extracted by the formamide method (8) and treated with restriction enzymes obtained from Bethesda Research Laboratories or New England Biolabs, using the conditions and buffers recommended by the supplier.

Southern blot analysis. Extraction of chromosomal or plasmid DNA, agarose electrophoresis, nitrocellulose transfers, nick translation, and probe hybridization were done by published procedures (27). RP3098 was used as the source of chromosomal DNA to avoid cross-hybridization to *tar* and *tap* sequences (see reference 3).

UV programming. Labeling of phage-directed proteins in UV-irradiated cells was done as described previously (5). Proteins were analyzed by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate as detailed previously (28).

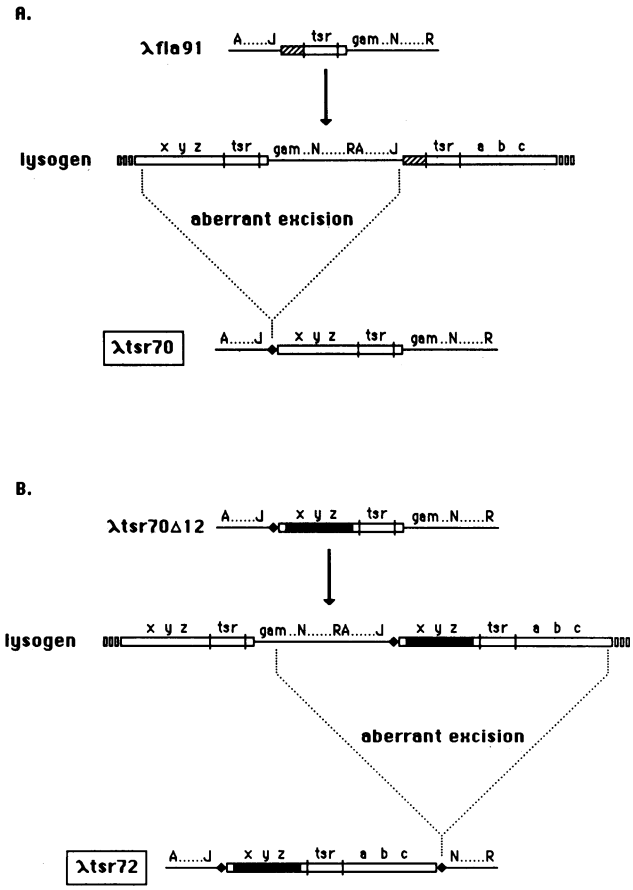

FIG. 1. Construction of λtsr transducing phages. Lambda sequences are indicated by thin lines, bacterial sequences by open boxes, plasmid sequences by cross-hatched boxes, and deleted material by solid boxes. Nonhomologous recombination between the indicated sites produces a novel fusion joint (represented by a solid diamond) in the excised transducing phage chromosome, which is drawn in its mature form after cutting at the *cos* sites between the *A* and *R* genes. (A) Derivation of λtsr70. The *tsr* insert of λfla91 is flanked on the left by pLC8-9 plasmid material that was not incorporated into λtsr70. (B) Derivation of λtsr72. The deletion of insert material on the left side of *tsr* in λtsr70Δ12 provided space for incorporation of host sequences adjacent to the right end of *tsr*.

TABLE 2. λtsr phage

Phage	Construction[a]	DNA content (% of wild type)
λfla91		82
λtsr91-1, 91-6	Method I	98
λtsr11-2, 11-7, 11-12, 15-8, 15-11	Method II	98
λtsr70	Method III	102
λtsr72, 73, 74	Method IV	95

[a] Construction details are given in the text.

RESULTS

Construction of specialized transducing phages. To search for other chemotaxis-related genes in the vicinity of the *tsr* locus, we constructed λ transducing phages that carried the *tsr* gene and as much as possible of the adjacent chromosomal material. The basic approach used in the construction schemes was to integrate a λ prophage at the *tsr* locus and then select for aberrant excision products that had picked up additional chromosomal material.

We began with λfla91 (25), which carries the *tsr* locus from *E. coli* but very little of the flanking regions (see below). The DNA content of λfla91 particles is about 82% of wild-type λ, providing sufficient packaging capacity to accommodate at least an additional 10 kilobases (kb) of host material. Although λfla91 has no site-specific integration system, it can integrate into the bacterial chromosome by homologous recombination with the *tsr* region of the host, leading to stable lysogens in which the prophage is flanked on both sides by a copy of the *tsr* locus. After induction of such a lysogen, the most common route of prophage excision is again through homologous recombination, which simply reforms the parental λfla91 chromosome. However, rare nonhomologous recombination events should lead to the formation of transducing phages that have incorporated additional host material adjacent to the *tsr* gene. Transducing particles that acquire new chromosomal material exclusively from one side of the prophage should have stable insertions, whereas those that pick up material at both prophage ends should segregate chromosomes of λfla91 size due to occasional homologous recombination between the duplicated *tsr* segments (Fig. 1).

Isolation of λtsr70. The bacterial insert of λfla91 was obtained from plasmid pLC8-9 and carries some plasmid-derived sequences at the left end of the *tsr* gene (Fig. 1A and see below). To eliminate those plasmid sequences, we first isolated a transducing phage that had incorporated material from the left end of the prophage, as shown in Fig. 1A. Three similar methods were used to look for excision events of this sort. (i) We introduced a *recA* mutation into a λfla91 lysogen to "lock in" the prophage as a means of increasing the relative frequency of aberrant excision events. Following induction, phage particles with large chromosomes were selected by plating the lysate on a Pel^- host, on which λ deletion mutants form plaques with low efficiency (9). (ii) A lysogen was constructed with a λfla91 derivative that carried a point mutation (*tsr-14*) at the left end of the *tsr* coding region. The integrative exchange positioned the mutant copy of *tsr* at the right end of the prophage in the lysogen. The subsequent lysate was screened for tsr^+ phages to identify those that had picked up the nonmutant copy of *tsr* at the left end of the prophage. (iii) Lysates were prepared from a $recA^+$ λfla91 lysogen and fractionated on CsCl equilibrium bouyant density gradients to enrich for particles with high DNA contents.

These three approaches yielded a total of 78 candidate phages. The stability of the insertion in each isolate was assessed by measuring the level of EDTA-resistant particles after several cycles of lytic growth. Most of the phages proved unstable by this criterion and were not studied further. The properties of eight independent stable isolates are summarized in Table 2. All of these phages retained the λ *gam* function present at the left end of the λfla91 prophage, indicating that they had probably picked up host sequences

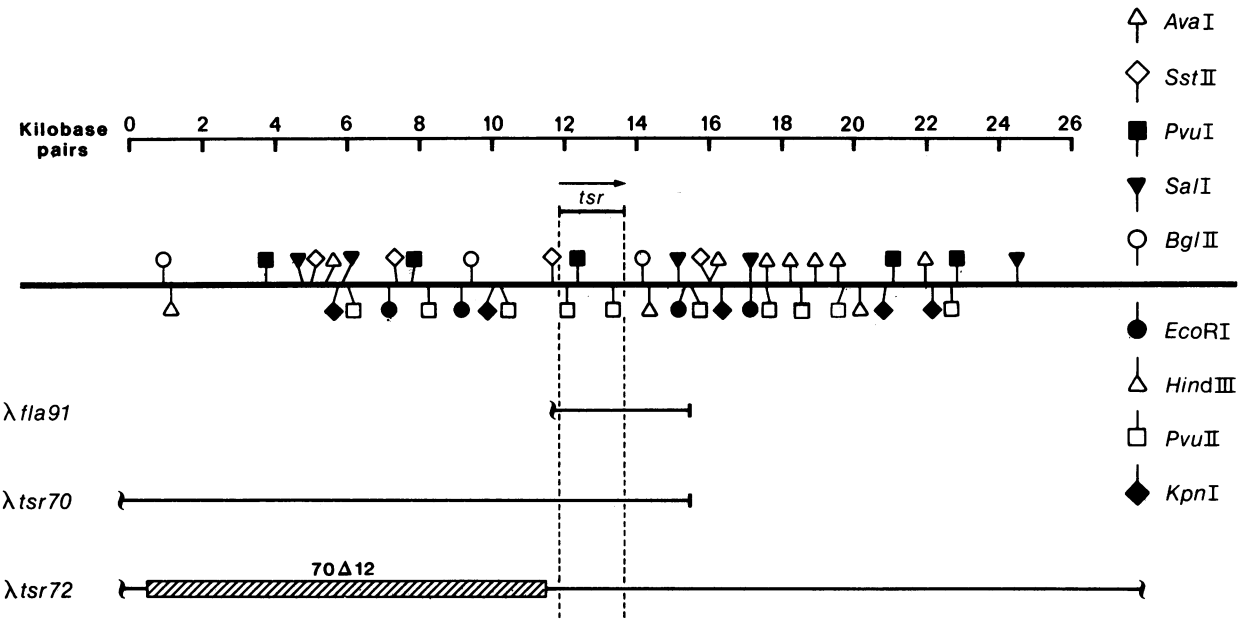

FIG. 2. Restriction map of the *tsr* region. Composite representation of the sites present in the bacterial inserts of λtsr transducing phages. The extent of insert material in each phage is shown below the map in the same orientation as in Fig. 1. The kilobase scale originates at a *Pvu*II site (not shown) in the *A-J* arm of λtsr70 and λtsr72, approximately 0.3 kb from the beginning of the insert. The position and orientation of the *tsr* coding sequence are inferred from the locations of *Pvu*I and *Pvu*II sites (2).

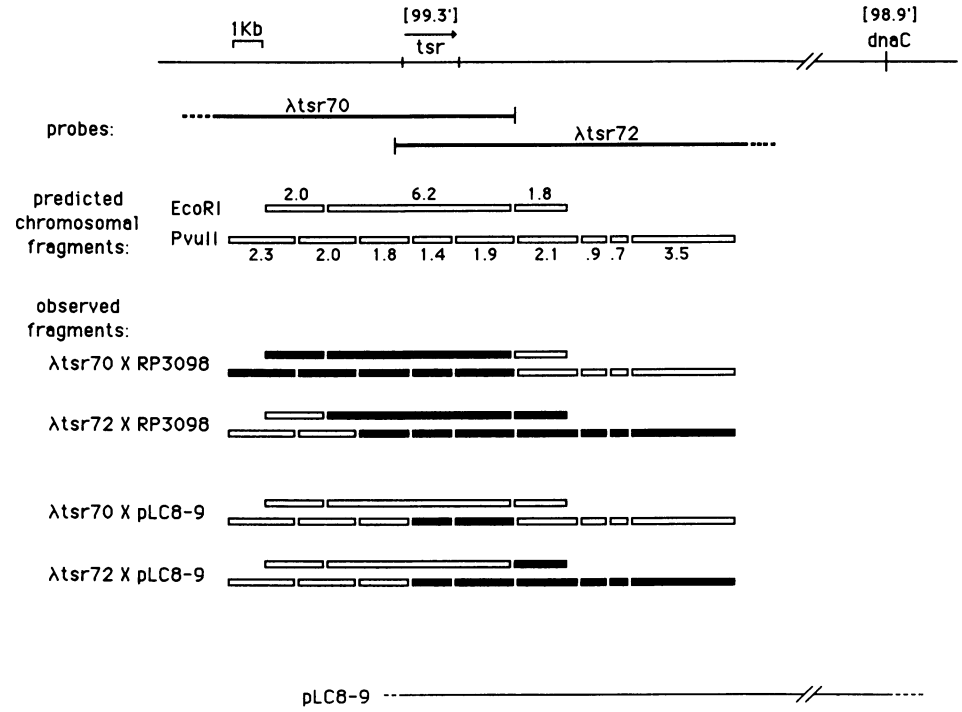

FIG. 3. Southern analyses of the *tsr* region. The *tsr* region is drawn to scale and in the same orientation as in Fig. 1 and 2. DNA from RP3098 and pLC8-9 was digested with *Eco*RI or *Pvu*II and probed with the insert sequences of λtsr70 and λtsr72. The predicted sizes and positions of fragments homologous to the insert material were based on the restriction map of Fig. 2. Solid bars indicate the restriction fragments detected in each experiment. The chromosomal sequences inferred to be present in plasmid pLC8-9 are represented by the line at the bottom of the figure. These results indicate that the *dnaC* gene, which is carried by pLC8-9, must be located to the right of *tsr*, as shown on the top line. The map positions (in minutes) of the *tsr* and *dnaC* loci are from Bachmann (1).

adjacent to the left end of the prophage, as shown in Fig. 1A. We chose λtsr70 for further characterization because it appeared to have the largest insertion.

Isolation of λtsr72. Transducing phages carrying host sequences at the right end of *tsr* were derived from λtsr70Δ12, a deletion mutant of λtsr70 lacking most of the insert material to the left of *tsr* (Fig. 1B). Like λtsr70, this phage should no longer carry the plasmid sequences present in λfla91. Lysates were prepared from a λtsr70Δ12 lysogen and plated on a host strain lysogenic for phage P2 to select Spi^- phages that had lost the λ *gam* function (32). Such phage should arise by aberrant excision events that delete the prophage *gam* gene, acquiring material at the right end of the prophage in the process. Three independent isolates were characterized; all three appeared to have lower DNA contents than wild-type λ (Table 2). We chose λtsr72 for further study.

Physical mapping of λtsr phages. The physical structures of λtsr70 and λtsr72 predicted by their isolation schemes (Fig. 1) were confirmed by restriction mapping. Both phages had small deletions of lambda material in the *A-J* arm corresponding to the excision joint in λtsr70 (data not shown). In addition, λtsr72 contained a 2-kb deletion of lambda material in the *N-R* arm corresponding to its unique excision joint and associated *gam* deletion (data not shown). A composite restriction map of the *tsr* region and the bacterial inserts in λfla91, λtsr70, and λtsr72 is shown in Fig. 2. The inserts in λtsr70 and λtsr72 proved to be about 15 kb in length. Although the insert in λfla91 was 5.6 kb in length, Southern analyses (not shown) demonstrated that only 3.3 kb of the λfla91 insert was homologous to host material; the remaining 2.3 kb corresponded to pLC8-9 sequences. These plasmid sequences were located next to the left end of *tsr* (Fig. 1A) and consequently were not present in λtsr70 or λtsr72. Since all three of the phages contained a functional *tsr* locus (as evidenced by the ability to complement a *recA tsr* host), the *tsr* gene must be located within the 3.3 kb of material common to all three inserts. The relative position and transcriptional orientation of the *tsr* gene within this region were deduced from the distribution of *Pvu*I and *Pvu*II sites, which are known to fall within the *tsr* coding sequence (2). As predicted by the derivation scheme, the additional insert material in λtsr70 was adjacent to the left (promoter-proximal) end of *tsr*, whereas that in λtsr72 was adjacent to the right (promoter-distal) end of *tsr*. Together the λtsr70 and λtsr72 inserts represent over 25 kb of *E. coli* chromosomal sequences centered on the *tsr* region.

Chromosomal orientation of the *tsr* locus. The restriction map shown in Fig. 2 was constructed by analyses of the bacterial inserts in transducing phages. To confirm that it was also an accurate representation of the chromosomal *tsr* region, we examined the distribution of *Eco*RI and *Pvu*II sites in bacterial DNA by Southern analyses with λtsr70 and λtsr72 as hybridization probes (Fig. 3). The sizes of three *Eco*RI and nine *Pvu*II fragments that lay entirely within the insert region could be predicted from the restriction map, and chromosomal fragments of those sizes were detected with the appropriate probes. In addition, we detected chromosomal fragments whose sizes could not be predicted in advance, which spanned the ends of the phage inserts (data not shown).

The transcriptional orientation of *tsr* was established by

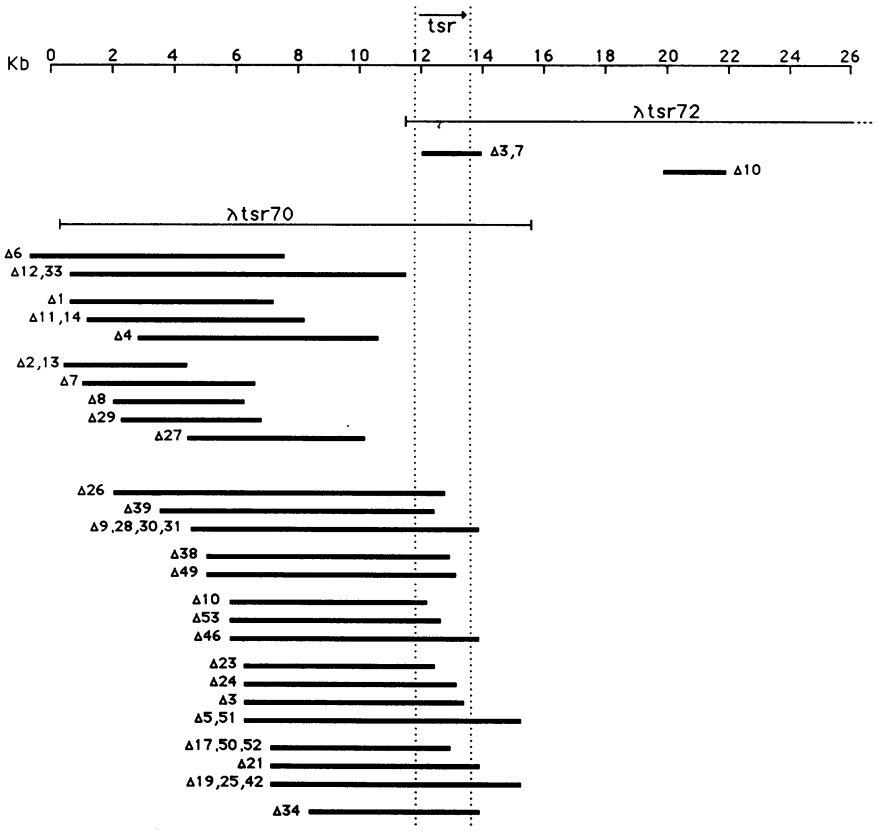

FIG. 4. Deletion derivatives of λtsr70 and λtsr72. Each deletion mutant was analyzed with a subset of the restriction enzymes listed in Fig. 2. The approximate position and size of the deleted material are indicated by thick lines, using the same scale as in Fig. 2. The relative positions of deletion endpoints within the *tsr* coding region were additionally based on mapping studies with *tsr* point mutations (see Fig. 5). For simplicity, deletions with similar endpoints have been grouped even though they are independent isolates and probably not identical.

Southern analyses of plasmid pLC8-9, which carries both the *tsr* and *dnaC* loci (6). As summarized in Fig. 3, the insert of pLC8-9 begins just before the promoter-proximal end of *tsr* and carries an extensive segment of the promoter-distal region. Neither λtsr70 nor λtsr72 was able to complement host *dnaC* mutations, so the *dnaC* gene must be located on the promoter-distal side of *tsr* beyond the end of the λtsr72 insert. Since *tsr* is located on the clockwise side of *dnaC*, it must be transcribed in the CCW direction on the genetic map.

Isolation of λtsr deletions. To confirm the position of the *tsr* locus within the λtsr70 and λtsr72 inserts, we isolated a series of deletion derivatives by selecting for spontaneous EDTA-resistant phage mutants (22). The location and extent of the deleted material were determined by restriction site mapping and by complementation and recombination tests with host *tsr* point mutations (Fig. 4). Since the bacterial sequences in these transducing phages are not essential for plaque formation, we expected that most of the deletions would remove portions of the insert material. This proved to be the case for λtsr70 but not for λtsr72. It appears that because the λtsr72 DNA molecule is somewhat shorter than the wild-type λ chromosome, relatively small deletions were able to render λtsr72 particles completely resistant to inactivation by EDTA. Many of those small deletions fell outside the insert region, evidently in nonessential phage sequences. In spite of this difficulty, we did obtain several λtsr72 deletions that entered the *tsr* coding region, and their physical positions as well as those of λtsr70 deletions were consistent with the previously established location of the *tsr* gene (Fig. 4).

Isolation and mapping of *tsr* point mutations. To map the *tsr* endpoints of transducing phage deletions and to obtain additional mutants for studies of *tsr* function, we subjected the insert of λtsr70 to mutagenesis by growth on a *mutD* host strain and screened for phage that could no longer complement a *tsr* tester mutant. Each mutation was then transferred into the host chromosome for mapping studies. All of the mutations fell into the *tsr* coding region and served to define 10 deletion segments (Fig. 5). These new *tsr* mutants exhibited a variety of chemotaxis phenotypes, including some with evident signaling defects. Several preliminary studies of these mutants have been published (5, 20, 23), and a more detailed account will be presented in a separate report.

Null phenotype of the *tsr* locus. Two initial observations indicated that *tsr* null mutants might exhibit the canonical Tsr^- phenotype previously defined with *tsr* point mutations. First, mutagenesis of wild-type cells with transposon Tn*5* yielded an insertion mutant with a Tsr^- phenotype. This mutant probably makes a completely nonfunctional Tsr protein, because the Tn*5* insertion was located in the promoter-proximal portion of the *tsr* coding sequence (*tsr-501* in Fig. 5). Second, we found that over 95% of the cells that survived prophage induction of strain RP498 had a Tsr^- phenotype. This strain was lysogenic for a λtsr prophage integrated at the *tsr* locus, and the survivors should represent cured derivatives that arose prior to the inducing treatment, most likely through spontaneous deletion of

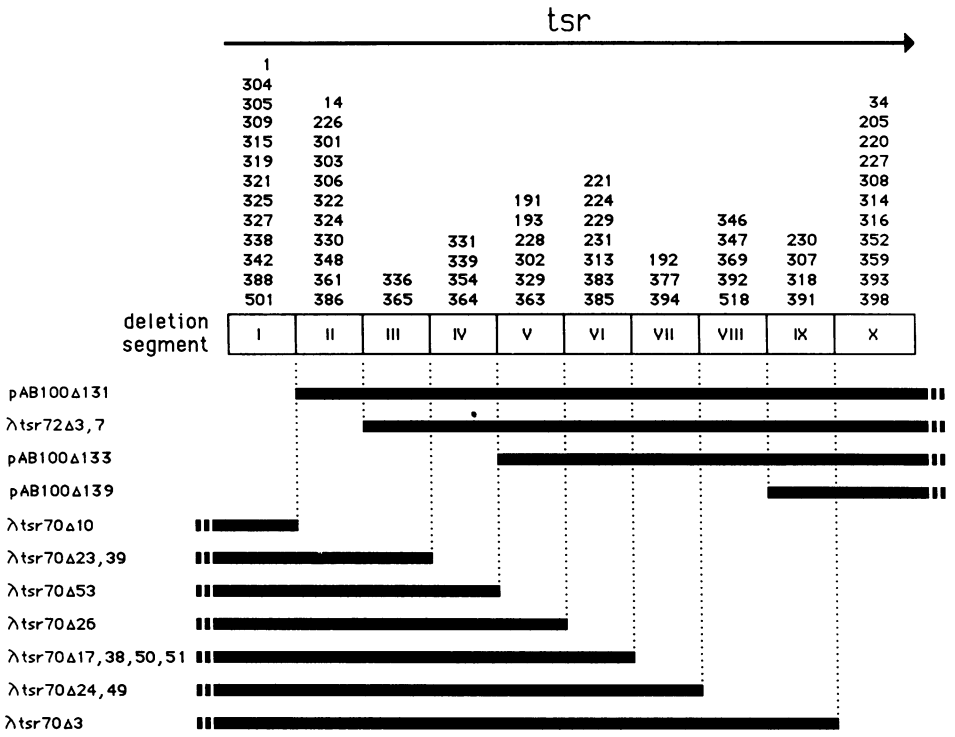

FIG. 5. Deletion map of the *tsr* gene. Allele numbers in the 205 to 398 range denote *tsr* point mutations isolated in the present study. The *tsr-501* allele (deletion segment I) is a Tn*5* insertion mutation, also isolated in this study. The other mutations are from previous studies (5, 19, 24).

prophage material. The high frequency of Tsr$^-$ cells among the survivors implies that many of these prophage deletions also remove adjacent *tsr* sequences. Although we have not studied these putative deletions in much detail, RP5543, which carries Δ*tsr-9101*, was routinely used as a host strain for propagating λtsr point mutant phages, and we never observed formation of *tsr*$^+$ recombinant phage in this strain. Thus, Δ*tsr-9101* probably removes the entire *tsr* coding region.

To construct better-defined null mutations of the *tsr* gene, we transferred *tsr* deletions from λtsr70 derivatives into the host chromosome. We chose λtsr70 deletions that retained some homology at both ends of the original insert (Fig. 4) and crossed them into the bacterial chromosome by homologous recombination. The chemotactic behavior of the resultant strains is summarized in Table 3. All were defective in serine and alanine taxis, responses that are known to be mediated by Tsr, but they showed normal swarm formation to aspartate, maltose, and ribose, which are handled by other transducers. These deletion strains formed typical Tsr$^-$ colonies on T swarm plates and exhibited normal swimming patterns in the absence of stimuli. Cell-tethering studies of strain RP5882, which carries Δ*tsr-7021*, confirmed that its unstimulated flagellar rotation pattern was identical to that of wild-type and Tsr$^-$ point mutants (data not shown). Thus, deletion of the *tsr* locus produces the standard Tsr$^-$ phenotype.

Protein products of the *tsr* region. Since all of the chromosomal *tsr* deletion strains exhibited a Tsr$^-$ swarm phenotype (Table 3), we conclude that there are no *fla*, *mot*, or *che* genes within the deleted material, because defects in such functions typically result in a general loss of motility or chemotaxis. The largest of these deletions, Δ*tsr-7026*, removed at least 10 kb of host sequences adjacent to the promoter-proximal end of *tsr* (Fig. 4). We were unable to conduct a similar analysis of the region promoter-distal to *tsr* due to difficulty in selecting large deletions in λtsr72. To examine this region for chemotaxis-related genes, we analyzed the proteins made by various λtsr phages in UV programming experiments. It is known that expression of most chemotaxis-related functions is dependent on the *flbB* and *flaI* loci, whose products are positive regulators of flagellar and chemotaxis promoters (13, 25). Thus, proteins made in a wild-type programming host (RP1508) but not in a *flbB-flaI* deletion host (RP1507) should represent chemo-

TABLE 3. Phenotype of *tsr* deletion strains[a]

Strain	*tsr* mutation[b]	T swarm phenotype	Chemotactic response[c]	
			Ser	Asp, Mal, Rib
RP5695	Δ*tsr-7026*	Tsr$^-$	−	+
RP5698	Δ*tsr-7028*	Tsr$^-$	−	+
RP5701	Δ*tsr-7030*	Tsr$^-$	−	+
RP5704	Δ*tsr-7039*	Tsr$^-$	−	+
RP5713	Δ*tsr-7024*	Tsr$^-$	−	+
RP5715	Δ*tsr-7049*	Tsr$^-$	−	+
RP5873	Δ*tsr-7050*	Tsr$^-$	−	+
RP5882	Δ*tsr-7021*	Tsr$^-$	−	+
RP5883	Δ*tsr-7023*	Tsr$^-$	−	+
RP5724	*tsr-501*::Tn*5*	Tsr$^-$	−	+
RP437	Wild type	Tsr$^+$	+	+

[a] All deletion strains had a wild-type swimming pattern.
[b] Chromosomal deletions derived from transducing phage are assigned allele numbers in which the first two digits designate the parental phage and the last two digits designate the deletion derivative of that phage (21).
[c] Ser, Serine; Asp, aspartate; Mal, maltose; Rib, ribose.

taxis-specific functions. The results of those programming experiments are shown in Fig. 6.

Three distinct FlbB/FlaI-dependent proteins were observed: the Tsr product, which characteristically runs as several closely spaced bands representing different methylation states (4), and two minor species of lower molecular weight. Synthesis of the minor proteins was correlated with the presence of Tsr. All three proteins were made by λfla91, λtsr70, and λtsr72, but were absent in λtsr70Δ50 and λtsr72Δ7, which carry *tsr* deletions. These findings imply that the minor proteins are either degradation products of Tsr or encoded in the region(s) immediately adjacent to *tsr*. We favor the former possibility for two reasons. First, the coding capacity of λfla91 is not sufficient to account for both proteins. Second, methyl-accepting chemotaxis proteins are notoriously sensitive to proteolysis (16, 30). We conclude, therefore, that there are no other FlbB/FlaI-dependent genes in the *tsr* region.

Several other proteins were observed in these experiments: λtsr70 made at least seven, and λtsr72 made a different set of at least six (Fig. 6). Although the *tsr* deletion controls, λtsr70Δ50 and λtsr72Δ7, exhibited the same band profiles as λtsr70 and λtsr72, larger deletion derivatives failed to make some of these proteins (data not shown). Moreover, λfla91, which has only a small bacterial insert, did not make any of them. These findings demonstrate that the additional proteins are insert specific and must be the products of bacterial rather than phage genes. However, the inserts do not have sufficient coding capacity to account for all of the observed proteins, and we suspect that some of them, particularly members of doublet bands (Fig. 6), could be related by posttranslational processing. Curiously, only a few of these proteins could be detected in RP1508, the wild-type host strain. Although the programming strains are nearly isogenic, the *flbB-flaI* deletion in RP1507 removes a substantial block of material, which could account for the differences seen in these experiments. RP1507 might lack a protein degradation activity or have an altered physiological response to UV irradiation. In fact, *ruv*, a locus involved in the SOS system, maps near the *flbB-flaI* loci and could be missing in the deletion. In any event, these proteins are unlikely to be chemotaxis related because they are made in the absence of FlbB and FlaI function.

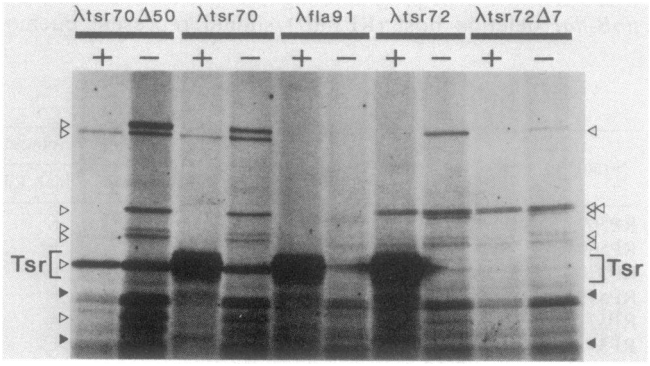

FIG. 6. Protein products of λtsr phage. Bacterial proteins made from the inserts of various transducing phages were labeled by UV programming in RP1508 (lanes +) and RP1507 (lanes −) host strains and analyzed on 8% acrylamide–0.2% bisacrylamide gels. Proteins with apparent M_r values in the 40,000 to 100,000 range are shown. Open arrowheads indicate FlbB/FlaI-independent bands made by λtsr70 or λtsr70Δ50 (left) or by λtsr72 or λtsr72Δ7 (right). Solid arrowheads indicate minor FlbB/FlaI-dependent bands that appear to be degradation products of the Tsr protein.

DISCUSSION

Two specialized transducing phages have been used to examine the genetic organization of the *tsr* region: λtsr70, which carries about 12 kb of chromosomal sequences adjacent to the promoter-proximal end of *tsr*, and λtsr72, which carries a similar amount of material adjacent to the promoter-distal end of *tsr*. Physical mapping and Southern hybridization analyses demonstrated that the positions of restriction sites in the phage inserts corresponded to those in the bacterial chromosome and that the *tsr* locus is transcribed in the CCW direction on the *E. coli* genetic map.

Several proteins made from the bacterial inserts of λtsr70 and λtsr72 were detected in UV programming experiments, but none of them appeared to be chemotaxis related. This conclusion is based on the premise that any chemotaxis or flagellar functions encoded in this region would require FlbB and FlaI activity for expression, as does the *tsr* gene. Two additional FlbB/flaI-dependent bands were seen in the programming experiments, but most likely represented degradation products of Tsr. Although we could have overlooked genes with relatively low levels of expression, there are probably no other chemotaxis loci in this segment of the chromosome. The other insert-specific proteins we observed were not FlbB/FlaI dependent. It is not clear whether any of them correspond to previously catalogued proteins (17), but the transducing phages and their deletion derivatives should prove useful in assigning protein bands to chromosomal loci in the *tsr* region.

These λtsr phages also provide tools for conducting more extensive genetic studies of the *tsr* locus itself. Like plasmid clones, specialized transducing phage are useful for localized mutagenesis of insert genes, and many new *tsr* mutations were isolated in this manner. Transducing phage mutations, unlike plasmid-borne alleles, can also be readily transferred to the host chromosome for further study. Deletions obtained from transducing phage derivatives were used to establish the *tsr* null phenotype and to construct a map of the *tsr* gene. Moreover, strains carrying deletions of the entire *tsr* coding region make ideal hosts for propagating mutant *tsr* plasmids or phages, which would otherwise accumulate unacceptably high levels of wild-type recombinants. These sorts of methods will enable us to carry out a detailed structure-function analysis of the Tsr transducer.

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