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Isolation and Characterization of Specialized ϕ80 Transducing Phages Carrying Regions of the *Salmonella typhimurium trp* Operon

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We have isolated a series of nondefective ϕ80 specialized transducing phage which carry segments of the *Salmonella typhimurium trp* operon. These phage were obtained from a lysogenic derivative of a merozygote constructed by transferring an *S. typhimurium trp* episome into an *Escherichia coli* strain which lacks the normal ϕ80 attachment site. The deoxyribonucleic acid (DNA) from one such phage was purified and employed in DNA-ribonucleic acid (RNA) hybridization studies. The results obtained show that, under our hybridization conditions, heterologous hybridization is less efficient than homologous hybridization. It was also observed that not all *S. typhimurium trp* messenger RNA can readily anneal to *E. coli trp* operon DNA. Heterologous hybrids consisting of *S. typhimurium trp* messenger RNA and *E. coli trp* operon DNA were estimated to have a dissociation constant 10-fold larger than that of homologous hybrids.

We recently described some of the properties of heterologous deoxyribonucleic acid-ribonucleic acid (DNA-RNA) hybrids consisting of *Salmonella typhimurium trp* messenger RNA (mRNA) and *Escherichia coli trp* operon DNA (4; R. M. Denney, Ph.D. thesis, Stanford University, Stanford, Calif., 1973). To extend our studies of sequence homology between the *trp* genes of these bacteria, we sought specialized transducing phage which carried *S. typhimurium trp* operon DNA. In this report we describe the isolation and characterization of ϕ80 phage-containing segments of the *trp* operon of *S. typhimurium*, and employ one such phage in DNA-RNA hybridization experiments with *S. typhimurium* and *E. coli trp* mRNA.

MATERIALS AND METHODS

Media. The minimal E medium described by Vogel and Bonner (22) was supplemented with glucose (0.1 to 0.5%). ACH medium is minimal plus glucose plus 0.5% tryptophan-free acid casein hydrolysate (Nutritional Biochemicals, Inc.). L-broth (11) and nutrient broth were supplemented with L-cysteine (60 μg/ml). Tryptone medium is 1% tryptone (Difco) and 0.5% sodium chloride. Agar, 1.5%, was used in solid media. Tryptone soft agar contains 0.7% agar.

Phage strains. ϕ80*h* is a host-range mutant of ϕ80 which plates on *tonB* mutants of *E. coli*. $\phi 80^{vir}$ cannot lysogenize any *E. coli* strains. These phage and *pt BA5-2* (Fig. 1) were generously provided by Naomi C. Franklin.

Bacterial strains. The principal strains used in this study are listed in Table 1. For convenience the *trp* genes of all strains are designated according to *E. coli* nomenclature (see Fig. 1). *E. coli* KB30 has the *E. coli* B *cysB-trp-suIII* region in an *E. coli* K-12 background and has been previously described (20). KB30Δ(*trp tonB* att^{80})-2 (hereafter called KB30Δ*trp2*) was obtained from a ϕ80 lysogen of KB30 (21) by selecting for simultaneous mutation to tryptophan auxotrophy and resistance to coliphage T1 (1, 6). Subsequent tests showed that, unlike the KB30(ϕ80) parent, KB30Δ*trp2* carried no *trp* DNA, no functional prophage, and lacked immunity to ϕ80*h* infection. Thus, the *trp2* deletion removed most or all of the ϕ80 prophage and, therefore, strain KB30Δ*trp2* either lacks att^{80} or has a hybrid att^{80} (Fig. 1).

K. E. Sanderson generously provided the *S. typhimurium trp* episomes F71 and F72 in *S. typhimurium trpE52 cysB12 pyrF146 ile78* (see footnote *a*, Table 1, for nomenclature of *trp* genes). F71, used in most studies reported here, is one of five *trp* episomes which were derived from *S. typhimurium* HfrB2 (18), a strain produced by transfer into *S. typhimurium* of the F-factor from *E. coli* (24). F71 carries *S. typhimurium* DNA extending an unknown distance to the right of the *trp* operon, and does not extend to the left as far as *cysB* (Fig. 1).

Episome transfer. Donor and recipient cells were grown to log phase in L-broth (ca. 5×10^8 cells/ml) with shaking at 37 C. The recipient (KB30Δ*trp2*) was heated in L-broth at 50 C for 20 min in an attempt to inactivate the host restriction system and increase the efficiency of interspecies transfer (16). Recipient (KB30Δ*trp2*) and donor (*S. typhimurium*/F71) cultures were mixed in ratios of 10:1 to 1:1 in L-broth and incubated for 40 min at 37 C without agitation.

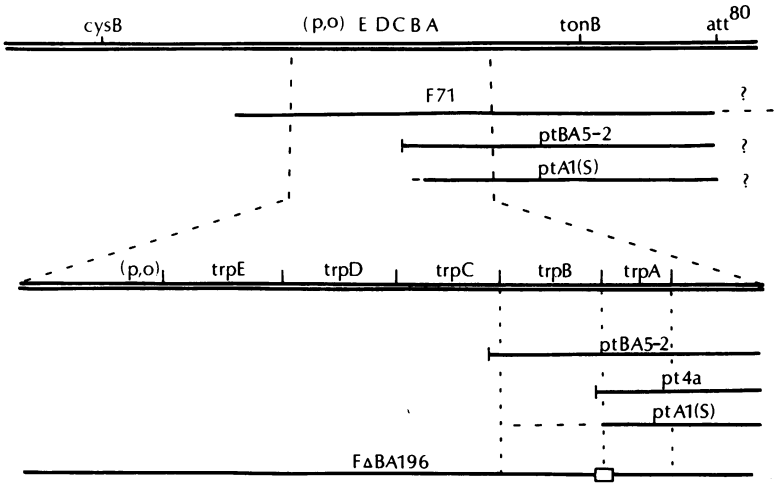

FIG. 1. *E. coli trp operon. The horizontal double line at the top is a schematic representation of the cysB-trp-tonB-att^{80} region of the E. coli chromosome. Immediately below we indicate the segments of bacterial DNA believed to be present in F71, pt A1(S), and pt BA5-2. F71 and pt A1(S) carry S. typhimurium trp operon DNA, and pt BA5-2 carries E. coli trp operon DNA. Below is an expanded diagram of the trp operon, followed by the segments of bacterial DNA carried by three transducing phage and an F-factor. F ΔtrpBA196 carries S. typhimurium DNA; pt 4a carries E. coli DNA. Where accurately known, the end points of the bacterial DNA of phage are indicated by vertical bars. Phage pt A1(S) may carry some but not all of S. typhimurium trp B. We do not know the right-hand end points of bacterial DNA in phage pt BA5-2, pt 4a, and pt A1(S), although, since the first two presumably arose by faulty excision of a prophage integrated at att^{80} in wild-type E. coli, their end points are presumably att^{80}. Phage pt A1(S) arose by excision of a prophage integrated to the right of the S. typhimurium trp operon of F71. The exact location of the φ80 prophage attachment site of F71 is not known; it may be at a location analogous to att^{80} in E. coli.*

TABLE 1. *Bacterial strains*

Strain	Genotype[a]	Description[b]
Salmonella typhimurium/F71	*trpE52 cysB12 pyrF146 ile78*/F71	Stock SU694, described by Sanderson and Hall (17), was generously provided by K. E. Sanderson.
S. typhimurium his519	Δ(*rfbB his*)	Generously provided by Bruce Stocker; isolated by Levinthal and Nikaido (11).
Escherichia coli KB30 Δ*trp2*	Δ(*att^{80} tonB trp*)-2	This strain is a *tonB-trp* deletion mutant of KB30 (20) in which the φ80 prophage and the entire *trp* operon were deleted.
E. coli Δ*trpAE1*/F71	Δ(*tonB trpAE1*) *str^r*/F71	The F71 episome was transferred from *S. typhimurium*/F71 to *E. coli* ΔtrpAE1. Δ*trpAE1* is a deletion of *tonB* and the entire *trp* operon.
E. coli Δ*trpAE1*/F71 *trpA7*	Δ(*tonB trpAE1*) *str^r*/F71 *trpA7*	The *trpA7* mutation[a] on the episome was isolated by UV mutagenesis and penicillin selection.
E. coli Δ*trpAE12*/F Δ*trpBA196*	Δ(*tonB trpAE12*) *str^r*/F72 Δ*trpBA196*	A deletion of parts of the *trpB* and *trpA* genes[a] of *S. typhimurium* (see Fig. 1) was crossed from *S. typhimurium* Δ*trpBA196* (provided by E. Balbinder) onto the *S. typhimurium trp* episome F72 (18).
*E. coli KB30*Δ*trp2*/F71	Δ(*att^{80} tonB trp*)-2/F71	The F71 episome was transferred from *S. typhimurium*/F71 to KB30Δ*trp2*.
*E. coli KB30*Δ*trp2*/F71 (*φ80h*)	Δ(*att^{80} tonB trp*)-2/F71 (*φ80h*)	*KB30*Δ*trp2* was made lysogenic for *φ80h*.
E. coli W1485	Wild type	
E. coli W3110	Wild type	

[a] The nomenclature of *trp* genes is that used for *E. coli* (see Fig. 1).
[b] All strains are from the collection of C. Yanofsky, unless otherwise stated.

Cells were centrifuged and resuspended in an equal volume of minimal medium lacking glucose, and 0.1 ml of this suspension was spread on minimal agar plates. Control plates consisted of 0.1 ml of the parental strains streaked on separate minimal agar plates. Plates were incubated for 4 to 7 days at 37 C.

Lysogenization with ϕ80h. Dilutions of ϕ80*h* sufficient to give 50 to 300 plaques per plate were plated in tryptone soft agar with approximately 2×10^8 bacteria from a fresh L-broth culture. Plates were incubated at 37 C for 2 to 3 days. Bacteria growing within plaques were isolated and purified by serial streaking and single colony isolation. The presence of the ϕ80*h* prophage in lysogenic bacteria was detected by replicating plates containing single colonies of the strain to be tested to tryptone soft agar seeded with ca. 2×10^8 cells of strain W1485, and incubating these indicator plates overnight at 37 C.

Induction of prophage with UV light. Log-phase cultures (5 ml) of KB30Δ*trp2*/F71(ϕ80*h*) (ca. 5×10^8 cells/ml) were centrifuged and resuspended in one-half volume of minimal medium (no glucose) and irradiated in open petri dishes with 400 to 800 ergs/mm^2 of ultraviolet (UV) light (18 cm from a General Electric G8T5 lamp for 10 to 20 s). The suspension was transferred to tubes containing 5 ml of tryptone broth and grown at 37 C on a reciprocal shaker. Cells and debris were pelleted by low-speed centrifugation and the supernatant was stored at 4 C with a few drops of chloroform. Lysates were titered in tryptone soft agar seeded with 2×10^8 cells of strain W1485.

Induction of prophage with mitomycin C. A 100-ml culture of KB30Δ*trp2*/F71(ϕ80*h*) was grown to 2.5×10^8 cells/ml at 37 C. Freshly dissolved mitomycin C (Nutritional Biochemicals, Inc.) in water was added to a final concentration of 10 μg/ml, and the cells were grown for 1 h, centrifuged at room temperature, and suspended in an equal volume of L-broth. After overnight growth, the culture was centrifuged, and 5 ml of chloroform was added to the supernatant. The lysate was stored in the cold and titered on W1485.

Selecting trp transducing phage. In initial experiments, 1×10^8 to 2×10^8 ϕ80*h* obtained by UV induction of KB30Δ*trp2*/F71(ϕ80*h*) were spread on ACH plates with 0.05 ml of an overnight L-broth culture of *E. coli* Δ*trpAE1*/F71 *trpA7*. (This strain carries an episome containing the *S. typhimurium trp* operon with a mutation in *trpA* [Table 1]). The plates were incubated for 4 to 7 days. Like all Trp$^-$ strains, Δ*trpAE1*/F71 *trpA7* cannot grow on ACH medium. A specialized transducing phage which carries *trpA* was detected on the plate by the growth of a colony surrounded by a cluster of small colonies (secondary transductants) and a zone of lysis. When plates were incubated at 37 C longer than 4 days, the transductant colonies in the cluster increased in number as the zone of lysis and transduction spread radially. Primary lysates were made by stabbing the zones of lysis with sterile toothpicks and transferring the phage to 1 ml of L-broth. After addition of a few drops of chloroform, the lysates were centrifuged and stored at 4 C.

In a later experiment, 10 ml of mitomycin C-induced lysate (ca. 5×10^9 phage/ml) in L-broth was incubated for 1 h in a 6-inch (15.2 cm) tube at 37 C on a reciprocating shaker to remove chloroform, and mixed with 1 ml of a fresh L-broth culture of *E. coli* Δ*trpAE12*/F Δ*trpBA196*. The F72 episome of this strain carries the *S. typhimurium trp* region with a deletion of parts of *trpB* and *trpA* (Table 1). After a further 20 min of incubation at 37 C with shaking to allow phage adsorption and infection, cells were sedimented at room temperature, resuspended in 1.1 ml of L-broth, and plated (0.2 ml/plate) on ACH medium. Plates were incubated at 37 C.

Purification of ϕ80h pt A1(S). A loopful of the primary stab lysate was streaked on ACH plates, previously spread with 0.05 ml of an overnight L-broth culture of Δ*trpAE1*/F71 *trpA7*. After 4 days of incubation at 37 C, zones of lysis around transductant colonies were stabbed and the phage was transferred to 1 ml of L-broth as described above. A dilution of this secondary stab lysate sufficient to give discrete plaques was plated in tryptone soft agar seeded with Δ*trpAE1*/F71 *trpA7*. Stab lysates were made from each of 30 plaques. A master plate was constructed by dipping a sterile toothpick into each lysate, and stabbing the surface of a tryptone soft agar plate seeded with Δ*trpAE1*/F71 *trpA7*. After overnight incubation at 37 C, the master was replicated to ACH plates seeded with 0.05 ml of fresh cultures of either Δ*trpAE12*/F Δ*BA196* or Δ*trpAE1*/F71 *trpA7*. These plates were incubated at 37 C for 3 days. Each of the 30 single-plaque isolates of purified ϕ80*h pt A1*(S) transduced Δ*trpAE1*/F71 *trpA7* to Trp$^+$. None of the isolates transduced Δ*trpAE12*/F Δ*BA196*, which indicates that the phage does not carry all of *trpB*$^+$.

Phage DNA isolation. DNA from ϕ80 *pt 4a* and ϕ80*h pt A1*(S) was isolated as described previously (17).

Pulse labeling and isolation of bacterial RNA. The basic procedure for pulse labeling and isolation of ^{3}H-labeled RNA has been described previously (4). Labeled RNA from *E. coli* W3110 and *S. typhimurium his519* was prepared as follows: log-phase cultures (ca. 5×10^8 cells/ml) growing at 37 C (*E. coli*) or 25 C (*S. typhimurium*) in minimal medium (plus 50 μg of L-histidine per ml for *S. typhimurium*) were derepressed for *trp* mRNA synthesis for 10 min by exposure to the tryptophan analogue 3-indolylacrylic acid (20 μg/ml) and labeled for 2 min (*E. coli*) or 1.5 min (*S. typhimurium*) with 20 μCi of [^{3}H]uridine per ml (28 Ci/mmol). RNA was prepared using two deoxyribonuclease treatments and two phenol extractions as described (4). Specific activity of the RNA was determined by (i) precipitating 2 to 10 μg of RNA with 5% trichloroacetic acid, collecting the precipitates on membrane filters (Millipore Corp.), and counting the filters in a toluene-based scintillation fluid in a Packard Tri-Carb liquid scintillation spectrometer with settings appropriate for ^{3}H (counting efficiency of [^{3}H]RNA on filters is about 25%); and (ii) determining the RNA concentration by measuring the absorbance at 260 nm, assuming an extinction coefficient for RNA of 24 cm^{-1} mg^{-2}.

DNA-RNA hybridization. ^{3}H-labeled RNA from

E. coli W3110 and *S. typhimurium his519* was annealed with 1 to 8 μg of denatured transducing phage DNA immobilized on filters (5) in a volume of 0.4 ml of hybridization buffer [0.01 M tris(hydroxymethyl)-aminomethane-hydrochloride, pH 7.5, at 25 C; 0.3 M KCl; 10^{-3} M ethylenediaminetetraacetic acid] for 18 h at 65 C. After hybridization, batches of filters were gently agitated for 15 min at 25 C with 3 ml of fresh hybridization buffer per filter, and then incubated for 1 h at 55 C in fresh hybridization buffer (1 ml/filter) with occasional stirring. After a final rinse with hybridization buffer, filters were dried, and the hybridized ^{3}H-labeled RNA was assayed by liquid scintillation counting as described above for acid precipitates.

RESULTS

Transfer of F71 to E. coli KB30Δtrp2. When cultures of *S. typhimurium* F71 and *E. coli* KB30Δ*trp2* are mixed and plated together on minimal medium (see Materials and Methods), prototrophic colonies appear at a frequency of two to five colonies per 10^7 KB30Δ*trp2* cells plated. These prototrophs presumably arise by interspecies transfer of F71 into KB30Δ*trp2*. To characterize the progeny from the above cross, a total of six Trp$^+$ colonies derived from three independent crosses were picked and purified by serial streaking on minimal medium. These and other related strains were tested for sensitivity to phages T6, T1, and φ80^{vir} (Table 2). Like the KB30Δ*trp2* parent, all prototrophic progeny from the above cross-tested strains were sensitive to phage T6. The *S. typhimurium* F71 parent (like wild-type *S. typhimurium*) was resistant to this phage. This is consistent with the proposed origin of the prototrophs by sexduction of KB30Δ*trp2*. Although both *S. typhimurium* F71 and KB30Δ*trp2* are resistant to phages T1 and φ80^{vir}, the progeny resulting from crossing the two strains is sensitive to both phages (Table 2). This is consistent with earlier observations in this laboratory that the presence of F71 confers T1 sensitivity on *E. coli* Δ*trpAE1* (C. Yanofsky, unpublished data). Both *E. coli* Δ*trpAE1* and KB30Δ*trp2* are resistant to phages T1 and φ80^{vir} because they lack *tonB*$^+$ (Table 1; Fig. 1). These data show that F71 carries a locus which can substitute for the *tonB* region of *E. coli;* thus, whatever the basis of the natural resistance of *S. typhimurium* F71 (and of wild-type *S. typhimurium*) to these phages, it is not due to lack of *tonB*$^+$. Further evidence that the progeny described are merozygotes of the type, KB30Δ*trp2*/F71, is presented below.

Does F71 carry an attachment site for the φ80 prophage? The presence on F71 of an *S. typhimurium* gene analogous to *tonB* raises the possibility that the episome might carry a φ80 attachment site (see Fig. 1). Since the KB30Δ*trp2* chromosome apparently lacks the normal φ80 attachment site (20), F71 might carry the only such site in the KB30Δ*trp2*/F71 merozygotes. To examine the effect of the presence of F71 on the ability to be lysogenized, we plated φ80*h* on lawns of KB30Δ*trp2* and KB30Δ*trp2*/F71, and, from plaques which contained visible bacterial growth ("colony-centered plaques"), we attempted to isolate stable lysogens. (We used φ80*h* instead of wild-type φ80 because only the host range mutant can adsorb to KB30Δ*trp2*.) We observed colony-centered plaques on lawns of both strains (15 to 20% of the plaques on KB30Δ*trp2* and 30 to 50% of the plaques on KB30Δ*trp2*/F71). We were able to isolate stable lysogens of φ80*h* from 7 of 8 colony-centered plaques on KB30Δ*trp2*/F71 and from 1 of 7 colony-centered plaques on KB30Δ*trp2* (Table 3). These data indicate that F71 facilitates stable lysogenization. In addition to obtaining one stable lysogen of KB30Δ*trp2* (isolate 2 in Table 3), we found two other isolates of this strain which even after extensive purification yielded some

TABLE 2. *Sensitivity of bacterial strains to phages φ80^{vir}, T1, and T6*[a]

Strain	Killing of bacteria by phage[b]		
	φ80^{vir}	T1	T6
W3110	+	+	+
Salmonella typhimurium F71	−	−	−
KB30Δ*trp2*	−	−	+
KB30Δ*trp2* T6^r	−	−	−
KB30Δ*trp2* × F71			
Cross 1			
Isolate 1	+	+	+[c]
Isolate 2	+[c]	+[c]	+
Cross 2			
Isolate 3	+	+[c]	+
Isolate 4	+	+[c]	+
Isolate 5	+[c]	+	+
Cross 3			
Isolate 6	+	+[c]	+
Δ*trpAE1*	−	−	+
Δ*trpAE1*/F71	+	+	+[c]

[a] A loopful of bacterial culture was streaked in a straight line on a nutrient agar plate. A loopful of phage lysate was placed on the streak and the position of the phage indicated by marking the bottom of the plate. Plates were incubated for 24 h at 37 C.

[b] Plates were scored by determining whether growth of the bacteria was inhibited by the phage.

[c] A few single colonies were observed on the plate where phage were applied, usually at the edges of the zone of lysis.

TABLE 3. *F71 facilitates lysogenization by φ80h*

KB30Δ*trp2*		KB30Δ*trp2*/F71	
Isolate	Fraction of colonies lysogenic[a]	Isolate	Fraction of colonies lysogenic[a]
1[b]	8/78	1	28/28
2	46/46	2	82/82
3	0/52	3	82/82
4	0/41	4	70/70
5	0/52	5	110/110
6	19/76	6	0/59
7	0/55	7	78/78
		8	76/76

[a] Lysogenic clones were isolated as described in Materials and Methods. After three successive streakings, a single colony from each clone was picked and grown up in L-broth. These cultures were washed twice with fresh L-broth by centrifugation to eliminate most free phage, and diluted to give colonies on nutrient agar plates. The figures in the table indicate the fraction of colonies which formed plaques when the plate was replicated to a φ80*h*-sensitive lawn of bacteria.

[b] Each cell line originated from a different colony-centered plaque.

phage-producing colonies (isolates 1 and 6 of KB30Δ*trp2*, Table 3). Presumably in these isolates the prophage is not stably integrated. The prophage in all KB30Δ*trp2*/F71(φ80*h*) isolates examined appears to be stably integrated, since all single colonies derived from such lysogens produce phage. The lysogenic merozygotes were tentatively designated KB30Δ*trp2*/F71(φ80*h*).

Isolation of trp transducing phage. If the φ80*h* prophage in KB30Δ*trp2*/F71(φ80*h*) is integrated at a site on the *S. typhimurium* F71 *trp* episome analogous to *att*[80] in *E. coli*, a φ80*h* lysate obtained by induction of the lysogenized merozygote might yield *trp* transducing particles. Since KB30Δ*trp2* lacks the entire *E. coli trp* operon, the only *trp* genes which could be carried by the phage would be derived from *S. typhimurium*. In the first experiment with an induced lysate of KB30Δ*trp2*/F71(φ80*h*) we obtained a phage (isolate 1, Table 3) which transduced Δ*trpAE1*/F71 *trpA7* to Trp$^+$. We purified this phage by serial streaking on Δ*trpAE1*/F71 *trpA7*, a strain which contains only *S. typhimurium trp* operon DNA. Characterization of this nondefective phage showed that it transduces *trpA* mutants, but not *trpB*, *trpC*, *trpD*, or *trpE* mutants (Table 4). The phage presumably carries the entire *trpA* gene because it efficiently transduces *E. coli trpA* mutants with operator-proximal and operator-distal mutations. In a later experiment, about 80 *trpB*$^+$*A*$^+$ transducing phage were detected in a mitomycin C-induced lysate of the same lysogenized merozygote (see Materials and Methods). The transduction recipient used in this latter experiment, *E. coli* Δ*trpAE12*/F Δ*trpBA196*, proved to be a better test strain than Δ*trpAE1*/F71 *trpA7*, because unlike the *trpA7* mutation Δ*trpBA196* does not revert. The frequency of *trpB*$^+$*A*$^+$ transducing phage in a mitomycin C-induced lysate was about 1 per 6 × 10^8 viable phage. The transduction patterns obtained with 13 of these phage are shown in Fig. 2. All of these phage transduce *E. coli* strains with mutations in *trpB* or *trpA*, since they were selected on a *trpBA* deletion mutant. One phage, number 73, is *trpA*$^+$*B*$^+$; one, number 21, is *trpA*$^+$*B*$^+$*C*$^+$; seven phage are *trpA*$^+$*B*$^+$*C*$^+$*D*$^+$; and four phage are *trpA*$^+$*B*$^+$*C*$^+$*D*$^+$*E*$^+$. Since these phage were obtained from the same induced lysate, some or all of the phage of a particular class may be identical. The pattern of operon segments carried by these transducing phage indicates that the φ80*h* prophage integrated into F71 on the *trpA* side of the *S. typhimurium trp* operon. Note in Fig. 2 that each of these phage which transduces an operator-distal mutation in any *trp* gene also transduces all operator-proximal mutations tested in that gene. This pattern is to be expected if the phage transduce by com-

TABLE 4. *Transducing capabilities of φ80h pt A1(S)*[a]

Transduction of *trp* mutants						
trpE22-1	*trpD1383*	*trpC782*	*trpB9578*	*trpA38*[b]	*trpA96*[c]	Δ*trpAE1*/F71 *trpA7*
−	−	−	−	+	+	+

[a] A loopful of the bacterial culture to be tested was streaked across an ACH plate, and a loopful of phage lysate was spotted at a known location on the bacterial streak. Plates were incubated at 37 C for 3 to 4 days. Transduction was revealed by extensive bacterial growth only where phage were applied.

[b] Most operator-proximal *trpA* mutation known.

[c] Most operator-distal *trpA* mutation known.

plementation but not by recombination. We expect transduction of *E. coli trp* mutants by recombination with a phage carrying *trp* DNA of *S. typhimurium* to be a rare event because of the low frequency of recombination between the *trp* genes of *E. coli* and *S. typhimurium* (3).

To demonstrate that the *trp* genes carried by these transducing phage are from *S. typhimurium*, the DNA of one phage, *ϕ80h pt A1*(S), was employed in DNA-RNA hybridization experiments. As can be seen in Table 5, 1.4 to 3.3-fold more *S. typhimurium trp* mRNA hybridizes to *ϕ80h pt A1*(S) DNA than to *ϕ80 pt 4a* DNA, depending upon the DNA-RNA ratio in the hybridization mixture. *ϕ80 pt 4a* carries *E. coli trpA*, about ⅓ of *trpB*, and bacterial DNA extending to the right of *trpA* towards att^{80} (Fig. 1). Conversely, 3.8- to 7.1-fold more *E. coli trp* mRNA hybridizes to *pt 4a* DNA than to *pt A1*(S) DNA. These results show that *S. typhimurium trp* mRNA hybridizes preferentially to *pt A1*(S) DNA and *E. coli trp* mRNA preferentially to *pt 4a* DNA.

Efficiency of annealing of trp mRNA to homologous and heterologous DNA. Not all *S. typhimurium trp* mRNA which can anneal to *trp* transducing phage DNA anneals in a single hybridization period of 24 or 48 h. The amount

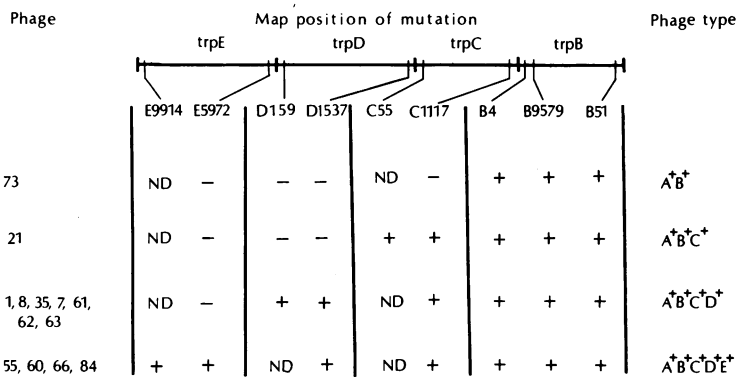

FIG. 2. *Transduction of E. coli trp mutants by ϕ80 trp phage. The procedure for transduction is described in the legend to Table 4. The approximate map positions of trp mutants are indicated on the diagram at the top. A plus indicates extensive transduction; a minus indicates no transduction; ND, not done. All phage shown are trpA+B+ because they were isolated on a trpBA deletion mutant (see text).*

TABLE 5. *ϕ80h pt A1(S) carries DNA of the S. typhimurium trp operon*

Source of RNA	Source of DNA	Hybridization[a]		
		1[b]	2[b]	3[b]
Salmonella typhimurium his519	*pt A1*(S)	168 ± 13	230 ± 7	323 ± 21
	pt 4a	56 ± 3	86 ± 12	200 ± 12
	Homologous [*pt A1*(S)]/ heterologous (*pt 4a*)	2.6–3.3[c]	2.3–3.2[c]	1.4–1.8[c]
Escherichia coli W3110	*pt A1*(S)	709 ± 122	1239 ± 89	2547 ± 153
	pt 4a	3975 ± 127	6649 ± 53	10176 ± 83
	Homologous (*pt 4a*)/ heterologous [*pt A1*(S)]	4.5–7.1[c]	4.8–5.6[c]	3.8–4.2[c]

[a] Labeled RNA from derepressed cells was prepared and hybridized to phage DNA as described in Materials and Methods. [^{3}H]RNA added per reaction: *S. typhimurium*, 5.5 × 10^5 counts/min; *E. coli*, 2.3 × 10^6 counts/min. All hybridizations were done in duplicate. The average low level of nonspecific binding of [^{3}H]RNA to *ϕ80* phage DNA (about 50 counts/min for *S. typhimurium*, 300 counts/min for *E. coli*) has been subtracted. Values are given as the mean counts per minute hybridized ± the sum of the deviations of specific and blank hybridizations. Values of hybridization are higher for *E. coli* because the specific activity of the radioactive RNA added to the hybridization reaction was higher.

[b] Micrograms of DNA per filter.

[c] The lower value is the minimum hybridization to homologous DNA divided by the maximum hybridization to heterologous DNA. The higher value is the maximum hybridization to homologous DNA divided by the minimum hybridization to heterologous DNA.

of *S. typhimurium trp* mRNA annealed to *pt BA5-2* after 48 h (4,324 counts/min, experiment I, Table 6) is only 13% greater than the amount annealed after 24 h (3,890 counts/min, experiment III, Table 6). Nevertheless, considerable RNA left in solution after one 48-h hybridization period anneals to the same phage DNA in a second 48-h hybridization period. In experiment I of Table 6, the ratio of *S. typhimurium trp* mRNA which hybridized to heterologous *pt BA5-2* DNA in the first round (H_1 = 4,324 counts/min) divided by the RNA left in the supernatant which subsequently hybridized to the same DNA in the second round (H_2 = 1,781 counts/min) is 2.42. The analogous ratio, H_1/H_2, of *S. typhimurium trp* mRNA annealed in first and second 48-h hybridization periods to homologous *pt A1*(S) DNA is 16.7 (8,032 counts/min to 482 counts/min, experiment II of Table 6). Thus, although the efficiency of annealing of *trp* mRNA to homologous DNA in one 48-h incubation under our conditions is not 100%, it is clearly greater than the efficiency of annealing to heterologous DNA.

Some *S. typhimurium trp* mRNA appears to be unable to anneal to *E. coli trp* operon DNA even when several successive hybridization periods are employed. This is shown by experiment III of Table 6. *S. typhimurium trp* mRNA was annealed three times for 24 h each to *pt BA5-2* DNA. Of the RNA which remained in the supernatant after the three hybridization periods, only 359 counts/min annealed in the fourth round, i.e., less than 10% of that which annealed in the first. Nevertheless, the supernatant RNA from the fourth hybridization yielded 1,780 counts/min when hybridized to homologous *pt A1*(S) DNA, compared to only 145 counts/min when hybridized a fifth time to heterologous *pt BA5-2* DNA. Yet a larger segment of the *trp* operon is present in *pt BA5-2* than in *pt A1*(S) (see Fig. 1).

DISCUSSION

From the merodiploid KB30Δ*trp2*/F71(φ80*h*), we have isolated specialized transducing phage which carry segments of the *S. typhimurium trp* operon. Thus, in the merodiploid, the prophage and the *trp* operon of the episome are linked. The pattern of transduction of *trp* mutants by the specialized transducing phage derived from one isolate of KB30Δ*trp2*/F71(φ80*h*) indicates that the prophage integrated into F71 on the operator-distal side of *trpA*. This site is probably analogous to att^{80} in *E. coli* (Fig. 1). The *cysB-trp* regions of *E. coli* and *S. typhimurium* are inverted on their respective chromosomes relative to outside markers (18), but the locations of the end points of the inverted regions are not known precisely. The ability of φ80 to integrate on the *trpA* side of the *S. typhimurium trp* operon suggests that the end point of the inversion on that side is beyond att^{80}.

TABLE 6. *Yield of S. typhimurium trp mRNA in successive hybridizations to homologous and heterologous trp operon DNA*

Expt	Hybridization of RNA at each step[a]										
	H_1		H_2		H_3		H_4		Test[d]		H_1/H_2
	DNA[b]	Δ[c]	DNA	Δ	DNA	Δ	DNA	Δ	DNA	Δ	
I (48 h)	E	4,324	E	1,781					E	546	2.42
									S	2,694	
II (48 h)	S	8,030	S	482							16.7
III (24 h)	E	3,890	E	2,138	E	1,400	E	359	E	145	1.79
									S	1,780	

[a] Pulse-labeled *S. typhimurium* RNA (13 μg, 4.5×10^6 counts/min) containing *trp* mRNA was hybridized in duplicate to 8 μg *pt BA5-2* DNA, *pt A1*(S) DNA, or φ80 DNA at 65 C. Hybridization times for steps H_1 to H_4 are given in parentheses under "Expt." After the first hybridization, each filter was drained and removed from its vial, 0.05 ml of water was added to the supernatant RNA to compensate for evaporation during the first hybridization, and a fresh DNA-filter was added. The incubation was then repeated. Third and fourth hybridization steps were performed in the same way.

[b] "E" represents DNA of phage *pt BA5-2*, which carries the *E. coli trpB* and *trpA* genes (Fig. 1); "S" represents DNA of phage *pt A1*(S), which carries the *trpA* gene of *S. typhimurium* (Fig. 1).

[c] Hybridization is expressed as counts per minute annealed, corrected for nonspecific binding of RNA to filters. Duplicates agreed to better than 10%.

[d] In experiments I and III, the supernatant RNA which remained after two or four successive hybridizations was annealed in the test hybridization for 24 h to *pt BA5-2* DNA or *pt A1*(S) DNA (E or S, respectively) to test for enrichment of the residual supernatant RNA in *Salmonella*-specific sequences.

Lavalle and De Hauwer (9) showed that the ratio, H_1/H_2, of the amount of *trp* mRNA which hybridizes to transducing phage DNA in one incubation (H_1), divided by the amount of residual *trp* mRNA left in the supernatant of the first incubation which hybridizes to an equivalent amount of the same DNA in a second incubation (H_2), is related to the dissociation constant of the hybrid by the equation $K_{diss} = [DNA]/(H_1/H_2) - 1$, where [DNA] is moles of DNA on a filter per liter of RNA solution tested. It is essential when employing this equation to estimate K_{diss} that each hybridization be allowed to reach equilibrium (8). (Under the conditions of our experiments, we estimate from measurements of the hybridization rate [4] that hybridization of *S. typhimurium trp* mRNA to *pt BA5-2* DNA is at least 90% complete in 48 h.) Applying the equation of Lavalle and De Hauwer (9), assuming a molecular weight for *pt BA5-2* and *pt A1*(S) DNAs equal to 3.1×10^7 (found for a similar transducing phage; reference 23), the calculated dissociation constants are about 0.4×10^{-10} and 4×10^{-10} liters/mol for the homologous and heterologous reactions, respectively. Lavalle and De Hauwer (9) obtained dissociation constants of 0.7×10^{-10} to 1.2×10^{-10} liters/mol for hybrids of *E. coli trp* mRNA and homologous DNA. Bishop (2) reported a dissociation constant of 3.2×10^{-11} for the hybrids of *E. coli* ribosomal RNA and DNA. Although our estimate of K_{diss} for the heterologous hybrid is somewhat inaccurate because equilibrium was not attained, failure to reach equilibrium cannot account for the large difference in K_{diss}.

Is *S. typhimurium trp* mRNA which anneals in the first incubation period more homologous to *E. coli trp* operon DNA than the RNA which anneals in subsequent incubation periods or the RNA which does not anneal during several successive incubations? Heterologous *trpA* mRNA which is left in the supernatant after hybridization forms hybrids with *E. coli trpA* DNA which are slightly less stable than hybrids formed during the first period of hybridization (12). This suggests that hybridization to heterologous DNA results in enrichment of supernatant RNA in *trp* mRNA sequences with lower homology to that DNA. The finding that exhaustive prehybridization of *S. typhimurium trp* mRNA with heterologous *pt BA5-2* DNA fails to remove all the RNA which can anneal to *pt A1*(S) DNA (experiment III, Table 6) is consistent with this view. Use of milder annealing conditions, e.g., lower temperature, might allow more *S. typhimurium trp* mRNA to anneal to heterologous DNA (13).

Mispairing of nucleotide sequences depresses the forward rate of annealing (4, 19, 21). Is the rate of the reverse reaction (dissociation of the hybrid) also altered by mispairing? *S. typhimurium trp* mRNA anneals approximately one-third as fast to phage DNA carrying the entire *E. coli trp* operon DNA as does *E. coli trp* mRNA (4). The annealing rate of *S. typhimurium trp* mRNA corresponding to the *trpBA* region, under the hybridization conditions reported here, is 1.7-fold slower than the average for total *S. typhimurium trp* mRNA (R. M. Denney, Ph.D. thesis, Stanford University, Stanford, Calif., 1973). The apparent 10-fold difference in equilibrium constants we observed for homologous and heterologous hybrids thus cannot be accounted for solely by the measured depression in the forward rate. Furthermore, *Aerobacter aerogenes trp* mRNA anneals to *E. coli trp* operon DNA with the same rate constant as does *S. typhimurium trp* mRNA (4). Nevertheless, the annealing efficiency of *A. aerogenes trp* mRNA to *E. coli trp* operon DNA is much lower than the annealing efficiency of *S. typhimurium trp* mRNA to the same DNA (R. M. Denney, Ph.D. thesis, Stanford University, Stanford, Calif., 1973). These data suggest that, in addition to decreasing the rate of annealing, mispairing increases the rate of dissociation of DNA-RNA hybrids.

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