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Isolation and Characterization of Nondefective Transducing Lambda Bacteriophages Carrying *fla* Genes of *Escherichia coli* K-12

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In *Escherichia coli* K-12, 11 *fla* genes and a *hag* gene are located between *his* and *uvrC*, making two clusters at map positions 42.5 and 43.0 min. Nondefective transducing lambda phages for these genes were isolated. Low-frequency-transducing donors were constructed starting from lysogens of λcI857 in which the prophage is integrated at a secondary attachment site at 44 min on the *E. coli* map. Two strategies were used to delete the region between the prophage and the *fla* genes. (i) Deletion mutants of the *supD* locus between *fla* and the prophage were isolated by selecting for loss of Su1$^+$, an allele of *supD*. (ii) A strain with a deletion starting within the prophage and ending at a position close to the *fla* genes was isolated from heat-resistant derivatives of the lysogen. A lysogen of λ*b*2 was then constructed in which the prophage had integrated at the site of the defective prophage by means of recombination with residual lambda deoxyribonucleic acid. From low-frequency-transducing lysate of the donor strains thus constructed, either directly or in combination with a procedure that extends the loci transduced, various λp*fla*'s were isolated. λp*fla*L1 carries all nine *fla* genes at 43 min, and λp*fla*H14 carries *hag* and two *fla* genes at 42.5 min.

A flagellum is a unique motility apparatus of bacterial cells consisting of three parts: filament, hook, and basal body (8, 9). The filament, which extends into the extracellular space, is connected by the hook to the basal body embedded in the cell membrane. The filament consists of a single kind of protein, flagellin, coded by the *hag* gene. Hook and basal body genes have not been defined.

The regulation of the formation and function of the flagellar apparatus has been subjected to extensive genetic studies especially in *Salmonella typhimurium*, *S. abortus-equi*, and *Escherichia coli* (for reviews, see references 1 and 12). More than 20 genes have been identified that are involved in the formation and/or functioning of flagella, i.e., *fla*, *hag*, *mot*, and *che* genes (1, 12, 14, 16, 26-29, 33, 36). These genes have been mapped in several regions of *E. coli* chromosome (Fig. 1). *fla* genes in the *his-uvrC* region were defined by Silverman and Simon (16-28) and by Kondoh and Ozeki (16). Although they can be aligned (Fig. 1), their correspondences have not been established.

The *fla* genes are defined by mutants lacking flagellar filament. Other parts of the flagellum also may be defective in the *fla* mutants. However, the actual role of each *fla* gene has remained largely unknown. Kondoh and Ozeki isolated mutants of *E. coli* that are defective in the synthesis of various *fla* gene products (16), and also attempted to derive specialized transducing phages that could supply gene products missing in the mutants. I report here the isolation of lambda transducing phages carrying the *fla* genes in the *his-uvrC* region. These phages were isolated as nondefective, plaque-forming phages.

(A preliminary account of this work has been presented [H. Kondoh and H. Ozeki, Jpn. J. Genet. 50:473, 1975].)

MATERIALS AND METHODS

Media. Lambda broth containing 1% tryptone (Difco) and 0.25% NaCl at pH 7.0 was routinely used for cultivation medium. Minimal medium was that of Davis and Mingioli (6) supplemented with 1 μg of thiamine per ml and 0.4% glucose. EMB-maltose was eosin-methylene blue medium (18) supplemented with 0.4% maltose. Solid agar media contained 1.2% agar (Wako), and the top agar contained 0.4% agar. Lambda motility agar was lambda broth supplemented with 0.3% agar. PCC motility agar contained 1% peptone (Difco), 0.5% Casamino Acids (vitamin free, Difco), 0.2% sodium citrate, and 0.28% agar.

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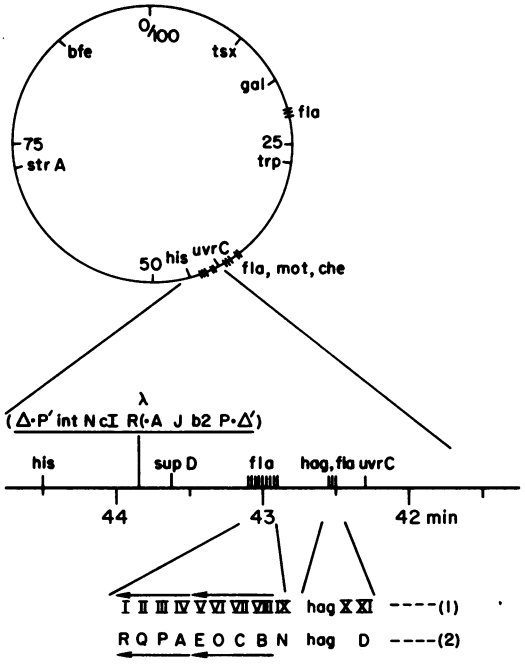

FIG. 1. *Genetic map of E. coli (4) with genetic markers relevant to the present study. Lambda prophage map in parentheses indicates location and orientation of prophages in KS899 and HK548. Arrows indicate transcriptional units and the direction. (1) fla genes defined by Kondoh and Ozeki (16; this work and unpublished data); (2) fla genes defined by Silverman and Simon (26-28).*

Bacteriophages. All of the specialized transducing phages for *fla* genes are the derivatives of lambda carrying a thermosensitive repressor gene *c*I857 (30). A mixture of λ*c*I and φ80*imm*λ*c*I was used to select the lambda lysogens. λ*b*2*c*I857*int*2 was employed in the course of the isolation of transducing phages carrying *fla* genes at 42.5 min. φ80pSu3$^+$ (2) was used to identify the amber mutations. T4*am*BU33 (10) was used for scoring Su1$^+$ activity. P1*vir* (13) was employed for generalized transduction by the procedure of Kondoh and Ozeki (16). Other bacteriophages are φ80*imm*λ (34), λ*h*, T6, and BF23.

Bacterial strains. The bacterial strains used are listed in Table 1. All are derivatives of *E. coli* K-12. The construction of several of these strains is described below.

(i) Strain HK548. An amber mutation in *tsx*, the gene for the receptor of T6, was introduced in strain W3623H *fla-95* (*his*$^-$ *trp*$^-$ *fla*II$^-$) to obtain strain HK510. This mutant was isolated from the spontaneously occurring T6-resistant mutants as one that exhibited very poor growth on agar medium containing φ80pSu3$^+$ and T6 as compared with growth on a medium containing φ80pSu3$^+$ alone. In the same way, strain HK511 was prepared as the *bfe*$^-$(Am) derivative of HK510. Strain HK521 was prepared by infecting HK511 with P1*vir* grown on CR63, selecting Fla$^+$ recombinants on PCC motility agar, and scoring Su1$^+$ activity by cross-streaking with T4*am*BU33 on lambda agar. HK521 is a Fla$^+$ Su1$^+$ joint transductant and is sensitive to both T6 and BF23 bacteriophages due to the amber suppressor. HK531 was a φ80*imm*λ lysogen of HK521 in which the prophage is integrated at the *att* site for φ80 closely linked to *trp*. HK531 [*his*$^-$ *trp*$^-$ *str*^{r}

TABLE 1. *Bacterial strains*

Strain	Genotype[a]									Prophage	Reference/ source
	his	*supD*	*fla*	*trp*	*gal*	*tsx*	*bfe*	*str*	Others		
W3623H *fla*	−	+		−	−	+	+	R		−	16
W3623 *del-4*	+	Δ	Δ[*fla*I-XI]	−	−	+	+	R		−	16
W3623 *del-92*	+	Δ	Δ[*fla*I-VII]	−	−	+	+	R		−	16
HK510	−	+	*95*	−	−	Am	+	R		−	This paper
HK511	−	+	*95*	−	−	Am	Am	R		−	This paper
HK521	−	Su1$^+$	+	−	−	Am	Am	R		−	This paper
HK531	−	Su1$^+$	+	−	−	Am	Am	R		φ80*imm*λ	This paper
HK548	+	Su1$^+$	+	+	−	Am	Am	R		λ*c*I857 at 44 min	This paper
NA12	+	Δ	Δ[*fla*I,II]	+	−	Am	Am	R		λ*c*I857 at 44 min	This paper
HfrH	+	+	+	+	+	+	+	+	HfrH	−	From H. Ozeki
KS899	+	+	+	+	Δ[*gal-att*λ-*bio*]	+	+	+	HfrH	λ*c*I857 at 44 min	16, 23 From K. Shimada
HK900 *del-52*	+	Δ	Δ[*fla*I-IX]	+	Δ[*gal-att*λ-*bio*]	+	+	+	HfrH	A segment of λ at 44 min	16
HK900 *del-92*	+	Δ	Δ[*fla*I-VII]	+	Δ[*gal-att*λ-*bio*]	+	+	+	HfrH	−	16
HK552	+	Δ	Δ[*fla*I-IX]	+	Δ[*gal-att*λ-*bio*]	+	+	+	HfrH	λ*c*I857 at 44 min	This paper
CR63	+	Su1$^+$	+	+	+	+	+	+	*lamB*	−	3; from H. Ozeki
W3110 (P2)	+	+	+	+	+	+	+	+		P2	From Y. Ryo
W3110 *polA*	+	+	+	+	+	+	+	+	*polA1*	−	7; from Y. Ryo
KY1340	−	+	−	Am	+	+	+	+	*recA met tyr*(Am) *arg*	−	20; from T. Yura

[a] Δ, Deletion mutation; Am, amber mutation; R, resistance.

bfe⁻(Am) *tsx*⁻(Am) Su1⁺ ϕ80*imm*λ] was mated with KS899, an HfrH derivative in which λc1857 is integrated between *his* and *supD* (16). His⁺ Trp⁺ recombinants were selected on minimal agar containing 250 μg of streptomycin per ml at 32°C. One such recombinant, lysogenic for λc1857, cured of ϕ80*imm*λ and inheriting Su1⁺, was designated HK548. Some of the heat-resistant derivatives from HK548 became resistant to both T6 and BF23 bacteriophages, presumably because the deletion of the *supD* locus leads to loss of Su1⁺ activity. This indicated that *tsx*⁻(Am) and *bfe*⁻(Am) mutations are inherited by strain HK548.

(ii) Deletion mutants of HK548. Deletion mutations were induced in strain HK548 by nitrous acid mutagenesis according to the procedure of Miller (18). Cultures of HK548 were treated with 0.05 M sodium nitrite in 0.1 M acetate buffer (pH 4.6) at 37°C for 15 min (the survival frequency of this treatment was about 10^{-4}). After growth of several generations at 30°C, a fraction was added to the mixture of the lysates of T6, BF23, λc1, and ϕ80*imm*λc1 to give a multiplicity of infection of more than 1 for each bacteriophage. These cells were plated in top agar on lambda agar to yield colonies at a frequency of about 10^{-3}. After incubation overnight at 32°C, most of the colonies were pale and mucoid. Compact colonies were picked, purified on EMB-maltose agar, and numbered with the prefix NA to be stored as the presumptive deletion mutants of the *supD* locus.

(iii) Strain HK552. Strain HK900 *del-52* is a heat-resistant derivative strain KS899 that contains a deletion starting from the inside of the prophage and extending through *fla*IX (16). HK552 was prepared by infecting HK900 *del-52* with λ*b2*c1857*int2* at a multiplicity of infection of about 10 and selecting cells immune to λc1 and ϕ80*imm*λc1.

Specialized transduction of Fla⁺ character. A culture of an *fla*⁻ recipient strain lysogenic for λh, to which $MgSO_4$ was added to 10 mM, was mixed with the lysate of *fla*-transducing lambda phages. The mixture was streaked on lambda motility agar and incubated at 37°C overnight. In some cases, the nonlysogenic recipient strain was infected simultaneously with both transducing phages and λc1857 (at a multiplicity of 10 for the latter) and incubated at 32°C. Abortive transductants produce "trails," whereas complete transductants produce "swarms" in motility agar. The complete transductants should consist of different classes: homozygotes in which the *fla* allele was replaced with that of the wild type carried by the transducing phage, heterozygotes in which transducing phage is inserted in *fla* genes by the aid of homologous recombination, and heterozygotes in which the transducing phage has recombined with λh prophage or λc1857 helper phage.

Assignment of *red* and *gam* characters. *Red* and *gam* characters of each lambda-derived phage were determined by examining the growth on *recA*⁻, *polA*⁻, and P2-lysogenic hosts according to the criteria shown in Table 2 (37, 38).

Measurement of buoyant density of phage particles in CsCl. A 1.4-ml portion of phage suspension was mixed with 1.5 ml of saturated CsCl solution at room temperature to give an average density of about 1.50 g/cm³. The mixture was centrifuged in a Spinco SW50-1 rotor at 21,000 rpm for 18 h at 20°C to obtain a nearly linear density gradient. Five-drop fractions collected from tubes punctured at the bottom were assayed for the phage titer. The center of the peak for each phage population was obtained from a Gaussian plot. Buoyant density was obtained from the peak position relative to those of λh and ϕ80c added as density references (1.508 and 1.493 g/cm³, respectively).

TABLE 2. *Assignment of red and gam characters of transducing lambda phages*[a]

Determination	*recA*	*polA*	(P2)
Wild type	+	+	−
red⁻	+	−	−
gam⁻	+	−	+
red⁻ *gam*⁻	−	−	+

[a] +, Plating efficiency was close to 1 relative to the case with the wild-type host; −, plating efficiency was less than 0.01.

Synthesis of bacteriophage-coded proteins in UV-irradiated cells (11). The procedures for the labeling of proteins by ^{14}C-amino acids and their analysis by electrophoresis were similar to those of Murialdo and Siminovitch (19) but with the following modifications. A culture of strain W3623H was irradiated by ultraviolet light (UV), infected, and incubated at 40°C. Proteins being synthesized were labeled from 5 to 30 min postinfection, solubilized in the sample buffer containing 1% sodium dodecyl sulfate by heating at 100°C for 5 to 20 min, and electrophoretically analyzed on sodium dodecyl sulfate slab gels.

RESULTS

Transducing phages carrying *fla* genes located at the map position of 43 min. (i) Construction of LFT donors. Deletion mutants of the region between the lambda prophage site and the *fla* genes were constructed to yield strains carrying *fla* genes contiguous with the prophage. One of the secondary attachment sites for lambda prophage is located at the map position of about 44 min (16, 23). It has also been shown that the *supD* locus is situated between *fla* genes at 43 min and the prophage (16; Fig. 1). Therefore, Su1⁺, one of the *supD* alleles with amber suppressor activity, could be used as a marker for the isolation of deletion mutants. Thus, strain HK548 was constructed with the characteristics Fla⁺ Su1⁺ *tsx*⁻(Am) *bfe*⁻(Am) and having the prophage λc1857 integrated at about 44 min. Strain HK548 has amber mutations in the genes for receptors of bacteriophages T6 and BF23 [*tsx*⁻(Am) and *bfe*⁻(Am)] which are suppressed by Su1⁺. This strain is sensitive to both phages but becomes resistant when it mutates into Su1⁺ by deletion. HK548 was treated with nitrous acid, a potent

mutagen for the generation of deletion mutations, and mutants resistant to T6, BF23, λcI, and φ80*imm*λcI were selected. The latter two phages were included to select the deletion mutants still retaining the prophage.

Three of 30 survivors of the phage infection, NA1, NA4, and NA12, yielded lysates that transduce the Fla$^+$ marker as anticipated, suggesting that the inactivation of the amber suppressor was the result of deletion mutations. The presence of deletions in two of these strains was confirmed by the fact that the *fla* genes I and II, the most proximate to *supD*, were deleted in NA4 and NA12. The properties of these low-frequency-transducing (LFT) donor strains are tabulated in Table 3.

NA12 is remarkable in two respects: lower burst size upon thermal induction compared with that of the parental strain HK548, and a high frequency of transduction of a Fla$^+$ marker by the lysate.

Although the small burst size of HK548 (ca. 10^{-3}) is expected since single lysogens at secondary attachment sites are characterized by inefficient *int-xis* functions (23), this factor alone cannot account for the magnitude of the observed decrease. It is quite conceivable that the deletion mutation starting from the inside of the *fla*II gene passes through *supD* and extends into the *b*2 region of the prophage, destroying the P·Δ′ structure required for the *int-xis* system to function (Fig. 2). In this circumstance, the prophage should be excised only through illegitimate recombination, which occurs at a very low frequency. The deletion mutation should have made the prophage and the remaining *fla* genes contiguous to each other (Fig. 2). The presence of the deletion as depicted was confirmed by heteroduplex analysis of the transducing phages (H. Kondoh and H. Yamagishi, unpublished data). Such a deletion mutation would also explain the high transduction efficiency of the lysate from NA12. Deletions of NA1 and NA4, on the other hand, should have left the P·Δ′ structure intact. We isolated *fla*-transducing phages from the lysate of NA12.

(ii) Isolation of plaque-forming transducing phages: N-series. When a culture of *fla*IV$^-$ (*am*75) lysogenic for lambda was infected with the induced phage lysate from NA12, a number of trails and swarms due to transductants were produced on motility agar. The agents that participated in the transduction should be hetero-

TABLE 3. *Properties of LFT donor strains*

LFT donor	*fla*	Complementation[a] with *fla* mutants: I	II	III	IV	VI	X	Phage yield per cell upon thermal induction	Frequency of abortive transduction of *fla*IV$^+$ per plaque-forming unit
HK548	+							2×10^{-3}	$<10^{-8}$
NA1	+							2×10^{-3}	2×10^{-7}
NA4	−	−	−	+	+	+	+	2×10^{-3}	5×10^{-7}
NA12	−	−	−	+	+	+	+	2×10^{-4}	5×10^{-4}

[a] Complementation test was carried out by P1-mediated transduction, as described by Kondoh and Ozeki (16). Representative *fla* point mutants were the donors of P1 lysate.

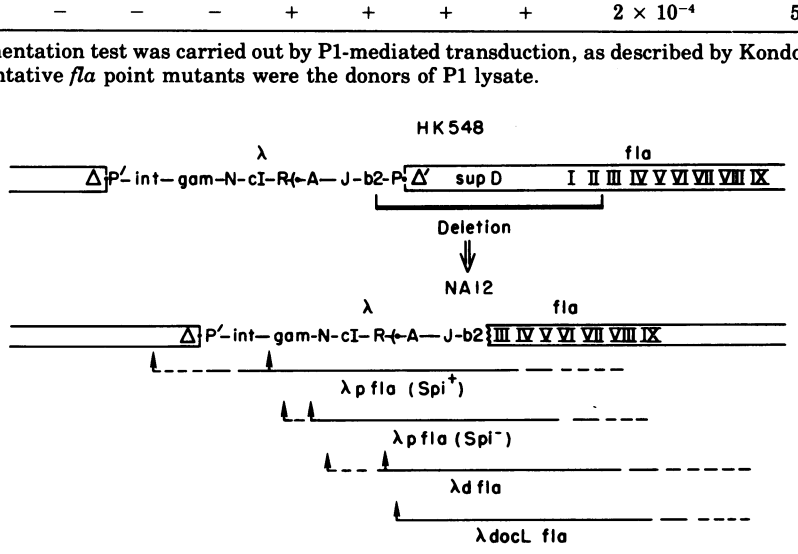

FIG. 2. *Mechanisms of the generation of the lysogenic strain NA12 and excision of fla-transducing phages.*

geneous and should consist of λp*fla*, λd*fla*, and λ*docL fla* (17). Nondefective λp*fla*'s were preferred because of the advantages in handling these phages. The contiguity of the *fla* genes and the prophage predicted that a considerable portion of the nondefective phages in the lysate would be λp*fla*. Phages in the lysate of NA12 were plated on the *fla*IV$^-$ (*am*75) indicator, and the cells at the plaque center were picked and stabbed in the lambda motility agar. One-eighth of the plaque centers produced trails and/or swarms, indicating that these plaques were those of λp*fla*'s carrying the *fla*IV gene. In this way, many transducing phages for the *fla* genes very close to the prophage were isolated as nondefective phages. However, this was not an efficient procedure to isolate transducing phages carrying a large number of *fla* genes. When a mutant of the *fla*VII gene (*fla-am*87) slightly more distant from the prophage than *fla*IV was employed as the indicator, only 1% of the plaques yielded transductants. The Spi phenomenon (38) was used to enrich for transducing phages carrying a larger number of *fla* genes. Wild-type lambda phage cannot form plaques on P2 lysogens, whereas *gam*$^-$ lambda phages escape from the P2 interference and form plaques. Transducing phages carrying a large number of *fla* genes would arise by substitution of a large area of the lambda genome including the *gam* gene (Fig. 2). From the lysate of NA12, several transducing phages carrying many of the *fla* genes at 43 min were isolated as plaques on a P2 lysogen [W3110(P2)]. One of them, λp*fla*N120, carried all of the *fla* genes at 43 min except for *fla*I and *fla*II, which had been lost in the original NA12.

(iii) Isolation of λp*fla*L1 carrying all of the *fla* genes at 43 min. The transducing phages isolated from NA12 carry transducing fragments for *fla* genes at 43 min with various right (*uvrC* side) ends but with a fixed left end point and lack *fla* genes I and II. An attempt was made to add *fla* genes I and II to the transducing fragment. An Fla$^+$ strain (HfrH) was infected with λp*fla*N120, and the lysogen occurring at a low frequency was isolated after selection for immunity to lambda. Since λp*fla*N120 lack all of the phage-coded recombination functions, λp*fla*N120 was integrated with the aid of the host *rec* function at the site of the host *fla* genes homologous to the transducing fragment on the phage (Fig. 3). The prophage that arose by integration of λp*fla*N120 should, therefore, be bracketed by the duplicated *fla* genes and should be excised mainly by the reverse process of integration. Such processes have been studied by Oka et al. (21) with φ80p*trp*. Since excision by the *rec* function is not very efficient, the lysate from the λp*fla*N120 lysogen was expected to contain at a relatively large number of phages that were excised by illegitimate recombination and that incorporated *fla*I and *fla*II genes (Fig. 3). In fact, the lysate was active in transducing *fla*I$^+$ and *fla*II$^+$. A procedure was devised to select for λp*fla*'s from a mixture of λp*fla*, λd*fla*, and λ*docL fla*, which mediate the transduction. A plate lysate was prepared from the phages induced in HfrH(λp*fla*N120) so that only nondefective phages grew several cycles. This procedure dilutes and inactivates defective phages. W3623 *del-92*, a strain with a deletion from *fla*I to *fla*VII, was then infected with the plate lysate and with λcI857, which may help the integration of λp*fla*'s. An Fla$^+$ complete transductant isolated on lambda motility agar was thermally induced and plated on an *fla*I$^-$ (*am*46) indicator. The observation that some of the plaques yielded Fla$^+$ transductants indicated that a novel transducing phage carrying *fla*I had been constructed. This transducing phage was denoted as λp*fla*L1.

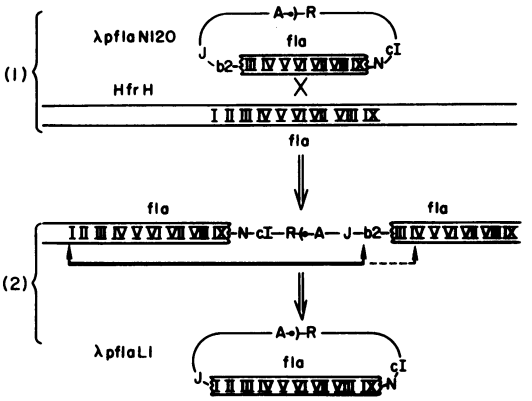

FIG. 3. *Mechanism of the generation of λpflaL1. The model involves two steps: (1) integration of λpflaN120 into the chromosome of HfrH; and (2) excision of λpflaL1 by illegitimate recombination at the sites indicated by arrows. The broken line indicates the case where the transducing fragment contains duplicated regions.*

If the novel transducing fragment of λp*fla*L1 was generated by the excision of the prophage as depicted in Fig. 3, the junction of the right end of the transducing fragment and the phage deoxyribonucleic acid (DNA) should be conserved; all of the *fla* genes at 43 min, from *fla*I to *fla*IX, should be carried by λp*fla*L1. In fact, λp*fla*L1 can transduce the Fla$^+$ character to a deletion mutant (*del-52*) lacking all of the *fla* genes at 43 min.

(iv) Construction of M-series transducing

phages. Once a transducing phage carrying *fla* genes I and II was isolated, it was easy to add the segment of *fla*I and *fla*II to the N-series transducing phages by recombination within the transducing fragment. Such recombinants were denoted by the numbers with prefix M (M-series transducing phages). For example, λp*fla*M1204 was constructed by the following procedures. An *h* mutant of λp*fla*L1 bearing a mutation in the J gene was isolated as a plaque on CR63. λ*fla*L1*h* was then crossed with a Gam$^+$ transducing phage λp*fla*N204, carrying *fla*III and *fla*IV, by mixed infection. Recombinants were isolated as those that could form plaques not only on CR63, but also on a *recA*$^-$ indicator strain on which λp*fla*L1 does not form plaques. Plaques on CR63 ensure that the left half of the phage genome originated from λp*fla*L1*h*, and those on a *recA*$^-$ strain indicate that the right half came from λp*fla*N204 (Fig. 4). Recombinants between the *h* and *gam* markers occurred with a frequency of about 3%, which was mostly attributed to recombination within the transducing fragment. This is in agreement with results of Kondoh and Yamagishi (unpublished data) indicating that the left end of the transducing fragment is very close to the J gene. Other M-series transducing phages carrying *fla*I and *fla*II were similarly constructed.

Transducing phages carrying *fla* genes at 42.5 min: H-series. Since deletion mutants isolated from strain HK548 were not useful for the transduction of *fla* genes at 42.5 min, another strategy was used to construct an LFT donor strain. Deletion mutants of *fla* genes were isolated as heat-resistant derivatives of KS899 in which λ*c*I857 is integrated at 44 min as a prophage (16). Some deletions ending at a point near the *fla* genes at 42.5 min may start from the inside of the prophage. This deletion should remove all of the killing functions of the prophage but not necessarily all of the prophage genome. Integration of lambda phage into the chromosome of such deletion mutants will be directed by the homology of lambda DNA sequences (Fig. 5), since the *fla*-deletion mutants from KS899 have deleted the primary attachment site for lambda as well. Lysogens of λ*b*2c1857*int*2 made from the deletion mutants of KS899 were examined for the ability to yield LFT lysates for the *fla*X gene. The *int*$^-$ mutant was used to prevent the integration at the secondary attachments sites, and the *b*2 deletion was used to help incorporation of a large transducing fragment into phage genome. It was found that one such lysogen (HK522) originating from HK900 *del-52* yields LFT lysate for the *fla*X marker. Thus, a part of the lambda prophage was retained in HK900 *del-52*. Considering the arrangement of prophage genes in KS899 (Fig. 1), the lambda segments retained in HK900 *del-52* should correspond to the *int*-side end. It should be remembered that all of the *fla* genes at 43 min are deleted, whereas the *fla* genes at 42.5 min remain intact in HK900 *del-52*.

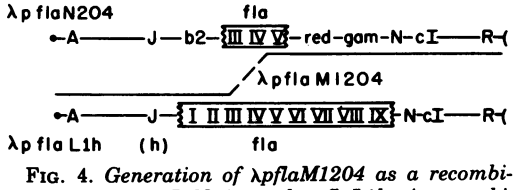

FIG. 4. *Generation of λpflaM1204 as a recombinant between λpflaN204 and λpflaL1h. A recombinant with Red$^+$ and h characters was isolated.*

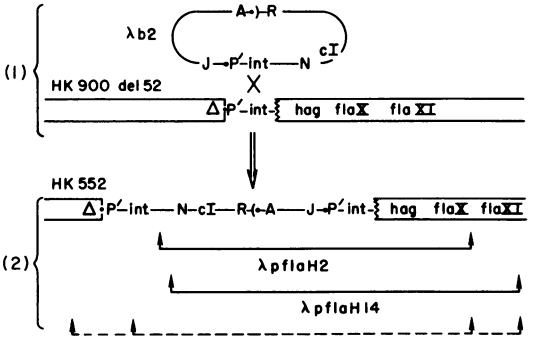

FIG. 5. *Mechanism of the generation of λpflaH2 and λpflaH14. The model involves two steps: (1) integration of λb2cI857int2 into the chromosome of HK900 del-52 at the int side of the lambda segment; and (2) excision of λpflaH2 and λpflaH14 by illegitimate recombination at the sites indicated by arrows. The broken line indicates the cases where transducing fragment is interposed between duplicated int-side lambda segments.*

A heat-induced lysate was prepared from strain HK552, and the phages were passed through several cycles of growth to yield a phage lysate. This procedure, as in the isolation of λp*fla*L1, was expected to enrich for nondefective transducing phages. A culture of the *fla*X$^-$ (*am*76) mutant was infected by the phages in this lysate and by λ*c*I857 added to facilitate the integration. A number of transductants were isolated on lambda motility agar. Phages were induced from the transductants, and the plaque centers on the *fla*X$^-$ indicator were examined for the ability to give rise to Fla$^+$ transductants. In this way, two λp*fla*'s, H2 and H14, were isolated. λp*fla*H2 carries *fla*X, and λp*fla*H14 carries both of the two *fla* genes, *fla*X and *fla*XI, at 42.5 min.

Characterization of λp*fla*'s. The *fla*-transducing phages were characterized with respect to the *fla* genes that they carry, the phage-coded general recombination genes, and buoyant density of the phage particles in CsCl. The results (Table 4) give an insight into the topology of the transducing phage genome.

The number of *fla* genes carried varied from one to nine depending on the transducing phage. Assuming that *fla* genes are incorporated into the transducing fragment from *his*-proximal genes in N- and H-series phages, *fla* genes were arranged linearly. Hence the gene order I-II-III-IV-V-VI-VII-VIII-IX-X-XI was established, in which orders III–IV, VI–VII, and X–XI had not been determined in the previous study (16). λp*fla*L1 carries all of the *fla* genes I to IX (43 min), whereas λp*fla*H14 carries both *fla*X and *fla*XI (42.5 min). Introduction of the both phages into a deletion mutant strain, W3623 *del-4*, which lacks all of the *fla* and *hag* genes of the *his-uvrC* region, restored flagellation and motility. Therefore, the regions transduced by λp*fla*L1 and λp*fla*H14 include not only all of the *fla* genes of the *his-uvrC* region, but also the *hag* gene. In fact, if λp*fla*H14 was generated by the mechanism depicted in Fig. 5, the *hag* gene should be carried by λp*fla*H14 and by λp*fla*H2.

The general recombination genes *red* (*exo* and *bet*) and *gam* are often deleted from the transducing phages, probably because of substitution by the transducing fragment. This is especially true in cases where a large number of *fla* genes are carried. This fact indicates that the transducing fragment is located in the central region of the phage genome. This is consistent with the mechanism of generation of λp*fla*'s proposed in Fig. 2 and 5.

The buoyant density of the phage particle gives an accurate estimate of the transducing phage genome size (5). Genome size varied from 85.9% (λp*fla*N111) to 103.3% (λp*fla*N156, λp*fla*N129) relative to wild-type lambda. It is known that the encapsulation of DNA is inefficient, and the resultant head is unstable in lambda-derived phages having genome sizes outside of this range.

The genome of λp*fla*N111 is reduced in size by as much as 14.1% compared with that of wild-type lambda. The observation that this transducing phage is *red*$^-$ *gam*$^-$ suggests that the *int-gam* region is deleted. The reduction in genome size estimated from deletion of the recombination region alone is from 10 to 15% (5, 32). Considering the three *fla* genes carried, λp*fla*N111 should have an additional deletion. Thus, the deletion of a part of the *b*2 region in the prophage of NA12 as proposed in the foregoing section is strongly supported.

Phage λp*fla*N120 carries the largest transducing fragment among the transducing

TABLE 4. *Characteristics of λpfla's*

λp*fla*	*fla* genes carried[a] at:											Recombination genes		Buoyant density in CsCl (g/cm^3)	Gain or loss of genome size relative to wild-type lambda[b] (%)
	43 min									42.5 min					
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	*red*	*gam*		
N156	−	−	+	−	−	−	−	−	−			+	+	1.5118	+3.3
N158	−	−	+	+	−	−	−	−	−			+	+	1.5023	−4.8
N151	−	−	+	+	−	−	−	−	−			−	−	1.4930	−12.5
N203	−	−	+	+	−	−	−	−	−			+	+		
M1203	+	+	+	+	−	−	−	−	−			+	+		
N111	−	−	+	+	+	−	−	−	−			−	−	1.491	−14.1
N202	−	−	+	+	+	−	−	−	−			+	+	1.5084	+0.4
N204	−	−	+	+	+	−	−	−	−			+	+		
M1204	+	+	+	+	+	−	−	−	−			+	+		
N201	−	−	+	+	+	−	−	−	−			−	+		
N121	−	−	+	+	+	+	−	−	−			−	−	1.4986	−8.0
N125	−	−	+	+	+	+	+	−	−			−	−	1.503	−4.2
N129	−	−	+	+	+	+	+	−	−			−	−	1.5118	+3.3
N112	−	−	+	+	+	+	+	−	−			−	−	1.5112	+2.7
N120	−	−	+	+	+	+	+	+	+			−	−	1.5104	+2.1
L1	+	+	+	+	+	+	+	+	+			−	−	1.5092	+1.0
H2										+	−	−	+	1.511	+2.6
H14										+	+	−	−	1.5076	−0.3

[a] +, At least a part of the gene is carried as judged by recombinant formation with a mutant of the gene; −, no recombinant.

[b] Calculated according to equation 6 of reference (5).

phages originating from NA12. This phage has a genome that is 102.1% that of wild-type lambda and is *red*$^-$ *gam*$^-$. The presence of another *red*$^-$ *gam*$^-$ phage, λp*fla*N129, having a smaller transducing fragment and larger genome size (103.3%) than λp*fla*N120 indicates that the right terminus of the transducing fragment substitution goes beyond the *gam* gene, since the *gam* gene occupies only 1% of the lambda genome (32). In addition, the deletion of a portion of the *b*2 region being considered, the size of the transducing fragment of λp*fla*N120 is estimated to be more than 15% that of the whole lambda genome.

In case of H-series transducing phages, the sizes of genomes are close to that of wild-type lambda, in spite of *b*2 and additional deletions in the region of recombination genes. Therefore, the transducing fragment should be larger than the *b*2 deletion.

Proteins synthesized in UV-irradiated cells. Proteins coded by the genes on the transducing phages can be labeled specifically with radioactive amino acids after infection of UV-irradiated cells and can be identified by sodium dodecyl sulfate-gel electrophoresis (11). Proteins labeled after infection of λp*fla*L1, λp*fla*H2, and λ*c*I857 were compared (data not shown). Six protein bands having apparent molecular weights of 48,000, 40,000, 38,000, 36,000, 30,000, and 27,000 were specific to λp*fla*L1. On the other hand, two bands having molecular weights of 60,000 and 26,000 were found with λp*fla*H2. The hypothesis that the protein of molecular weight 60,000 is flagellin is supported by the observation that heating the sample at 100°C for more than 10 min created the three bands characteristic of flagellin (15).

DISCUSSION

A prerequisite for the generation of specialized transducing phage from a lysogen is that the gene to be transduced must be close to the integration site of the carrier prophage (for a review, see reference 22). Distant genes can be made contiguous with the prophage by transposition of either the genes or prophage. One way of accomplishing the transposition utilizes the secondary attachment sites for lambda phage scattered throughout the chromosome of *E. coli* (23). Having failed in an attempt to insert lambda prophage into *fla* genes, I utilized the lysogens in which thermoinducible λ*c*I857 is integrated at one of the secondary attachment sites at 44 min near to the *fla* genes (16; Fig. 1). The *fla* genes at 43 and at 42.5 min were, however, still too distant from the prophage to be transduced. I, therefore, isolated a mutant with a deletion of the region between the prophage and the *fla* genes.

Two techniques were used to delete the region between the prophage and the *fla* genes. (i) Deletion mutants of the *supD* locus between *fla* and the prophage were isolated by selecting for loss of Su1$^+$, an allele of *supD*. (ii) A strain with a deletion starting within the prophage and ending at the position close to the *fla* genes was isolated from heat-resistant derivatives of the lysogen. Then, a lysogen of λ*b*2 was constructed in which the prophage had integrated at the site of the original prophage by means of recombination with residual lambda DNA.

From the LFT donors thus constructed, a number of transducing lambda phages carrying *fla* genes were isolated as nondefective phages (λp*fla*'s). They carry the *fla* genes of the two clusters between *his* and *uvrC*. All of the *fla* genes in the cluster at the map position of 43 min are carried by λp*fla*L1, and those in the cluster at 42.5 min are carried by λp*fla*H14. Other transducing phages carry parts of these clusters.

The essential part of the isolation procedures of λp*fla*L1, λp*fla*H2, and λp*fla*H14 involves the two steps: (i) integration of the circular genome of the infecting phage by a single recombination event, which is directed by the homology between the phage and host DNAs; (ii) excision of a new phage by illegitimate (nonhomologous) recombination. In the case of λp*fla*L1, the first integration process took place by recombination between the transducing fragment of λp*fla*N120 and the homologous region of the host genome, whereas in the case of λp*fla*H2 and λp*fla*H14, the homology between a segment of λ*b*2 and a residual lambda DNA sequence in HK900 *del-52* provided the site of recombination. The prophage genome integrated by such a homologous recombination event should be interposed between the duplicated homologous DNA sequences (Fig. 3 and 5). When the prophage genome is excised by illegitimate recombination to give rise to a new nondefective transducing phage, there would be a chance that the excised phage also includes the duplicated homologous DNA sequences (Fig 3 and 5, broken lines). Such transducing phages should contain a transducing fragment bracketed by homologous DNA sequences and, hence, would be genetically unstable in that they lose the transducing fragment during the course of phage growth; the transducing fragment would be excised from the phage genome by homologous recombination. Such a phenomenon has been reported for φ80pSu3$^+$ (2, 34, 35).

The nondefective transducing phages gener-

ated as described above and carrying transducing fragments bracketed by homologous DNA sequences are characterized by the following two points. (i) The genome of the new transducing phage is larger than the genome of the original phage. (ii) Excision of the transducing fragment creates a phage genetically identical to the original phage, except for the possible exchange of the mutations. The following facts suggest that λp*fla*L1, λp*fla*H2, and λp*fla*H14 do not carry duplicated regions bracketing the transducing fragment. The genome of λp*fla*L1 (101.0% of lambda) is smaller than that of λp*fla*N120 (102.1% of lambda), whereas λp*fla*H2 and λp*fla*H14, which are *red*$^-$, do not segregate Red$^+$ phage at a detectable frequency (less than 10^{-4} in phage stocks). The genome structures without duplicated regions were confirmed by heteroduplex analysis of transducing phages (Kondoh and Yamagishi, unpublished data).

More than half of the *fla* genes are located in the *his-uvrC* region (Fig. 1); these have been subjected to extensive genetic analysis (16, 26–28). Specialized transducing phages are, of course, beneficial in the more detailed analysis, especially of the regulatory elements. The results of the genetic analysis with λp*fla*'s will be presented in another paper.

The importance of the *his-uvrC* region is emphasized by the inclusion of the *hag* gene, the structural gene for flagellin. Regulation of flagellin synthesis is complex and must be coordinated with the flagellum morphogenesis. This is suggested by the observation that flagellin-specific messenger ribonucleic acid was not detected in mutants of a number of different *fla* genes (31). Genetic evidence in this paper argues that the *hag* gene is carried by λp*fla*H2 and λp*fla*H14. Supporting this is the synthesis of flagellin-like protein observed in the UV-irradiated cells infected with λp*fla*H2. These transducing phages could be a powerful tool for the study of the *hag* gene by providing specific *hag* DNA useful as template for transcription in vitro and for hybridization experiments.

After this work was completed, papers appeared reporting the construction in vitro of transducing phages carrying some of the flagellum-related genes (24, 25). Synthesis of flagellins in UV-irradiated cells was also reported.

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