JOURNAL OF VIROLOGY, June 1977, p. 654-661
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Vol. 22, No. 3
Printed in U.S.A.

Isolation of Specialized Lambda Transducing Bacteriophages for Flagellar Genes (*fla*) of *Escherichia coli* K-12

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Received for publication 23 November 1976

Specialized transducing lambda phages carrying the region III flagellar genes (*fla*) of *Escherichia coli* K-12 were isolated by a new method. A strain carrying both a cryptic λ prophage near the *his* genes and a deletion of the *att*λ gene was used as a starting strain. The lysogen of λ*cI857*p*gal8-bio69* was isolated in which the prophage was integrated within the λ cryptic genes by means of recombination with the residual λ DNA. The strains with deletions starting within the prophage and ending in these *fla* genes were selected from among the heat-resistant survivors of the lysogen. They were then infected with heat-inducible and lysis-defective λ phages and, thus, specialized transducing phage lines for *hag* and *fla* were obtained. High-frequency transfer lines of rare phages carrying the *fla* genes were isolated by inducing a strain carrying a heat-inducible λ prophage near the *his* genes and selecting by transduction of a *fla* deletion strain. Preliminary characterization of these transducing phages is also reported.

Various specialized transducing phage lines have been established and used by a number of groups in order to study the in vitro mechanism of gene expression. Such a phage line would be also useful for the identification of the products of the genes for flagellar formation, *fla*, most of whose functions are still obscure (3, 4). The *fla* genes in *Escherichia coli* K-12 were assigned to three regions called regions I, II, and III by Silverman and Simon (17). The region I *fla* genes were mapped near the *pyrC* gene, those of the region II were mapped in the region between the *aroD* and *uvrC* genes, and those of the region III were mapped in the region between the *uvrC* and *his* genes in the *E. coli* chromosomal map.

In this paper, we report the isolation of λ specialized transducing phages carrying the region III flagellar genes of *E. coli* K-12, *fla* and *hag*, controlling the formation of flagella and flagellin, respectively. Genes to be transduced by a specialized transducing phage should be close to the integration site of the prophage. Such a location can be established by transposition of either the gene or the prophage. We devised the latter method to insert the *att*λ gene within a close vicinity of the genes in question. To this end, a strain carrying the *att*λ gene near the genes of interest was isolated from the strain that contained both a deletion of *att*λ gene and a cryptic λ prophage, infected with λ*cI857*p*gal8-bio69* (6). Then, the DNA length between the *att*λ gene and these genes was shortened by selection of heat-resistant survivors carrying some prophage gene deletions that extend into the host genes. We think that this method will be applicable to other genes as well, because λ lysogens with a prophage integrated in a secondary attachment site are easily isolated (14). We also report λp*fla* isolation directly from a λ lysogen that has a λ prophage near the *his* gene.

Independent isolations of flagellar transducing phages carrying similar regions of *E. coli* have been carried out by others (15; H. Kondoh, personal communication).

MATERIALS AND METHODS

Bacteria and bacteriophage strains. The origin and genotype of the *E. coli* K-12 strains used are listed in Table 1. Table 2 lists the bacteriophages used. Stock lysates of the phage strains without *cI857* mutations were prepared by a plate method (1). Lysates of λ strains carrying the *cI857* allele were prepared from appropriate lysogens by heat induction at 42°C for 15 min followed by incubation at 37°C for 1 h. The lysates were concentrated by the method of Yamamoto et al. (20). Phages without amber mutations were titered on strain W3350 and those with amber mutations were titered on strain Ymel. λ Lysogens were identified by cross-streaking with high-titer lysates (more than 10^9 PFU/ml) of λ*vir* and λ*b2cI* on tryptone (Difco) agar plates.

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Strains that were lysed by λ*vir* but not by λ*b2cI* were classified as λ lysogens.

Media and growth conditions. The standard rich media used were L broth and L broth agar (LA) (8). The L broth medium contained (per liter of water): 10 g of tryptone (Difco), 5 g of yeast extract (Difco) and 5 g of NaCl. EMB agar, nutrient gelatin agar (NGA), R agar, and R top agar were prepared as described previously (7). Tryptone broth contained (per liter of water): 10 g of tryptone (Difco) and 5 g of NaCl. Tryptone agar and top agar, made from tryptone broth and containing 1.2 and 0.3% agar, respectively, were used for the preparation of λ phage. Cultures were grown with aeration at 30°C unless otherwise specified.

Scoring and selection of *fla*. The flagellar character was ordinarily identified by using NGA (5). Colonies on LA were picked with toothpicks, gridded on NGA, and incubated for 6 h. Motile clones made swarms and nonmotile ones did not.

Transductions. P1-mediated transductions were performed as described previously (8). λ-Mediated transductions were carried out as follows: recipient cells were grown overnight in L broth containing 0.2% (wt/vol) maltose and 0.01 M $MgSO_4$. They were collected by centrifugation, resuspended in an equal volume of a 0.01 M $MgSO_4$ solution, and incubated for 1 h at 37°C for starvation. For transduction, a 0.1-ml portion of phage suspension and an equal volume of bacterial suspension as above were mixed, giving a multiplicity of infection (MOI) of 5. The mixtures were incubated for 30 min for adsorption and plated on NGA.

High-frequency transducing (HFT) lysates were identified by a rapid screening test. Each well of MicroTest II dishes (96 wells, Falcon Plastics) contained 0.05 ml each of lysates (10^{10} PFU/ml) and recipient cells as above (2×10^9 cells/ml). The mixtures were plated on NGA after incubation for 30 min at room temperature. HFT lysates gave confluent swarms on NGA after 9 h at 30°C.

RESULTS

Isolation of specialized transducing phage lines carrying *fla* genes. In an initial step, we thought that an *E. coli* K-12 strain, KS899 (9, 14, 19), which had a λ*cI857* prophage integrated between the *his* genes and the region III *fla* genes (17) in its chromosome, would be useful for the isolation of the region III *fla*-transducing phages (Fig. 1). Accordingly, we attempted to isolate λ*fla* phages from the heat-induced lysates of strain KS899 by the method described for the isolation of λ*gal*- or λ*bio*-transducing phages (2). However, the attempt was unsuccessful, probably because the location of the prophage was too distant from the region III *fla* genes (9, 19). Therefore, we devised a new method for isolating transducing phages that carry parts of the *fla* and *hag* genes from derivatives of strain KS899.

First, we isolated heat-resistant survivors of strain KS899 that were derived from cells in which most of the prophage genes had been deleted. One of them carried λ genes *F* through *J* near the *his* genes and was designated strain KS650 (Fig. 1 and 2; reference 14). Although the deleted chromosomal region of strain KS650 was not determined, this strain makes swarms on NGA (5) and should have a complete set of *fla* genes for flagellar assembly and function. To introduce the *att*λ gene into the chromosome of strain KS650, we inserted the λ*cI857*p*gal8*-

TABLE 1. *Bacterial strains used*

Strain	Relevant genotype	Source/reference
KS650	HfrH Δ(*gal att*λ *chlA bio uvrB*)[a] *thi* carrying λ genes (*F L I J*) near *his*	#60-11 (14)
EJ757	λ*cI857*p*gal8-bio69* lysogen of KS650	This study
KS899	HfrH Δ(*gal att*λ *chlA bio uvrB*) *thi* (λ*cI857* near *his*)	Single lysogen of #60 (14)
EJ800	HfrH Δ(*gal att*λ *chlA bio uvrB*) *thi* Δ(*flaP Q R*)	Heat-resistant clone of KS899
Ymel	F⁺*mel, supE, supF*	H. Ikeda
Ymel(P2)	P2 lysogen of Ymel	This study
EJ710	F⁻*leu trp his met ilvA argG thi thyA xyl mtl strA nalA uvrC*	Y. Komeda
EJ971	*flaA* of EJ710	Y. Komeda
EJ972	*flaB* of EJ710	Y. Komeda
EJ974	*flaC* of EJ710	Y. Komeda
EJ975	*flaD* of EJ710	Y. Komeda
EJ976	*flaE* of EJ710	Y. Komeda
EJ977	*hag* of EJ710	Y. Komeda
EJ980	*flaI* of EJ710	Y. Komeda
EJ982	*flaP* of EJ710	Y. Komeda
EJ983	*flaQ* of EJ710	Y. Komeda
EJ984	*flaR* of EJ710	Y. Komeda
MS1861	F⁻*his argE thyA galU thi strA flaN*	(18)
MS1862	F⁻*his argE thyA galU thi strA flaO*	(18)
W3350	F⁻*galT galK*	Y. Hirota

[a] Δ denotes deletion.

TABLE 2. *Bacteriophages*

Strain	Comments and source
λ*cI857susS7*	T. Icho
λ*imm21int29*	Shimada et al. (14)
λ*vir*	Shimada et al. (14)
P2	T. Yura
P1*kc* derivative	Y. Komeda et al. (8)
λ*cI857*p*gal8-bio69*	K. Shimada (6)
λ*cI857susA.B*	YS122 of K. Shimada
λ*c*⁺*susF96*	YS46 of K. Shimada
λ*cI857susJ*	YS18 of K. Shimada
λ*imm434susN7*	YS189 of K. Shimada
λ*imm434susP3*	YS192 of K. Shimada
λ*imm434susQ21*	YS191 of K. Shimada
λ*Imm434susR.R*	YS193 of K. Shimada

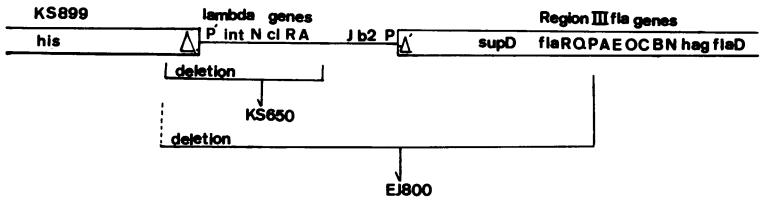

FIG. 1. *Genetic map of E. coli K-12 KS899 near fla. Only the relative sequence of the genes is shown. Thin lines indicate DNA originated from λ phage. P·P' in λ genes indicates λ phage attachment site. Δ·Δ' indicates a secondary attachment site of E. coli (14).*

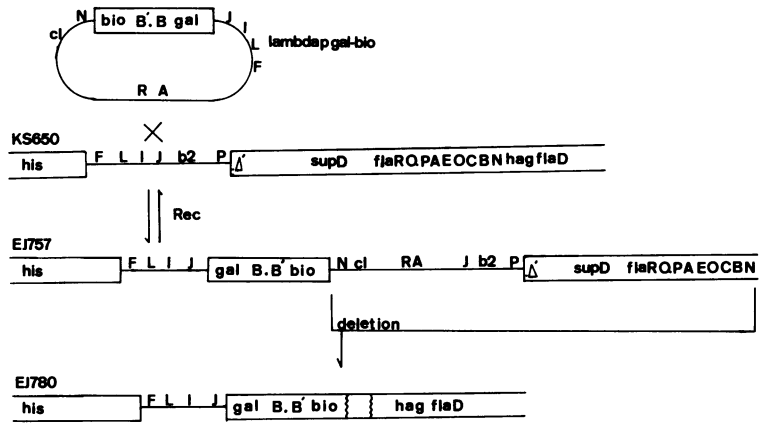

FIG. 2. *Possible process of isolation of strain EJ780. The gap between wavy lines indicates an undefined chromosomal deletion.*

bio69 phage (6) genome into the cryptic λ genes by isolation of *gal*$^+$ lysogens on EMB galactose plates. The λ*cI857*p*gal8-bio69* phage has a deletion in the part of the λ genome, (*b2-int-xis-red*)^{del}, and contains instead bacterial genes (*gal-att*λ*-bio*)$^+$. When strain KS650 is lysogenized with λ*cI857*p*gal8-bio69*, the resulting lysogens presumably carry the prophage integrated within the cryptic λ genes, because this strain has been deprived of the normal λ attachment site and because there is a high degree of homology between the λ*cI857*p*gal8-bio69* genes and the cryptic λ genes carried by the mutant. One of the lysogens thus obtained was designated strain EJ757. A possible genome structure of the prophage carried by this strain is depicted in Fig. 2.

The DNA length between the transposed *att*λ gene and *fla* genes in strain EJ757 was accordingly shortened by introducing various deletions as follows. Most cells of a λ*cI857* lysogenic culture are killed at high temperature (40°C), because phage functions lethal to the host are induced. The rare heat-resistant survivors are often derived from cells in which the phage genes specifying or controlling the lethal functions have been deleted (12, 13). The deletion may extend into adjacent bacterial genes (13). An overnight culture of strain EJ757 was incubated at 42°C for 15 min and was plated on EMB galactose plates. The plates were incubated at 40°C overnight. Galactose-fermenting colonies were picked, and among them nonmotile clones were selected by stabbing NGA. Twenty-four such clones were isolated independently and were designated strains EJ774 through EJ797 (Table 3). Their defective genes were examined by complementation tests utilizing a P1*kc* derivative prepared on various region III *fla* mutants (17) as donors (Table 1). The results are summarized in Table 3. Most of the *fla* mutants (21 of 24) have deletion end points between *hag* and *flaD*. None has the deletions extending into *flaD*. The fact that we could isolate *fla* deletion mutants clearly shows that the λ*cI857*p*gal8-bio69* prophage is located at the chromosomal site near the *fla* genes (Fig. 2).

One of the mutants, strain EJ787, has a *fla* mutation that is complemented by transduced DNA of all the *fla* mutants used here. This strain might be a *fla* point mutant that simultaneously had a *fla* mutation in region I or II (17) and a deletion of the prophage gene. How-

ever, this is unlikely for the following reason. This strain did not show any reversion to fla^+ after prolonged incubation on NGA for 3 days. We selected mutants that had deletions of the prophage gene and then examined them for *fla* mutations. The spontaneous frequency of *fla* mutations is usually 10^{-6} per cell per generation, whereas we could isolate *fla* mutants at a frequency of 1% of the heat-resistant survivors that we examined. Since we could isolate an HFT phage from this strain, as shown below, it should have a deletion mutation near the *fla* genes. Therefore, these two mutations might occur as a single event, and strain EJ787 would be a *fla* deletion mutant. A plausible explanation for this result is that there is another gene(s) controlling the Fla^+ phenotype, which is located between the *flaR* and the prophage genes. Otherwise, if strain EJ787 has a deletion end point in the *flaR* gene, it could transcribe the *flaR* cistron and make a partial product of *flaR*, as suggested by the transcription sequence described by Silverman and Simon (18). The partial *flaR* product could then be complemented by the *flaR* product of EJ984 (*flaR*). We need more *flaR* mutants to clarify this point.

All of the mutants could be lysogenized with λ*cI857susS7* phages at about the normal frequency previously described (14). Therefore, each of the strains EJ774 through EJ797 was deduced to have an *att*λ gene located close enough to the region III *fla* genes so that transducing phages carrying these genes might be isolated by the standard method (2; see Fig. 3).

We chose one of them, strain EJ780, and prepared a lysate from its lysogen by heat induction. When strain EJ977, a *hag* mutant, was starved and infected with this lysate, hag^+ transductants appeared at a frequency of 10^{-8} on NGA. Thirteen hag^+ and temperature-sensitive transductants showing immunity to λ phage superinfection were picked and purified by single-colony isolation. Each lysate prepared from these transductants by heat induction was tested for ability to transduce the *hag* gene. One of the lysates was found to demonstrate the HFT character for the *hag* gene. When induced in liquid cultures, the transductant gave titers of 1×10^9 to 2×10^{10} PFU/ml on a Ymel indicator lawn and *hag* transducing titers of 1×10^7 to 1×10^9 per ml as assayed by swarm formation of the *hag* mutant (EJ977). The transductant clone that produced this HFT lysate for *hag* was designated strain EJ977-11, and the transducing phage prepared from this strain was designated λ*fla#7-11*.

In a similar way, the lysate from EJ787 was used to infect a *flaQ* mutant, EJ983. $flaQ^+$ transductants appeared at a frequency of 10^{-9}. Fifty temperature-sensitive and immunity-plus clones were picked and examined for ability to yield HFT lysates. One lysate harbored this character, and the phage was designated λ*fla#14-41*. The transductant from which the lysate of λ*fla#14-41* was obtained was designated strain EJ983-41.

Isolation of other *fla* specialized transducing phages. First, we isolated various *fla* deletion strains from strain KS899 by the method described by Kondoh and Ozeki (9). The culture of strain KS899 grown overnight in L broth was incubated at 42°C for 15 min and plated on LA. The plates were incubated overnight at 40°C. Heat-resistant surviving clones appeared at a frequency of 10^{-6}/cell. Among them, we sought nonmotile clones on NGA. One of the nonmotile derivatives was found to have a deletion extending from *flaR* to *flaP* in the *fla* cluster and was designated strain EJ800. This mutant served as the starting strain in the following experiments.

Lysates prepared from strain KS899 by heat induction of L broth cultures yielded phage titers of only 10^4 PFU/ml. Several independently prepared lysates were therefore concentrated, and the resultant phage lysate (10^7 PFU in the

TABLE 3. *Characterization of fla deletion strains*[a]

Strain	Complementation[b]											
	flaR	*Q*	*P*	*A*	*E*	*O*	*C*	*B*	*N*	*hag*	*D*	*I*
EJ774 through EJ779	−	−	−	−	−	−	−	−	−	−	+	+
EJ780	−	−	−	−	−	−	−	−	−	+	+	+
EJ781 through EJ786	−	−	−	−	−	−	−	−	−	−	+	+
EJ787	+	+	+	+	+	+	+	+	+	+	+	+
EJ788 through EJ791	−	−	−	−	−	−	−	−	−	−	+	+
EJ792	−	−	−	−	−	−	−	−	−	+	+	+
EJ793 through EJ797	−	−	−	−	−	−	−	−	−	−	+	+

[a] Mapping was carried out by P1-mediated transduction. Standard *fla* mutants listed in Table 1 were used as donors.

[b] +, Existence of complementation; −, no complementation.

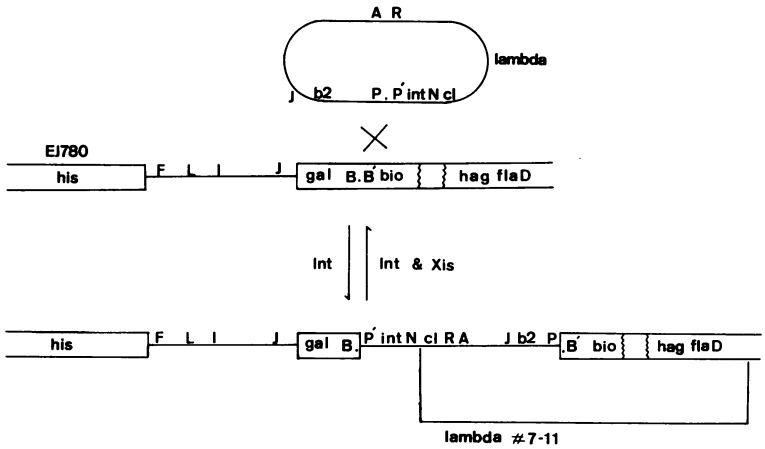

FIG. 3. *Possible origin of λcI857susS7dfla#7-11 phage from strain EJ780.*

total) was plated with a starved culture of the recipient strain EJ800 on NGA. Twenty-two motile transductants were obtained. Of these, 20 were temperature-sensitive for growth and had immunity. However, none of the transductants yielded plaque-forming λ phages upon heat induction, suggesting that they carried defective λ*fla* prophages. The defective transducing phages derived from strain KS899 could most likely have arisen by chromosomal deletion followed by an abnormal excision, because the DNA between the prophage λ genome and the *fla* genes is too large to be packed in a λ phage particle in a single event (Fig. 4). One of the *fla*$^+$, *imm*λ transductants thus obtained was designated strain EJ734. This strain is thought to carry part of the λ genome, including the *cI857* gene, in the *fla* cluster (Fig. 4). Therefore, we analyzed the remaining prophage markers by spotting 10^6 to 10^7 particles of a λ*sus* mutant or a λ*sus*$^+$ control on tryptone broth agar plates containing about 10^7 cells from an exponential-growth-phase culture of strain EJ734. The presence of a given allele resulted in confluent lysis at the location of the phage spot. This strain had λ gene *P*, *Q*, *R*, *A*, *B*, *F*, or *J*, but did not have the λ gene *N*. The presumed chromosomal sequence of strain EJ734 around the region III *fla* genes is shown in Fig. 4.

In order to isolate *fla* phages from strain EJ734, we introduced a λ*imm21int29* phage into this strain. The lysogens might possibly carry the λ*imm21int29* genes within the cryptic λ genes, because strain EJ734 had been deprived of the *att*λ gene. One of the λ*imm21int29* lysogens was purified. The presumed chromosomal sequence around the *fla* genes is shown in Fig. 4 (I) and (II). The prophage was induced with mitomycin C (0.5 μg/ml) at 42°C. A class of transducing phage, carrying bacterial DNA previously adjacent to the right end point of an integrated prophage, can be selected indirectly by ability to grow on a phage P2 lysogen (10). This variant (called Spi$^-$) has lost phage genes from the left end of the prophage map. From the known orientation of the prophage genes in the chromosome of strain KS899 (Fig. 4 and references 9 and 19), it is conceivable that some of the phages showing an Spi$^-$ phenotype may possess the *fla* genes. Therefore, the resultant lysate was plated on a Ymel derivative lysogenized with P2 phage. Of plaques appearing on the Ymel strain, clear plaques were found with a frequency of 2×10^{-7}. λ Phages of these plaques were picked with a metal loop and suspended in 2 ml of L broth. Each suspension was tested for the ability to transduce the *flaQ* gene into strain EJ983 (*flaQ*). Two (λ*pfla#3* and λ*pfla#36*) of the 40 Spi$^-$ phages tested were able to transduce the *flaQ* genes. These plaque-forming specialized transducing phages were found to have λ immunity, because these phages could make plaques on the λ*imm21* lysogen but not on the λ lysogen. A possible scheme for the derivation of these phages is depicted in Fig. 4. We can propose two gene orders for the chromosomal sequence of strain EJ734 carrying the λ*imm21int29* prophage, as a result of the λ*imm21int29* integration by means of Rec-mediated recombination. Scheme (I) shows recombination at a left-hand point of *cI* and (II) shows recombination at a λ gene located between *N* and *J* genes, as in Fig. 4. Because we selected Spi$^-$ phages carrying the *flaQ* gene and they had λ immunity, scheme (I) is more likely.

Characterization of the specialized trans-

ducing phages. The *fla* genes carried by λp*fla#3*, λp*fla#36*, λ*fla#7-11*, and λ*fla#14-41* were determined by transduction into various *fla* mutants (Table 1) nonlysogenic or lysogenic for λ. Since trail formations are recognized as criteria for complementation among *fla* genes (15, 16), we used them for examination of the *fla* genes carried by the HFT phages. And, if we use a λ lysogen as a recipient, the trails should be formed only when the HFT phage has a promoter of the *fla* genes. The results are summarized in Table 4. λp*fla#3* formed trails on nonlysogenic *flaE*, *A*, *P*, *Q*, and *flaR* mutants, and λp*fla#36* formed trails on *flaN*, *B*, *C*, *O*, *E*, *A*, *P*, *Q*, and *flaR*. Similarly, λ*fla#7-11* formed trails on nonlysogenic *flaD* and *hag* mutants, whereas λ*fla#14-41* formed trails on *flaQ* and *flaR* mutants. When lysogenic recipients were used, λp*fla#3* formed trails on *flaA*, *P*, *Q*, and *R*; λp*fla#36* formed trails on *flaN* through *flaR*, and λ*fla#7-11* formed trails on *flaD* and *hag* mutants. However, λ*#14-41* could not form

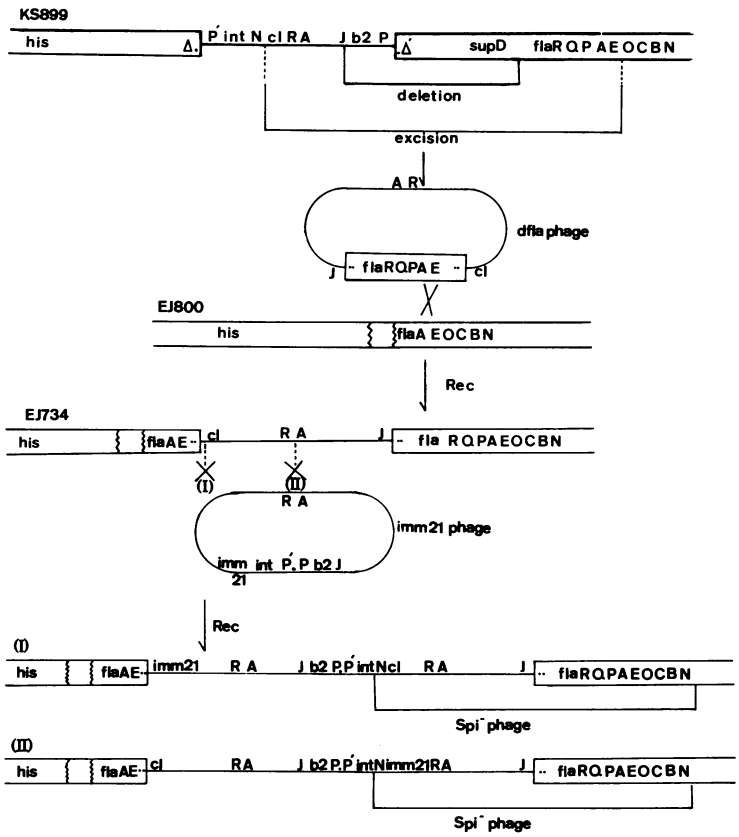

FIG. 4. *Possible origin of defective fla-transducing phages isolated from transductants of EJ800. (· ·) Undefined excision end points.*

TABLE 4. *Characterization of fla-transducing lambda phages* [a]

Strain	Complementation [b]											
	flaR	*Q*	*P*	*A*	*E*	*O*	*C*	*B*	*N*	*hag*	*D*	*I*
λp*fla#3*	⊕	⊕	⊕	⊕	+	−	−	−	−	−	−	−
λp*fla#36*	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−
λd*fla#7-11*	−	−	−	−	−	−	−	−	−	⊕	⊕	−
λd*fla#14-41*	+	+	−	−	−	−	−	−	−	−	−	−

[a] Mapping was carried out using *fla* mutants (Table 1) nonlysogenic or lysogenic for λ as recipients on NGA. Complementation was scored by trail formation (16).

[b] ⊕, Complementation with lysogenic and nonlysogenic recipients; +, complementation with only nonlysogenic recipients; −, no complementation.

trails on any of the strains tested.

To determine which parts of the phage genes were replaced by bacterial DNA in λ*fla#7-11* and λ*#14-41* phages, the following experiments were performed. The λ*fla#7-11* phage was used to infect nonlysogenic strain EJ977 (*hag*) at an MOI of 10^{-4}. Two percent of the motile transductants retained λ immunity but did not yield plaques on a lawn of Ymel (*supE, supF*). Hence, λ*fla#7-11* was a defective phage. It carries *cI857* and *susS7* and therefore can be designated as λ*cI857susS7*d*fla#7-11*. We then carried out a marker rescue experiment to see which of the λ genes remained intact in this transducing phage line. A single λ*cI857susS7*d*fla#7-11* lysogen was first superinfected with λ*susA*, λ*susJ*, or λ*imm434susN*, and then heat-induced. The resultant lysate from such a culture was plated on W3350 (Su^-) and Ymel (Su^+) as indicator strains. With λ*susA* and λ*susJ* as superinfecting phages, the progeny phages growing on strain W3350 were observed at a frequency of 5% of the parental phages growing on a permissive strain (Ymel). However, when λ*imm434susN* was used as a superinfecting phage, the progeny appeared at a frequency of less than 10^{-7} of the parent growing on the latter strain. These results indicate that λ*cI857*d*fla#7-11* harbors intact *A* and *J* genes but no intact *N* gene. Similarly, λ*fla#14-41* was also found to be defective in the *N* gene and can be designated as λ*cI857susS7*d*fla#14-41*.

DISCUSSION

Transducing phages carrying the region III flagellar genes *fla* and *hag* of *E. coli* K-12 have been isolated. With these phages, it may be possible to determine the polypeptides synthesized by the individual *fla* genes by analyzing the proteins directed by the phage DNA in vivo (11, 16) or in vitro (22). Preliminary in vivo experiments using the method described (16) were carried out with λp*fla#36* and λ*#7-11*. So far, we could identify the production of flagellin (molecular weight, 54,000) from λ*#7-11* bacteriophage (Y. Komeda, unpublished data). Biophysical methods for locating genes (21) may also be applicable to these *fla* genes. Analyses using these methods will provide more precise information on gene arrangement and interaction. The results shown in Table 4 are interpreted as follows: *flaE* of λp*fla#3* and *flaQ* or *flaR* of λ*fla#14-41* do not have their own promoters. Therefore, these genes are expressed only by read-through from the λ promoter. This interpretation of the complementation analysis with these specialized transducing phages conforms with that of the transcription sequences described by Silverman and Simon (18).

In principle, all the genes of interest could be inserted into the λ phage genome with lysogens carrying a secondary λ attachment site as reported by Shimada et al. (14), followed by induction of an extensive deletion in the chromosome and by introduction of a new *att*λ gene by infecting with λ*cI857*p*gal8-bio69* phage. If an indispensable gene is deleted, a strain carrying the gene in question on an F′ element can be used.

We also demonstrated that the abnormal lysogen could produce transducing phages for the genes almost 1 min apart from the location of the prophage (9) on the *E. coli* map. These phages may be produced by an abnormal excision after a deletion of the chromosomal DNA between the prophage genes and the *fla* genes. If strict selection of transductants is performed by making use of a deletion strain as a recipient, we can obtain rare transducing phages.

Strains carrying the *att*λ gene adjacent to the *fla* genes were constructed in this study. These strains are of value for deletion mapping of regions II and III *fla* genes of the *E. coli* chromosome. If gal^+ survivors of a λ*cI857* lysogenic derivative are selected at 40°C, they probably retain the *att*λ gene adjacent to their deletion end points. By repeating this cycle, we were able to isolate *fla* deletion strains that had deletions extending into the region II *fla* genes. This kind of strain will be useful in genetic studies of other *E. coli* genes.

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